Imparting size, shape, and composition control of materials for nanomedicine

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This *tutorial review* presents an overview of strategies for the synthesis and fabrication of organic nanomaterials, specifically those with potential for use in medical applications. Examples include liposomes, micelles, polymer–drug conjugates and dendrimers. Methods of driving shape *via* "bottom-up" synthetic approaches and thermodynamics and kinetics are discussed. Furthermore, methods of driving shape *via* "top-down" physical and engineering techniques are also explored. Finally, a novel method (referred to as PRINT) used to produce nanoparticles that are shape-specific, can contain any cargo, and can be easily modified is examined along with its potential future role in nanomedicine.

Introduction

The design and exploitation of materials and structures where at least one dimension is measured in the nanometer range broadly defines the term "nanotechnology."[1] Under the umbrella of nanotechnology, a variety of research disciplines are necessarily involved, ranging from the fabrication of nanomachines, to the application of nanolithography, to the development of nanoparticles. Nanoparticles both organic and inorganic based, have applications in a variety of fields including catalysis, photovoltaics, and coatings as well as in the emerging field of nanomedicine where they can be used as imaging agents and drug-delivery vectors. This review is intended as an introduction to the current methods of synthesis and fabrication of organic nanoparticles and their particular application in nanomedicine.

Inorganic nanoparticles are traditionally synthesized using nucleation and arrested growth strategies from a solution.[2–6] To produce structures that are not solely spherical, researchers typically use additives or sacrificial templates to yield exotic shapes. The peripheral surface of the resultant inorganic materials produced can be further modified. Alternatively,

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Larken E. Euliss

Larken E. Euliss was born in Greensboro, North Carolina. In 1999 she obtained her B.S. degree in chemistry with distinction under the direction of James D. Martin at North Carolina State University. Her undergraduate research included the synthesis of metal halide analogs of zeolite frameworks. She then attended the University of California at Santa Barbara for her Ph.D. under the direction of Galen D. Stucky and Timothy J. Deming where her dissertation encompassed the principles employed by Nature in the biological assembly of biomaterials as inspiration for developing completely synthetic and novel composite materials. During her graduate career she also worked with Christopher B. Murray as an IBM Research Intern Fellow applying these principles. She completed her doctorate work in 2004. Presently, she is post-doctoral fellow working under the direction of Joseph M. DeSimone at the University of North Carolina at Chapel Hill on the combination of imaging and targetable controlled delivery of nanomaterials employing the PRINT platform.

Julie A. DuPont

Julie A. DuPont was born in Worcester, Massachusetts. In 2000, she obtained her B.A. in Chemistry from the College of the Holy Cross in Worcester. Her undergraduate research project investigated the preparation of molybdenum and tungsten dipeptide complexes with Schiff base ligands under the guidance of Dr Richard S. Herrick. She then attended the University of Delaware in Newark where she worked in the group of Dr Charles G. Riordan. During the tenure of her graduate career, she investigated the coordination chemistry of thioether-supported, low-valent cobalt complexes. She completed her Ph.D. in 2005. Currently she is working as a post-doctoral fellow at the University of North Carolina at Chapel Hill where her research is centered on the design and fabrication of functionalized polymeric nanoparticle platforms that combine targeted drug delivery with imaging by utilizing the techniques from imprint lithography.

Table 1 The methods outlining the positive and negative attributes of each of the primary techniques of bottom-up and top-down synthesis of nanoparticles

Method	Material	Size	Functionalizable?	Loadability	Scalable?
Nucleation[2–6]	Inorganic	<100 nm	Minimal	No	Yes
Synthetic[8–40]	Organic	10 nm–μm	Yes	Minimal	Yes
Microfluidics[41,42]	Organic composites	μm	Minimal	Minimal	Perhaps
Top-down (photolithography) lithography[44,46]	Organic composites	nm–μm	Yes	Yes	No
Imprint lithography PRINT[51,52]	Organic composites	nm–μm	Yes	Yes	Yes

organic based nanoparticles are usually produced *via* emulsion and inverse emulsion techniques or are 'grown' in a "bottom-up" fashion using synthetic methodologies. These materials can traditionally be modified with ease and cargos can be kinetically 'trapped' into or covalently attached to the cores of the structures. In addition to the bottom-up approaches, "top-down" approaches using microfluidics and lithography have been used to produce organic nanomaterials as well. Recently, research exploiting imprint lithography techniques borrowed from the electronics industry offers the ability to control size, shape, cargo, matrix composition, and functionalization.

Nanomaterials: An overview of synthesis and fabrication

Nanotechnology can be defined as technology that is developed at the atomic, molecular, or macromolecular scale.[1] This size range (from 1–1000 nanometers) allows for the creation and use of structures, systems and devices that have novel properties with atomic-level control. This type of fine control has been exercised by Nature for centuries. Take for example the abalone shell,[7] this living organism controls the organization of inorganic constituents on the molecular, and even atomic scale by using electrostatic interactions, hydrogen bonding, disulfide bonding, and other interactions that can be present between supramolecular organic assemblies and inorganic components. Many scientists would like to reproduce this type of fine control in the laboratory by fabricating nanomaterials and devices that possess atomic precision on every level such as cargo, surface chemistry, shape, size, and matrix. Therefore, one important current trend in materials development is toward the control over these aspects of novel nanomaterials. It is no surprise that considerable effort has been devoted to the design and fabrication of such materials, but it is clear that the scientific community has only "scratched the surface" with respect to nanostructure complexity, composition, and function. Traditional approaches simultaneously combine synthesis, processing, and shape control to develop materials that have advanced capabilities in sensors, electronics, and information processing applications. The growing convergence of physical sciences with biology offers extraordinary biomedical research opportunities and may have a revolutionary impact in the medical field. In this review, we will present a synopsis of the various methods of organic nanomaterial synthesis which can be broadly split into two main techniques, "bottom-up" and "top-down" nanomaterials. The methods are summarized in Table 1 which outlines both the advantages and limitations of each of these techniques.

Stephanie Gratton

Stephanie Gratton was born in 1981 in Peterborough, Canada. In 2000 she obtained her BSc degree in chemistry from McMaster University (Canada). Stephanie is currently studying for a PhD in polymer science at the University of North Carolina at Chapel Hill. Her research centers on the cellular delivery of biocompatible, monodisperse, shape-specific polymeric nanogels produced using PRINT technology.

Joseph DeSimone

Joseph DeSimone, Ph.D., William R. Kenan Jr. Distinguished Professor of chemistry at the University of North Carolina at Chapel Hill (UNC-CH) and professor of chemical engineering at North Carolina State University, is a leading researcher in material science, especially as it pertains to the translation of breakthroughs in the basic sciences into practical applications. DeSimone has published over 200 peer-reviewed scientific articles and has almost 100 patents in his name. In 2005 DeSimone was elected into the National Academy of Engineering and the American Academy of Arts and Sciences. DeSimone was also the recipient of the 2005 ACS Award for Creative Invention. Dr DeSimone received his B.S. Degree in Chemistry in 1986 from Ursinus College in Collegeville, Pennsylvania and his Ph.D. Degree in 1990 from the Department of Chemistry at Virginia Polytechnic Institute and State University where he worked with Professor James E. McGrath. At UNC-CH, his research efforts are focused on developing synthetic pathways for polymer synthesis and processing in liquid and supercritical carbon dioxide. DeSimone recently launched Liquidia Technologies along with former members of his laboratory, to commercialize these recent breakthroughs from his laboratory for use in micro- and nanofluidics, soft lithography and nano-fabrication of colloidal particles and displays. These results from DeSimone's laboratory most recently became a foundation for the Carolina Center for Cancer Nanotechnology Excellence funded by the National Cancer Institute. DeSimone is the co-PI of this newly established Center.

Organic nanoparticles: Limited control over size and shape

The use of micro- and nanoscopic ''vessels'', such as micelles, vesicles, liposomes, and hollow spheres are the subject of intense research, particularly in the emerging field of nanomedicine, ranging in applications from gene delivery, drug delivery,[8] and waste removal.[9] In this section, we will touch on the cutting edge methods for the synthesis of organic nanoparticles, which will include: supramolecular self-assembled aggregates (such as lipid micelles and vesicles), polymeric nanoparticles, DNA–polymer conjugates, and dendrimers. Micelles and vesicles typically consist of surfactants or block copolymers, where the intrinsic differences in chemical potential of the coordinated polymer fragments permit the stabilization of the interface between the solvent medium and the final structure.[10] These components have been used to organize a wide array of highly stable and responsive vesicles that can be used for the transport and delivery of therapeutic agents. While several approaches exist for the synthesis of such organic-based nanoparticles, they are all based on ''bottom-up'' synthetic methods which rely on concepts of self-assembly. One important advantage of these materials is that the composition can be controlled and modified with ease and cargos can be partitioned into the cores of the structures. Liposomes, and more broadly, micelles, have been produced by a range of both natural and synthetic amphiphilic polymers leading to nano-scale structures and, specifically the use of liposomes in medical applications has received a great deal of attention in recent years. These membrane structures, composed of a phospholipid bilayer surrounding an aqueous or hydrophilic core, show exceptional biocompatibility and thus a great potential for clinical use as pharmaceutical carriers, particularly in the treatment of cancer. Indeed, several liposome-based drugs are currently in clinical trials or already on the market such as Doxil®, Ambisome®, and Daunoxome®. The seminal work by Bangham demonstrated that when placed in an aqueous system, phospholipids will self-assemble to form bilayer or multilayer structures.[11–13] Since then, several models have been introduced for the synthesis of liposomes. Szoka *et. al.*[14,15] describe the formation of liposomes *via* reverse-phase evaporation. Here, vesicles were formed by introduction of an aqueous phase to a mixture of phospholipid and an organic solvent which is subsequently removed *via* evaporation. Furthermore, Finer *et al*. demonstrated a bilayer rearrangement into vesicles by using a sonication procedure. He described the breakdown of multilamellar particles of egg yolk lecithin by sonication into fragments which then reaggregated to form single shelled vesicles.[16] Research in this area has led to many important improvements in the synthesis of liposomes such as the ability to increase circulation time, augment cargo load, and tether pendants to the surface.[17] Possible methods to attain long-circulating liposomes and therefore increased drug accumulation in the desired target areas, include coating the surface of the liposome with polyethylene glycol (PEG),[18] which is a commercially available and biocompatible polymer that promotes an enhanced retention and permeability (EPR) effect.[19] Recent research by Zalipsky *et al*. has focused on the synthesis of a PEG-coating that can be separated from the liposome at low pH levels as found in tumor cells.[20,21] Additionally, chemistries have been developed to modify liposomes *via* surface conjugation with proteins, peptides, or other biologically-relevant molecules. Reactions between a carboxyl group and an amino group, for example, leads to the formation of amide bonds on the surface of liposomes allowing for surface interaction with relevant proteins.[22] Moreover, other surface ligands, such as folate, have been used to modify the surface of liposomes.[23] For example, the anticancer drug, doxorubicin loaded into folate-modified liposomes, has been successfully delivered into tumor cells *via* a receptor-mediated method and demonstrated higher cytotoxicity.[24]

Related to liposomes, shell-cross linked knedel (SCK) structures have been shown to be biocompatible, stable, and able to carry a pharmaceutical cargo.[25,26] Wooley *et al*. report on the modification of SCK's with functional groups (azido or alkynyl) on the shell or core domains of the micelles and SCK's. The introduction of reactive groups to these nanostructures allows for further interaction with biologically-relevant substrates (Fig. 1A).[27] The SCK's were produced *via* addition of water to a THF solution of polymer followed by dialysis against water yielding SCK's with a narrow size dispersion. Further functionaliztion was carried out by a condensation reaction of an azido of an alkynyl-functionalized primary amine with the acrylic acid residues within the PAA shell of the micelles.[27] Shen *et. al.* report on a correlated group of organic nanoparticles composed of amphiphilic brush copolymers. These core–surface cross-linked micelles were produced *via* a solvent displacement method with an acetone–water system. The stability of these brush-copolymer derived micelles is due to the partial cross-linking on the core surface.

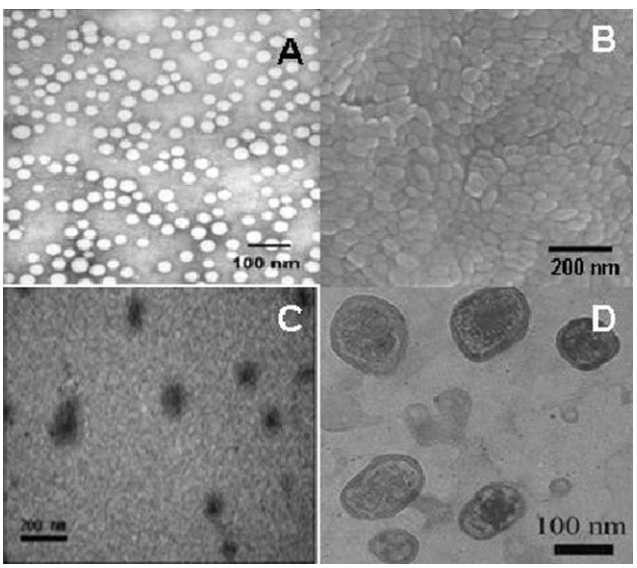

Fig. 1 Conventional delivery vectors. A) Shell cross-linked knedels (SCKs); B) non-spherical nanoparticles synthesized *via* a ''miniemulsion'' technique; C) 200 nm cationic nanogel delivery vectors produced *via* inverse microemulsion polymerization. Conventional delivery vectors: TEM micrograph of liposomes. Reprinted with permission from ref. 17, 27, 39 and the author (Fig. 1B). Copyright (2002, 2005) American Chemical Society.

The surface properties of these micelles can be readily modified for enhanced targeting for the drug delivery applications of these nanoparticles.[28]

The methods described yield liposomes, micelles, and vesicles that permit the encapsulation of various pharmaceutically-relevant cargos and allow modification of the periphery with various targeting ligands. Despite the many advantages posed by these fabrication techniques, some significant challenges still exist. These include restricted payload size, lack of robustness, fast elimination from the blood, and accumulation in the liver. Additionally, these self-assembled structures are limited to spherical shapes and offer limited control over size and dispersity. Although liposomal vectors exhibit promising *in vitro* transfection efficiencies when used in gene therapy applications, they often exhibit poor *in vivo* pharmacokinetics profiles and formulation instability. The pharmacokinetics of the *in vivo* administration of cationic liposome–DNA complexes indicate that the complexes are rapidly eliminated from plasma.[29] The elimination is triggered by serum protein binding to the vector followed by reticuloendothelial system uptake. Covalent addition of hydrophilic flexible polymers, such as poly(ethylene glycol) (PEG), to the surface of these liposomes decreases protein binding, increasing the *in vivo* circulation times. Many of these systems, however, still have unacceptable formulation stability. In addition to these contemporary delivery systems, the ability to incorporate a variety of imaging beacons that are both shape and site specific, while simultaneously monodisperse, has proven to be unattainable.

While the spherical shape of micelles and liposomes is determined by external forces such as surface tension, several methodologies are currently being investigated for the synthesis of non-spherical polymer nanoparticles. Huck and coworkers describe the synthesis of the first non-spherical liquid-crystalline polymeric nanoparticles utilizing a miniemulsion technique. Here, a main-chain liquid crystalline polymer (MCLCP) was dissolved in chloroform and mixed with water and a surfactant and followed by ultrasonication to form a miniemulsion. A suspension of polydisperse nanoparticles ranging from 30–150 nm was formed after the evaporation of the solvent (Fig. 1B). These particles spontaneously take on an ellipsoidal shape with high aspect ratios. This shape can be further controlled by altering the temperature.[30]

Additional directions in the search for the ideal organic nanoparticle are the design and synthesis of polymer conjugates. Polymer–drug (or protein) conjugates are hybrid structures that tend to be water soluble (due to the control of the chemical composition of the polymer), can be tumor specific *via* the EPR effect, and tumor targeting ligands can decorate the polymer portion of the conjugate, and can be captured by endosomal cellular uptake.[31,32] The typical synthetic strategy for the fabrication of polymer-conjugates has involved the modification of polymer chain ends after polymerization to form reactive end groups. Recent research in this area has led to the development of a more straighforward method which involves utilizing protein-reactive initiators in the polymer synthesis.[33,34] For example, Bontempo and Maynard report on a new synthetic strategy using strepavidin as an initiator for polymerization of *N*-isopropylacrylamide to prepare a modified polymer that is conjugated to strepavidin at the biotin binding sites only.[34] These nanomaterials offer additional alternatives in the field of nanomedicine but they also struggle with some challenges. For example, the conjugates need to be comprised of a high molecular mass, biodegradable polymeric matrix so they can better exploit the EPR effect. Also, the drugs to be delivered need to be covalently attached to the polymeric carrier, which sometimes requires a slight variant of the desired drug to facilitate such covalent conjugation. In addition, the linker between the drug and the carrier needs to be degraded to release the drug at the right time or in the desired location in order to optimize the efficacy of the system.

Dendrimers are yet another class of polymeric nanoparticles that have found use in biological applications ranging from drug delivery to tissue repair. Dendrimers are polymers that are branched (as opposed to conventional linear polymers) where the branches radiate in a symmetric fashion from a central core.[35] While they can be made from many different polymeric materials, dendrimers found in biological applications are usually based on polyamidoamines, polyamides, carbohydrates or polypeptides.[35] Dendrimers are typically grown in a step-wise fashion and generally, are synthesized using either a divergent or convergent fabrication method. Tomalia *et al*. comment on the fabrication of starburst dendrimers *via* the divergent method resulting in exponential-like growth.[36] A convergent technique was used by Frechet and Gitsov where dendrons were grown first and then attached to the core in a subsequent step.[37] Dendrimers can supply a valuable architecture to the realm of nanomedicine, however these conjugates lack complete control over shape, size and mondispersity. Specifically, higher generations of dendrimers tend toward an increase in polydispersity due to defects upon formation. Additionally, the ability to load large amounts of therapeutics, targeting moieties, and imaging modalities has proven difficult.[38]

Inverse microemulsion techniques have been employed in the synthesis of polymeric nanoparticles for the ability to create submicron hydrophilic polymer particles with improved polydispersities for use in drug delivery applications. The DeSimone group[39] utilized inverse microemulsion polymerization techniques to synthesize stable, biocompatible polymeric nanogels less than 200 nm in size, for antisense and gene delivery to HeLa cells *via* the exploitation of charge (Fig. 1C). These spherical particles showed a narrow size distribution with polydispersity of less than 10% for the non-ionic hydrogels (Fig. 1C).[39] Furthermore, Horgan and Vincent describe a method of producing 5–15 nm sterically stabilized organic nanoparticles *via* an inverse microemulsion technique. This method allows for facile surface modification as well, leading to the possibility of these particles to be used for drug-delivery and other biomedical applciations.[40]

Utilizing engineering techniques to control size and shape of materials

The ability to control the matrix material, as well as the size and shape of nanomaterials is an important goal as materials science approaches the nanometer regime. Organic based

nanomaterials have the ability of highly diverse matrices as well as the ability to tether pendants to the surface and/or encapsulation of materials into the polymeric cores but little control over shape and size. In this section, we will present the various engineering methods that material scientists have exploited to control the shape, size and matrix material of the nanomaterials.

The use of ''top-down'' synthesis enables the engineering of fabrication techniques that can produce a myriad of shapes and sizes composed of a variety of materials. Microfluidics offers the ability to control the synthesis of non-spherical particles (Fig. 2). For example, Doyle and co-workers[41] have utilized shearing forces of a photopolymer in a continuous water phase at a specifically-designed microfluidics junction (fabricated by pouring poly(dimethylsiloxane) (PDMS) on a silicon wafer containing positive-relief channels patterned in a SU-8 photoresist) to produce non-spherical uniform polymer particles on the micron scale (Fig. 2B and 2C). By varying the speed of the 'shearing' liquid, one can control the dimensions and shape of the resulting droplets. The polymeric (PEG based) disc-shaped particles produced are on the order of 16 μm with a diameter of 40 μm. Additionally, Whitesides *et al.*[42] have applied a similar method to control the size of monodisperse particles (20 to 1000 μm) by utilizing a microfluidics device to not only photopolymerize the particles but thermally 'set' the particles into their defined shape based on the speed of the shearing material. By utilizing this method, they can also produce multi-component polymer-based beads. These beads have cargos that include: copolymers, fluorescent dyes, inorganic nanoparticles (CdSe), liquid crystals, and microporous particles. The loadablity of these polymeric particles provides the ability to use these materials as carriers of medicine.

In addition to microfluidics, materials scientists are using lithography for the fabrication of biologically relevant

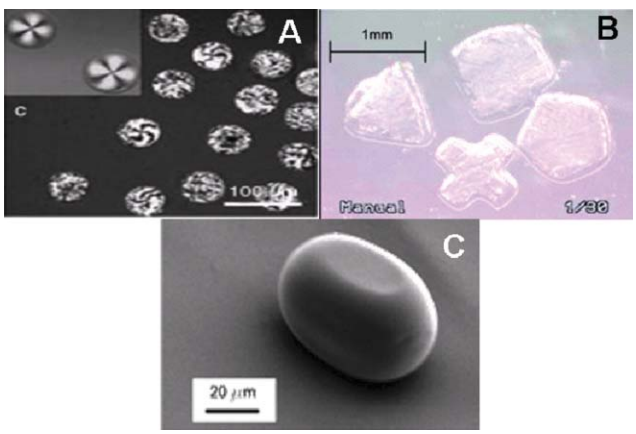

Fig. 2 Exemplary particles made using top-down techniques. A) Polarization microscopy image of 4-cyano-4′-pentylbiphenyl tripropyleneglycol diacrylate microspheres. Inset shows particle morphology at low polymerization rate. B) Poly(ethylene glycol) hydrogel with the incorporation of a designated sensing moiety. C) Non-spherical colloidal poly(ethylene glycol) hydrogel particle fabricated *via* microfluidics using a t-junction. Reprinted with permission from ref. 41, 44. Copyright (2004, 2005) American Chemical Society and Wiley 2005 (ref. 42).

nanomaterials and devices such as biosensor arrays. Lithography has traditionally used light (photolithography) generated by lasers or other sources of various wavelengths (365 nm, 248 nm, and 193 nm) to create images or patterns in formulated polymer films known as photoresists.[43] Once these patterns are generated, they are typically ''transferred'' into the underlying substrate using aggressive processing conditions including reactive ion etching processes.[43] Willson *et. al.*[44] have utilized this platform of photolithography to engineer discrete shapes (square, circle, triangle, cross) in the micron-to-millimeter regime. UV irradiation through a photomask was used to pattern photopolymerizable liquids into hydrogel materials that have discrete shapes. Unpolymerized materials were washed away, leaving freestanding cross-linked millimeter-size objects behind that corresponded to the shapes on the photomask. These objects were composed of a PEG hydrogel with the incorporation of a designated biosensing moiety and complimentary binding to the bioarray was monitored using fluorescence.

In the world of integrated circuits, photolithography has been the benchmark technique for pattern generation; however, it is becoming increasingly difficult for this technique to keep pace with the doubling of the number of transistors on an integrated circuit every 18 months as predicted by Moore's Law.[45] This is especially true now, as the feature sizes on integrated circuits are already far below the wavelength of the light used to make the images. To make patterns having extremely small feature sizes, researchers have had to make remarkable advances in photolithography that include using shorter and shorter wavelengths of light (*e.g.* 193 nm ArF and 157 nm F_2 lasers, extreme UV light and even X-rays), phase-shift masks, and immersion lithographic means. However, all of these approaches are enormously expensive and indeed it is expected that next generation photolithography tools will costs upwards of $30 million per tool. A typical electronics fabrication factory, referred to as a foundry, will require many such tools to generate the multilevel electronic devices in use today. This fact requires electronics companies, such as IBM and Intel, to invest billions of dollars on new factories. This method of photolithography to produce 'top-down' nanomaterials is neither cost-effective, nor user friendly, nor even necessary for use in nanomedicine.

In contrast, a new lithographic technique, imprint lithography, is being applied for the precise fabrication of next generation nanoparticles, integrated circuits and other electronic and photonic devices with sub-100 nm features.[29,45–48,51] Here, a very simple molding process is used in which a mask that contains shaped cavities is brought into direct contact with curable liquids to create features and patterns on surfaces or other functionalized substrates (Fig. 2). In principle, imprint lithography is orders of magnitude less expensive than traditional photolithographic methods used to make features that are smaller than the imaging exposure wavelengths available today. Hence, there is tremendous excitement over imprint lithography as a replacement for photolithography. However, one ubiquitous drawback of imprint lithography is the so-called flash layer or ''scum'' layer which interconnects all features made using imprint lithography (Fig. 3).[29,45–48] In microelectronics applications, the ''scum'' layer is typically

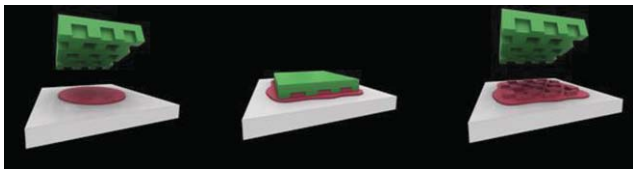

Fig. 3 Illustration representing imprint lithography with the resulting "scum layer".

eliminated by using harsh etching processes, such as reactive ion etching from an oxygen plasma (O_2-RIE) which works by bombarding a surface with an anisotropic stream of high energy particles that chemically ablate the resist uniformly to remove the scum layer.[49] While these processing methods are well established in the semiconductor industry where hard, robust, inorganic materials are the norm, they are certainly incompatible with delicate organic materials and those that contain biologically-derived moieties.

Simultaneous control over size, shape, function, and cargo: PRINT (particle replication in non-wetting templates)

The top-down approach of imprint lithography offers an engineering alternative to produce monodisperse shape-specific nanocarriers. In 2004, DeSimone and colleagues in the Chemistry Department at UNC reported a breakthrough in the materials and methods used in imprint lithography which enables the generation of sub-50 nm features.[51] They have shown that specifically-designed, photochemically curable perfluoropolyether-based elastomers (PFPEs)[51] could perform accurate nanometer-scale molding when used *in lieu* of traditional materials, such as quartz, glass, silicon and silicones, that are presently used in imprint lithography. PFPEs have such a low surface energy that removal of molded materials made from PFPE-based molds is facile. The performance of PFPEs in imprint lithography was first demonstrated using replica molds generated from master templates created at IBM's Almaden Research Center in California that had features with a width of 140 nm, a depth of $\sim$50 nm and a separation of 70 nm. The molds cast using the PFPE-based fluoroelastomer materials (0.4 nm roughness factor) maintained preservation of the nanoscale features of the patterned silicon wafer master. The features on the PFPE-based mold as determined by AFM had an average height of 51 nm, which was in excellent agreement with the measured 54 nm height of the features in the silicon master.

As mentioned previously, one of the drawbacks of imprint lithography is the ubiquitous scum layer that plagues this technique when it is based on traditional materials such as poly(dimethylsiloxane) (PDMS) (Fig. 3).[29,47,48] PFPE-based molds on the other hand, being so highly fluorinated, have surface energies that were measured to be 12 dynes cm^{-1}, far lower than that of PDMS (20 dynes cm^{-1}).[51] With such non-wetting, non-swelling characteristics, PFPE-based materials enable the generation of harvestable, scum-free objects, or particles, using what is referred to as particle replication in non-wetting templates or PRINT (Fig. 4). PRINT enables the

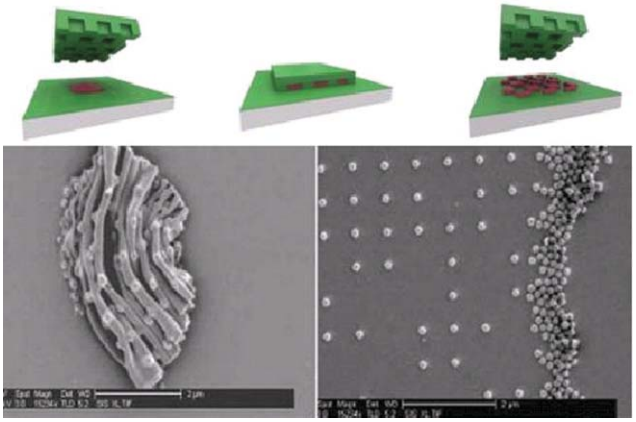

Fig. 4 Inset: Schematic depicting the PRINT technology. PRINT is able to produce isolated, harvestable "scum-free" objects because the highly fluorinated elastomeric mold and the substrate are both non-wetting producing isolated nanostructures. (Left) SEM image of the attempted harvesting of 200 nm particles made using traditional imprint lithography which results in the formation of a scum layer that eliminates the fabrication of free standing objects or particles. (Right) Particles made using PRINT and their harvesting using a doctor's blade demonstrating the fabrication of isolable, discrete objects. Reprinted with permission from ref. 52. Copyright (2005) American Chemical Society.

fabrication of monodisperse particles. PRINT utilizes the non-wetting properties of the highly fluorinated elastomeric mold and the substrate to produce isolated, harvestable objects with applications ranging from photovoltaics to drug delivery.[52] This is accomplished by taking advantage of the reversible seal that is formed between the mold and the substrate as slight downward force is applied. This is particulary important for the organic liquid to be molded has a contact angle greater than 90° with the fluorinated substrate. Under these conditions, the organic liquid to be molded is either confined within the shaped cavities of the mold or forced out due to the low surface energy of both the mold and the surface (Fig. 4). This methodology is a versatile and flexible method for the direct fabrication and harvesting of monodisperse, shape-specific nano-biomaterials. Unlike other particle fabrication techniques, PRINT is delicate and general enough to be compatible with a variety of important next generation cancer therapeutic, detection and imaging agents, including various cargos (*e.g.* DNA, proteins, chemotherapy drugs, biosensor dyes, radiomarkers, contrast agents), targeting ligands (*e.g.* antibodies, cell targeting peptides) and functional matrix materials (*e.g.* bioabsorbable polymers, stimuli responsive matrices, *etc*). PRINT is the first *general, singular* method capable of forming particles that: are monodisperse in size and uniform shape; can be molded into any shape; can be comprised of essentially any matrix material; can be formed under extremely mild conditions (and therefore is compatible with delicate cargos); is amenable to post functionalization chemistry for the bioconjugation of targeting ligands; and which initially fabricates particles in an addressable array (which opens up combinatorial approaches since the particles can be "bar-coded" using methods similar to DNA array technologies.[50] In contrast to the present methods that utlize microfluidics techniques for

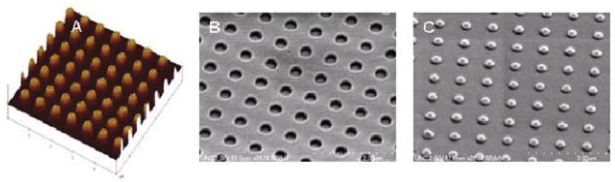

Fig. 5 A) Atomic force micrograph of a 160 nm post master; B) scanning electron micrograph of an unused, empty PFPE mold with 160 nm features (aspect ratio = 1 : 1); and C) a scanning electron micrograph of harvested PEG-composite particles on the medical adhesive sacrificial layer.

particle fabrication, PRINT has the ability for more breadth of sizes (>100 nm) and is more amenable to scalability.

In the PRINT process, a photocurable perfluoropolyether (PFPE) precursor is cast against the patterned master to make an elastomeric polymer mold.[52] The PFPE mold is brought into contact with a liquid precursor on a non-wetting substrate and pressure is applied to create a seal between the mold and the substrate. The monomer precursor is polymerized, and monodisperse, shape-specific polymer particles are obtained. The key to making uniform particles of a specific shape using PRINT is to have robust master templates that contain the repetitive features of interest. Currently, the repetitive rectangular features have sizes ranging anywhere from 500 μm to 70 nm, where the length can be varied in all three dimensions. The features are placed far enough apart so that sufficient room is left in between features in order to ensure enough room is available to manage excess liquid in the final PRINTing process when using liquids that have a contact angle greater than 90° with the fluorinated substrate. This is also balanced with the goal of closely packing as many features into an area as possible to increase the throughput of PRINT.

To demonstrate the scale-up possibilities with the PRINT process, a permanently etched master was made by transferring a repetitive, uniform shape from an epoxy based resist onto a silicon wafer using conventional photolithography and reactive ion etching processes. The pattern, now permanently etched into the wafer with well resolved entities (Fig. 5A), can be used repeatedly to make a large number of identical elastomeric PFPE replica molds by photochemically curing the dimethacrylate functionalized PFPE oligomer (Fig. 5B).[52] The PFPE replica molds were used to fabricate individual, monodisperse particles using the PRINT process (Fig. 5C), which were then harvested to produce colloidal suspensions.

To date, *monodisperse* particles from a wide range of particle matrix materials have been fabricated using PRINT. PRINT can be used to make such particles from poly(D-lactic acid) (PLA) and derivatives thereof such as poly(lactide-*co*-glycolide) (PLGA). It is well known that PLA and PLGA have had a considerable technological impact on the drug delivery and medical device industries because they are bioabsorbable and non-toxic.[53] Monodisperse PLA particles using PRINT were fabricated by treating a small amount of the cyclic lactide monomer, (3*S*)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione, with the FDA-approved polymerization catalyst, stannous octoate, at 110 °C in a PFPE mold designed to fabricate 200 nm particles. After polymerization was achieved, the PFPE mold and the flat, non-wetting substrate can be separated to reveal an array of monodisperse 200 nm trapezoidal particles (Fig. 6, middle).

Additionally, monodisperse, shape-specific 200 nm trapezoidal particles from poly(pyrrole) (Ppy) were generated. Ppy has been used in a variety of applications, ranging from electronic devices and sensors to cell-scaffolds.[54] The Ppy particles were fabricated in a one-step polymerization by placing a drop of a 1 : 1 v/v solution of THF–pyrrole and perchloric acid into the molding apparatus, followed by vacuum evaporation of the solvent. Monodisperse 200 nm Ppy trapezoidal particles as well as 3 μm arrows were fabricated and harvested (Fig. 6, right).

As stated previously, PEG is a material of tremendous interest to the biotechnology community due to its commercial availability, non-toxic nature, and biocompatibility (Fig. 7). PRINT can be used to produce monodisperse, nanometer and larger scale PEG particles in a wide range of compositions (*e.g.* with various crosslink densities of the hydrogel, with incorporation of cationically charged monomers, linking groups, *etc.*) by molding PEG–diacrylate liquid monomer

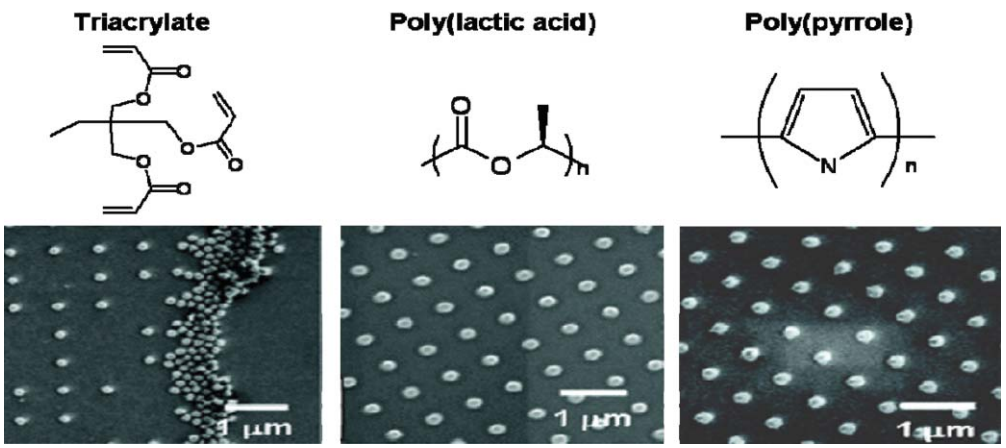

Fig. 6 SEM image of 200 nm monodisperse, shape-specific particles made from a highly cross-linked triacrylate resin (left); bioabsorbable poly(lactic acid) (middle), and conducting poly(pyrrole) (right). Reprinted with permission from ref. 52. Copyright (2005) American Chemical Society.

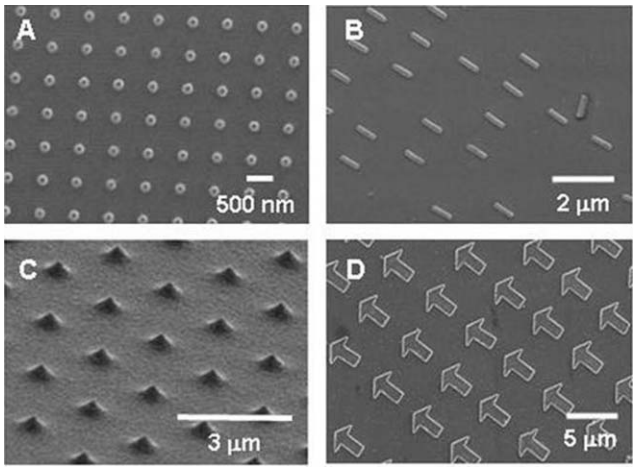

Fig. 7 Manipulation of shape using PRINT; A) 200 nm trapezoidal PEG particles; B) 200 nm × 800 nm bar PEG particles; C) 500 nm conical PEG particles that are < 50 nm at the tip; D) 3 μm arrow PEG particles. Reprinted with permission from ref. 52. Copyright (2005) American Chemical Society.

followed by room temperature photopolymerization. Because the morphology of the particles is controlled by the master, it is possible to generate any of the aforementioned monomer systems into particles on a variety of length scales.

By taking advantage of the delicate nature of PRINT, it is possible to incorporate a myriad of materials into the precursor PRINT solution prior to particle formation, including imaging contrast agents (superparamagnetic iron oxide particles), therapeutics (doxorubicin/paclitaxel/bortezomib), organic dyes (rhodamine), antibodies, proteins, and/or DNA (Fig. 8). These cargos may be encapsulated within the matrices of the PRINT particles using the gentle, non-reactive methods for forming particles *via* solvent evaporation.

The biological activity of specific cargos can be maintained during PRINT encapsulation. This has been confirmed by performing biotin-binding experiments with avidin-containing particles. Specifically, fluorescently-labeled avidin (CY-3 fluorescent dye) was encapsulated in 500 nm conical PEG–acrylate PRINT particles (70% PEG–diacrylate, 30% PEG–monomethacrylate) as described previously. The PEG–monomethacrylate was added to the particle formulation to increase the mesh size of the particles, which enhances the biotin diffusion rate. These avidin-containing PRINT particles were subsequently exposed to a fluorescein-labeled biotin solution for 30 min, washed several times with water to remove any unbound biotin, and then observed using confocal microscopy. As shown in Fig. 8, CY-3-labeled avidin (red) was colocalized with fluorescein-labeled biotin (green), which indicates preferential binding of biotin to the avidin-containing particles. Control experiments with PRINT particles that were identical in all regards, with the exception that they did not contain encapsulated avidin, showed no binding of biotin. These results suggest that biological biotin/avidin recognition is preserved during PRINT photoencapsulation, consistent with a variety of previous studies demonstrating the preservation of biological activity of proteins and DNA after UV photopolymerization within PEG–acrylate-based hydrogels.[55]

Previous literature[39] has shown that non-viral delivery vehicles used in *in vivo* applications required sizes at or below 200 nm in diameter for increased systemic circulation, providing less likelihood of the particle being trapped in the liver or spleen. Using NIH 3T3 cells, the effect of upper particle size on cellular uptake was established. Fig. 9 shows 5 μm (diameter) PRINT PEG–composite particles that were not taken up by these macrophage cells, while the 3 μm (diameter) PRINT PEG–composite particles were taken up by the embryonic cells. Furthermore, there was a noticeable increase in the amount of particle uptake with the sub-200 nm PRINT particles.[56] Based on results obtained from cell uptake studies, the nanoparticle matrix can be varied in order to obtain improved cellular delivery of genetic material as well as the release of the genetic material inside the nucleus.

Conclusions

Nanomedicine will be extensively exploited in the clinic once a nanoparticle system attains targeted delivery of a therapeutic agent as well as localization of the therapeutic agent within the cell. Indeed, great progress has already been made toward this goal. For example, liposomal nanocarriers such as Doxil®, Ambisome®, and Daunoxome® are already in clinical use

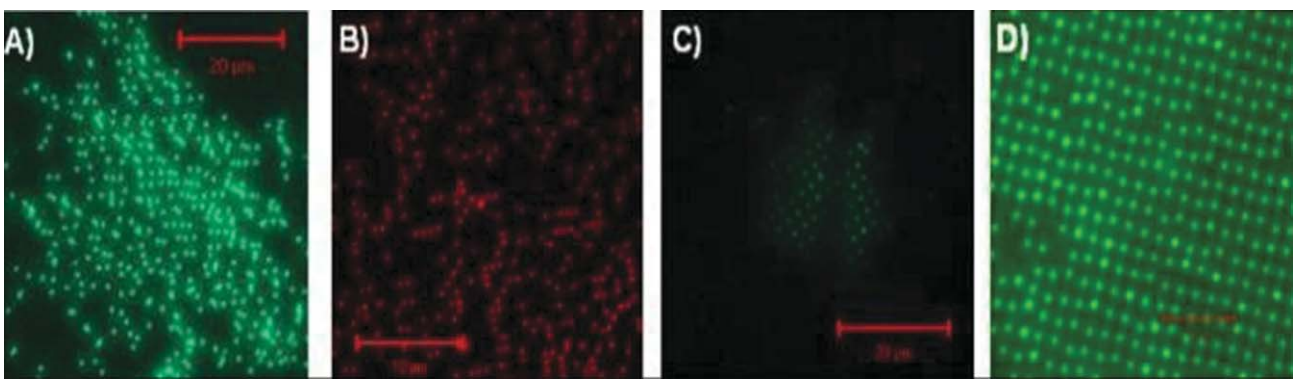

Fig. 8 Confocal images demonstrating the encapsulation of fluorescently-labeled A) avidin (66 000 Da); B) DNA; C) adenovirus (AAV); and D) doxorubicin. Reprinted with permission from ref. 52. Copyright (2005) American Chemical Society.

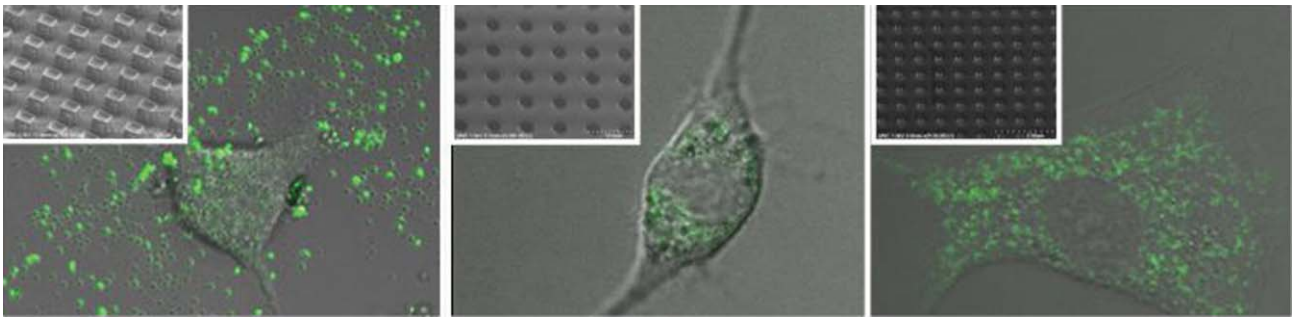

Fig. 9 Cellular (NIH 3T3 cells) uptake studies of fluorescently-tagged cationic hydrogel particles made *via* PRINT. (Left): 5 μm disc shaped particles showing no cellular uptake; (middle): 3 μm disc shaped particles showing extensive cellular uptake; (right): 160 nm cylindrical particles showing extensive cellular uptake. Inset: SEMs of the identified particles on the harvesting layer are also shown.[56]

in lieu of their free-drug counterparts in part because they offer enhanced effectiveness and lower side effects.

As with any newly emerging technology, there are important questions that must be addressed as this technology progresses. For example, ''what role does size and shape play on the biodistribution of these nanoparticles?'' and, ''How can size, shape and/or composition influence the efficacy of nanocarriers *in vivo*?'' Perhaps most importantly, the questions regarding the safety of delivery of nanoparticles *in vivo* need to be investigated thoroughly due to the fact of complete control over dispersity in size and shape. Industry-wide, this need has been recognized as indicated by the formation of a voluntary program which aims at collecting data on existing nanomaterials and subsequently assessing their risks.[57] Strategies are being discussed to fully and consistently characterize all aspects of nanoparticles such as size, shape, dispersity, composition, surface chemistry and more.[58] Not only does such a strategy need to be reliably implemented, but researchers from across the scientific spectrum, from materials and engineering to pharmacology and toxicology must fully collaborate to evaluate the safety and efficacy of these nanomaterials.

Nanotechnology brings exciting new possibilities to the field of medicine. One can envision nanocarriers that can be targeted to a specific tissue or cells to simulataneously detect and diagnose diseases as well as to treat them through the delivery of therapeutics. It is our expectation that nanomedicine will lead to more effecacious detection, diagnosis, and treatment of disease strategies than traditional methods in use today. The ideal nano-carrier will be one that is size- and shape-specific, has the ability to encapsulate fragile cargos, and has the flexibility to be functionalized with surface targeting ligands. Bottom-up approaches intrinsic to the synthesis of organic materials lack precise control over shape but offers excellent control over functionality. The top-down approach of microfluidics and photolithography can offer some shape control but with limited opportunities for shapes below 1 μm in size. Alternatively, the new emerging technique, PRINT combines some of the best elements from both bottom-up and top-down synthesis strategies, offering a highly versatile method for the production of isolated, monodisperse organic particles of nearly any size and shape that can contain delicate organic functional agents.

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