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(54) **Title**: PARTICLES FOR THE EFFECTIVE FABRICATION AND UTILIZATION OF MICRO- AND NANOARRAYS

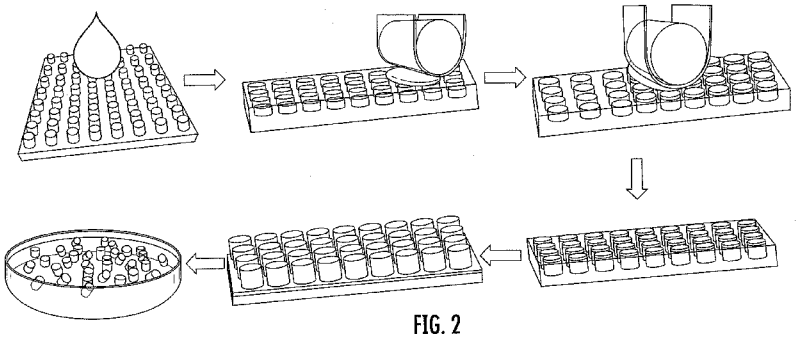


FIG. 2

(57) **Abstract**: Particles that may be used for the fabrication of micro- and nanoarrays are provided. The particles may comprise one or more target molecules to be delivered to cavities on a substrate. Methods for preparing the particles and for introducing target molecules to the cavities using these particles to produce a micro- or nanoarray are also presented. Additionally, methods of using these micro- or nanoarrays are provided.

PARTICLES FOR THE EFFECTIVE FABRICATION AND UTILIZATION OF MICRO- AND NANOARRAYS

FIELD OF THE INVENTION

Embodiments of the present invention relate to engineered particles that can be used to load target molecules into a micro- or nanoarray.

BACKGROUND OF THE INVENTION

Micro- and nanoarrays of biomolecules immobilized on solid surfaces are important tools for biological research, including genomics, proteomics, disease diagnosis, drug development, and cell analysis. Micro- and nanoarrays spatially sort molecular species so that that multiple species can be independently addressed. The most common arrays are DNA micro- and nanoarrays, made up of oligonucleotide probes attached to a solid surface that can be exposed to complementary targets in an unknown sample. DNA arrays may allow for the simultaneous quantification of thousands of DNA sequences in parallel. Protein micro- and nanoarrays use similar concepts and principals to DNA arrays. Other micro- and nanoarrays with similar formats include cell arrays, chemical compound arrays, and tissue arrays.

All typical micro- and nanoarray fabrication techniques target the same objective: efficient distribution of uniform, dense arrays of small droplets of target molecules. According to the spot formation techniques, methods are often categorized as “contact printing” and “non-contact printing.” Contact printing includes pin printing and microstamping, while non-contact printing, generally newer techniques, includes photochemical methods, inkjet, and electrospray deposition. The ideal fabrication technique for the micro- and nanoarrays must be versatile in sequence design and easy to fabricate in a reproducible manner while minimizing cost, solution volume, and impurities.

Contact printing techniques can be used to form arrays by directly contacting the printing device with the substrate. These techniques include a variety of pin printing, including nano-tip printing, and microstamping. Pin printing is a widely used technique for fabricating arrays for both small-scale laboratory and large-scale industrial use. Spot uniformity and positional accuracy are key, and affected by multiple factors including sample viscosity, pin contact area, surface properties of both

substrate and pin, substrate planarity, and the fabrication environment -- all which make reproducibility more difficult to achieve. Slight changes in hydrophilicity can change the spot size and shape to 50% or greater. Contamination and dust must be controlled to produce high-quality arrays with little risk of pin clogging. Nano-tip printing with scanning probe microscopes (SPM) allows the production of sub-μm spots achieving higher densities. Whether traditional pin printing methods or SPM-based methods are used, all methods of pin printing suffer commercially because they are serial printing techniques which are time consuming. An alternative to pin printing is microstamping, where hundreds of spots are printed in parallel using a polymeric stamp. While the microstamping process is simple and inexpensive, the amount of sample transferred from the stamp to the substrate is difficult to control reproducibly, depending on the amount and dispersion of ink transferred to the stamp, the contact pressure, and the control of solution concentrations.

In contrast, non-contact printing techniques can be used to form arrays without directly contacting the substrate. A variety of approaches have been developed for non-contact printing. For example, inkjet technology shows promise for inexpensive, high-throughput fabrication. However, there are significant drawbacks with the limitations of substrates to be used and the splashing and satellite droplets that cause contamination and irregular spot size. Electrospray deposition suffers from similar drawbacks, with the additional disadvantage that biologic species can be sensitive to the electric fields. Printing oligonucleotides using photolithography can produce very efficient high density arrays, although the method can be time consuming for patterning longer sequences and the failure of photodeprotection at any stage terminates the surface oligonucleotide. Microfeatures down to 16 μm^2 have been created using this technique.

Although there are a variety of micro- and nanoarray fabrication techniques that have emerged, no single technique has provided both an excellent quality array (uniform, high density spots) and significant throughput at reasonable cost.

One alternative means for the production of a micro- or nanoarray is to start with a two-dimensional surface comprising multiple microscale or nanoscale cavities such as that disclosed in U.S. Patent No. 7,476,503 to Turner et al. (2009), incorporated herein by reference in its entirety. The desired target molecule or molecules can be

loaded into the cavities to produce a micro- or nanoarray of target molecules. Although this method has advantages over other techniques for the production of micro- or nanoarrays, the efficiency with which the target molecule or molecules may be loaded into the cavities may be low. Only a relatively small percentage of the cavities in the array may be utilized. Further, some cavities may contain multiple target molecules, whereas others contain none. It would be advantageous to develop a means by which single target molecules or a plurality of molecules could be more effectively deposited into a cavity or cavities of a plate or other substrate to produce a more uniform micro- or nanoarray.

BRIEF SUMMARY OF THE INVENTION

Compositions and methods for loading target molecules into the cavities of micro- and nanoarrays are provided herein. Compositions of the present invention include both particles comprising one or more target molecules and tailored micro- and nanoarrays.

In one aspect of the invention is provided a method of introducing one or more target molecules into one or more cavities of a micro- or nanoarray, comprising the steps of preparing nanoparticles by placing a material comprising a liquid and further comprising one or more types of target molecules into a recess in a mold, hardening the material to make a particle, and removing the particle from the recess; delivering said particles into the cavities of the nanoarray, wherein the nanoarray itself can be another mold having predetermined cavities; and dissolving the matrix material comprising the particle, releasing the target molecule in the cavity. In some embodiments, the concentration of a target molecule in the liquid material to be placed into the recesses of the mold is configured to result in one target molecule per recess. In other embodiments, the liquid material is first cast into a thin layer, before being introduced to the mold, which further helps control the number of available target molecules per recess.

According to other embodiments, after the particles are removed from the mold recesses, the particles are screened to determine if they include zero, one, or more than one target molecule. In some embodiments, because the particles have highly uniform monodispersity, they can be analyzed by weighing, ultragradient centrifugation, chromatography, electrophoresis, micro- or nanofluidic separation, flow cytometry, combinations thereof, or the like to determine the difference in number of target

molecules per particle. In other embodiments, after the particle is formed, a larger molecule can be reversibly attached to the target molecule(s) of the particle to exemplify the difference of particles having no, one, or more than one target molecule associated therewith. In certain embodiments, after determining and sorting out the particles with only one target molecule associated therewith, the larger molecule can be removed and the particles can be utilized with the micro or nanoarray.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Having thus described the invention in general terms, reference will now be made to the accompanying drawings, which are not necessarily drawn to scale. The drawings are exemplary only, and should not be construed as limiting the invention.

FIG. 1 illustrates examples of particle geometries tailored to cavity geometries; and

FIG. 2 is a schematic view of a system capable of fabricating particles in accordance with various embodiments of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the inventions are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

The present subject matter broadly describes methods of delivering one or more target molecules of interest to cavities within micro- and nanoarrays. In particular, the

method involves using engineered particles comprising one or more target molecules to introduce the one or more target molecules to cavities within the micro- or nanoarray. The present subject matter further relates to two-dimensional surfaces that may be engineered such that they contain a plurality of cavities with specific sizes and/or shapes. The various sizes and/or shapes of the cavities and the engineered particles may be designed such that certain particles may be dispersed within certain cavities and not within others. The sizes and/or shapes of the cavities and particles may also be engineered to ensure that only one particle or a set maximum number of particles may reside in any single cavity, facilitating the delivery of a specified number of target molecules to the cavities.

In some embodiments of the present invention are provided particles, which may have predetermined geometries and sizes. Controlling the geometry and size of the particles may determine which cavity or cavities within a micro or nanoarray a given particle may enter. For example, a cylindrical particle would fit into a circular hole of a slightly larger diameter, and a cubic particle would fit into a square hole of a slightly larger size. In some embodiments, the cavity shapes and sizes and particle shapes and sizes are engineered such that more than one particle may enter a cavity. According to some embodiments, the plurality of particles includes a monodisperse plurality of particles wherein the broadest dimension of the particles in the monodisperse population can be any linear dimension between about 5 nm and about 200 nm; for example, from about 5 to about 50 nm, about 50 to about 100 nm, about 100 to about 150 nm, or about 150 to about 100 nm. In some embodiments, the particles have a linear dimension less than about 50 nm, less than about 100 nm, less than about 150 nm, or less than about 200 nm. For example, the broadest linear dimension can be selected to be about 20nm and accordingly the monodisperse plurality of such particles will have a broadest linear dimension of about 20 nm with less than about 25 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles may have less than about 20 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have less than about 15 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have less than about 10 percent difference in the broadest linear dimension between particles of the population. In alternative

embodiments, the particles can have less than about 5 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have less than 2 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have less than 1 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have between about 1 and 20 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have between about 1 and about 15 percent difference in the broadest linear dimension between particles of the population. In alternative embodiments, the particles can have between about 1 and about 10 percent difference in the broadest linear dimension between particles of the population. In some embodiments, particles are fabricated in an array of substantially congruent recesses thereby forming a plurality of substantially congruent particles. The linear dimension of the particle can be a predetermined dimension, a cross-sectional diameter, a circumferential dimension, or the like.

FIG. 1 illustrates various embodiments of engineered particles in accordance with the present disclosure and representative cavity shapes into which these particles may fit. The geometries and sizes of the particles are not limited. For other examples of particles comprising various geometric shapes that may be used according to the present invention, see for example U.S. Patent Application Serial No. 11/594,023 filed November 7, 2006, incorporated herein by reference in its entirety. The particles may comprise any material. For example, the particles may be prepared from a mixture comprising a polymer, a liquid polymer, a solution, a monomer, a plurality of monomers, a polymerization initiator, a polymerization catalyst, an inorganic precursor, an organic material, a natural product, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a ligand, a cell penetrating peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, a charged species, combinations thereof, and the like.

The particles may be fabricated by any means known in the art. In some embodiments, a particle of the present invention is fabricated in a polymer or non-wetting polymer mold. The mold may have cavities of a substantially predetermined shape. In some embodiments, a nanoparticle is fabricated by introducing a liquid composition into a mold cavity. A particle is formed from the composition in the

cavity by curing the liquid composition as described more fully below. The particles may then be extracted from the mold cavity. The molds, materials, and methods are described in greater detail below and in the references incorporated herein. In some embodiments, the non-wetting molds are fabricated from low surface energy polymer materials, such as, but not limited to fluorinated elastomer-based materials, fluoropolymers, perfluoropolyether, fluoroolefin, acrylates, silicone materials, polydimethylsiloxane, fluorinated styrene, triazine fluoropolymer, vinylidene fluoride, tetrafluoroethylene, hexafluoropropylene, 1,2,3,3,3-pentafluoropropene, chlorotrifluoroethylene, vinyl fluoride, and the like, including the methods and materials disclosed in the applicant's co-pending patent applications disclosed elsewhere herein and incorporated in their entirety by reference herein.

In preferred embodiments, fabrication of the particles involves a top-down micro- and nano-fabrication technique PRINT™ (Particle Replication in Non-wetting Templates), as generally shown in FIG. 2. See U.S. Publication No. 2009/0028910 to DeSimone *et al.*, filed December 20, 2004, which is incorporated herein by reference in its entirety. PRINT™ is a platform technology that enables the generation of engineered micro- and nano-particles having precisely controlled size, shape, chemical make-up and functionality. PRINT™ is the first scalable top-down fabrication process useful for making organic and inorganic, shape-controlled, engineered particles and two-dimensional arrays of particles. PRINT™ is amenable to continuous roll-to-roll fabrication techniques that can enable the scale-up of these new materials to practical levels for the building of various prototype devices. In this regard, unique particle shapes may be designed and fabricated using this continuous roll-to-roll nano- and micro-fabrication process. In some instances, the shape-specific engineered particles may comprise target molecules.

PRINT™ technology allows for predetermined engineering of the parameters of an ideal nanoparticle for a given application. PRINT™ technology utilizes liquid polymers or Fluorocur™ (Liquidia Technologies, Inc., Research Triangle Park, N.C.) to replicate micro or nano sized structures on a master template. The polymers utilized in the PRINT™ method can be liquid at room temperature and can be photochemically cross-linked into elastomeric solids that enable high resolution replication of micro- or nano-sized structures. The liquid polymer is cured while in contact with the master template, thereby forming a replica image of the structures on the master template.

Upon removal of the cured liquid polymer from the master template, the cured liquid polymer forms a patterned template that includes cavities or recess replicas of the micro or nano-sized features of the master template and the micro or nano-sized cavities in the cured liquid polymer can be used for high-resolution micro or nanoparticle fabrication. For more detailed description of the materials used to fabricate the molds of the present invention and methods of molding micro- or nanoparticles in these molds, see U.S. Patent Application Serial Nos.: 10/583,570, filed June 19, 2006, and 11/594,023 filed November 7, 2006; and PCT International Patent Application Serial Nos.: PCT/US04/42706, filed December 20, 2004; PCT/US/06/23722, filed June 19, 2006; PCT/US06/34997, filed September 7, 2006; PCT/US06/43305, filed November 7, 2006; and PCT/US07/02476, filed January 29, 2007; each of which is incorporated herein by reference in its entirety. See also, U.S. Provisional Patent Application Serial Nos.: 60/531,531, filed December 19, 2003; 60/583,170, filed June 25, 2004; 60/604,970 filed August 27, 2004; 60/691,607, filed on June 17, 2005; 60/714,961, filed September 7, 2005; 60/762,802, filed January 27, 2006; 60/798,858, filed May 9, 2006; 60/734,228, filed November 7, 2005; 60/757,411, filed January 9, 2006; 60/799,876, filed May 12, 2006; 60/833,736, filed July 27, 2006; and 60/828,719, filed October 9, 2006; each of which is incorporated herein by reference it its entirety. As the particles produced reflect the shape and size of the recesses of the molds, the molds may be specifically engineered such that particles of the desired shape and/or size are produced.

As shown in FIG. 2, the master template (grey) is fabricated using advanced lithographic techniques. A unique liquid fluoropolymer (green) is then added to the surface of the master template and photochemically crosslinked (top row, left), then peeled away to generate a precise mold having micro- or nanoscale cavities (upper middle). The low surface energy and high gas permeability of the PRINT mold enables liquid precursors (red) to particles to fill the cavities (top row, right) through capillary rise. The inter-connecting "flash" layer of liquid wetting the land area between the cavities is not formed (bottom row, right). Once the liquid in the mold cavities is converted to a solid, the array of particles (red) can be removed (bottom row, middle) from the mold (green) by bringing the mold in contact with an adhesive layer (yellow).

In some embodiments, the particles comprise one or more "target molecules." In preferred embodiments, each particle contains only about one target molecule. The

target molecules may be anything desirable of being delivered to a micro- or nanoarray. For example, the target molecules may be, but are not limited to, such molecules as DNA, RNA, or enzymes such as DNA or RNA polymerases. The target molecule or molecules may be attached to, contained therein or associated with the particle in any way. For example, the target molecule or molecules may be conjugated onto the surface of the particle or contained within the particle. In one embodiment, the target molecule or molecules may be physically entrapped within the particle due to interactions such as Van der Waals forces, electrostatic, hydrogen bonding, other intra- and inter-molecular forces, combinations thereof, and the like. The target molecule-containing particles may be prepared as described above. For example, in one embodiment, a population of particles may be prepared such that each has an average of one binding site, which may be any type of binding site (e.g., an amine functionality). Such particles may be prepared via the PRINT™ process described herein, using a matrix material which has included therein or thereon only one binding site. This population of particles may be reacted to attach the target molecule.

In some embodiments, the functionalized particle can be a particle that has been harvested from mold cavities and can be either an isolated free flowing three dimensional object or a particle coupled with or bound to a particular location on a film. In alternative embodiments, the functionalized particle can be a particle that remains within a mold cavity of a mold, such as the molds described elsewhere herein and in the patents and applications incorporated in their entirety by reference herein.

In some embodiments, the functionality of the particles can be a result of including a functional monomer, such as for example, an amine, into or in association with the particle. In alternative embodiments, the functionality of the particles can be achieved by including an amine functional monomer in the particle composition. In some embodiments, the functionality of the particles can be achieved by including an amine functional monomer with an acrylate or methacrylate polymerizable group in the particle composition. In other embodiments, the functionality of the particles can be achieved by including a carboxylate functional monomer in the particle composition. In some embodiments, the functionality of the particles can be achieved by including a carboxylate functional monomer with an acrylate or methacrylate polymerizable group in the particle composition. In some embodiments the functionality of the particles can be a surface functionality or a bulk material functionality.

In some embodiments the particle composition includes polymer component, a cross-linking agent, and an amine functionalizing agent. According to the present invention, the particle composition can mean an isolated particle after harvest from the mold cavity or the particle composition in the mold cavity prior to harvesting. In some embodiments, the polymer component includes a polymer selected from the group consisting of, but not limited to, poly(methyl methacrylate) (PMMA), acrylamide, or the like. In some embodiments, the amine functionalizing agent includes, but it not limited to, methacrylamide hydrochloride or the like. In some embodiments, the cross-linking agent includes, but is not limited to, methylenebisacrylamide. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 85:10:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 10 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 80:15:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 15 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 75:20:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 20 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 70:20:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 20 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 65:30:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 30 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 60:35:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 35 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 55:40:5 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 40 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 50:40:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 40

percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 40:50:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 50 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 30:60:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 60 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 25:65:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 65 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 20:70:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 70 percent amine functionality. In some embodiments, the polymer:amine functionalizing group:cross-linking agent components includes a ratio of about 10:80:10 polymer to amine functionalizing group to cross-linking agent and can result in a particle with about 80 percent amine functionality.

In some embodiments, the particle composition substantially includes only an amine functionalizing group and a cross-linking agent. In such embodiments the components includes a ratio of about 95:5 amine functionalizing group to cross-linking agent and can result in a particle with about 95 percent amine functionality. In such embodiments the components includes a ratio of about 90:10 amine functionalizing group to cross-linking agent and can result in a particle with about 90 percent amine functionality. In such embodiments the components includes a ratio of about 85:15 amine functionalizing group to cross-linking agent and can result in a particle with about 85 percent amine functionality. In such embodiments the components includes a ratio of about 80:20 amine functionalizing group to cross-linking agent and can result in a particle with about 80 percent amine functionality. In such embodiments the components includes a ratio of about 75:25 amine functionalizing group to cross-linking agent and can result in a particle with about 75 percent amine functionality. In such embodiments the components includes a ratio of about 70:30 amine functionalizing group to cross-linking agent and can result in a particle with about 70 percent amine functionality.

In some embodiments, a target molecule-containing particle may be prepared as follows. The wells of a two-dimensional mold such as that disclosed in, for example, PCT International Application Serial No. PCT/US2006/023722, filed June 19, 2006, may be filled with a mixture comprising a curable liquid of a solidifiable material and a dilute concentration of a target molecule. By solidifiable material is meant any material that is in liquid form, but which may be cured by some method to produce a particle, including, but not limited to liquid polymers or polymer precursors, including Fluorocur™. In certain embodiments, the solidifiable material is a material such as that disclosed in, for example, U.S. Application Serial Nos. 10,583,570, filed March 5, 2007, 11/633,763, filed December 4, 2007, or 12/250,461, filed October 13, 2008; PCT International Application Serial No. PCT/US2006/023722, filed June 19, 2006; or PCT International Application Serial No. PCT/US2009/041559, filed April 23, 2009, each incorporated herein by reference in their entireties. In some embodiments, the solidifiable material may be designed to be degradable, such as the materials disclosed in PCT International Appl. Serial No. PCT/US2009/041652, filed April 24, 2009, or PCT International Application Serial No. PCT/US2009/041559, filed April 23, 2009, both incorporated herein by reference. Controlling the concentration of target molecule in the liquid allows for control over the number of target molecules per particle produced. In preferred embodiments, the concentration of target molecules is such that each particle should comprise only one target molecule. When the curable liquid containing the target molecule is partitioned into the wells of the array, the desired number of target molecules per particle may be obtained. The target molecule-containing particles may then be harvested from the mold.

In certain embodiments, there is likely to be some distribution of particles containing different numbers of target molecules. In some embodiments, the particles may be sorted before distributing to the micro- or nanoarrays. The particles may be sorted by any method known in the art. For example, in one embodiment, flow cytometry may be used to sort and select particles based on the number of molecules associated with or contained within the particles. In another embodiment, ultragradient centrifugation may be used to sort and select particles based on the number of molecules associated with or contained within the particles on the basis of weight. Ultragradient centrifugation can be used to separate cell organelles, lipids, proteins, and other biomolecules that vary only slightly in molecular weight. See, for example, Scanu et al., *J. Lipid Research* 25: 630 (1984); Nixon et al., *J. Gen. Microbiol.* 21: 569

(1959); and Leighton et al., *J. Cell Biology* 91: 482 (1968), all incorporated herein by reference. In some embodiments, the change in weight by the addition of the target molecule will be sufficient to separate particles comprising different amounts of target molecules. In other embodiments, the change in weight by the addition of the target molecule will be insufficient; in such cases, a larger molecule may be attached to the target molecules to add weight to the particle and amplify the effect of having zero, one, or multiple target molecules per particle. For example, where the target molecule is a DNA polymerase, a large DNA molecule may be attached. In other embodiments, column chromatography or electrophoresis may be used to separate the particles. Particles having zero, one, or more target molecules attached to the surface may have different affinities to a solid substrate and may therefore be expected to separate as they pass through a column or capillary.

In preferred embodiments, the particles may be sorted to provide a plurality of particles with a given number of target molecules associated therewith or attached thereto. For example, in one preferred embodiment, a plurality of particles is provided, each having only one target molecule associated or attached thereto. In preferred embodiments, the plurality of sorted particles comprises greater than about 80%, greater than about 85%, greater than about 90%, greater than about 95%, greater than about 98%, or greater than about 99% particles containing the desired number of target molecules.

In certain embodiments, the particle comprises a matrix material that may be selectively dissolved once the particles have been delivered to the cavities of the micro- or nanoarray. Accordingly, in some embodiments, one or more particles may be delivered to the cavities of a micro- or nanoarray and the matrix material comprising the particle is selectively dissolved, leaving the target molecule or molecules that were associated with that particle behind in the cavity. For example, in one embodiment, the matrix material of the particles may be a water-soluble material and a non-solvent such as chloroform or isopropanol may be used to float the particles into the cavities of an array using fluidic self-assembly. Once the particles are in the cavities, the matrix materials may be dissolved with water to release the target molecules in the cavities. In another embodiment, the matrix material of the particles may comprise a water-insoluble material at pH=7, but which will dissolve at a different pH to release the target molecule into the cell. These foregoing examples are not intended to be limiting;

any combinations of solvent and non-solvent for a given composition may be used according to the described method.

In some embodiments, the target molecule that has been deposited in one or more cavity of a micro- or nanoarray may be conjugated to a surface of the cavity, preferably to the bottom surface of the cavity. The target molecule may be conjugated to the surface of the cavity by any means known in the art.

In some embodiments, the particles may further comprise one or more additional cargos. The additional cargo may be, but is not limited to, for example, small molecule ionic molecules, nucleic acids, proteins, therapeutic agents, diagnostic agents, and imaging agents as well as organic nanoparticles which may encapsulate a wide range of therapeutic, diagnostic, and imaging agents. Cargos may include but are not limited to small molecule pharmaceuticals, therapeutic and diagnostic proteins, antibodies, DNA and RNA sequences, imaging agents, and other active pharmaceutical ingredients. Further, such cargo may include biologically active agents. In addition, the cargo may include a polynucleotide. The polynucleotide may be provided as an antisense agent or interfering RNA molecule such as an RNAi or siRNA molecule to disrupt or inhibit expression of an encoded protein. Other cargo may include, without limitation, MR imaging agents, contrast agents, gadolinium chelates, gadolinium-based contrast agents, or radiosensitizers. In certain embodiments, the additional cargo is a dye. The dye may be associated with or incorporated into the particle by any means known in the art. For example, the dye may be coordinated with a target molecule conjugated to the surface of the particle or may be dissolved in the particle. In some embodiments, the particles may further comprise one or more magnetic materials as a cargo. For example, the particles may comprise magnetite. The presence of a magnetic material associated with or contained within a particle may allow for the use of magnetics to facilitate the loading of the particles into the cavities of the micro- or nanoarray during the fluidic self-assembly process. In some embodiments, the particles may further comprise one or more labels to surface label the particles. The surfaces may be labeled, for example, on one face or on one end by conjugating chemical groups onto the particles before removing them from the mold. The particles may comprise Janus particles or patchy particles. Janus particles or patchy particles may be particularly useful for delivering site specifically one type of target molecule and/or multiple different target molecules. In some embodiments, this delivery may be performed in a stoichiometric fashion. The foregoing examples of cargos that can be

added to the particles are not intended to be limiting; the particles may comprise any type of cargo or multiple types of cargo desirable for a given application.

The cargos associated with particles may provide a number of functions. In some embodiments, for example, the cargos may be added to help to facilitate the efficient loading of particles into the cavities of a micro- or nanoarray. In some embodiments, the cargos may be added to provide a handle for later analysis. For example, where a dye molecule is associated with or incorporated into a particle, the distribution after self-assembly of the particles into the cavities of a micro- or nanoarray can be monitored.

In some embodiments, the loading of target molecules into the cavities of the array via particles may be higher than that achieved by conventional loading techniques. For example, the target molecules may be loaded into greater than about 30% of the cavities, greater than about 40% of the cavities, greater than about 50% of the cavities, greater than about 60% of the cavities, greater than about 70% of the cavities, greater than about 80% of the cavities, greater than about 90% of the cavities.

In preferred embodiments, the number of target molecules per cavity can be controlled. In certain preferred embodiments, the cavities of the nanoarray contain only one target molecule. The size and/or shape of the cavity and the size and/or shape of the particles may be precisely engineered to target the introduction of one particle per cavity. The particles may be further engineered such that each particle comprises a particular number of target molecules. In preferred embodiments, the particles each comprise only one target molecule.

In another embodiment of the present invention is provided a micro- or nanoarray comprising a surface, wherein the surface comprises a plurality of cavities. The micro- or nanoarray may be prepared by any method known in the art. For example, the micro- or nanoarray may comprise a SMRT™ chip (Pacific Biosciences, Menlo Park, CA). See, for example, U.S. Patent No. 7,476,503, filed September 16, 2005 and Eid et al., Real-Time DNA Sequencing from Single Polymerase Molecules, *Science* 32: 133-38 (2009), both incorporated herein by reference in their entireties. In an alternative embodiment, the micro- or nanoarray may be a mold as previously described herein or as disclosed in, for example, WO2008/127455, filed December 5, 2007, incorporated herein by reference in its entirety.

In some embodiments, the cavities may be arranged in a predetermined density. In some embodiments, each cavity may have a predetermined geometry and size. For example, the cavity geometries may be, but are not limited to, being selected from a circle, square, rectangle, triangle, or any polygonal shape and combinations thereof. According to some embodiments, the cavities described herein are less than about 1 μm in a dimension. According to some embodiments the cavities are between about 1 nm and about 500 nm in a dimension. According to other embodiments, the cavities are between about 10 nm and about 200 nm in a dimension. In still further embodiments, the cavities are between about 80 nm and 120 nm in a dimension. According to other embodiments, the cavities are between about 20 nm and about 120 nm in dimension. In further embodiments, the cavities have a maximum cross-sectional dimension of less than about 1 micrometer. In some embodiments, the cavities have a maximum cross-sectional dimension between about 5 nanometers and about 1 micrometer. In some embodiments, the cavities have a maximum cross-sectional dimension between about 10 nanometers and about 1 micrometer. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 800 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 750 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 500 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 300 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 250 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 200 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 150 nanometers. In some embodiments, the cavities have a maximum cross-sectional dimension less than about 100 nanometers. The dimension of the cavities can be a predetermined dimension, a cross-sectional diameter, or the like.

An array may comprise cavities with about the same geometries and sizes or may comprise cavities with a range of different geometries and sizes. Controlling the geometry and size of the cavities may determine which particles may access a given cavity. In preferred embodiments, a plurality of different cavity shapes and/or sizes are provided, which may accommodate a plurality of different particle shapes and/or sizes.

The sizes of the cavities may also be predetermined to allow certain cavities to accommodate one or multiple particles.

In some embodiments, a plurality of different types of particles may be loaded into a plurality of cavities. For example, particles with varying sizes and/or geometries and carrying different numbers or types of target molecules may be combined and distributed onto a micro- or nanoarray comprising cavities with varying sizes and/or geometries. The particles may self-assemble into matching cavities, providing an engineered array with a known distribution of particles. The particles may thus be suitable for distributing different target molecules to tailored locations within the micro- or nanoarray. In one embodiment, each type of particle has a different dye molecule associated with or conjugated to it. A series of different dye-labeled particles may be mixed and fluidically self assembled into the cavities of the micro- or nanoarray. Such a method would allow determination of which target molecule is in which cavity by correlating the dye associated with each type of particle with which dye is observed in each cavity.

In one embodiment, the resulting micro- or nanoarray may be used, for example, for DNA sequencing. The ability to effectively isolate one polymerase molecule per cavity may allow for single-molecule, real-time DNA sequencing. See, for example, Eid et al., Real-Time DNA Sequencing from Single Polymerase Molecules, *Science* 323:133-38 (2009) and Korlach et al., Long, Progressive Enzymatic DNA Synthesis Using 100% Dye-Labeled Terminal Phosphate-Linked Nucleotides, Nucleosides, Nucleotides, and Nucleic Acids 27(9): 1072-82 (2008) incorporated herein by reference in its entirety. For other related references, see U.S. Patent Nos. 7,405,434; 7,427,343; 7,462,452; 7,476,503; 7,485,424; 7,486,865; and 7,563,574 and U.S. Patent Application Publications Nos. 2009/0247426; 2009/0061430; 2009/0087850; 2009/0105094; 2009/0176233; 2009/0181396; 2009/0208957; and 2009/0246791, all incorporated herein by reference in their entireties.

The applications of the micro- or nanoarray prepared according to the methods herein are not limited to DNA sequencing, and can be used for any technique in which isolation of target molecules is desirable.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same

extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

EXAMPLES

Preparation of an Array and Particles Obtained Therefrom

A patterned layer of low surface energy perfluoropolyether was prepared as disclosed in the applicants co-pending patent applications incorporated herein by reference. Also as disclosed in the applicants co-pending patent applications incorporated herein by reference the patterned cavities were filled with a monomer solution of 90 mole% N-(3-Aminopropyl)methacrylamide hydrochloride and 10 mole% N,N′-Methylenebisacrylamide crosslinker in an aqueous solvent. The monomer solution was then UV cured to polymerize. The process resulted in the discrete cavities of the patterned layer being filled with an amine functionalized hydrogel material of N-(3-Aminopropyl)methacrylamide. Each cavity was separated by the low surface energy polymer between the filled cavities.

In alternate experiments, the same process was followed with a monomer solution of 20 mole% N-(3-Aminopropyl)methacrylamide hydrochloride, 70 mole% acrylamide, and 10 mole% N,N′-Methylenebisacrylamide crosslinker in an aqueous solvent being introduced into the cavities of the patterned perfluoropolyether. The monomer solution was UV cured in the cavities of the low surface energy polymer, resulting in discrete filled cavities with an amine functionalized material.

Isolated particles were formed by placing a cover sheet over the patterned and filled substrates and passing the sandwich through a roller to laminate the hydrogel filled in the cavities to the cover sheet. The cover sheet was then removed resulting in discrete particles of the hydrogel material contained in the cavities attached to the surface of the cover sheet. The particles were finally collected in suspension by washing with an aqueous solvent.

THAT WHICH IS CLAIMED:

1. A method of introducing one or more target molecules into one or more cavities of a nanoarray, comprising:

preparing nanoparticles by placing a material, comprising a liquid and further comprising one or more types of target molecules, into a recess in a mold, hardening the material to make a particle, and removing the particle from the recess;

delivering said particles into the one or more cavities of the nanoarray; and

dissolving the matrix material comprising the particle, releasing the target molecule in the cavity.

2. The method of claim 1, further comprising, after preparing the nanoparticles, analyzing the nanoparticles to determine which nanoparticles include a single target molecule.

3. The method of claim 2, wherein analyzing the nanoparticles comprises reversibly binding a larger molecule with the target molecule.

4. The method of claim 3, further comprising, analyzing the particles with larger molecules bound with the target molecule of the particles to determine the number of target molecules associated with the particles.

5. The method of claim 4, further comprising, selecting the particles with one target molecule associated therewith, removing the larger molecule, and delivering the selected particles into a micro- or nanoarray.

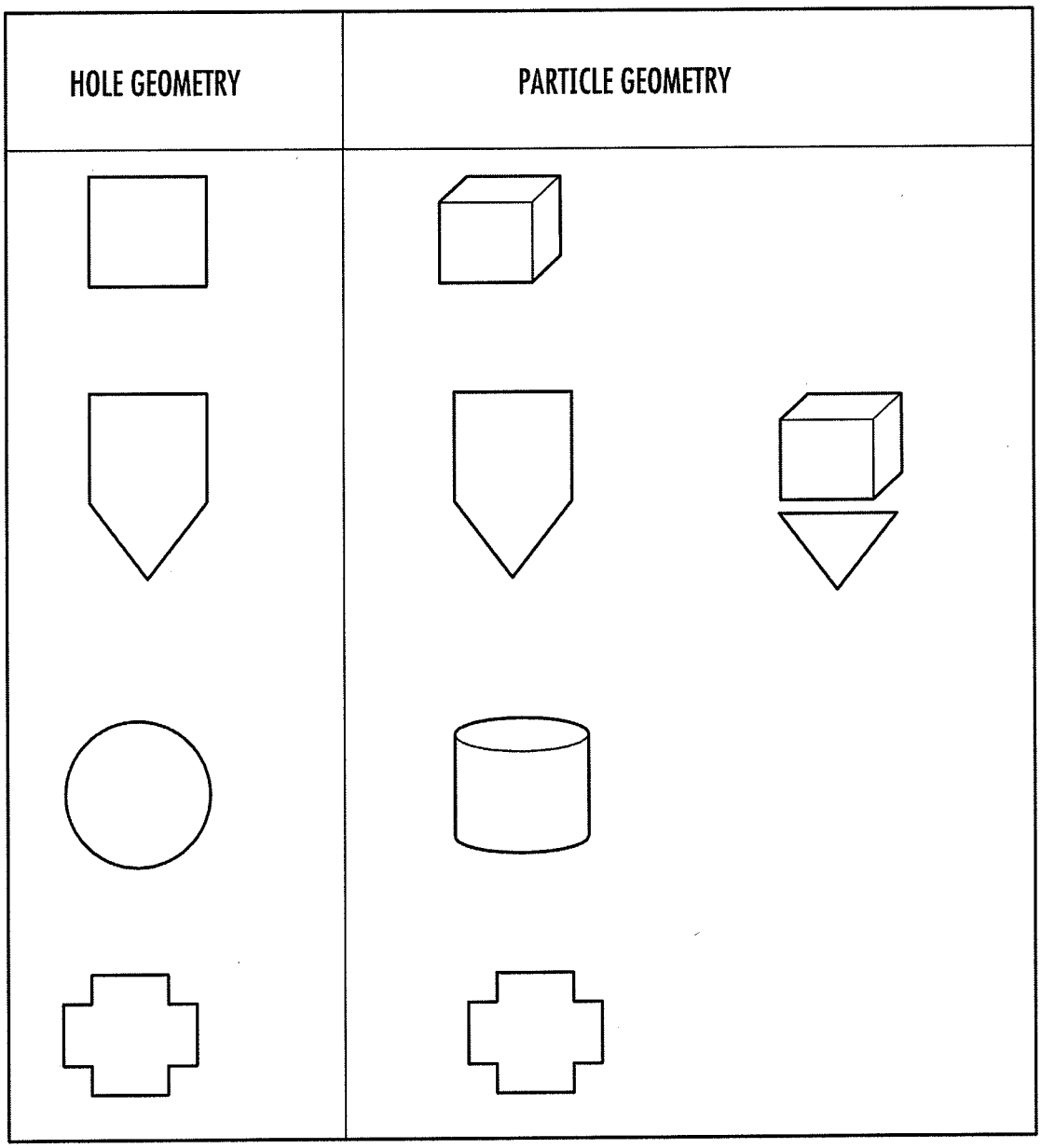


FIG. 1

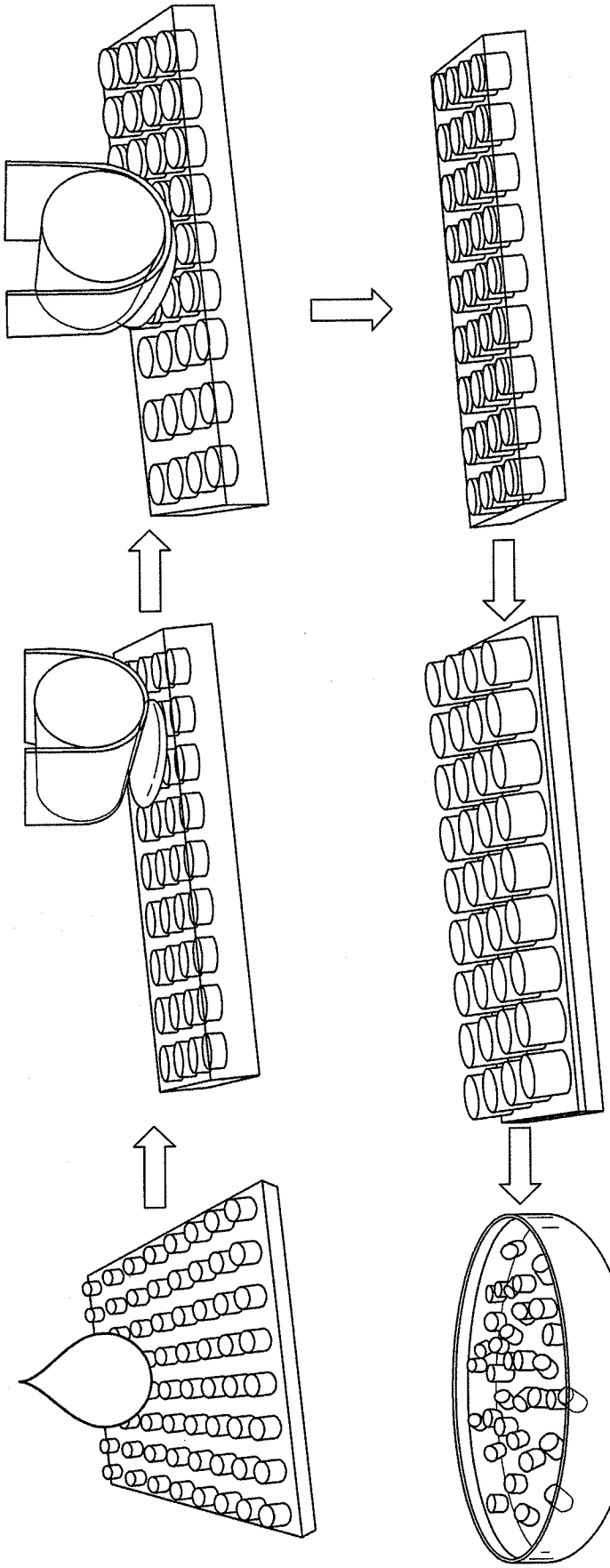


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/53473

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/00 (2010.10)
USPC - 435/4; 977/702; 977/795
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 435/4; 977/702; 977/795

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PubWEST (PGPB, USPT, EPAB, JPAB); Google Scholar: nanoarray, fabrication, nanoparticle, cavity, mold, harvest, PRINT (Particle Replication In Non-wetting Templates)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/127455 A2 (ROTHROCK et al.) 23 October 2008 (23.10.2008) pg 31 para 4, pg 32 para 3-5, pg 43 para 2, pg 55 para 2, pg 56 para 1	1-5
Y	US 7,476,503 B2 (TURNER et al.) 13 January 2009 (13.01.2009) col 8 ln 12-14, col 18 ln 48-48, col 25 ln 10-17	1-5

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 November 2010 (29.10.2010)	13 JAN 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)