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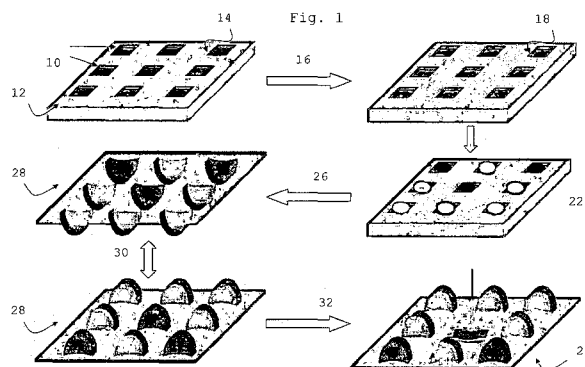
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(54) Title: PROCESS FOR MANUFACTURE OF A MICRO-CHAMBER ARRAY



(57) Abstract: There is provided a process for making a micro-chamber array comprising (i) applying a first layer on a substrate having an array of micro-wells disposed on said substrate; (ii) loading cargo into the layered micro-wells of the substrate; (iv) applying a sealing layer to the loaded cargo to encapsulate the cargo therein between said first layer and said sealing layer; and (v) removing the substrate from the first layer to form the micro-chamber array having plural micro-chambers, each of said micro-chambers comprising an outer layer defining a micro-chamber interior having said cargo therein. The highly ordered pattern of micro-chambers allows release of said cargo from selected micro-chambers in a programmed way.



PROCESS FOR MANUFACTURE OF A MICRO-CHAMBER ARRAY**Technical Field**

The present invention generally relates to a process
5 for making a micro-chamber array. The present invention
also relates to a micro-chamber array.

Background

Advanced drug formulations deal with the elaboration
10 of delivery systems that provide controlled release of
bioactive materials or a target cargo. In general,
immobilizing (patterning) of the target cargo can be
classified into four categories, namely a) covalent
binding, b) physical adsorption of target molecules onto
15 solid supports, c) entrapment inside pore microspheres or
hydrogels, and d) confinement within a semi-permeable
membrane.

There are several disadvantages associated with the
method of covalent binding and the method of physical
20 adsorption of target molecules onto solid supports.
Firstly, such methods often have limited flexibility in
terms of the choices of materials that can be loaded onto
the solid supports or that can be covalently bonded with
the support. Secondly, such methods often result in the
25 detrimental effects of binding forces (matrix effects) due
to the contact of the target cargo with another surface.
Furthermore, the target cargo of such methods is often
exposed to harsh environment conditions such as oxidation,
UV irradiation, and pH etc. On the other hand, one of the
30 problems associated with the method of entrapment of the
cargo inside pore microspheres or hydrogels is the
possibility of leakage of the cargo into the surroundings.

One of the current methods for immobilizing a target
cargo is membrane confinement. In membrane confinement,

the target cargo is encapsulated within the polymer matrices or in the core-shell structures. In the latter, the shell degradation rate determines the release rate of the target cargo. Such a method may be utilized in the delivery of a variety of biologically active agents as the films are dissolvable upon application of the required external stimulus to release the biological agents. However, the films produced by such a method are usually thin and fragile. When the thin films are subjected to stress, partial or even complete rupture of the membrane may result. This may cause damage to the cargo or undesired leakage of the cargo contained within the membrane. Furthermore, such a method has limited applicability with regard to the polymer species used to produce the films and often harsh conditions have to be used for film decomposition or polymerization. Such harsh conditions for film decomposition include low pH, oxidizing agents, organic solvents and elevated temperatures.

Another approach is to load microcapsules with the cargo and then immobilize the microcapsules into the micro-wells. In this approach, the microcapsules can unload their content depending on the environmental conditions. However, such a method has only limited applicability with regard to the polymer species used to produce the microcapsules and the environment in which the films are exposed. Disadvantageously, such a method has limited applicability with regard to the type of cargo as the microcapsules are maintained in an environmental condition that allows for the loading and unloading of only a single type of cargo. Furthermore, in the known method for loading microcapsules, the microcapsules have to be formed around a core which is then removed by dissolution or degradation, which is very time consuming

and increases the time and expense of manufacturing the micro-array.

Accordingly, there is a need to provide a micro-chamber array that overcomes, or at least ameliorates, the disadvantages mentioned above. Therefore, it is an object of the present invention to provide systems having controllable or adjustable loading as well as release properties.

Summary

According to a first aspect, there is provided process for making a micro-chamber array comprising (i) applying a first layer on a substrate having an array of micro-wells disposed on said substrate; (ii) loading cargo into the layered micro-wells of the substrate; (iii) applying a sealing layer to the loaded cargo to encapsulate the cargo therein between said first layer and said sealing layer; and (iv) removing the substrate from the first layer to form the micro-chamber array having plural micro-chambers, each of said micro-chambers comprising an outer layer defining a micro-chamber interior having said cargo therein

Advantageously, the disclosed process may be highly reproducible and may be used to fabricate different types of micro-chamber arrays that may be used for containing a wide variety of cargo in the chambers.

Advantageously, the disclosed process enables rapid fabrication of the microchambers compared to prior art methods. This leads to a lower relative cost of manufacture. Additionally, because the cargo is loaded directly into the micro-well, there is more accuracy and less errors associated with the disclosed process. More advantageously, the walls of the microchambers or micro-wells do not necessarily have to possess tunable

permeability towards a cargo to load and successively release it or indeed be supplied with the cargo as in a known process. Hence, the present method significantly broadens the variety of cargo that could be loaded.

5 According to a second aspect, there is provided a process for releasing the cargo from at least one micro-chamber in the micro-chamber array made in the process of the first aspect, comprising at least one of the following steps: (i) rupturing at least one of the first layer and
10 the sealing layer of the at least one micro-chamber; and (ii) changing an environmental condition to render at least one of the first layer and the sealing layers of the at least one micro-chamber permeable to said cargo.

Advantageously, by rupturing or changing the
15 environmental condition, one or more chambers of the disclosed micro-chamber array may be configured to release the cargo contained therein. The one or more chambers may be selected independently from the other chambers to release the cargo contained therein. The one or more
20 chambers may be selected based on its position in the micro-chamber array. The release of one or more chambers of the disclosed micro-chamber array may be carried out in a time specific manner. In one embodiment where laser is used to release the cargo, the user can decide when to
25 switch on the laser, thus releasing the contents of the target chamber. This then defines the time when the cargo loaded in this chamber will be released in one pulse. In another embodiment, the size of the hole made in the roof of the micro-chamber can be controlled, thus delaying the
30 release of the cargo from the target chamber and hence controlling the duration of the release.

According to one embodiment, there is provided a micro-chamber array comprising a surface, a plurality of micro-chambers on the surface, wherein each of said micro-

chambers comprises an outer layer defining a micro-chamber interior having cargo therein; and a thermal conductor in thermal communication with said outer layer and being configured to rupture said micro-layer on application of heat.

Advantageously, the micro-chamber array may be used to store and contain a wide variety of cargo.

Advantageously, the presence of the thermal conductor may aid in the release of the cargo from a specific micro-chamber on the micro-chamber array. This may allow for the selected release of a desired cargo.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "cargo" is to be interpreted broadly to refer to any substituent material, liquid or gas that can be contained in the micro-chamber(s). The cargo may include substituent materials selected from the group consisting of proteins, antibodies, antibody fragments, enzymes, peptides, growth factors, drugs, bioactive agents, DNA fragments, RNA fragments, RNA, polysaccharides, colloidal particles, nanocrystals, precipitates, and mixtures thereof. The cargo may include liquids such as aqueous solutions, oils, oil-based liquids or solutions containing micelles.

The term "bioactive agents" is to be interpreted broadly to refer to molecules or compounds that are capable of altering, antagonizing or promoting biological or chemical events. The bioactive agent may work by having either an agonistic or antagonistic effect on a receptor and hence, may affect the activities of that receptor such that the bioactive agent may be used for treating or preventing diseases which is mediated by that receptor.

Hence, in one embodiment, the term "bioactive agent" may refer to a drug.

The term "environmental condition" is to be interpreted broadly to refer to any external condition, that when applied to the micro-chamber array, is capable of causing the first layer or sealing layer (or both) making up the micro-chamber to release the cargo contained in the micro-chamber. The external condition may be applied to the micro-chamber to at least partially rupture the first layer and/or sealing layer or may be configured to tune the permeability of the first layer and/or sealing layer such that the cargo therein may be released from the micro-chamber while keeping the first layer and/or sealing layer structurally intact. The environmental condition may be selected from the group consisting of pH, temperature, ionic strength, magnetic field, ultrasound, light, presence or concentration of a solvent, concentration of a trigger compound, and a combination thereof.

The term "nanoimprint lithography" is to be interpreted broadly to include any method for printing or creating a pattern or structure on the micro/nanoscale on the surface of a substrate by applying a mold with the defined pattern or structure on the surface at certain temperatures and pressures. A method of nanoimprint lithography can be referred from US Patent Number 5,772,905.

The term "microscale" is to be interpreted to include any dimensions that are in the range of about 1 (μm) to about 100 μm . The terms "microwells" or "micro-chambers" as used herein, refer to corresponding wells or chambers comprising "microscale" features.

The term "nanoscale" is to be interpreted to include any dimensions that are below about 1 μm .

The term "plasma treatment" is to be interpreted broadly to include any exposure of a surface to plasma such that organic contaminants on the surface are at least partially destroyed. Generally, such plasma is a low-pressure oxidative plasma such as oxygen (O₂), argon, and mixtures of oxygen and argon, generated with a radio frequency (RF) or microwave source.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically

disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a process for making a micro-chamber array will now be disclosed.

The process for making a micro-chamber array may comprise the steps of (i) applying an first layer on a substrate having an array of micro-wells disposed on said substrate; (ii) loading cargo into the layered micro-wells of the substrate; (iii) applying a sealing layer to the loaded cargo to encapsulate the cargo therein between said first layer and said sealing layer; and (iv) removing the substrate from the first layer to form the micro-chamber array having plural micro-chambers, each of said micro-chambers comprising an outer layer defining a micro-chamber interior having said cargo therein.

The process may comprise the step of providing a substrate which serves as a template for deposition of the first layer and sealing layer. The template may be a sacrificial template in which the template may be destroyed during manufacture of the micro-chamber array

such that the substrate does not form part of the resultant micro-chamber array.

The substrate may be a polymer substrate although any material that is capable of being imprinted by a mold can be used. For example, in other embodiments, the substrate may be silicon or silicon based materials such as glass. In another embodiment, the substrate may be Gallium (III) arsenide. In a further embodiment, the substrate may be sapphire.

Advantageously, the process comprises the step of selecting a thermoplastic polymer as the polymer substrate. In one embodiment, the process comprises the step of selecting the monomers to form the thermoplastic polymer from the group consisting of acrylates, phthalamides, acrylonitriles, cellulose, styrenes, alkyls, alkyls methacrylates, alkenes, halogenated alkenes, amides, imides, aryletherketones, butadienes, ketones, esters, acetals, carbonates and combinations thereof. In one embodiment, the thermoplastic polymer is at least one of a polystyrene, a polymethyl methacrylate and a polycarbonate. Exemplary monomers to form the thermoplastic polymer may be selected from the group consisting of methyls, ethylenes, propylenes, methyl methacrylates, methylpentenes, vinylidene, vinylidene chloride, etherimides, ethylenechlorinates, urethanes, ethylene vinyl alcohols, fluoroplastics, carbonates, acrylonitrile-butadiene-styrenes, etheretherketones, ionomers, butylenes, phenylene oxides, sulphones, ethersulphones, phenylene sulphides, elastomers, ethylene terephthalate, naphthalene terephthalate, ethylene naphthalene and combinations thereof.

In another embodiment, the polymer substrate may be a photoresist material. Suitable photoresist materials include epoxy based negative resists such as SU-8™ resist

available from MicroChem Corp. of Newton, Massachusetts, United States of America.

The polymer substrate may be a polymer composite whereby particles may be added to or incorporated with the polymer. These particles may be selected from the group consisting of calcium carbonate, carbon filler, glass filler, fibers, glass fibers, carbon fibers, carbon nanotubes and mixtures thereof.

The substrate may comprise a plurality of imprints on the surface. In one embodiment, the imprint may be a micro-well. The process may comprise the step of forming the imprints or micro-wells on the substrate. The imprints may be formed on the surface of the substrate by employing e-beam lithography, nanoimprint lithography, photolithography, or some other suitable lithographic techniques. In one embodiment, the step of forming the imprints or micro-wells on the substrate comprises using nanoimprint lithography to form the micro-wells.

Nanoimprint lithography comprises the steps of applying a mold under pressure to a polymer substrate that is above its glass transition temperature or melting temperature to form the micro-wells thereon; cooling the polymer substrate to a temperature below its glass transition temperature or melting temperature; and demolding the polymer substrate.

The mold may have a defined imprint forming surface and may be applied onto the substrate at a defined temperature and pressure for a defined period of time. The temperature, pressure and time duration to be applied will be dependent on the polymer substrate used and the imprints to be formed on the surface of the polymer substrate.

The mold may be made of any suitable material that is chemically inert and may be harder than the softened

substrate when used at the applying temperature. The mold may have a low surface energy so that any demolding can be carried out without destroying the imprinted structure. Hence, the mold may be selected from the group consisting of silicon, metal, glass, quartz, ceramic and combinations thereof.

The imprint forming surface on the mold may be made up of columns such that corresponding holes are formed in the substrate. The columns may have defined heights and widths in the micro/nanoscale in which the minimum dimension of the height or width (diameter) of the columns is 500 nm. The columns may be spaced apart from each other.

The mold may be treated with an anti-stiction agent such as a silane-based anti-stiction agent before applying the mold to the substrate.

The temperature for nanoimprint lithography may be selected to be above the glass transition temperature (T_g) or melting temperature of the polymer substrate. The imprint temperature may be higher than the glass transition temperature by at least about 40°C, at least about 50°C, at least about 60°C, at least about 70°C, at least about 80°C, at least about 90°C, or at least about 100°C. At this imprint temperature, the polymer substrate softens and may conform to the shape of the mold such that an imprint is created on the surface of the polymer substrate whereby the pattern of the imprint may be complementary to the pattern on the mold when the polymer substrate is cooled and subsequently hardened. For example, if the mold has columns, when the mold is applied onto the surface of the polymer substrate, the columns on the mold may result in corresponding holes on the polymer substrate. In other words it is negative replica of the mold.

The imprint temperature may be selected from the group consisting of about 120°C to about 200°C, about 140°C to about 200°C, about 160°C to about 200°C, about 180°C to about 200°C, about 120°C to about 140°C, about 120°C to about 160°C and about 120°C to about 180°C. In one embodiment, the imprint temperature is about 140°C. It is to be noted that the imprint temperature should be higher than the glass transition temperature, but lower than the temperature, at which decomposition of selected polymer starts.

The imprint pressure used may be selected from the group consisting of about 10 bars to about 50 bars, about 20 bars to about 50 bars, about 30 bars to about 50 bars, about 40 bars to about 50 bars, about 45 bars to about 50 bars, about 10 bars to about 20 bars, about 10 bars to about 30 bars, about 10 bars to about 40 bars and about 10 bars to about 45 bars. In one embodiment, the imprint pressure is about 40 bars. The higher the pressure, the faster is the imprint process.

The time period used when applying the mold to a surface of the polymer substrate may be in the range of about 4 minutes to about 20 minutes, about 8 minutes to about 20 minutes, about 12 minutes to about 20 minutes, about 16 minutes to about 20 minutes, about 4 minutes to about 8 minutes, about 4 minutes to about 12 minutes and about 4 minutes to about 16 minutes. In one embodiment, the time period used is about 5 minutes.

The pressure may be released after cooling the polymer substrate to a temperature below its glass transition temperature or melting temperature. The pressure release temperature may be lower than the glass transition or other relaxation temperature, or melting temperature of the polymer substrate.

The process may comprise the step of plasma etching the micro-wells after the demolding step.

The shape of the imprints may be of a defined shape when viewed from the top of the imprint. The shape of the imprint may be substantially circular, oval-shaped, square-shaped, trapezoidal-shaped or rectangular. Hence, when referring to the dimension of the imprint, this may be interpreted to as referring to the longest dimension of the imprint, such as the diameter or length of the imprint, as appropriate, depending on the shape of the imprint. In other embodiments, the shape of the imprint may not be defined, that is, the imprint may have an irregular shape, when viewed from the top of the imprint.

The dimension of the imprint may be in the range selected from the group consisting of about 0.5 microns to about 100 microns, about 5 microns to about 100 microns, about 10 microns to about 100 microns, about 20 microns to about 100 microns, about 30 microns to about 100 microns, about 40 microns to about 100 microns, about 50 microns to about 100 microns, about 60 microns to about 100 microns, about 70 microns to about 100 microns, about 80 microns to about 100 microns, about 90 microns to about 100 microns, about 0.5 microns to about 5 microns, about 0.5 microns to about 10 microns, about 0.5 microns to about 20 microns, about 0.5 microns to about 30 microns, about 0.5 microns to about 40 microns, about 0.5 microns to about 50 microns, about 0.5 microns to about 60 microns, about 0.5 microns to about 70 microns, about 0.5 microns to about 80 microns and about 0.5 microns to about 90 microns.

The depth of the imprint may be in the range selected from the group consisting of about 0.5 microns to about 100 microns, about 5 microns to about 100 microns, about 10 microns to about 100 microns, about 20 microns to about 100 microns, about 30 microns to about 100 microns, about

40 microns to about 100 microns, about 50 microns to about 100 microns, about 60 microns to about 100 microns, about 70 microns to about 100 microns, about 80 microns to about 100 microns, about 90 microns to about 100 microns, about 0.5 microns to about 5 microns, about 0.5 microns to about 10 microns, about 0.5 microns to about 20 microns, about 0.5 microns to about 30 microns, about 0.5 microns to about 40 microns, about 0.5 microns to about 50 microns, about 0.5 microns to about 60 microns, about 0.5 microns to about 70 microns, about 0.5 microns to about 80 microns and about 0.5 microns to about 90 microns.

The aspect ratio of the imprint, defined as the ratio between the diameter or length of the imprint to the depth of the imprint, may be in the range selected from the group consisting of about 0.5 to about 5 (that is, about 1:2 to about 5:1), about 1 to about 5, about 2 to about 5, about 3 to about 5, about 4 to about 5, about 0.5 to about 1, about 0.5 to about 2, about 0.5 to about 3 and about 0.5 to about 4.

The imprints may be arranged in a regular array such as for example, a rectangular array, a pentangular array, a circular array or may be arranged in an irregular array such as a trapezoidal array.

The number of imprints (such as micro-wells) per mm^2 of the substrate may be in the range selected from the group consisting of 1 to about 10^6 imprints, about 100 to about 10^6 imprints, about 1000 to about 10^6 imprints, about 10^4 to about 10^6 imprints, about 10^5 to about 10^6 imprints, 1 to about 10 imprints, 1 to about 100 imprints, 1 to about 10^3 imprints, 1 to about 10^4 imprints, 1 to about 10^5 imprints and 1 to about 10^6 imprints.

About 70% to about 100% of the imprints (such as micro-wells) may be filled with the cargo, or at least about 70% to about 75%, about 70% to about 80%, about 70%

to about 85%, about 70% to about 90%, about 70% to about 95%, about 75% to about 100%, about 80% to about 100%, about 85% to about 100%, about 90% to about 100% and about 95% to about 100% of the imprints may be filled with the cargo.

The imprints may define a region for storing or containing the cargo in the region. In one embodiment, the imprint region may be the depressed region (that is, the base) of a micro-well.

The applying step (iii) may comprise the step of assembling a plurality of layers of alternating charge on the substrate having an array of micro-wells disposed on the substrate. The assembling step may comprise alternatively exposing the substrate to solutions of cationic polymer and anionic polymer.

The layers of alternating charges may be deposited to form a first layer on the surface of the polymer substrate. The layers of alternating charges may be termed as a polyelectrolyte membrane (PEM) layer or film. Each of the layers to be deposited may comprise a polymer or may comprise a nanoparticle. At least two different types of polymers of opposite charges may be used to form at least two layers successively, each layer comprising one type of polymer. The nanoparticle may be a thermal conductor such as a metal. The metal may be selected from the group consisting of silver, copper, gold, aluminum, iron, platinum, and an alloy of any of the foregoing.

The first layer may be deposited onto the polymer substrate using a layer-by-layer deposition technique. In this technique, the polymer substrate may be subjected to a series of dip-coating steps in which the polymer substrate may be dipped into alternating solutions of an anionic polymer and a cationic polymer. After each dipping, the polymer substrate may be washed to remove any

non-adsorbed components. These steps of dip-coating and washing may be repeated for a number of times in order to achieve the desired thickness of the first layer. A bi-layer includes two layers of alternating charge. In one
5 embodiment, the number of bi-layers deposited may be at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, more than 50 and more than 55.

The minimal number of bi-layers or the critical
10 thickness of first layer needed to prevent collapse of the microchamber depends on the size and shape of micro-chamber and the Young's modulus of the first layer. The larger is the micro-chamber, the thicker should be the first layer and vice versa. The higher the Young's modulus
15 of the first layer is, the thinner the first layer can be. In one embodiment where the first layer is made up of alternating layers of PAH-PSS, the Young's modulus of this first layer is in the range 300-700 MPa. The critical thickness is about 400 nm for cylindrical micro-chambers
20 having 7 microns in diameter. The upper limit to the number of bi-layers is the ratio between the first layer thickness and the diameter of the micro-well. If the first layer fills the well completely, the micro-chambers will not have any room for housing the cargo.

25 The thickness of the first layer may be in the range selected from the group consisting of about 400 nm to about 600 nm, about 400 nm to about 420 nm, about 400 nm to about 440 nm, about 400 nm to about 460 nm, about 400 nm to about 480 nm, about 400 nm to about 500 nm, about
30 400 nm to about 520 nm, about 400 nm to about 540 nm, about 400 nm to about 560 nm, about 400 nm to about 580 nm, about 420 nm to about 600 nm, about 440 nm to about 600 nm, about 460 nm to about 600 nm, about 480 nm to about 600 nm, about 500 nm to about 600 nm, about 520 nm

to about 600 nm, about 540 nm to about 600 nm, about 560 nm to about 600 nm and about 580 nm to about 600 nm. In one embodiment, the thickness of the coating may be about 500 nm.

5 The thickness of each layer may be controlled by adjusting the pH or ionic strength of the dipping solution so that the thickness of each layer may be adjusted between about 0.5 nm and about 80 nm. Hence, the dip-coating technique may result in a first layer in which the individual layers have a different thickness from each other.

The anionic solution may comprise an anionic polymer. The anionic polymer may be formed from anionic monomers selected from the group consisting of acrylic acid, methacrylic acid, ethacrylic acid, maleic acid, itaconic acid, 2-acrylamido-2-methylpropanesulphonic acid, vinylsulphonic acid, vinylsulphuric acid, vinylphosphonic acid, vinylacetic acid, styrenesulphonic acid, styrenesulphuric acid, crotonic acid, allylacetic acid, and 4-methyl-4-pentonic acid, acrylamido methyl propane sulphonic acid, ammonium and alkali metal salts thereof and combinations thereof.

25 The anionic polymer may be an anionic polyelectrolyte. The anionic polyelectrolyte may be selected from the group consisting of gum arabic, anionic polysaccharide, alginate, pectin, agar, carrageenan, poly(2-acrylamido-2-methyl-1-propanesulphonic acid), poly(2-acrylamido-2-methyl-1-propanesulphonic acid-co-acrylonitrile)acrylonitrile, poly(4-styrenesulphonic acid), poly(4-styrenesulphonic acid-co-maleic acid), poly(sodium acrylate), poly(sodium 4-styrenesulphonate), sodium poly(vinylsulphonate), sodium polyanetholesulphonate and combinations thereof. In one

embodiment, the anionic polyelectrolyte is poly(sodium 4-styrene sulfonate) (PSS).

In one embodiment, the anionic solution may be a colloidal solution of gold nanoparticles.

5 The cationic solution may comprise a cationic polymer. The cationic polymer may be formed from cationic monomers selected from the group consisting of dimethyldiallyl ammonium chloride, ethyleneamine, ethyleneimine methacrylamido propyl trimethyl ammonium
10 chloride, 2-methacryloyloxyethyl trimethyl ammonium chloride, 2-methacryloyloxyethyl trimethyl ammonium methosulfate, diquateryary ionene and combinations thereof. In one embodiment, the cationic polymer is poly(ethyleneimine) (PEI).

15 The cationic polymer may be a cationic polyelectrolyte. The cationic polyelectrolyte may be selected from the group consisting of gelatin (such as gelatin A or gelatin B), chitosan, whey protein, whey
20 protein, albumin, beta-lactoglobulin, potato protein, faba bean legumin, soybean protein, poly(acrylamide-co-diallyldimethylammonium chloride), poly(allylamine hydrochloride), poly(diallyldimethylammonium chloride) and combinations thereof. In one embodiment, the cationic polyelectrolyte is poly(allylamine hydrochloride) (PAH).

25 In one embodiment, a cationic polymer such as poly(ethyleneimine) is deposited on the polymer substrate to form a first layer thereon. Subsequently, alternating layers of a cationic polyelectrolyte such as poly(allylamine hydrochloride) and an anionic
30 polyelectrolyte such as poly(sodium 4-styrene sulfonate) are successively deposited on the PEI layer.

In another embodiment, a colloidal solution of gold nanoparticles may be used to form one or more negatively charged layer(s) instead of PSS. As an example, a layer of

gold nanoparticles may be deposited after each tenth PSS-PAH bi-layer.

The concentration of the anionic or cationic solution may independently be from the range selected from the group consisting of about 1 mg/ml to about 10 mg/ml, about 1 mg/ml to about 3 mg/ml, about 1 mg/ml to about 5 mg/ml, about 1 mg/ml to about 7 mg/ml, about 1 mg/ml to about 9 mg/ml, about 3 mg/ml to about 10 mg/ml, about 5 mg/ml to about 10 mg/ml, about 7 mg/ml to about 10 mg/ml, and about 9 mg/ml to about 10 mg/ml.

The layers may also be deposited using techniques such as spray coating, inkjet printing, brush coating, roll coating, spin coating, soft lithography, microcontact printing, multi-layer transfer printing, polymer-on-polymer printing or combination thereof.

The process may comprise the step of sonicating the polymer substrate before the depositing step. The sonicating step may aid to remove any air bubbles that may be trapped inside the micro-wells in the polymer substrate. This may ensure that the layers are deposited in a uniform manner on the polymer substrate, without any discontinuity or breakage in the layer that may occur if a bubble were present.

The process may comprise the step of loading cargo into the layered micro-wells of the substrate. The loading step may comprise loading one or more different cargo types into respective micro-wells. The cargo may be a gas, liquid or a substituent material. The cargo may be tagged with a fluorescent label in order to identify desired cargo and hence, aid in the location-specific release of the desired cargo.

The cargo may be a liquid such as aqueous solutions, oil-based liquids or solutions containing micelles.

The cargo may be a gas. The gas may be encapsulated in the micro-chambers during fabrication of the micro-chamber array in a gaseous environment comprising the particular gas to be encapsulated.

5 The cargo may be a substituent material such as a protein, a recombinant protein, a purified protein, an antibody, an antibody fragment, a recombinant antibody, an enzyme, a peptide, an amino acid, a growth factor, a drug, a bioactive agent, an immunological agent, a nucleic acid,
10 a polysaccharide, a colloidal particle, a nanocrystal, a precipitate, and mixtures thereof.

 The drug may be a therapeutic, diagnostic, prophylactic or prognostic agent. The drug may be an antibiotic, an anti-viral agent, an anesthetic, a
15 steroidal agent, an anti-inflammatory agent, an anti-neoplastic agent, an antigen, a vaccine, an antibody, a decongestant, an antihypertensive, a sedative, a birth control agent, a progestational agent, an anti-cholinergic, an analgesic, an anti-depressant, an anti-
20 psychotic, an adrenergic blocking agent, a diuretic, a cardiovascular active agent, a vasoactive agent, a non-steroidal anti-inflammatory agent, a nutritional agent, or combinations thereof.

 The drug may be a protein-based drug such as an
25 enzyme inhibitor, a colony-stimulating factor, a plasminogen activator, a polypeptide hormone, insulin, a myelin basic protein, a collagen S antigen, calcitonin, angiotensin, vasopressin, desmopressin, a LH-RH (luteinizing hormone-releasing hormone), somatostatin,
30 glucagon, somatomedin, oxytocin, gastrin, secretin, a h-ANP (human atrial natriuretic polypeptide), an ACTH (adrenocorticotrophic hormone), a MSH (melanocyte stimulating hormone), beta-endorphin, muramyl dipeptide, enkephalin, neurotensin, a VIP (vasoactive intestinal

peptide), a CCK-8 (cholecystokinin), a PTH (parathyroid hormone), a CGRP (calcitonin gene related peptide), endothelin, a TRH (thyroid releasing hormone), an interferon, a cytokine, streptokinase, urokinase, and a growth factor.

The nucleic acid may be a DNA, a DNA fragment, a RNA, a RNA fragment, a sequence of nucleotides or a sequence of oligonucleotides.

The fluorescent label may be accomplished by using a chemically reactive derivative of a fluorophore. The reactive derivative may be selected from an amine reactive isothiocyanate derivative (such as Fluorescein isothiocyanate and Tetramethyl Rhodamine Iso-Thiocyanate), amine reactive succinimidyl esters (such as NHS-fluorescein), sulphydryl reactive maleimide activated fluors (such as fluorescein-5-maleimide) or a derivative of perylene, 3,4,9,10-tetra-(hectoxy-carbonyl)-perylene.

It is to be appreciated that more than one type of cargo can be stored or contained in the micro-well. Hence, each micro-well may contain the same type of cargo or may contain a plurality of different types of cargo.

The cargo may be loaded into the micro-wells by a variety of processes which depend on the type of cargo to be loaded. For example, if the cargo is a powder, the powder may be mechanically spread over the micro-chambers. In another example, if the cargo is a dispersion of particles such as colloidal particles, the dispersion may be loaded in the micro-wells by way of template assisted self assembly.

The process may comprise the step of applying a sealing layer to the loaded cargo to encapsulate the cargo therein between the first layer and the sealing layer. The sealing layer may be formed of a polymeric material. The polymeric material may be another set of plurality of

layers that form the sealing layer. The individual layers of the sealing layer may be applied directly onto the loaded cargo using the layer-by-layer deposition technique, or other techniques, as mentioned above.

5 Alternatively, the sealing layer may be formed on another (second) substrate. Here, the second substrate, together with the sealing layer thereon, is placed on top of the polymer substrate such that the sealing layer contacts with the first layer and encapsulates the cargo between
10 the first layer and sealing later. To facilitate adhesion between the two layers, the top layer of each layer that is exposed to each other may be of an opposite charge. The micro-wells may be sealed by applying a force to both of the substrates or applying heat to promote annealing of
15 the polymer layers present in the first and sealing layers. The two substrates may be heated when placed in a solution such as water or a sodium chloride solution to promote annealing of the polymer layers present in the first and sealing layers. During heating, the annealing of
20 the polymer layers present in the first and sealing layers may be promoted and thus ensure that the two layers seal adequately. Although some cargo like microparticles may not need very well sealed microchambers, additional annealing at high temperature and ionic strength may be
25 necessary for proper sealing of chemicals with average molecular weight.

The heating temperature may be in the range selected from the group consisting of about 60°C to about 80°C, about 60°C to about 65°C, about 60°C to about 70°C, about
30 60°C to about 75°C, about 65°C to about 80°C, about 70°C to about 80°C and about 75°C to about 80°C. In one embodiment, the heating temperature is about 70°C.

The heating temperature may be applied for a time period in the range selected from the group consisting of

about 40 minutes to about 120 minutes, about 40 minutes to about 60 minutes, about 40 minutes to about 80 minutes, about 40 minutes to about 100 minutes, about 60 minutes to about 120 minutes, about 80 minutes to about 120 minutes and about 100 minutes to about 120 minutes. In one embodiment, the time period in which the heating temperature is applied for is about 60 minutes.

At least one of the first layer and polymeric sealing layer may be permeable to the cargo. The at least one of the first layer and polymeric sealing layer may be permeable to the cargo when exposed to an environmental condition.

At least one of the first layer and polymeric sealing layer may be impermeable to the cargo. The at least one of the first layer and polymeric sealing layer may be impermeable to the cargo when exposed to an environmental condition.

The permeability of the first layer and/or polymeric sealing layer may be tuned or adjusted depending on the environmental conditions of the micro-chamber array. In an embodiment where the cargo is an oil-based solution, the cargo may be loaded into the micro-chambers via a solvent-exchange method. First, the sealed micro-chambers are introduced to an organic solvent, for example toluene that is used for dissolving the PMMA template. Then the cargo such as sunflower oil or oil-based solution is added to the organic solvent. The sunflower oil or oil-based solution is fully miscible with the organic solvent, but has much higher viscosity and diffuses slowly into the micro-chambers. After about an hour, excess oil is washed away with a few drops of toluene. The oil micro-droplets remain entrapped in the micro-chambers.

At least one of the first layer and sealing layer may comprise the thermal conductor capable of rupturing at

least one of the respective first layer and sealing layer upon application of local heat.

The process may comprise the step of removing the polymer substrate from the first layer to form the micro-chamber array having plural micro-chambers, each of the micro-chambers comprising an outer layer defining a micro-chamber interior having the cargo therein.

The volume of the interior of the micro-chamber may be in the range selected from the group consisting of about 0.1 pL to about 0.5 pL, about 0.1 pL to about 0.2 pL, about 0.1 pL to about 0.3 pL, about 0.1 pL to about 0.4 pL, about 0.2 pL to about 0.5 pL, about 0.3 pL to about 0.5 pL, about 0.4 pL to about 0.5 pL and about 0.15 pL to about 0.17 pL. In one embodiment, the volume of the micro-chamber may be about 0.16 pL.

The substrate removing step may comprise the step of dissolving the polymer substrate or the step of peeling the substrate and first layer from each other. The substrate removing step may also comprise the step of freezing the polymer substrate such that the polymer substrate can be cut away from the micro-chamber array. The polymer substrate may be freezed until the temperature of liquid nitrogen.

The dissolving step may comprise the step of selecting an organic solvent that selectively dissolves the polymer substrate but does not dissolve the polymer(s) or component(s) making up the coating layer and the sealing coat. The organic solvent may be an aromatic solvent such as toluene or benzene. The organic solvent may be an aliphatic solvent such as chloroform, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), dimethyl sulphoxide (DMSO), N-methyl-2-pyrrolidone (NMP), dichloromethane (DCM) or tetrahydrofuran (THF).

In an embodiment where the polymer substrate is PMMA, the organic solvent used is toluene. The process may comprise the step of removing the organic solvent. The removing step may comprise the step of drying the micro-well array in order to remove the organic solvent.

After the polymer substrate is removed, the resultant product is then the micro-chamber array made up of the first layer and sealed with the sealing layer as mentioned above. The micro-chambers in the array may each contain one or more cargo as mentioned above.

The fill-rate of the micro-chambers (defined as percentage of micro-chambers that are filled with the cargo) depends strongly on the loading method and the type of cargo to be loaded. Solvent-exchange method may result in a nearly 100% fill-rate while template-assisted assembly of colloids may give about 98% of loading for melamine formaldehyde particles. The fill-rate may be about 40% or even lesser for glass beads.

The process may comprise the step of releasing the cargo from at least one micro-chamber in the micro-chamber array. Hence, there is provided a process for releasing the cargo from at least one micro-chamber in the micro-chamber array made as disclosed above. The process for releasing the cargo may comprise at least one of the following steps: (a) rupturing at least one of the first layer and the sealing layer of the at least one micro-chamber; and (b) changing an environmental condition to render at least one of the first layer and the sealing layers of the at least one micro-chamber permeable to said cargo.

The releasing step may at least partially rupture all of the micro-chambers or may selectively at least partially rupture a selected micro-chamber. The rupturing

step may be undertaken under conditions to selectively release the cargo from the micro-chamber array

The environmental condition may be selected from the group consisting of pH, temperature, ionic strength, magnetic field, ultrasound, light, presence or concentration of a solvent, concentration of a trigger compound, and a combination thereof.

When the environmental condition is pH, the pH to be used depends on the polyelectrolyte couple and ionic strength. When the ionic strength is high (higher than about 150 mM), the high ionic strength weakens the electrostatic interactions between the oppositely charged polyelectrolytes in the first layer or in the sealing layer. Thus, the permeability of at least one of the first layer or the sealing layer increases. The film composed of polyallylamine/poly(sodium 4-styrene sulfonate) disassembles at pH higher than 10 and release the content of the micro-chambers.

When the environment condition is ultrasound, high power ultrasonic treatment may cause the mechanical rupture of the chambers' walls or the sealing layer. It is to be noted that the ultrasonic power and frequency, and ultrasound duration depend on the stiffness of the first layer and/or sealing layer and have to be determined each time.

When the environmental condition is magnetic field or light in the presence of the thermal conductor, the environmental condition causes relaxation in the polyelectrolyte multilayer network making up the first layer and/or sealing layer and substantially increases the permeability (magnetic field, light) or causes chamber's rupture (light). The environmental conditions, such as pH, ionic strength, ultrasound, and magnetic field affect a group of chambers.

The environmental condition may be light such as laser. By means of a focused laser beam, each chamber can be addressed individually. The laser may be selected from the group consisting of visible (light) laser, infrared laser, ultraviolet laser and neodymium-doped yttrium aluminium garnet (Nd-YAG) laser. In one embodiment, the laser is Nd-YAG laser operating at a wavelength of 532 nm. When laser is used to rupture the micro-chamber, at least one of the first layer and the sealing layer may contain a thermal conductor. The rupturing step may comprise heating at least one of the first layer and the sealing layer in the presence of the thermal conductor. The heating step may comprise applying a laser beam to at least one of the first layer and the sealing layer.

In one embodiment, the at least one of the first layer and the sealing layer may contain a thermal conductor such as a metal nanoparticle. The thermal conductor may be selected from the group of chromophores in the same wavelength as the laser consisting of silver or gold nanoparticles, gold nanorods, natural and synthetic organic dyes and an alloy of any of the foregoing. The at least one of the first layer and the sealing layer may comprise gold nanoparticles that have adsorption band in the same wavelength as the laser. In the first layer and/or the sealing layer, one layer of gold nanoparticles may be deposited after each tenth polymeric bi-layer. Hence, if there are 40 polymeric bi-layers, four layers of gold nanoparticles form part of the first layer and/or the sealing layer. As the laser is shone onto the selected micro-chamber, the light adsorbed by the nanoparticles dissipates as heat, resulting in a highly localized increase in temperature that destroys the surrounding the first layer and/or the sealing layer and thereby releases the cargo therein. As the heating is

localized, the cargo inside that micro-chamber remains undamaged. In addition, the heat only affects that particular micro-chamber while leaving the other micro-chambers intact. A microscope may be used to direct the laser beam onto the selected micro-chamber.

The micro-chamber may be ruptured by applying a mechanical force at a selected speed. For example, the diamond tip of a Nanoindenter machine or a needle may be used to mechanically pierce the first layer and/or sealing layer at a selected speed making up the selected micro-chamber. The mechanical force used may be applied at a speed in the range selected from the group consisting of about 0.7 mN/s to about 15 mN/s, about 1.5 mN/s to about 15 mN/s, about 10 mN/s to about 15 mN/s. In one embodiment, the mechanical force used may be applied at a speed about 0.7 mN/s or about 1.5 mN/s.

There is provided a micro-chamber array comprising a surface; a plurality of micro-chambers on the surface, wherein each of the micro-chambers comprises an outer layer defining a micro-chamber interior having cargo therein; and a thermal conductor in thermal communication with said outer layer and being configured to rupture the outer layer upon application of heat.

Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 is a schematic diagram illustrating the process of fabricating an array of polyelectrolyte

multilayer (PEM) micro-chambers, loading them with cargo and releasing this cargo.

Fig. 2 shows the Scanning Electron Microscope (SEM) images of PEM film with array of empty microchambers: tilted view on (a) $2 \times 4 \mu\text{m}$ pillars, Scale bar - $1 \mu\text{m}$, (b) $25 \times 25 \times 12 \mu\text{m}$ bricks, Scale bar - $10 \mu\text{m}$, and (c) $7 \times 4 \mu\text{m}$ discs, Scale bar - $5 \mu\text{m}$ and (d) cross-section of $7 \times 4 \mu\text{m}$ discs, Scale bar - $10 \mu\text{m}$.

Fig. 3 shows SEM images of arrays of PEM micro-chambers sealed on the glass support, illustrating (a) 10, (b) 20, and (c) 40 bi-layers of PEM. Scale bar - $10 \mu\text{m}$.

Fig. 4 shows an SEM image of the cross-section of an individual PEM micro-chamber of 40 bi-layers. Scale bar - $1 \mu\text{m}$. Fig. 5 shows an SEM image of PMMA wells coated with PEM (40 bi-layers) and filled with $1.87 \mu\text{m}$ melamine formaldehyde (MF) colloidal particles. Scale bar - $5 \mu\text{m}$.

Fig. 6 shows a fluorescence microscope image of the PEM chambers (40 bi-layers) filled with FITC particles.

Fig. 7 shows the optical microscope images of PEM chambers loaded with MF particles before (a) and after several sequential pulses of focused Nd-YAG laser (b) to (d).

Fig. 8 shows the CRM image of the micro-chambers filled with oil: top view (c) and cross-sectional view (d). Color code of the image: red = oil (1660 cm^{-1} band is assigned to double bond, C=C stretching); green = PEM (1604 cm^{-1} band is assigned to C-C stretching in the aromatic ring). All scale bars - $5 \mu\text{m}$.

Fig. 9 shows the time-resolved fluorescence microscope images showing site-specific release-on-demand of oil-soluble fluorescent dye into water by opening a specific micro-chamber with a focused laser beam: just before laser pulse (a) 0s; (b) 2.5s, (c) 3.75s, and (d) 5s after the pulse. Scale bar - $5 \mu\text{m}$.

Fig. 10 shows the SEM images of micro-chambers after probing with a Nanoindenter tip at (a) 0.33 mN/s, (b) 0.67 mN/s, and (c) 1.33 mN/s loading speed. Scale bar - 5 μm .

5

Detailed Description of Drawings

Fig. 1 describes a process of fabricating an array of polyelectrolyte multilayer micro-chambers, loading them with cargo and releasing this cargo. Referring to Fig. 1, in the first step, an array of imprints 10 of a desired dimension and arrangement is fabricated on the surface of a polymer film 12. This imprinted polymer film 14 serves as a sacrificial substrate to support the fabrication of micro-chambers. Next, a coating step 16 is carried out on the imprinted polymer film, wherein the film is coated with PEM using layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes to form an imprinted polymer film coated with PEM 18. In the LbL assembly, a thermal conductor in the form of gold particles is present in the polymer film layers and hence gold is present in the walls of the microchambers. Then in the loading step 20, the desired cargo 22 is loaded into the micro-chambers 24. In the following step 26, the openings of micro-wells are sealed with another polymer film (not shown) and the sacrificial polymer film 14 is dissolved. Accordingly, the array of sealed micro-chambers loaded with desired cargo 28 is obtained. In the next step 30, the array of sealed micro-chambers loaded with desired cargo is flipped over. Finally in the last step 32, the cargo 22 from each individual micro-chamber 24 can be released by a number of means such as mechanically breaking it using a focused laser beam 34 which heats the microchamber walls due to the presence of the gold or a micro-tip (not shown) which literally breaks the wall of the micro-chamber.

30

Examples

Non-limiting examples of the invention and a comparative example will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Materials

10 Poly(allylamine hydrochloride) (PAH, Mw = 15 000), poly(sodium 4-styrene sulfonate) (PSS, Mw = 70 000), poly(ethyleneimine), branched (PEI, Mw = 25 000, Mn = 10 000) were purchased from Aldrich. 1H,1H,2H,2H-perfluorodecyltrichlorosilane (FDTS), 96% was purchased
15 from Alfa Aesar. Fluorescein isothiocyanate (FITC), >90% (HLPC) was purchased from Fluka. Sodium chloride, p.a. and toluene, AR were purchased from Merck. Hydrochloric acid, 37% and sulfuric acid, 95-97% were purchased from Honeywell. Hydrogen peroxide, 31% was purchased from MGC
20 Pure Chemicals Singapore Pte. Ltd. All chemicals were used as received. Colloidal gold was purchased from BioRad. Aqueous dispersion of melamine formaldehyde (MF) colloidal particles (1.87 μm in size) was purchased from Microparticles GmbH (Germany). Polymethylmethacrylate
25 (PMMA) coil (0.05 mm thick, 600 mm width, 1 m length) was purchased from Goodfellow Cambridge Ltd. Sylcard 184 Silicone elastomer kit was purchased from Dow Corning Co. LM-ETFE sheet 25 μm thick was kindly provided by Asahi Glass Co. Deionized (DI) water from Milli-Q (Millipore)
30 water purification system was used to make all solutions.

Example 1

Fabrication of the wells pattern on the surface of free-standing polymer films

In this example, a pattern is fabricated on the surface of a polymer film using Nanoimprint Lithography (NIL). A silicon mold with cylindrical pillars (7 μm in diameter and 4 μm in height) is processed by lithography and etching processes, which yield a high definition of structures on the surface of the mold. It is understood that the type of imprinted structure is dependent on the mold design used to form the polymer film. As seen in Fig. 2, a variety of micro-chamber shapes can be made depending on the mold design. Fig. 2(a) illustrates a PEM film with array of 2x4 μm pillars, Fig. 2(b) illustrates a PEM film with array of 25x25x12 μm bricks, and Fig. 2(c) illustrates a PEM film with array of 7x4 μm discs while Fig. 2(d) shows the cross-section of the 7x4 μm discs.

The silicon mold is cleaned in a piranha solution (a 3:1 mixture of 96% sulfuric acid and 30% hydrogen peroxide) at 140°C for 30 minutes, rinsed with Deionized (DI) water, dried in a flow of dry argon, and placed in a clean oven at 100°C for 1 hour. The mold is further treated with a fluorosilane release agent through an overnight vapor deposition of FDTS. The NIL process is performed using an Obducat nanoimprinter. A polymethylmethacrylate (PMMA) sheet with a thickness of 50 μm is cut slightly larger than the mold size and placed between the cleaned silicon wafer and the mold. The assembly is heated to 140°C and a pressure of 40 bars is applied for 5 minutes to let the PMMA flow into the trenches of the mold. The imprint process is completed by cooling the temperature to 80°C and releasing the pressure. Finally, the assembly is

demounted and the imprinted PMMA sheet subsequently detached from the mold.

Fabrication of the PEMs coating on the surface of imprinted PMMA sheet.

The imprinted PMMA sheet described above is then coated with PEM using layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes. The LbL assembly is performed using a dip-coating robot machine (Riegler & Kirstein GmbH, Germany). Prior to dip-coating, the PMMA sheet is sonicated in water for 5 minutes to remove air bubbles that may be trapped inside the wells. After that, said sheet is exposed for 15 minutes to 2 mg/ml solution of the corresponding polyelectrolyte that contains 2M NaCl. Poly(ethyleneimine) (PEI) is applied to form a first layer (its pH is adjusted to approximately 5 by HCl prior to dip-coating). Further alternating layers of poly(sodium 4-styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) are applied. At least three washings are conducted after each adsorption step to ensure the removal of all non-adsorbed macromolecules.

Referring to Fig. 3, there is shown the SEM images of the array of PEM micro-chambers sealed on the glass support. It can be seen that the ability of the micro-chambers to keep their shape improves substantially with the number of bi-layers that defines the thickness of the PEM film. With only 10 bi-layers deposited, dissolution of PMMA and further drying result in the complete collapse of the micro-chambers as seen in Fig. 3(a). Referring to Fig. 3(b), it can be seen that with 20 bi-layers deposited, the micro-chambers exist but they are significantly distorted. In contrast, the PEM film made of 40 bi-layers is strong enough to keep the micro-chambers' shapes as shown in Fig. 3(c), albeit with a minor roof sagging and a tilted wall.

Fig. 4 shows a cross-sectional view of the individual PEM chamber made of 40 bi-layers. The chamber walls are approximately 500 nm thick. The inner volume of the chamber is about 0.16 pL. The inner surface of the chamber has much larger roughness than the outer one.

Loading of PEM chambers with aqueous dispersion of colloids

Micro-particles are introduced into the imprinted wells using Template-Assisted Self Assembly (TASA) process during de-wetting of the slurry which is confined within a fluidic cell comprised of two substantially parallel glass substrates. Prior to assembly of the fluidic cell, the top glass substrate is rinsed with acetone, and dried in a flow of dry argon. The imprinted PMMA sheet coated with PEM described above is placed onto the bottom glass substrate.

Next, a thin frame of LM-ETFE film (25 μ m thick) is sandwiched between said PMMA sheet and the top glass substrate. After that, the cell is tightened with clips. A small hole (approximately 2 mm in diameter) is drilled into the top glass substrate using a laser, and a glass tube (approximately 5 mm in diameter) is glued to this hole with epoxy.

After the fluidic cell is assembled, an aqueous dispersion of melamine formaldehyde (MF) colloidal particles is injected into the cell through the glass tube. Once the slurry has completely filled the void space between the top glass substrate and the imprinted PMMA sheet, it is forced to move slowly with dry argon flow. Both capillary forces and gravity push the colloidal particles into the template wells. Next, the fluidic cell is disassembled. Particles stuck in between the wells are removed with a wet tissue.

Referring to Fig. 5, there is shown the micro-chambers coated with PEM and filled with 1.87 μm MF colloidal particles. It can be seen that the particles are statistically distributed among the wells, such that some wells contain fewer particles and some are fully filled with the particles.

Sealing

The imprinted PMMA film coated with PEM and with MF beads entrapped in the wells as described hereinabove, are placed onto a glass substrate pre-coated with PEM and held together with clips. It is important to note that the terminal layers of both PEM should have the opposite charge for better adhesion. Then, this assembly is immersed into a beaker filled with toluene, and after complete dissolution of PMMA, this assembly is removed from the beaker, washed carefully with excess of toluene and allowed to dry. Further sealing is performed by heating the assembly in water or a NaCl solution to promote annealing of the PEMs.

Next, the film is turned over and placed onto a glass substrate covered with a mixture of Sylcard 184 silicone elastomer base and curing agent (wells openings towards the PDMS precursor). This assembly is heated at 70°C for 3 hours. Finally, the PMMA film is dissolved in toluene.

Selective release of the cargo from individual micro-chamber

A second harmonic of a pulsed Nd-YAG laser, Lotis Tii, wavelength 532nm, is used for opening the micro-chambers. The collimated laser beam is focused onto the sample film through a Nikon microscope objective, 50x magnification, N.A.=0.45. The size of the laser spot on the sample film may be about 10 μm . The laser fluence

incident to the objective lens may be up to $1 \text{ mJcm}^2\text{pulse}^{-1}$. The sample film is positioned in the field of view of the same objective using an XYZ stage. By moving the stage in the XY plane, one can selectively aim the laser beam onto a specific chamber. Observation of the film is made using the same objective in transmission mode or in fluorescence mode. For transmission mode, a 150 W white light source is used. To excite the fluorescence, the sample film is directly irradiated by a collimated beam of a CW AOTK diode laser (50 mW, wavelength 532nm or 473 nm depending on a dye used). The images are recorded by a CCD camera connected to a computer.

Example 2

15 Loading of PEM chambers with dry powder

Fluorescein isothiocyanate (FITC) powder (1-3 μm crystals) is mechanically spread over the openings of the PEM-coated micro-chambers as described hereinabove.

Referring to Fig. 6, there is shown the fluorescence microscope image of the PEM chambers (40 bi-layers) filled with FITC particles. Intense fluorescence is detected inside the chambers filled with FITC particles 50 in contrast to the empty chamber 52. No fluorescence is detected in the surrounding water. In contrast, breaking or mechanical damage of the micro-chambers with a needle causes immediate coloring of the surrounding water with the fluorescent dye. This demonstrates that the chambers walls are properly sealed and impermeable towards FITC molecules (molecular weight 389).

30 Fig. 7 shows a series of optical microscope images before and after the laser pulse irradiation. The opening of individual micro-chambers and release of their content may be performed by irradiation with a focused beam of the pulsed Nd-YAG laser (532 nm). 4 layers of gold

nanoparticles having adsorption band at this wavelength are incorporated into the PEM film to make it sensitive towards this laser (1 layer of Au nanoparticles after each tenth PSS-PAH bi-layer). The laser irradiation is conducted within the plasmon absorption band of gold nanoparticles, which were incorporated into the micro-chamber walls. The presence of the gold nanoparticles inside the micro-chambers is confirmed by UV-vis spectroscopy (data not shown). Light adsorbed by the Au nanoparticles dissipates as heat resulting in a highly localized increase in temperature that destroys the surrounding PEM film. However, the cargo in the micro-chambers remains undamaged as the increase in temperature is localized or restricted only to the PEM film.

Referring to Fig. 7(a), there is shown the sealed PEM chambers containing MF particles are obtained via covering the wells with glass pre-coated with 20 PEM bi-layers followed by PMMA dissolution in toluene and drying. A circular hole appears in the PEM film upon irradiation as seen in Fig. 7(b). The diameter of the hole varies from 12 to 18 μm , which is slightly larger than the laser beam spot. Referring to Fig. 7(c) and 7(d), it can be seen that one micro-chamber may be opened at a time, leaving the neighboring micro-chambers undamaged. Simultaneous release of the loaded MF balls is achieved by burning of the chambers. The MF particles are released from the opened micro-chambers as indicated by the arrows in Fig. 7(b)-(d).

Example 3

Loading of PEM chambers with sunflower oil

The imprinted PMMA sheet coated with PEM described in Example 1 is loaded with sunflower oil using a solvent-exchange method. Referring to Fig. 8, there is

shown the CRM image of the micro-chambers filled with oil: top view (c) and cross-sectional view (d). It can be seen that the oil droplets are specifically located within the PEM micro-chambers and completely fill the micro-chambers. Raman spectrum taken from the PEM film between the chambers indicates the presence of mere traces of oil.

Referring to Fig. 9, there is shown a series of fluorescence microscope images taken before and after the single laser pulse irradiation shows the release of oil-soluble low-molecular fluorescent dye into surrounding water. Fig. 9(a) illustrates the fluorescence microscope image of the micro-chamber just before laser pulse irradiation. Fig. 9(b) to (d) shows the opening of the micro-chamber after laser pulse irradiation at 2.5 s, 3.75 s, and 5 s after the pulse respectively. It can be seen that the neighbouring micro-chambers remain undamaged.

Example 4

In this example, selective opening of the individual micro-chambers is performed using mechanical piercing, for example, with a sharp diamond tip of Nanoindenter machine.

Fig. 10 shows the SEM images of PEM chambers after their probing with a tip at different forces. As seen in Fig. 10(a), a probe of 5 mN force on the micro-chambers results in just a scratch on the micro-chamber roof. In contrast, a probe of 10 mN force results in an approximately 1 μm size hole as seen in Fig. 10(b). It can be seen in Fig. 10(c) that a bigger hole results from a probe of higher force of 20 mN. This provides for a precise control over the release of cargo from individual chambers.

Applications

The disclosed process may be applied in numerous industrial applications, not least in therapeutic applications, pharmaceutical compositions. The fabrication of microcapsules on the surface of polymer film may also be used for immobilizing proteins and performing cascade enzymatic reactions.

Advantageously, the disclosed process may be highly reproducible and may be used to fabricate different types of micro-chamber arrays that may be used for containing a wide variety of cargo in the chambers. The disclosed micro-chambers are well-sealed and may be impermeable towards low molecular weight compounds, thereby allowing the micro-chamber array to be used to store and contain a wide variety of cargo.

More advantageously, the disclosed micro-chamber array have controllable or adjustable loading and release properties of the encapsulated cargo. When exposed to an environmental condition, one or more chambers of the disclosed micro-chamber array may be configured to release the cargo contained therein. The one or more chambers may be selected independently from the other chambers to release the cargo contained therein.

Advantageously, the presence of the thermal conductor may aid in the release of the cargo from a specific micro-chamber on the micro-chamber array. The cargo inside the specific micro-chamber remains undamaged during release and the other micro-chambers also remain intact during the release of the cargo from a specific micro-chamber.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such

modifications and adaptations come within the scope of the appended claims.

Claims

1. A process for making a micro-chamber array comprising:
 - 5 (i) applying a first layer on a substrate having an array of micro-wells disposed on said substrate;
 - (ii) loading cargo into the layered micro-wells of the substrate;
 - (iii) applying a sealing layer to the loaded cargo
10 to encapsulate the cargo therein between said first layer and said sealing layer; and
 - (iv) removing the substrate from the first layer to form the micro-chamber array having plural micro-chambers, each of said micro-chambers comprising an outer
15 layer defining a micro-chamber interior having said cargo therein.
2. A process as claimed in claim 1, wherein said applying step comprises assembling a plurality of
20 layers of alternating charge on a substrate having an array of micro-wells disposed on said substrate.
3. A process as claimed in claim 2, wherein said assembling step comprises alternately exposing said
25 substrate to solutions of cationic polymer and anionic polymer.
4. A process as claimed in any one of the preceding
30 claims, comprising before step (i), the step of forming the micro-wells in the substrate.

5. A process as claimed in claim 4, wherein said forming step comprises using nano-imprint lithography (NIL) to form said microwells.

5 6. A process as claimed in claim 5, wherein said NIL comprises the steps of:

(v) applying a mold under pressure to a polymer substrate that is above its glass transition or melting temperature to form said micro-wells therein;

10 (vi) cooling said polymer substrate to a temperature below its glass transition or melting temperature; and

(vii) demolding the polymer substrate.

15 7. A process as claimed in claim 6, wherein said process comprises the step of plasma etching the micro-wells after said demolding step.

20 8. A process as claimed in claim 1, wherein said loading step comprises loading one or more different cargo types into respective micro-chambers.

25 9. A process as claimed in any one of the preceding claims, wherein the sealing layer is formed of a polymeric material.

30 10. A process as claimed in claim 9, wherein at least one of the first layer and the polymeric sealing layer is permeable to the cargo.

11. A process as claimed in claim 10, wherein at least one of the first layer and the polymeric sealing

layer is permeable to the cargo when exposed to an environmental condition.

12. A process as claimed in claim 9, wherein at least one of the first layer and the polymeric sealing layer is impermeable to the cargo.

13. A process as claimed in claim 12, wherein at least one of the first layer and the polymeric sealing layer is impermeable to the cargo when exposed to an environmental condition.

14. A process as claimed in any one of the preceding claims, wherein at least one of said first layer and sealing layer comprises a thermal conductor capable of rupturing at least one of said respective first layer and sealing layer upon application of heat.

15. A process as claimed in claim 14, wherein said thermal conductor is a metal.

16. A process as claimed in claim 15, wherein said metal is selected from the group consisting of silver or gold nanoparticles, gold nanorods, natural and synthetic organic dyes and an alloy of any of the foregoing..

17. A process as claimed in any one of the preceding claims, wherein said removing step comprises dissolving said substrate.

18. A process as claimed in any one of claims 1 to 16, wherein said removing step comprises peeling said substrate and said first layer from each other.

19. A process as claimed in any one of the preceding claims, comprising the step of releasing the cargo from the micro-chambers.

20. A process as claimed in any one of claims 2 to 19, comprising the step of applying at least 20 bi-layers to said substrate.

21. A process as claimed in claim 20, comprising the step of applying at least 40 bi-layers to said substrate.

22. A process for releasing the cargo from at least one micro-chamber in the micro-chamber array made in the process as claimed in any one of claims 1 to 21, comprising at least one of the following steps:

(i) rupturing at least one of the first layer and the sealing layer of the at least one micro-chamber; and

(ii) changing an environmental condition to render at least one of the first layer and the sealing layers of the at least one micro-chamber permeable to said cargo.

23. A process as claimed in claim 22, wherein at least one of the first layer and the sealing layer contains a thermal conductor and the rupturing step comprises heating at least one of the first layer and the sealing layer in the presence of said thermal conductor.

24. A process as claimed in claim 23, wherein said heating step comprises applying a laser beam to at least one of the first layer and the sealing layer.

5 25. A process as claimed in claim 22, wherein said rupturing step comprises piercing the first layer.

26. A process as claimed in claim 22, wherein said rupturing step comprises piercing the sealing layer.

10

27. A process as claimed in any one of claims 22 to 26, wherein said rupturing step is undertaken under conditions to selectively release said cargo from said microcapsule array.

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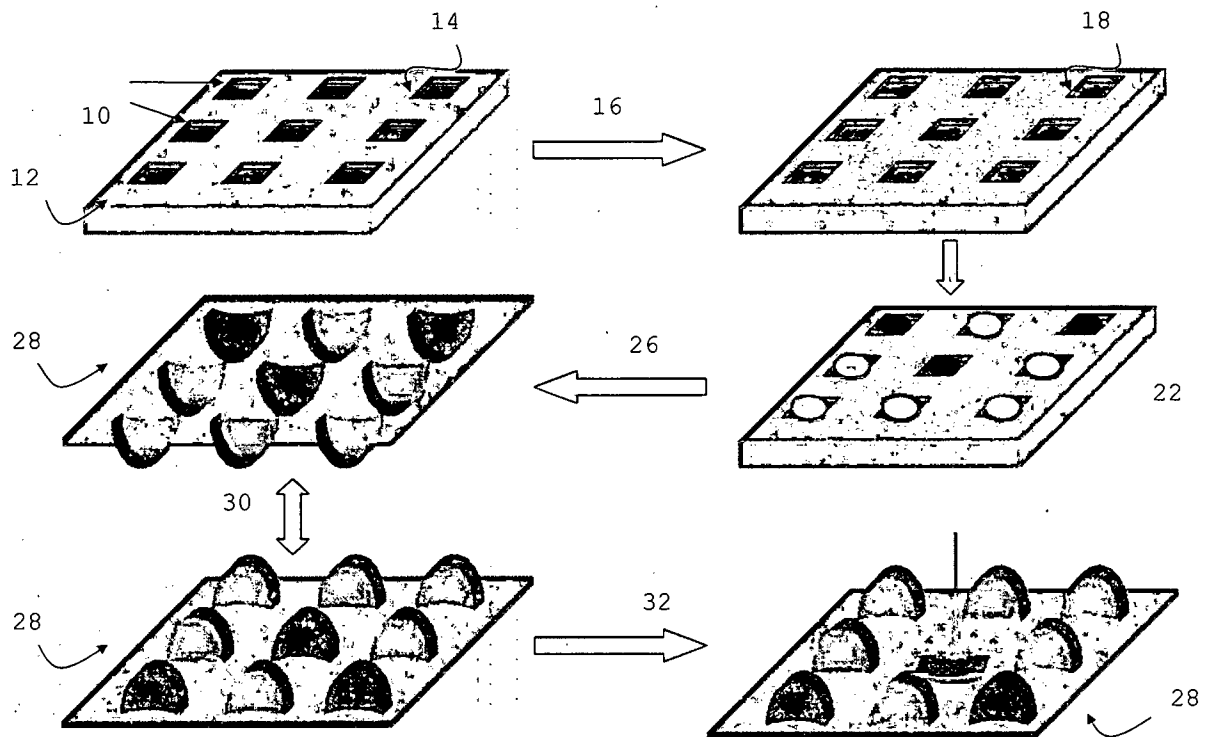


Fig. 1

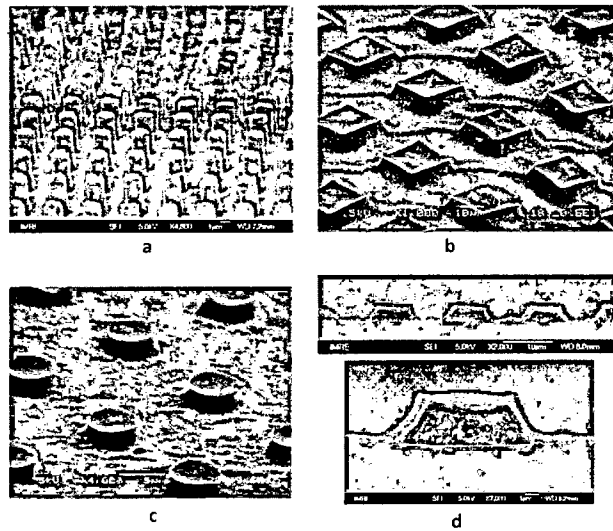
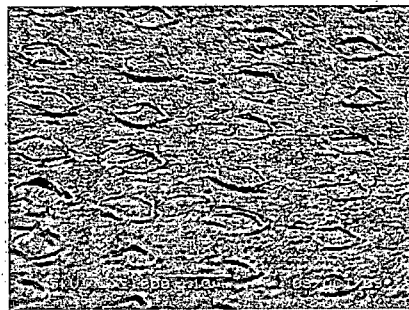
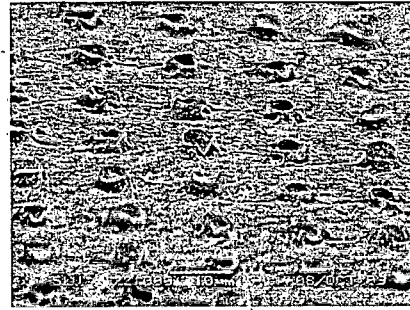


Fig. 2

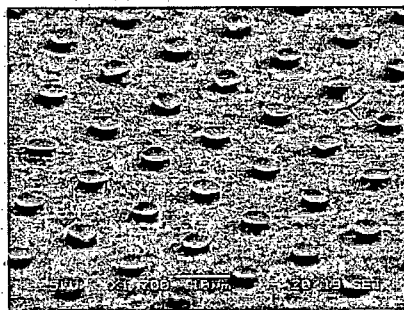
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(a)



(b)



(c)

Fig. 3

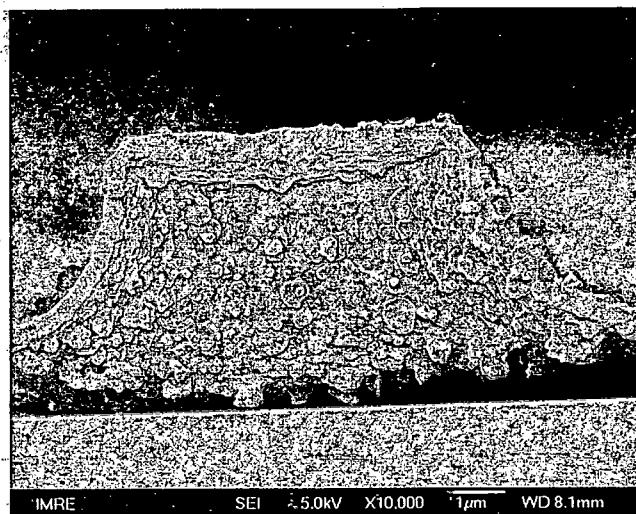


Fig. 4

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Fig. 5

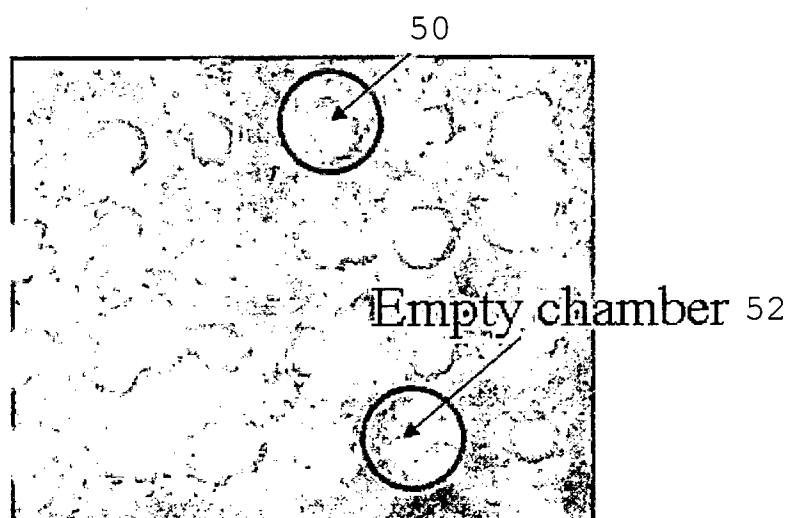


Fig. 6

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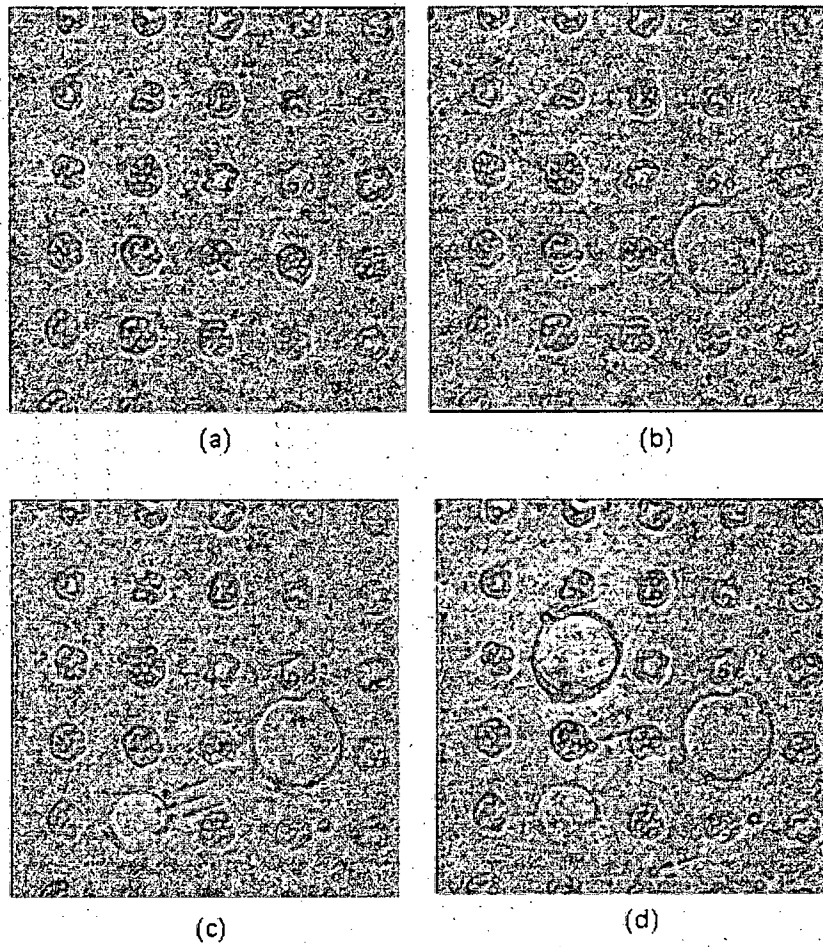
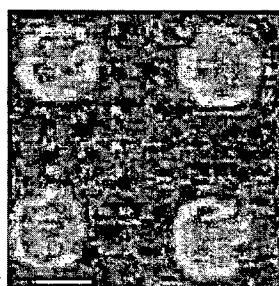


Fig. 7

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c



d

Fig. 8

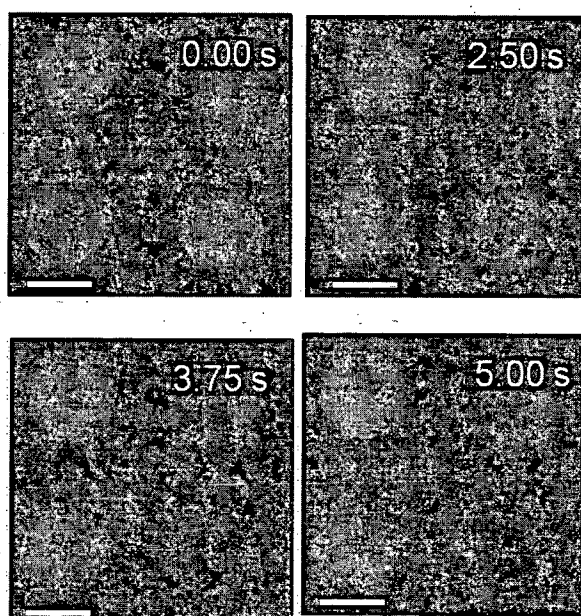


Fig. 9

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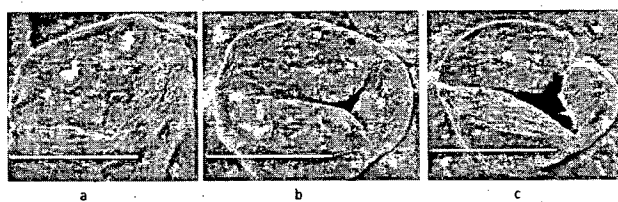


Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2011/000071

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

B32B 3/26 (2006.01)**A61J 3/00** (2006.01)**B81B 7/04** (2006.01)

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)

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)

WPI, EPODOC with keywords: microchamber, microreservoir, microwell, micromold; array; polymer; layer, membrane, sheet; drug, cargo; substrate, template, scaffold; seal, cover, lid, enclose, wrap; break, rupture, release; and similar terms

Google Scholar with keywords: polymer microreservoir sheet; microchamber peel, lift-off

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRAYSON, A. 'A Resorbable Polymeric Microreservoir Device for Controlled Release Drug Delivery', MIT PhD. Thesis, 2003 [retrieved on 31 March 2011] Retrieved from the internet <URL: http://dspace.mit.edu/bitstream/handle/1721.1/17036/54763982.pdf?sequence=1 > Section 2.2.2.1.2 and 2.2.2.2	1 - 27
A	US 7070592 B2 (SANTINI JR, ET AL.) 4 July 2006 Abstract and figures 1 - 3	1 - 27
A	US 2009/0298712 A1 (KIRYUKHIN ET AL.) 3 December 2009 Abstract and figure 1	1 - 27
A	WO 2005/010240 A2 (MICROCHIPS, INC) 3 February 2005 Abstract and figures 1 - 5	1 - 27



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search
05 April 2011Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2011/000071

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
US	7070592	AU	45131/01	AU	2005234663
		EP	1235560	EP	1688132
		US	6808522	US	2004248320
		WO	0141736	CA	2393603
US	2009298712	NONE			
WO	2005010240	US	2005050859	US	7114312
				US	2007036835
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					