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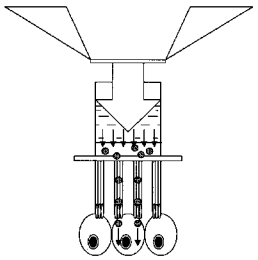


FIG. 14b

(57) **Abstract**: The present invention provides a process of making a nanopipe array comprising spaced bundles of nanopipes and a process of making individual nanopipe bundles. The array or bundle can be used in a device for the delivery of a substance, for example, the delivery of a substance into a patient for the treatment of a disease or condition.

NANONEEDLES

Technical Field

The present invention relates to the field of nanotechnology, such as nanopipes and processes for creating nanopipes.

Background Art

It is expected that the use of biopharmaceuticals such as peptides and genetic drugs, such as plasmid DNA, siRNA and miRNA (for gene therapy), will play an increasing role in medical treatment in the future. However, these macromolecules are too large, too labile and too hydrophilic to cross biological membranes by simple diffusion. Consequently the current method of administering such macromolecules to mammals is by injection. This increases the cost of therapy as it requires trained personnel, and decreases patient compliance due to the pain experienced during injection.

In order for these macromolecules to be able to permeate cell membranes *in vitro*, it is necessary to use electroporation. However, electroporation has a negative effect on cell viability.

Stem cells are an example of a biotarget into which it is desirable to transfect molecules such as DNA and RNA, because stem cells are able to self-renew. If, for example, a faulty gene in a stem cell is replaced by a 'correct' gene, each new generation of stem cells originating from the 'corrected' stem cell should contain the correct gene. Accordingly, if a correct gene is inserted into stem cells *in vitro* and those stem cells are then grafted into a patient, the patient should have a lifetime supply of cells with the corrected gene, originating from those stem cells.

This is a particularly attractive option of therapy for cells that have a high turnover, and are constantly being lost and replaced. Examples of these include the hematopoietic and epithelial systems. Hematopoietic stem cells are

multipotent and can become any cell of the blood system. 'Corrections' made to stem cells make the need for repeated treatment for a condition, e.g. replacing a faulty type of cell, redundant. As stem cells are able to develop into several different types of cells, the 'corrected' gene will only be expressed in the appropriate type of cell. Further, in some diseases and conditions stem cells are largely replaced. An example of this is the replacement of the pluripotent stem cells in the bone marrow in aplastic anaemia.

However, it is difficult to carry out genetic modification of stem cells (among others). The most successful methods of carrying out this process known so far have negative side effects on cell viability. Electroporation causes a substantial increase in the permeability of the cell membrane by applying an external electrical field, allowing a substance to be introduced into a cell. However, this method can be highly cytotoxic.

Topical drug delivery refers to a form of administration in which therapeutic agents or vaccines are transferred across the epithelium, such as the skin and mucosal epithelia. Skin is a readily accessible location for administration and capillaries lie only tens of μm beneath its surface. However, one of the main functions of the skin is to protect the body.

Topical drug delivery has been hampered by the presence of the topmost layer of dead skin cells (the *stratum corneum*). Though only 10-20 μm thick, this layer provides an effective barrier to diffusion of substances across the skin, with keratin-rich cells embedded in lipid lamellae. Only low molecular weight molecules with moderate oil and water solubility are able to diffuse through this barrier. This layer represents the rate-limiting step in diffusion of a drug across the skin.

Proposals have been made for using nanomaterials for biomedical applications.

Summary of the Invention

The invention is set out in the claims. In particular, the invention relates to bundles of nanopipes composed of amorphous carbon and their manufacture. The term 'bundle' or 'clump' of nanopipes is used to describe a plurality of nanopipes, which are located grouped together in close proximity to each other, with a spacing of between, for example, approximately 0nm to approximately 100nm, or for example approximately 10nm to approximately 50nm, or for example approximately 20 to approximately 40nm, between neighbouring nanopipes.

A special template is used to create the nanopipes, leading to an ordered vertical arrangement of nanopipes, and a two-step masking procedure is used to define the nanopipe bundles. These nanopipes may for example be used in a medical device.

Accordingly, a process for producing a more uniform nanopipe array is provided. Further, a homogeneous, densely-packed array of nanopipes which is not suitable for some applications, such as in a medical device, is avoided. In particular, a process for making an array with spaced clumps suitable for skin and/or penetration is provided.

The carbon pipes themselves are formed by high temperature chemical vapour deposition (CVD) of amorphous carbon in the pores of an anodic aluminium oxide (AAO) template using known methods. Conventionally using this CVD approach, a uniform carpet of closely spaced, aligned nanopipes is produced.

However, such dense arrays are not suitable for drug delivery. Previously existing techniques can be used to control the size and width of the template, the diameter, the spacing of the pores and the thickness of the deposited carbon, but not to control "clumping", i.e. obtaining an array with a non-continuous arrangement of nanopipes. After the CVD stage, the template matrix can be easily removed using wet chemical etching to leave behind the carbon nanopipes.

One of the key advantages of the present invention is the ability to controllably pattern the AAO template prior to CVD so that carbon is selectively deposited in only some of the pores. This patterning can be achieved in several ways:

In one embodiment, a pore blocking material consisting of a layer of metal is deposited on both surfaces of the AAO template in order to block the entrances to selected pores. By this means, deposition of carbon is constrained to only those pores that remain open. The present invention achieves this patterning using sputtering or thermal metal evaporation under high vacuum, and a patterned mask, for example made of copper, which is placed between the deposition source and the AAO template.

One challenge in achieving desired patterns of unblocked pores is that the ideal configuration will consist of regularly spaced islands of open pores surrounded by an adjoining region of a blocking material, such as a metal. This arrangement cannot be obtained by deposition through a single mask. The present invention solves this problem by using two-stage deposition through a grid pattern, rotating the mask through 90° for the second deposition. The result is a pattern of unblocked pores grouped into islands on the template. Island diameter is approximately equal to the width of the masking members and inter-island spacing is equal to the spacing between the members.

The success of the selective blocking is dependent on the accurate positioning of the mask with respect to the AAO template such that the metal deposition on both sides of the template is in register. That is, the openings of pores that are blocked by the deposited metal layer on one side of the template should also be blocked on the opposed surface of the template. If the alignment of the mask on the two opposed surfaces of the template is not in register, then carbon will be deposited in the pore through the unblocked entrance and the desired patterning of the resulting nanopipe array will not be achieved. In the present invention, a supporting rigid, high precision metal frame is used to hold the template in the apparatus to ensure accurate and repeatable alignment.

The blocking material layer, for example a metal layer, provides a double function: during CVD, it serves to pattern the deposition of carbon by blocking selected pores. After CVD, once the AAO template has been etched away, the metal layer provides mechanical support for the patterned carbon nanopipe array. The layer, which may for example be made of metal, also provides an impermeable barrier for containing a fluid reservoir.

A second method of patterning the array is to use a focused ion beam (FIB) combined with a metal vapour deposition system in an electron microscope to directly apply a pattern of blocking metal to the surface of the alumina template.

A third possibility is to use a mechanical plough, for example implemented using an Atomic Force Microscope (AFM) to directly remove template material in a controlled pattern.

Finally, other methods of electrochemical deposition on AAO templates could be used to allow controlled pattern.

Brief Description of the Figures

Embodiments of the invention will now be described, by way of example with reference to Figures 1-8, which show the various stages of preparation of the nanopipes according to the invention; Figures 9 and 10, which show the nanopipe bundles; and Figures 12 – 14 show the 'nanoneedles' in a medical device.

Figure 1 shows a schematic view from above of a plain AAO template prior to undergoing CVD. Pore size and spacing are exaggerated for illustration purposes.

Figure 2 shows a schematic view from above of a mask being positioned on the template to cover some of the pores.

Figure 3 shows a schematic view from above of metal deposited on and hence blocking the exposed pores of the template.

Figure 4 shows a schematic view from above of the template after the mask has been removed, with the pores that were covered by the mask remaining open.

Figure 5 shows a schematic view from above of the mask rotated by 90° with respect to the template.

Figure 6 shows a schematic view from above of a second metal deposition on the template, blocking the pores not covered by the mask.

Figure 7 shows a schematic view of the template from above with mask removed, revealing the pattern of pores that have not been blocked off by either the first or the second metal deposition on the template.

Figure 8 shows a schematic view from above of the pattern of islands of nanopipes, created in the pores of the template by CVD, the metal coating

having then been chemically removed, and the AAO template having been etched away.

Figure 9 shows a schematic cross-sectional view of the islands of nanopipes, supported on one side by the deposited metal coating

Figure 10 shows a cross-section of nanopipes created in an AAO template without masking or pore blocking.

Figure 11 shows a schematic cross-sectional view of the islands of nanopipes, supported on one side by the deposited metal coating, and a liquid reservoir.

Figures 12aand 12b show a schematic representation of a nanoinjector for *in vivo* applications and a mechanism for drug release.

Figures 13a and 13b show a method for loading a nanoinjector with drug and/or nanoparticle solution, and subsequent release of the drug.

Figures 14a and 14b show a mechanism of controlling insertion in a number of cells with a micromanipulator.

Figures 15a, 15b and 15c are a series of micrographs showing the surface of a cell being penetrated by a bundle of nanopipes.

Detailed Description of the Invention

In overview the invention relates to fabricating bundles of nanopipes.

Nanotubes are hollow nanoscale tubes made of carbon. Two types of nanotubes exist, each having a different process of manufacture and different properties. The first type are single-, or multi-wall carbon nanotubes, which have a fullerene molecular structure. The second type are carbon nanopipes, which are created by chemical vapour deposition (CVD) of amorphous carbon in pores that span the thickness of the membrane of an alumina template. The

present invention is particularly concerned with the second type of carbon *nanopipes*.

Membranes comprising an array of aligned nanopipes running substantially parallel from one surface of the template to the opposed surface – i.e. spanning the thickness of the membrane - are known. The following known method can be used to produce such an array:

Firstly aluminium foil is electrochemically etched in acid, creating the anodic aluminium oxide (AAO) template, which has monodisperse nanopores in a dense hexagonal 'honeycomb' pattern. Densities of these pores can be as high as 10^{11} cm^{-2}.

To create the nanopipes, the pore walls are lined with carbon, using CVD. The AAO template is placed in a furnace and a hydrocarbon gas is flowed over the template at an elevated temperature, for example 700 °C, for several hours, allowing carbon to be deposited on the surface of the template. Accordingly, the pores spanning the thickness of the membrane are lined with carbon, creating nanopipes. Nanopipes produced by this method will consist at least partly of amorphous carbon.

Described below is a technique according to the invention by which bundles of nanopipes are created.

The area of an AAO template **1** as shown in Figure 1 is typically $1cm^2$. The template is a porous membrane, whose pores **2** span the thickness of the membrane. Pore density is typically 10^9 cm^{-2} and can be between 10^8 cm^{-2} and 10^{11} cm^{-2}. Pore diameter is typically 100nm.

The fabrication process involves a series of fabrication steps as follows:

A mask **3** is positioned on the template **1** to cover some of the pores **2**. The mask used may, for example, be made of metal, such as copper, and consists of one or more members **4** accurately positioned over the template so that some of the pores are exposed and others are obscured. The one or more members of the mask may for example be in the form of bars that may be substantially parallel to each other. The width of the members of the mask, shown on Figure 2 as distance **A**, determines the ultimate spacing between the nanopipe bundles. The spacing between the members of the mask, shown on Figure 2 as distance **B**, determines the ultimate size of the nanopipe bundles.

A metal **5**, such as gold, chromium, or aluminium, is deposited onto the exposed pores that are not covered by the mask by evaporation under high vacuum or by sputtering, as shown in Figure 3.

The mask is removed from the template to reveal the pattern of deposited metal. The pores that were covered by the mask are now exposed **7** and the pores that were not covered by the mask are blocked by the metal coating **8**, as shown in Figure 4. The metal layer is sufficiently thick to completely block the entrances of the pores of the template.

The mask **3** is rotated by 90° with respect to the template, as shown in Figure 5. The metal deposition step described above is repeated. Again, the pores that are not covered by the mask are blocked by the deposited metal layer **8**, as shown in Figure 6.

Figures 7 shows the 'islands' of pores **9** on the template that have not been blocked off by a layer of metal, and the pattern of deposited metal **10** blocking off the remaining pores of the template. The resulting pattern of pores on the template arises from the areas of the template that were not covered by the mask in both the first-, and second metal deposition step. The islands of open

pores **9** are surrounded by the metal layer that blocks the openings to the remaining pores.

The two-stage metal deposition patterning process described above is then optionally repeated on the opposite side of the template. A frame allows accurate alignment of the mask on both faces to ensure the pattern of deposited metal is the same on both sides. That is, the same pores are blocked on both sides of the template.

The 'islands' of unblocked pores obtained during the two-step deposition and masking procedure ultimately result in the nanopipe bundles.

The next stage is the deposition of carbon by CVD. A layer of amorphous carbon **11** is deposited on the surface of the open pores and the external faces of the template. The entire internal surface length of the pores and the spaces between the pores are coated in a layer of carbon.

The metal coating is then chemically removed from one or both sides of the template, and the matrix of the AAO template is etched away, leaving islands of nanopipes **12**, created in the pores of the template by CVD, as shown in Figure 9. As the metal coating will be covered in a layer of carbon, the carbon can be removed by brief exposure to oxygen plasma or by mechanical polishing, prior to the metal coating being removed.

If a different metal is used on the two sides of the template, then one metal layer can selectively be chemically removed and the other left intact, for example, if the metal on a first side of the template is chemically inert, and the metal on the second side of the template is easily etched. This way, bundles of nanopipes, or 'nanoneedles' **12** supported by a layer of surrounding metal **8** as shown in Figure 9 can be obtained. The presence of the metal layer adds strength and creates a non-permeable, fluid-tight barrier – i.e. all liquid must

flow through the nanopipes, as it cannot permeate the metal layer surrounding the nanopipes.

Whether two different metals are used, or if the same metal is used, the metal on both sides of the template can be removed, in either one or two stages, depending on which metal or metals were used, leaving independent clumps or bundles of nanopipes.

Although off-the-shelf templates can be used, nanopipes grown on commercially available templates have been found by the applicant to demonstrate an irregular morphology. The stated inner diameters of the pores were often found to apply only to the final few μm of the end of far longer and wider channels - typically 50 μm long, and >200nm wide. Further, the resulting pore shape was often found to be highly variable on one side, with a wide distribution of aperture sizes and a strong tendency to provoke pore blocking during CVD.

Therefore according to a preferred aspect AAO templates are prepared to ensure regularly spaced pores of uniform inner diameter along the entire length of the pores, e.g. <100nm between pores, by the following method:
High purity aluminium plates were mechanically polished until a mirror-like finish was obtained. Electropolishing of the plates was then carried out. This was followed by a two-step anodising procedure in 0.5M oxalic acid at 40V, which was used to obtain the template. The aluminium substrate was the removed, followed by a pore opening and widening treatment. The membranes obtained by this method show well-ordered arrays of pores with a diameter of 62±4nm and distance between pore centres of 102±1nm, and a membrane thickness, corresponding to a pore length of 78±2μm (Figure 10).

PRODUCING NANOPIPES BY USING CVD

Carbon nanopipes for transporting fluids are grown in the AAO templates manufactured as described above using a variation of known CVD methods:

The AAO template is cut to the desired size using a scalpel, for example approximately 9mm x 9mm, then annealed between two quartz plates for 4 hours at 675°C, in order to prevent curling during subsequent CVD, ramping up to and down from the annealing temperature slowly. The template is then heated in a tube furnace at the natural ramp of the furnace (Vecstar with 25mm quartz tube) in a 100sccm stream of argon gas (Pureshield 99.995%, British Oxygen Company). When the temperature of the template stabilises at 675±3°C, a mixture of ethylene and helium gas (30% : 70% ratio; premixed; Scott Speciality Gases) is flowed over the template for 2 hours, at 60sccm. During this step, the template is supported using tungsten wires in a ceramic boat, with the longitudinal axis of the pores being perpendicular to the direction of gas flow. The template is then allowed to cool at the natural ramp of the furnace in a stream of hydrocarbon gas, for example 100sccm. The flow rate of the hydrocarbon gas may be varied to control wall thickness.

Average pore diameter was assessed by Scanning Electron Microscopy (SEM) (LEO 1525 Gemini with Field Emission Gun) to be 43±3nm by calibrated micrographs. SEM and Transmission Electron Microscopy (TEM) were also used to confirm nanopipe uniformity and evenness of carbon deposition.

ETCHING AWAY AAO

After the carbon nanopipes have been created inside the pores of the AAO template by CVD, the AAO template can be etched away to yield carbon nanopipes. This is achieved by sonicating the template, or a part of it, at low power (Jencons cleaning bath) in 2M $NaOH_{(aq)}$ for 30 minutes, then washing

three times in de-ionised water with centrifugation at 13,000rpm. The obtained suspension of nanopipes is dried on a 300mesh holey carbon film grid.

Gentler sonication, with more dilute NaOH and shorter process times, may be used to remove the alumina template matrix while still leaving the carbon nanopipes organised in an intact array.

Nanopipe wall thickness and uniformity was assessed by Transmission Electron Microscopy (TEM) (Jeol FX2000). The average inner diameter of the nanopipes was 46±3nm and the carbon wall thickness 6±1nm.

The nanopipes are uniform in length within a template and are in the range of approximately 50-1000 μm in length, for example 50-250μm, for example 50-100 μm, depending on the thickness of the AAO template. The nanopipes have a uniform internal diameter, which may be of approximately 20-500nm, for example approximately 20-150nm, for example approximately 20-100nm. The thickness of the nanopipe walls can be controlled in the range of 5-20nm.

Within the array, nanopipes are grouped together into bundles, which may for example be arranged parallel to each other. Each bundle of nanopipes may comprise up to several hundred individual pipes. For example, the bundles may comprise 5-200, 5-100, or 10-30 individual pipes.

The edge length of a bundle may for example be 200-2500nm, with the spacing between bundles being, for example, 1000-100,000nm.

The overall array comprising a plurality of nanopipe clumps, may have an area up to several hundred mm^2, for example up to $500mm^2$, for example up to $200mm^2$, for example up to $100mm^2$.

BIOMEDICAL APPLICATIONS

The clumps-, or bundles of nanopipes are considerably stronger than individual pipes by themselves. This is because clumps or bundles of nanopipes are less flexible compared to other nanotubes. Graphitic nanotubes are too flexible to be inserted into a cell and single nanotubes bend when in contact with a cell membrane, thus not allowing proper insertion under nanomanipulation. This gives a nanopipe bundle a mechanical advantage, allowing it to penetrate cell membranes, as well as intervening tissue overlaying target cells. Alternatively, single nanopipes arrays may be used when accuracy is required in applications such as injecting into a cell or sampling from a living cell or cells and/or its compartments (i.e. nuclei, lysosomes). "Single nanopipe array" may mean either an array with only one nanopipe bundle, or an array where each 'island' consists of only one nanopipe.

The fabrication method described above can be used to form, for example, a medical device, incorporating the nanobundles. Such a device can be used for *in vitro* and/or *in vivo* applications.

For these devices, the ability to control the size of the clumps as well as the spacing between them, with the aim of fabricating devices that can deliver drugs painlessly, with minimal trauma and more efficiently than using existing alternatives, is paramount. Deployment can be in the form of a patch for direct application to mucosa, to epithelial layers, skin, or to cells on culture plates.

In the context of *in vitro* applications, these nanopipe arrays provide a means of delivering therapeutic agents, for example, proteins, peptides, RNA or DNA sequences directed at the cytosol or to the nuclei of cells, across cell membranes.

Due to the arrayed format, the device offers the ability to penetrate the cell membrane of cells in culture, and transfer molecules, such as proteins, peptides, DNA, RNA, pharmaceutically active drugs, nanoparticles, and the like, into the cells. This method of delivering particles into cells *in vitro* allows a minimum contact time with cells and the ability to control the depth of cell penetration. The method further causes minimum cell damage compared to electroporation. A further advantage is that controlled release of administered agent is possible.

Administration via an *in vivo* device results in minimum tissue invasion and no adverse effects on the carbon nanomaterial are observed. The device has the ability to transfer all types of drugs across skin or mucosae.
Further advantages of the device include the ability to control the depth of penetration of the nanoneedles into the tissue. The device also provides the ability to control the duration of the drug administration and provides controlled release characteristics.

The applicant has undertaken mechanical force testing of the carbon nanopipe bundles and found them to be stronger than the smallest silicon microneedles and less susceptible to breaking inside the body of a patient. Using AFM to measure the buckle strength (compression testing) for carbon nanopipe arrays, individual bundles have been consistently shown to withstand a force greater than 75μN.

Silicon microneedle arrays, whose needles are up to 1000μm in length and a few (25-100) μm in diameter, are known. The smallest reported silicon microneedle is 25 μm in diameter and 500 μm in length. It was reported that when this silicon microneedle was inserted into the skin, it induced only 5-10%

of the pain of a 26 gauge hypodermic needle (*Adv Drug Deliv Rev*, M.R. Prausnitz, 2004, 56, 581-587).

The bundles of nanopipes, or 'nanoneedles' of the present invention are no more than approximately 100 μm in length. Therefore, the nanoneedles do not penetrate the skin deeply enough to reach nerve cells. The nanopipes are each of approximately 70-200nm in diameter. In both the *in vivo* and the *in vitro* device, the small size of the nanoneedles (for example 5 carbon nanopipes each with a 200nm diameter), and the spacing between the bundles, results in trauma to the target cells being minimised. The average diameter of a cell is 20 μm. Accordingly, a nanopipe bundle having a 1μm diameter is small enough to allow penetration of one individual cell. In an array, nanoneedles will be spaced with a minimum distance of 20 μm from each other to allow penetration of each cell in the area. Thus, when using an array of nanoneedles, each one of adjacent cells, for example skin cells, can be penetrated by one nanoneedle (carbon nanopipe bundle) of the array. Thereby, due to the dimensions of the nanoneedles of the present invention, *in vivo* applications can be carried out with the patient experiencing practically no pain.

Silicon microneedle arrays have the disadvantageous tendency to fracture inside the skin. A further advantage of the bundles of carbon nanopipes of the present invention is that they are stronger due to their 'bundle' structure. This enables the nanopipes and nanoneedles to withstand fracture.

Accordingly, the nanoneedles, or nanobundles, nanopipes or nanopipe arrays, of this invention enable both *in vivo* and *in vitro* cellular transfection to be carried out, as well as achieving a reduction in the pain associated with administration.

Manufacture of a device that makes use of these nanopipes, or nanobundles, is as described above – up to and including removal of metal and alumina template by chemical etching, either on both sides of template or only on one side. The nanobundles or the array of nanopipes is/are then attached to an injector device. This device could, for example, have tips in a 96-well format, akin to the well plates used for cell culture. The distance between the nanopipe bundles is calibrated using corner pillars. The bundles of nanopipes act as nanoneedles and these terms will be used interchangeably when describing the medical uses of the invention.

Several methods can be used to deliver drugs or other molecules to target cells using the nanopipe array. An impermeable barrier **13**, such as a metal layer, on one side of the array roots the clumps of nanopipes **12** in position and allows for the possibility of a reservoir of fluid **14** behind the array to be delivered to the target cells through the central pores of the nanopipes, as shown in Figure 12a. Using the reservoir mechanism described above, combined with a means of creating pressure in the fluid – for example mechanically, by heating, or using a piezo actuator – a drug can be injected directly into the cells through the nanopipe channels, as shown in Figure 12b.

Alternatively, simple passive loading of the nanopipes can be accomplished by exposing the open tips of the nanopipe bundles to a solution or suspension of the therapeutic agent, as shown in Figure 13a. Wetting and capillary forces will cause the drug to enter pores of the nanopipes and to become attached to their outer surface. Such passive loading can take place from either end of the nanopipes. This interaction can be rationally modulated by altering the surface chemistry of the carbon walls, for example using chemical methods or by means of reactive plasma to enhance flow through the nanopipes. Once in

contact with the target cells, passive diffusion, or active injection leads to controlled release of the agent, as shown in Figure 13b.

Accordingly, a delivery device such as a patch can be produced, which comprises the bundles of nanoneedles separated from a reservoir **15** containing an active pharmaceutical. The active pharmaceutical reservoir is for example, covered by a semi-permeable membrane **16**, or a non-permeable, but frangible membrane. The active pharmaceutical may be provided in a freeze-dried state. Alternatively, the active may be provided in the device as a solution. A separate water reservoir **14** is located adjacent to the drug reservoir, far from the nanoneedles, and also separated from the drug by a semi-permeable membrane. When the patch is used, the water in the water reservoir is brought into contact with the pharmaceutical active, and the water is drawn into the drug reservoir through the membrane **16** by osmosis. The drug is then able to flow through the pores of the nanopipes. If the patch with nanoneedles is placed on, for example, skin or mucosal cells of a mammal, the nanoneedles will penetrate the cell membrane and the drug will be delivered into the cell.

A similar set up can also be used with a micromanipulator, or a microinjector, as shown in Figure 14. Such a device would be placed onto, for example, skin cells of a mammal, or used on cells in culture, and the nanoneedles are guided with the aid of the micromanipulator to penetrate the cell membranes at defined depths. The pharmaceutical active may be provided in a freeze-dried state, or as a solution. The drug could for example be separated from the reservoir by a semi-permeable membrane, or a non-permeable, but frangible membrane, and be brought into contact with the contents of the reservoir, for example water, if the upper surface (remote from the needles), or an injector located upon the water reservoir of the drug solution, is pressed down on the device. The drug, or drug solution, would then be 'flushed' into the penetrated cells.

The nanoneedles of the microinjector are also able to take up drug molecules. For example, if the free ends of the nanoneedles are placed into a solution containing drug molecules, these molecules will be taken up into the nanoneedles. The drug can then be administered to e.g. a mammal by penetrating the appropriate cell membranes with the nanoneedles. By applying pressure, the drug will then flow out, or be pushed out of the nanoneedles, into the cells.

Such a patch or microinjecting device can be used to transfer therapeutics or vaccines across skin and/or mucosal epithelia, or across cell membranes of cells in culture. The difficulty of getting macro molecules across the stratum corneum layer of the skin is discussed above. The nanoneedles of the present invention are able to permeate this barrier with ease. The length of the nanoneedles allows them to traverse the stratum corneum and target dermis where blood capillaries end.

Injection into a cell using a nanoneedle is shown in Figures 15a, 15b and 15c. Figure 15a is a micrograph of a bundle of carbon nanopipes **17**, welded to the tip of a tungsten needle **18**. Figure 15b is a micrograph of a human kidney cell **19**. Figure 15c shows the nanoneedle **17** penetrating the surface of the cell **19** to a depth of several micrometers. The nanoneedle was subsequently withdrawn and then used to penetrate the cell a second time.

In one embodiment of a device of the present invention, the nanopipe bundles (comprising for example 5 carbon nanopipes each with a 200nm diameter, or 10 carbon nanopipes each with a diameter of 100nm) should, for example, measure up to about 1μm in total diameter. The device thus offers a painless,

simple method for delivering drugs and other large molecules across the skin or mucosal layer, or into cells in culture.

It will be understood that any appropriate CVD and masking techniques can be used in the present invention.

Claims

1. A process of making a nanopipe array comprising spaced bundles of nanopipes, comprising partially covering a porous membrane, whose pores span the thickness of the membrane, with a mask; depositing a blocking material onto a surface of the porous membrane blocking the entrances of the pores that are not covered by the mask; removing the mask; and depositing nanopipe precursor material on the unblocked portions of the membrane.

2. A process according to claim 1, wherein the nanopipe array is made of carbon.

3. A process according to claim 1 or claim 2, wherein the porous membrane in an anodic aluminium oxide (AAO) template.

4. A process according to any one of claims 1 to 3, wherein the deposited blocking material is a metal.

5. A process according to any one of claims 1 to 4, wherein the blocking material is deposited onto the porous membrane by evaporation under high vacuum or by sputtering.

6. A process according to any one of claims 1 to 5, wherein after blocking material is deposited on the porous membrane in a first deposition step, the mask is rotated by 90° with respect to the porous membrane, and subsequently blocking material is deposited on the porous membrane in a second deposition step, blocking the entrances

of the pores that are not covered by the mask in the rotated position of the mask.

7. A process according to any one of claims 1 to 6, wherein the deposition of the blocking material is repeated on the opposite surface of the porous membrane.

8. A process according to any one of claims 1 to 7, wherein a frame is used to ensure accurate alignment of the mask with respect to the porous membrane and to ensure the blocking of the entrances of the same pores on both sides of the porous membrane.

9. A process according to any one of claims 1 to 8, wherein chemical vapour deposition is used to deposit carbon on the porous membrane and in the pores of the porous membrane.

10. A process according to any one of claims 1 to 9, wherein the first and second deposition steps are each carried out with a metal, such as one or more of gold, chromium, aluminium.

11. A process of making a carbon nanopipe bundle by chemically etching away the matrix of the AAO template of the array of any one of claims 1 to 10.

12. A process of making a carbon nanopipe bundle according to claim 11, wherein the blocking material deposited on the areas of the porous membrane that were not covered by a mask in both the first and second deposition steps is chemically removed from one surface or from both surfaces of the porous membrane.

13. An array made by the process of any one of claims 1 to 10.

14. A nanopipe bundle comprising a plurality of nanopipes of uniform dimensions, and both ends of each nanopipe being open, each nanopipe being 50-1000μm, for example 100-500μm in length and having an inner diameter of 20-450nm, for example 20-100nm, for example 20-80nm, and an external diameter of 50-500nm, for example 100nm.

15. A nanopipe array comprising at least one nanopipe bundle, wherein each bundle comprises a plurality of nanopipes, and a support from which the or each bundle extends.

16. A nanopipe array according to claim 15, wherein the support is made of a metal.

17. A nanopipe array according to claim 16, wherein the support is a layer made of a metal selected from one or more of gold, chromium, aluminium.

18. A nanopipe array according to any one of claims 15 to 17, wherein the support is continuous except for the open ends of each nanopipe.

19. A device comprising one or more nanopipe bundles that each comprise a plurality of nanopipes, and a first reservoir containing a substance to be delivered through the nanopipes, and a delivery component allowing delivery through the nanopipes.

20. A device as claimed in claim 19, wherein the device allows delivery of the substance through the nanopipes into a cell, either *in vivo* or *in vitro*.

21. A device as claimed in claim 19 or 20, wherein the device comprises a second reservoir containing a fluid.

22. A device as claimed in claim 21, wherein the fluid in the second reservoir is water.

23. A device as claimed in any one of claims 19 to 22, wherein the first reservoir is separated from the nanopipes by a semi-permeable membrane.

24. A device as claimed in any one of claims 19 to 23, wherein the lower surface of the second reservoir is separated from the upper surface of the first reservoir by a further semi-permeable membrane.

25. A device as claimed in any one of claims 19 to 23, wherein the lower surface of the second reservoir is adjacent to the upper surface of the first reservoir and the first and second reservoir are separated by a distance.

26. A device as claimed in any one of claims 19 to 23, wherein the lower surface of the second reservoir is separated from the upper surface of the first reservoir by a non-permeable, but frangible membrane, which membrane can be broken by means located on the upper surface of the first reservoir, which faces the lower surface of the

second reservoir, by applying a downwards force on the upper surface of the second reservoir.

27. A device according to any one of claims 19 to 26, wherein the delivery component is a means which causes the substance to be delivered to be pushed through the nanopipes of the device.

28. A device according to any one of claims 19 to 27, wherein the delivery component is an injecting means.

29. A device according to any one of claims 19 to 28 for use in medicine.

30. A device according to any one of claims 19 to 28, for use in treating or preventing a disease or condition, by delivering a compound, a pharmaceutical composition, or a macromolecule such as peptides, proteins, DNA, RNA, or a plasmid, across a cell membrane into a cell.

31. A method of delivering a compound, a pharmaceutical composition, or a macromolecule such as peptides, proteins, DNA, RNA, or a plasmid, across a cell membrane into a cell.

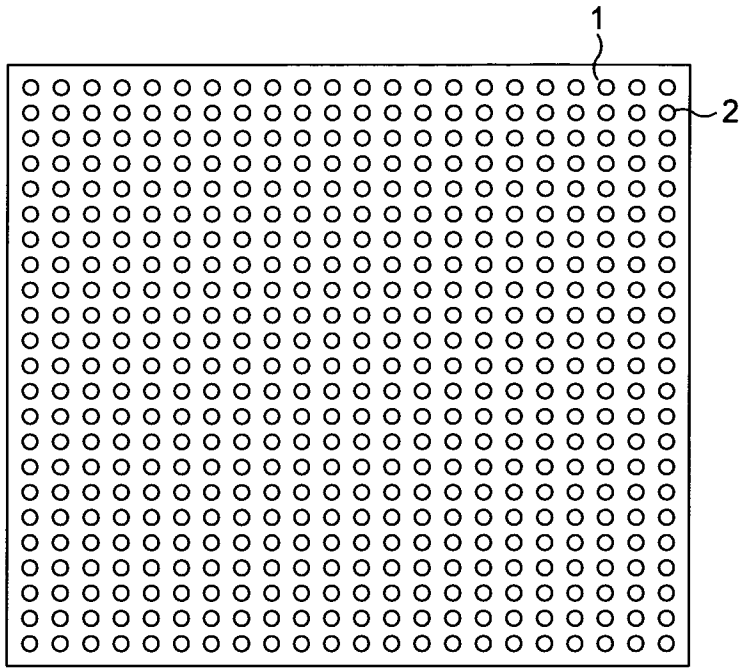


FIG. 1

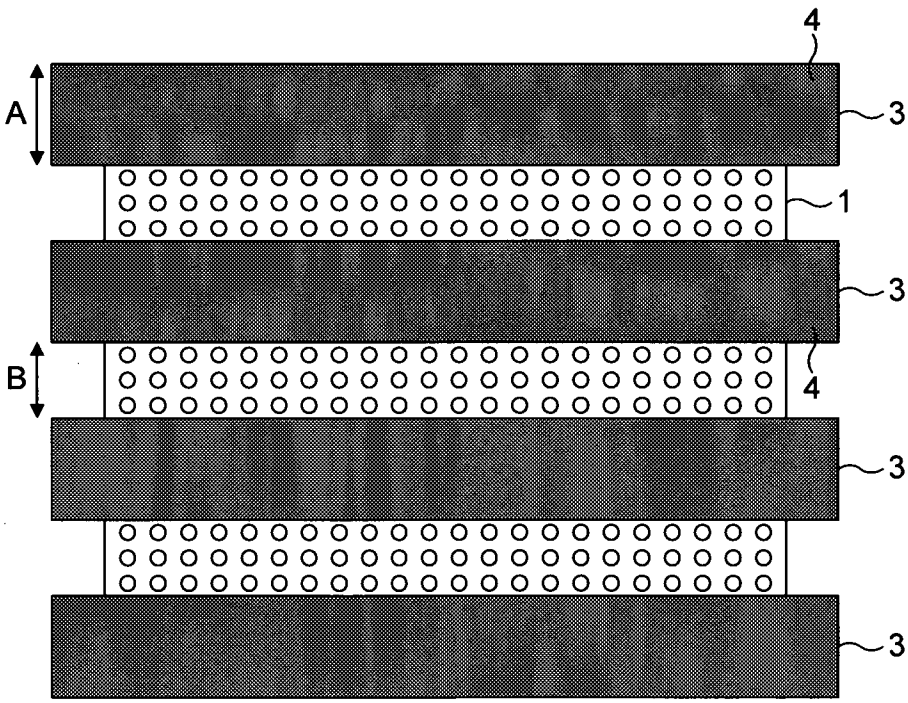


FIG. 2

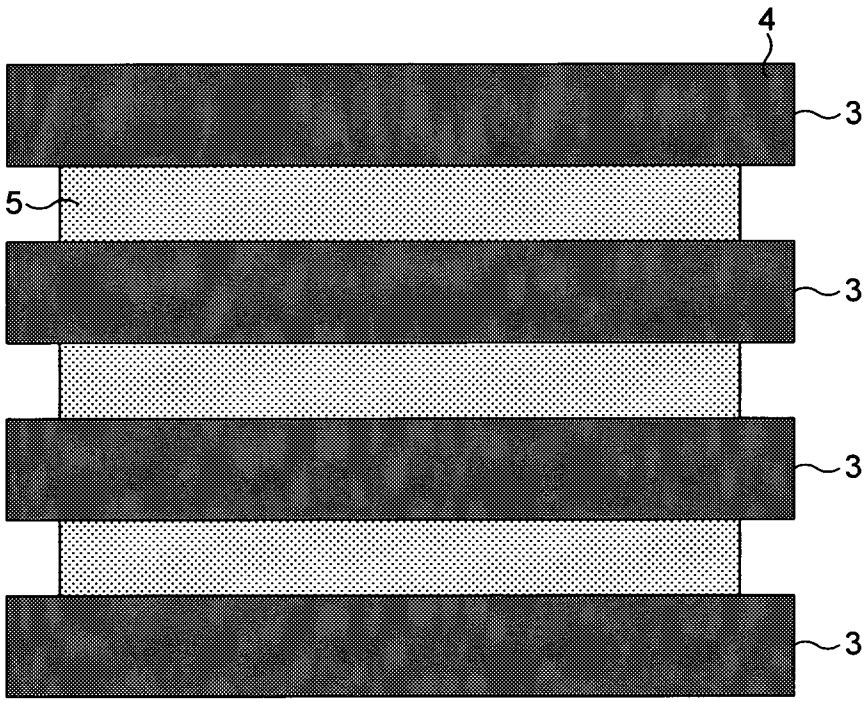


FIG. 3

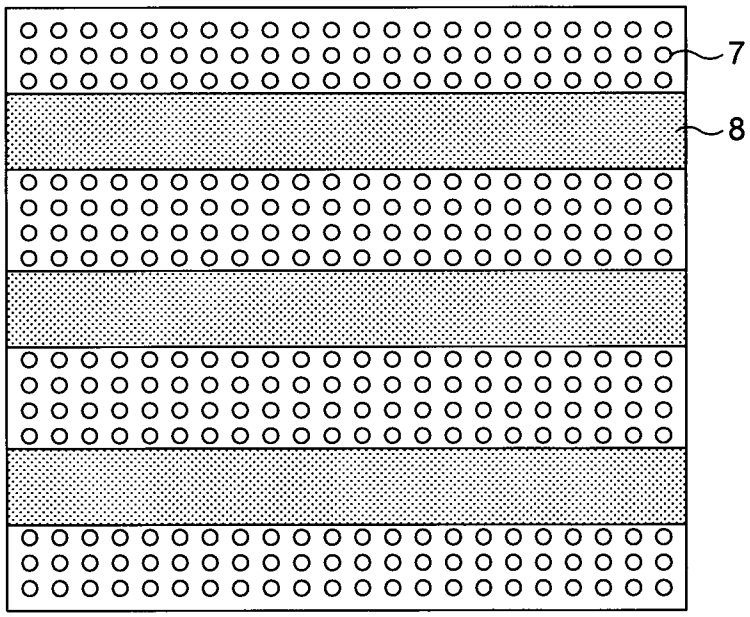


FIG. 4

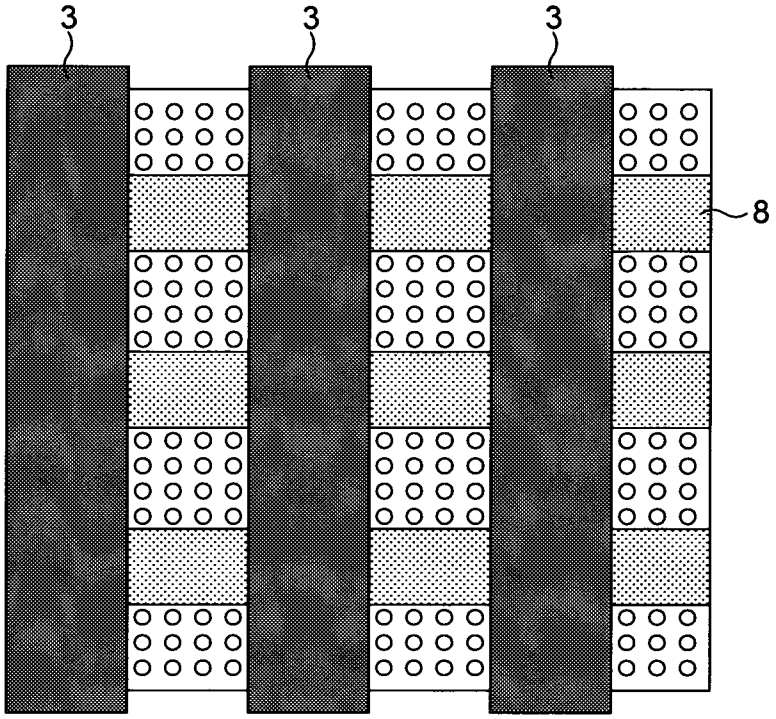


FIG. 5

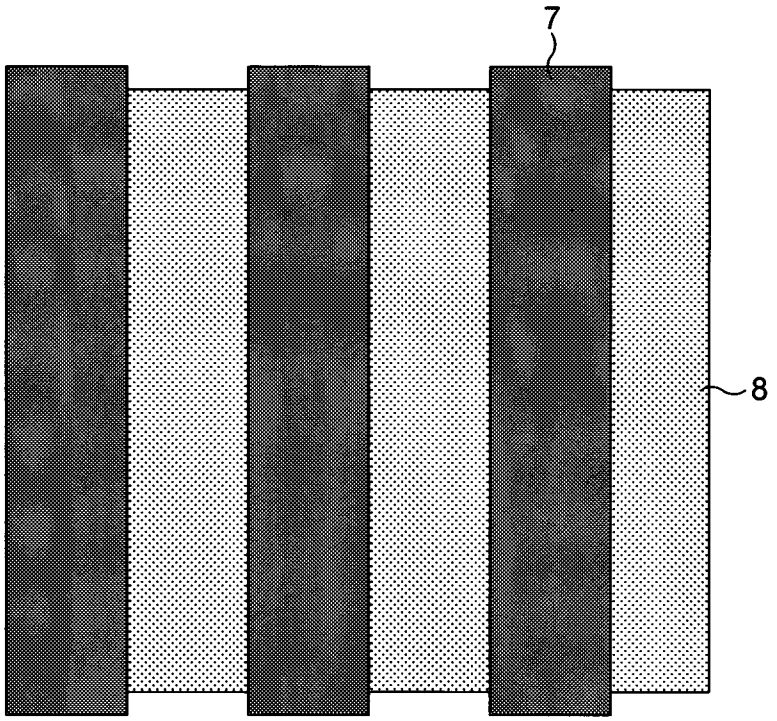


FIG. 6

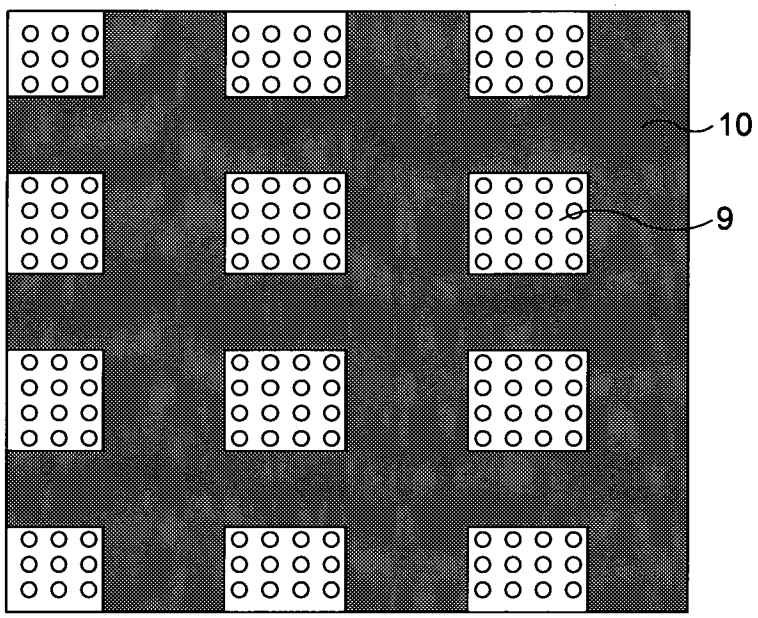


FIG. 7

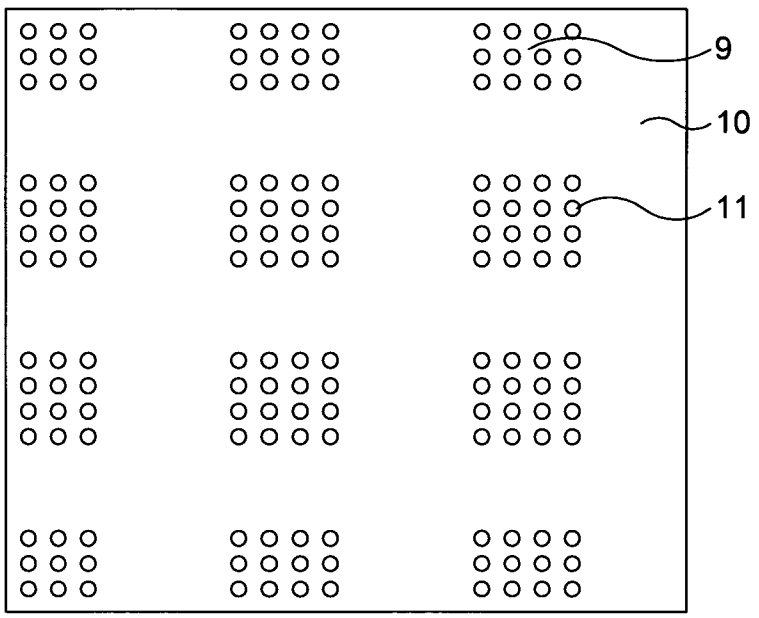


FIG. 8

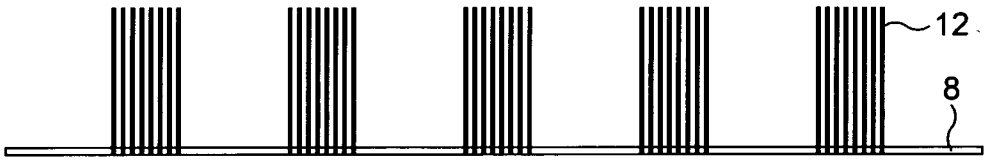


FIG. 9

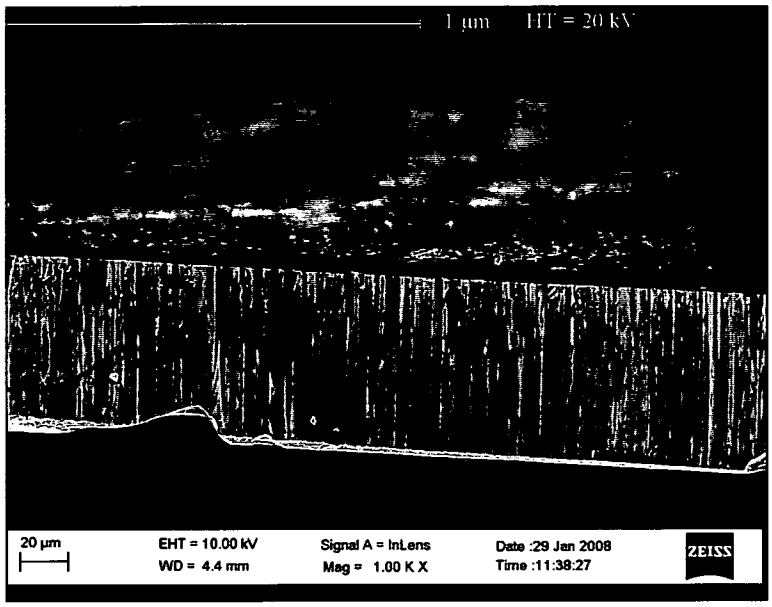


FIG. 10

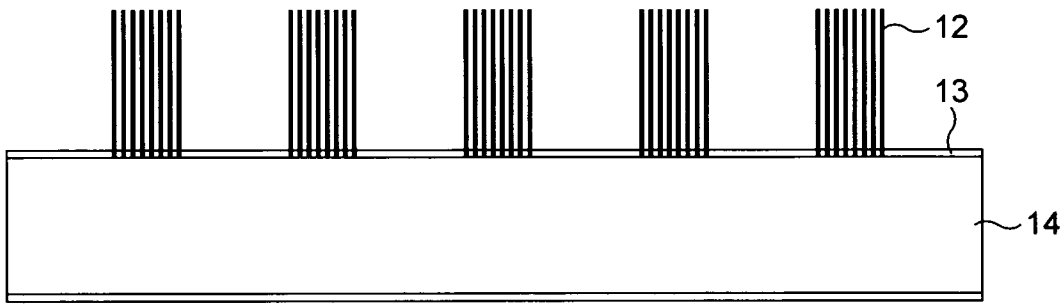


FIG. 11

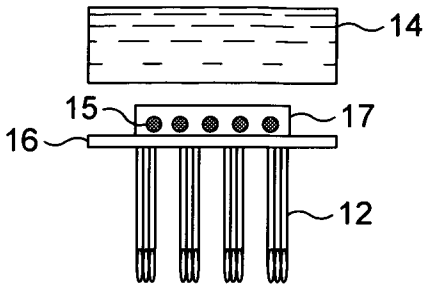


FIG. 12a

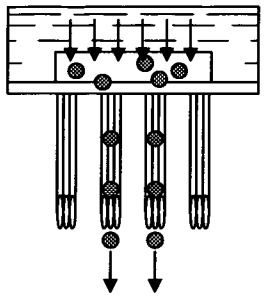


FIG. 12b

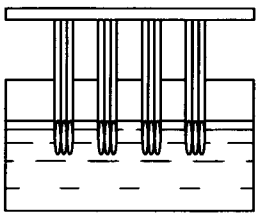


FIG. 13a

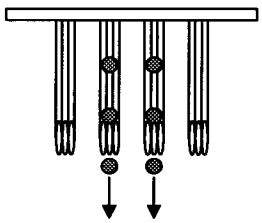


FIG. 13b

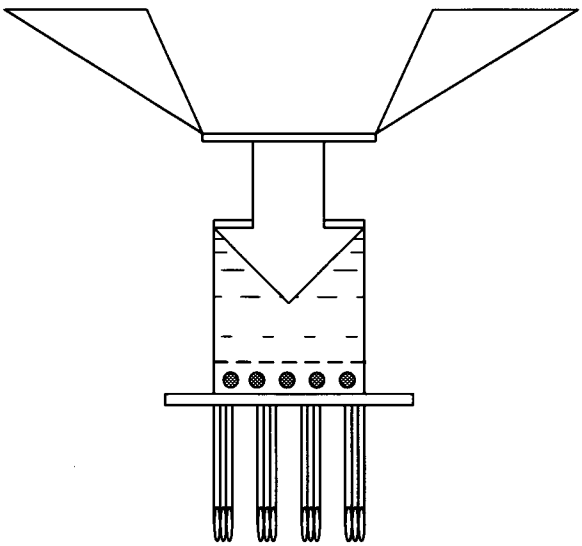


FIG. 14a

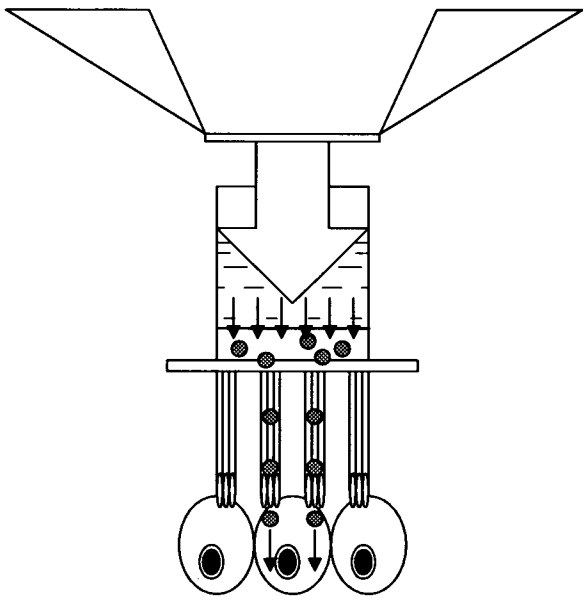


FIG. 14b

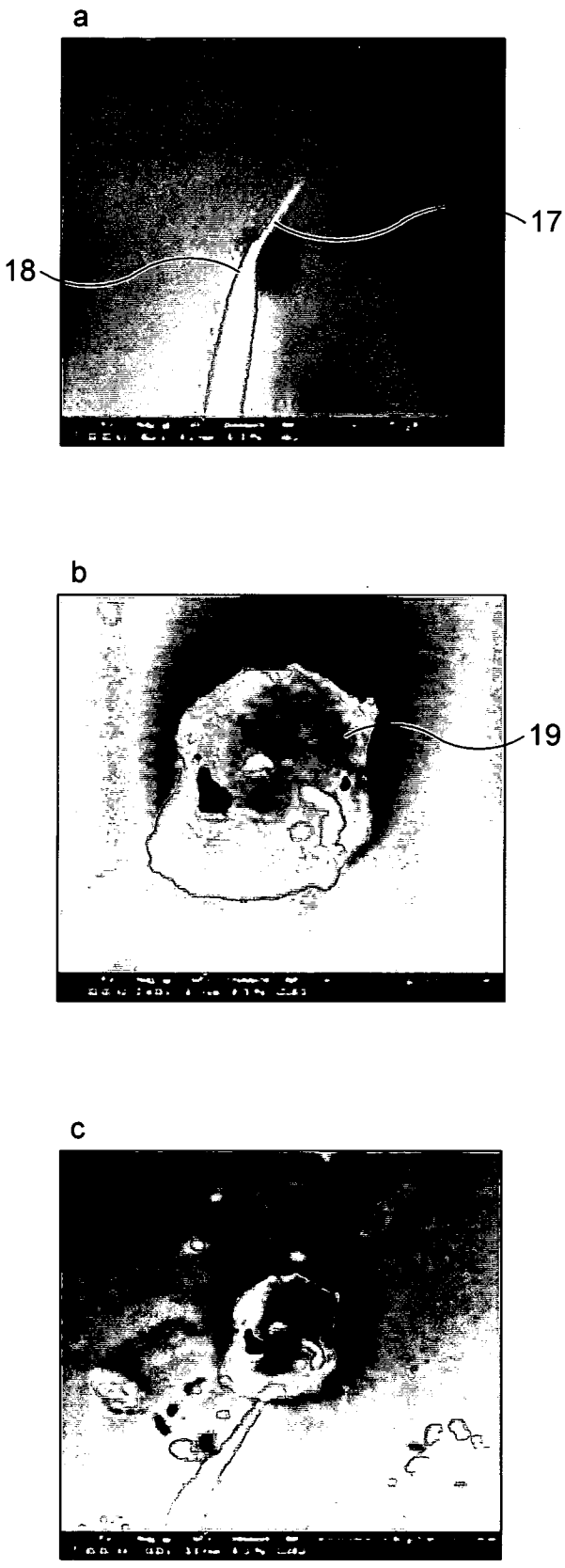


FIG. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/000090

A. CLASSIFICATION OF SUBJECT MATTER
INV. C01B31/02

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)
C01B C23C H01L

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 practical, search terms used)
EPO-Internal, WPI Data, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WAN JUN YU ET AL: "Patterned carbon nanotube field emitter using the regular array of an anodic aluminium oxide template; Patterned carbon nanotube field emitter using the regular array of an anodic aluminium oxide template" NANOTECHNOLOGY, IOP, BRISTOL, GB, vol. 16, no. 5, 1 May 2005 (2005-05-01), pages S291-S295, XP020091054 ISSN: 0957-4484 page S292, paragraphs 1,2,4 page S293, paragraphs 2,3; figure 3	13,15
A	the whole document	1-12,14, 16-18

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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 document 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
17 November 2009	03/03/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Follens, Lana

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/000090

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WHITBY M., ET AL.: "Enhanced fluid flow through nanoscale carbon pipes" NANOLETTERS, vol. 8, no. 9, 15 August 2008 (2008-08-15), pages 2632-2637, XP002553147 page 2633, paragraph 3-6 page 2634, paragraph 3; figure 1	13-15
A	the whole document	1-12, 16-18

X	EUN JU BAE ET AL: "SELECTIVE GROWTH OF CARBON NANOTUBES ON PRE-PATTERNED POROUS ANODIC ALUMINUM OXIDE" ADVANCED MATERIALS, WILEY-VCH VERLAG, WEINHEIM, DE, vol. 14, no. 4, 19 February 2002 (2002-02-19), pages 277-279, XP001132505 ISSN: 0935-9648 page 278, paragraph 2; figures 1-3	13,15
A	the whole document	1-12, 14-18

X	WO 2008/105809 A (RENSSELAER POLYTECH INST [US]; TALAPATRA SAIKAT [US]; KAR SWASTIK [US]) 4 September 2008 (2008-09-04) paragraphs [0006], [0008], [0012], [0020] - [0022], [0025], [0027], [0031]; figures 2A,4	13,15-17
A	the whole document	1-12,14, 18

X	MILLER S.A., ET. AL: "Electroosmotic flow in template-prepared carbon nanotube membranes" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 123, 10 November 2001 (2001-11-10), pages 12335-12342, XP002553148 page 12336, paragraph 2-4 page 12337, paragraph 6; figure 2	13,15,18
A	the whole document	1-12,14, 16,17

A	US 2007/275499 A1 (CORDERMAN REED ROEDER [US] ET AL) 29 November 2007 (2007-11-29) the whole document	1-12

A	US 6 858 197 B1 (DELZEIT LANCE D [US] ET AL) 22 February 2005 (2005-02-22) the whole document	1-12

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/000090

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/097275 A (MOLECULAR NANOSYSTEMS INC [US]; PAN LAWRENCE [US]; FORNACIARI BERT [US) 14 August 2008 (2008-08-14) the whole document	1-12
A	LI X., ET AL.: "Photolithographic approaches for fabricating highly ordered nanopatterned arrays" NANOSCALE RES. LETT., vol. 3, 30 October 2008 (2008-10-30), pages 521-523, XP002554031 the whole document	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2009/000090

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

claims 1-18

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18

This first invention concerns a process to make a nanopipe array (claims 1-10, 13 and 15-18). This process can comprise a further step to remove the AAO template to form a nanopipe bundle (claims 11-12 and 14). The problem solved by the first invention is to provide a process to generate bundles of nanopipes in the form of an array or in the isolated form.

---

2. claims: 19-30

A device to deliver a compound comprising one or more nanopipe bundles, a first reservoir and a delivery compound. The problem solved by the second invention is to provide an apparatus to deliver a compound painlessly across the cell membrane to a cell by minimising the trauma to the target cell.

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3. claim: 31

The current claim 31 is very broad and concerns any method of delivering a compound across a cell membrane into a cell.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/000090

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008105809 A	04-09-2008	NONE	
US 2007275499 A1	29-11-2007	US 2009214851 A1	27-08-2009
US 6858197 B1	22-02-2005	NONE	
WO 2008097275 A	14-08-2008	US 2008292835 A1	27-11-2008