Abstract:
The present invention relates to a device comprising a patterned polymer layer with high aspect ratio to be used in contact with biological solutions or fluids, as well as the method of production of said layer, comprising a phase of polymerization moulding, that uses an acrylic and/or epoxy polymerizable material with a mold of organic or inorganic nature.
5 The present invention relates in general to a patterned polymer layer with high aspect ratio to be used in contact with biological solutions or fluids.

More particularly, the present invention relates to a patterned polymer layer, comprising a microstructure of grooves or wells delimited by walls with a high aspect ratio, i.e. with a ratio of height to thickness greater than or equal to five. The present invention relates also to a method of production of such a patterned polymer layer.

PRIOR ART

In the biomedical sector, devices are known for the filtration of biological solutions or biological fluids containing biological components, such as proteins, enzymes, antibodies, antigens, DNA, cells, platelets, and so on. Said devices comprise a series of grooves or wells of micrometric dimensions delimited by walls with a high aspect ratio.

Such devices are typically made by techniques of dry etching of substrates of silicon or of some other inorganic material.

For example, patent US 6,841,339 describes a process for the preparation of a micro-mold of silicon using a plasma anisotropic erosion process (Bosch process) described in US 5,501,893 or another process of the "etch-and-coat" type, in which the erosion process runs in parallel with a process of passivation, i.e. of protection, of the walls in the course of formation.
Patent US 5,256,360 describes the use of an EDM apparatus (EDM: electric discharge machining) to make a filter mold with a plurality of rod-like protrusions and suggests the use of techniques of laser-beam micro-machining or electron-beam micro-machining.

Alternatively, other techniques of micro-erosion are known for obtaining microstructures with high aspect ratio. For example, US 6,582,890 describes processes that use exposure to X-rays, known by the acronyms LIGA and SLIGA, from the German "Lithographische Galvaniformung Abformtechnik". These processes are critical owing to their cost and the danger of exposure of the operators to X-rays and a process is suggested for the preparation of a multilayer microstructure with high aspect ratio using a series of depositions and exposures of photosensitive layers, using a "lost" mold.

A first disadvantage of the devices made with the processes of the prior art is that the production techniques are very expensive.

Moreover, the devices produced on substrates of silicon or of some other inorganic material are very fragile and have poor mechanical properties, so that they are easily broken.

The surface of silicon or of inorganic material is, moreover, poorly wettable by water or aqueous solutions.

The devices made with these materials therefore require surface treatments with chemical agents, for example, polyethylene glycols and/or functionalizing agents, which may alter the geometry of the device, or with physical agents, such as plasma treatments based on nitrogen or forming gas, which do not guarantee their long-term durability. Such treatments can be difficult and expensive owing to the conditions in which they are carried out (high temperature, high vacuum).

Applicants have determined that the use of the techniques of polymerization molding, where a liquid monomer or prepolymer is
poured into a mold and then polymerized, does not lend itself to the production of micrometric structures, because removal of the polymer from the mold is difficult and subject to breakage. Alternatively, in the case when dissolution of the mold is employed, the method becomes particularly expensive for microstructures with high aspect ratio, as it is necessary to make a mold for each device.

SUMMARY OF THE INVENTION

The applicant has found that the use of conventional materials and techniques does not meet the requirements imposed on modern devices for filtration of biological solutions or biological fluids.

The applicant in fact observed that devices for the filtration of biological solutions or biological fluids should be made of a material that is more resistant and less fragile, displaying good characteristics of intrinsic wettability, by a less expensive method.

The applicant has now found that it is possible to overcome the aforementioned disadvantages with a method of polymerization moulding that uses an acrylic and/or epoxy polymerizable material with a mold made of organic material, for example photosensitive polymer materials, or inorganic material, for example silicon or metals, whose surface has been treated with a suitable polymerization inhibitor.

The present invention therefore relates to a device comprising a patterned polymer layer with high aspect ratio to be used in contact with biological solutions or fluids, as well as the method of production of said layer, comprising a phase of polymerization moulding that uses an acrylic and/or epoxy polymerizable material with a mold of organic or inorganic nature.

BRIEF DESCRIPTION OF THE DRAWINGS
Fig. 1 shows a sectional view of the mold-polymerizable formulation-substrate system during the method of production of a patterned polymer layer according to the present invention, characterized by a series of parallel channels. Fig. 1(a) shows the empty mold, Fig. 1(b) shows the mold filled with the liquid polymerizable formulation, Fig. 1(c) shows the positioning of the substrate and polymerization, Fig. 1(d) shows separation of the substrate-patterned polymer layer system from the mold.

Fig. 2 shows a sectional view of the mold-polymerizable formulation-substrate system during the method of production of a different patterned polymer layer according to the present invention, represented by a cover for the patterned polymer layer of Fig. 1. Fig. 2(a) shows the empty mold, Fig. 2(b) shows the mold filled with the liquid polymerizable formulation, Fig. 2(c) shows the positioning of the substrate and polymerization, and Fig. 2(d) shows the separation of the substrate-patterned polymer layer system from the mold.

Fig. 3 shows a sectional view of the patterned polymer layer-cover system during the method of assembly. Fig. 3(a) shows assembly of the two components, and Fig. 3(b) shows the finished product.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a device comprising a patterned polymer layer with high aspect ratio to be used in contact with biological solutions or fluids, as well as the method of production of said layer, comprising a phase of polymerization moulding that uses an acrylic and/or epoxy polymerizable material with a mold of organic or inorganic nature.
The patterned polymer layer obtained with the process of the present invention comprises a microstructure of grooves or wells delimited by walls with a high aspect ratio, i.e. with a ratio of height to width greater than or equal to five. The term microstructure refers to a three-dimensional structure having at least one dimension (height, width, or length) of micrometric dimensions, i.e., lower than 500 µm, preferably lower than 100 µm.

The molds for use in the present invention can be made of a considerable variety of materials. It is possible to use molds made of inorganic material, for example silicon and metal, or organic materials, for example photosensitive polymers (SU-8). Preferably, molds made of silicon are used.

The mold can be produced starting from a flat, smooth surface by various techniques, such as etching, hot embossing, laser, laser blasting, micromachining, and so on.

An example of a mold for structures with high definition can be constituted of a silicon wafer on which fine structures are produced by dry etching. Examples of methods for making the high-definition mold to be used in the process of the present invention are described in the aforementioned patents US 5,501,893 and US 6,841,339.

As shown in Fig. 2, if required, using the method of the present invention it is possible to produce, by means of suitably patterned molds 1', covers 4' for devices containing the microstructures mentioned above. These can be glued to the device so as to obtain device 10 for the filtration of biological solutions or biological fluids containing biological components, illustrated in Fig. 3. Device 10 comprises suitable inlet and outlet openings, not shown in Fig. 3, for feed and exit of the fluid to be filtered.

The formulation used for obtaining the patterned polymer layer with high aspect ratio can be acrylic or epoxy. Preferably, acrylic
formulations are used, so as to obtain high polarity of the surface of the patterned polymer layer.

The type of formulation used has an influence on the process and the materials used for making the mold and the substrate as well as the process used for production of the patterned polymer layer of the present invention.

The formulation used must, after polymerization, provide a polymer material with very low adhesion to the surface of the mold, but with very high adhesion to the surface of the substrate. In this way it will be possible to separate the patterned polymer layer and the mold without causing breakages and/or defects in the finished article.

The applicant found that the use of the aforementioned acrylic and/or epoxy formulation makes it possible to obtain a patterned polymer layer with good antifouling properties, i.e. particularly resistant to the formation of scale, and biocompatible, i.e. without interactions with the biological fluids with which it comes in contact, without any release or absorption of components from the layer or from the biological fluid, respectively.

Therefore, the use of the acrylic and/or epoxy formulation means it is possible to avoid all surface treatments, such as thermal, corona, plasma treatments and so on, that are normally used for improving the antifouling properties and biocompatibility.

Furthermore, the applicant found that the use of the aforementioned acrylic and/or epoxy formulation makes it possible to obtain better wettability of the surface of the polymer layer.

The acrylic formulation preferably comprises a mixture of at least one polyl tri(meth)acrylate monomer, and at least one polyalkylene glycol mono(meth)acrylate monomer.

The polyl tri(meth)acrylate monomer is preferably selected from triacrylates such as ditrimethylolpropane triacrylate (DiTMPTTA), tris-(2-
hydroxyethyl)-isocyanurate triacrylate (TEICTA), dipentaerythritol triacrylate (DiPETA), trimethylol propane triacrylate ethoxylate (TMPEOTA), trimethylolpropane triacrylate propoxylate (TMPPOTA), pentaerythritol triacrylate ethoxylate (PETEOIA), glyceryl triacrylate propoxylate (GPTA), pentaerythritol triacrylate (PETA), trimethylolpropane triacrylate (TMPTA) and modified pentaerythritol triacrylate; and trimethacrylates such as triethyleneglycol trimethacrylate (TIEGTMA), tetraethylene glycol trimethacrylate (TTEGTMA), polyethylene glycol trimethacrylate (PEGTMA), trihydroxyhexane trimethacrylate (HTTMA), bisphenol A trimethacrylate ethoxylate, trimethylolpropane trimethacrylate (TMPTMA).

Polyol tri(meth)acrylate ethoxylate or propoxylate monomers are preferably used. The use of polyol tri(meth)acrylate ethoxylate or propoxylate monomers improves the wettability of the surface of the resultant patterned polymer layer of the present invention.

Moreover, the use of polyol tri(meth)acrylate ethoxylate or propoxylate monomers, normally liquid with low viscosity, permits the viscosity of the formulation to be reduced, so that there is then better filling of the microcavities of the mold.

The preferred polyol tri(meth)acrylate ethoxylate or propoxylate monomers include, but are not restricted to, trimethylolpropane triacrylate ethoxylate (TMPEOTA), trimethylolpropane triacrylate propoxylate (TMPPOTA), pentaerythritol triacrylate ethoxylate (PETEOIA), glyceryl triacrylate propoxylate (GPTA), bisphenol A trimethacrylate ethoxylate, and trimethylolpropane trimethacrylate ethoxylate (TMPETMA), commercially available for example from IGM Resins, with the tradename Omnimer™.

The acrylic formulation preferably comprises from about 40% to about 90%, by weight, based on the total weight of the acrylic formulation, of polyol tri(meth)acrylate ethoxylate or propoxylate...
monomers. According to a preferred embodiment, the acrylic formulation preferably comprises from about 50% to about 80%, by weight, based on the total weight of the acrylic formulation, of polyol tri(meth)acrylate ethoxylate or propoxylate monomers.

The polyalkylene glycol mono(meth)acrylate monomer is preferably selected from (meth)acrylates such as polypropylene glycol monomethacrylate, polyethylene glycol monomethacrylate, polyethylene glycol-polypropylene glycol monomethacrylate, polypropylene glycol monoacrylate, polyethylene glycol monoacrylate, polypropylene glycol polytrimethylene monoacrylate, polyethylene glycol polytetramethylene glycol monomethacrylate, methoxypolyethylene glycol monomethacrylate, perfluoroalkylethyl polyoxyalkylene monomethacrylate, and combinations thereof. The aforementioned (meth)acrylates are commercially available, for example, from Nippon Oils & Fats Co., with the tradename Blemmer PP series (polypropylene glycol monomethacrylates), Blemmer PE series (polyethylene glycol monomethacrylates), Blemmer PEP series (polyethylene glycol-polypropylene glycol monomethacrylates), Blemmer AP-400 (polypropylene glycol monoacrylate), and Blemmer AE-350 (polyethylene glycol monoacrylate).

The acrylic formulation preferably comprises from about 5% to about 35%, by weight, based on the total weight of the acrylic formulation, of polyalkylene glycol mono(meth)acrylate monomers. In a preferred embodiment, the acrylic formulation preferably comprises from about 10% to about 30%, by weight, based on the total weight of the acrylic formulation, of polyalkylene glycol mono(meth)acrylate monomers.

The epoxy formulation can comprise any polymerizable epoxy resin known in the art, such as the reaction products of condensation of epichlorohydrin with polyalcohols or polyphenols (in particular bisphenol A), cycloaliphatic epoxy resins, epoxy resins derived from diolefins or
polyolefins or a copolymer thereof, composite epoxy resins obtained by copolymerization with glycidyl-methacrylate, epoxy resins obtained from glycerides of unsaturated fatty acids, and so on.

The epoxy formulation preferably comprises (i) cycloaliphatic epoxy resins, for example, 2,4-epoxycyclohexylmethyl-(3,4-epoxy)cyclohexane carboxylate (ERL4221™, Union Carbide Plastics Company, or Araldite™CY1 79, Ciba Products Company), 3,4-epoxycyclohexylmethyl-(3,4-epoxy)cyclohexane carboxylate (Cyracure™UVR-61 05, Dow Chemical), bis(3,4-epoxy-6-methylcyclohexylmethyl) adipate (ERL4289™, Union Carbide Plastics Company, Araldite™CY178, Ciba Products Company), bis(3,4-epoxycyclohexylmethyl) adipate, (EHPE3150, Daicel Chemical Industries), vinylcyclohexene dioxide (ERL4206™, Union Carbide Plastics Company), bis(2,3-epoxycyclopentyl) ether resin (ERL4205™, Union Carbide Plastics Company), 2-(3,4-epoxy)cyclohexyl-5,5-spiro(3,4-epoxy)-cyclohexane-m-dioxane (Araldite™ CY1 75, Ciba Products Company), (ii) epoxy resins of polyphenolic glycidyl ethers, for example, diglycidyl ether bisphenol A epoxy resins (Epon™826, Epon™828, Epon™830, Epon™1001, Epon™1002, Epon™1004, Shell Chemical Company), polyglycidyl ether novolac epoxy resins (DEN™431, DEN™438, and DEN™439, Dow Chemical Company); (iii) epoxy resins of glycidyl esters, for example diglycidyl phthalate (ED-5661, Celanese Resins Company), diglycidyl tetrahydrophthalate (Araldite™CY1 82, Ciba Products Company) and diglycidyl hexahydrophthalate (Araldite™CY1 83, Ciba Products Company or ED-5662, Celanese Resins Company); and mixtures thereof.

Polymerization of the acrylic and/or epoxy formulation can take place according to methods that are known in the art, for example thermally and/or photochemically. Preferably polymerization takes place photochemically. If the substrate is not transparent to radiation, it will be
necessary to use thermal initiators instead of photoinitiators for promoting polymerization of the formulation.

The acrylic and/or epoxy formulation comprises from about 1% to about 25%, more preferably from about 2% to about 20 wt.% relative to the total weight of the formulation, of a thermal initiator or an initiator that is sensitive to UV and/or blue radiation (photoinitiator). The term "photoinitiator" denotes a compound that is able to convert the energy of blue and/or UV radiation to free radicals. The term "thermal initiator" denotes a compound that is able to convert thermal energy to free radicals. The presence of free radicals starts a chain reaction that converts the reactive monomer to oligomers, and finally to polymers.

Examples of suitable photoinitiators include, but are not restricted to, 2,2'-(2,5-thiophenediyl)bis(5-tert-butylbenzoxazole); 1-hydroxycyclohexyl phenyl ketone; 2,2-dimethoxy-2-phenylacetophenone; xanthone; fluorenone; anthraquinone; 3-methylacetophenone; 4-chlorobenzophenone; 4,4'-dimethoxybenzophenone; 4,4'-diaminobenzophenone; Michler's ketone; benzophenone; benzoin propyl ether; benzoin ethyl ether; benzyl dimethyl ketal, 1-(4-isopropylphenyl)-2-hydroxy-2-methylpropan-1-one; 2-hydroxy-2-methyl-1-phenylpropan-1-one; methylbenzoyl formate thioxanthone; diethylthioxanthone; 2-isopropylthioxanthone; 2-chlorothioxanthone; 2-methyl-1-(4-(methylthio)phenyl)-2-morpholinopropan-1-one; and 2,4,6-trimethylbenzoyldiphenylphosphine oxide.

Preferably, multifunctional photoinitiators are used in the acrylic formulation. Examples of suitable multifunctional photoinitiators include, but are not restricted to, Esacure KIP 100 (a mixture of 70 wt.% of an oligomeric a-hydroxy acetophenone and 30 wt.% of dimethyl hydroxy acetophenone), KIP 150, Esacure KTO-46 (mixture of trimethylbenzophenone, polymeric hydroxy ketone, and
trimethylbenzoyldiphenyl phosphine oxide), and Esacure ONE (a-hydroxyketone Afunctional photoinitiator), all commercially available from Lamberti S.p.A., Gallarate, Italy).

Advantageously, suitable thermal initiators include, but are not restricted to, organic peroxides such as diacyl peroxides, per oxybicarbonates, alkyl peresters, dialkyl peroxides, per ketals, ketone peroxides and alkyl hydroperoxides, and azo compounds. Specific examples of said thermal initiators are dibenzoyl peroxide, t-butyl per benzoate, t-butyl peroxide, methyl ethyl ketone peroxide, and azobisisobutyronitrile.

Preferably, photoinitiators of the cationic type are used in the epoxy formulation. The use of cationic photoinitiators is preferred because photoinitiators of this type are not sensitive to oxygen and the reaction can be carried out in normal atmospheric conditions. The cationic photoinitiators are selected from those that release a Lewis acid or a Brønsted acid on irradiation, for example aryl diazonium salts, diaryliodonium salts, triaryl sulphonium salts, dialkyl phenacyl sulphonium salts, dialkyl-4-hydroxyphenyl sulphonium salts, Fe-arene compounds, and silanol-aluminium complexes. The preferred examples of aromatic photoinitiators are aromatic iodonium salts and aromatic sulphonium salts, for example, triarylsulphonium hexafluorophosphate (Cyracure™ UVI-6992, Dow Chemical Company), triarylsulphonium hexafluorophosphate (Cyracure UVI-6976, Dow Chemical Company), and arylsulphonium hexafluorophosphate (Esacure 1064, Lamberti, Italy).

The patterned polymer layer according to the present invention is preferably attached to a substrate. Adhesion between the patterned polymer layer and the substrate is effected during polymerization of the acrylic or epoxy formulation.
The substrate for use in the present invention can be constituted from a variety of materials, such as silicon, polymers (PMMA, PC, COC, COP, and PS), photopolymers such as SU8 (MicroChem Corp.), Ordyl SY314 (Elga Europe), and TMMR 2000 (Tokyo Ohka Kogyo Co.) and metals such as aluminium, copper, tantalum, and nickel. The substrate has the function of providing support for the patterned polymer layer, by developing strong adhesion with said layer.

During the final phase of separation of the patterned polymer layer from the mold, the strength of adhesion of the polymer layer with respect to the substrate must be greater than that developed with respect to the mold. Preferably, to reduce costs and for better adhesion, a substrate of polymethylmethacrylate (PMMA) is used.

Preferably, the substrate is transparent to UV and/or blue radiation, to permit polymerization by means of the aforementioned photoinitiators. If the substrate is not transparent to UV and/or blue radiation, advantageously thermal initiators will be used to promote the polymerization of the formulation.

The process of the present invention comprises:

(a) a surface treatment of a mold previously formed,
(b) pouring of the liquid polymerizable formulation in the mold,
(c) optionally, a surface treatment of a substrate,
(d) positioning of said substrate on the liquid polymerizable formulation,
(e) polymerization of the liquid polymerizable formulation, and
(f) separation of the mold from the patterned polymer layer.

Advantageously, the mold and the substrate undergo surface treatments.
The purpose of the surface treatment is to modify the characteristics of adhesion of the polymerized formulation with respect to the mold and to the substrate.

The surface treatment carried out on the mold has the purpose of reducing the adhesion of the polymerized formulation to the surface of the mold.

In the case of acrylic formulations, whose polymerization is inhibited by the presence of oxygen, a layer of molecular oxygen is physisorbed (i.e., made to undergo physisorption or physical adsorption) on the surface of the mold by various techniques, including a low-power oxygen plasma treatment.

Advantageously, formation of the layer of molecular oxygen on the surface of the mold inhibits the polymerization of the acrylic formulation at the interface with the surface of the mold, reducing the adhesion of the patterned polymer layer to the mold and favoring its removal.

Advantageously, formation of the layer of molecular oxygen on the surface of the mold also makes said surface more polar, improving the wettability of the liquid acrylic formulation with respect to the surface and consequently improving the filling of the microcavities of the mold.

In the case of epoxy formulations, the surface of the mold is treated in various ways depending on the type of polymerization used. In the case of anionic polymerization, the surface of the mold is treated with a plasma of Lewis acids, such as BF$_3$. In the case of cationic polymerization, the surface of the mold is treated with aminosilanes or ammonia.

Conversely, the surface treatment performed on the substrate has the purpose of increasing the adhesion of the polymerized formulation to the surface of the substrate.
In the case of acrylic formulations, the surface of the substrate is submitted to a plasma treatment with nitrogen, forming gas, or rare gases (avoiding the use of oxygen).

In the case of epoxy formulations, the surface of the substrate is treated in various ways depending on the type of polymerization used. In the case of cationic polymerization, the surface of the substrate is treated with a plasma of rare gases or oxygen, avoiding the use of plasmas containing nitrogen (forming gas or nitrogen). In the case of anionic polymerization, the surface of the substrate can be treated with a plasma of rare gases or oxygen, but also with a plasma of forming gas or nitrogen.

In the case when the substrate is polymeric (for example PMMA or PC), increase in adhesion between polymer layer and substrate can be promoted with suitable solvents which, when introduced in the formulation, attack the surface of the polymer material of the substrate, promoting migration of the monomers of the formulation into said substrate.

Advantageously, the presence of suitable solvents in the formulation promotes, in the pouring phase, migration of the monomers within the substrate. During the subsequent polymerization phase, a very high strength of adhesion develops between polymer layer and substrate, as the interface between the two materials disappears.

Advantageously, the formulations then comprise organic solvents of various kinds, such as ketones (acetone, methyl ethyl ketone), alcohols (ethanol, methanol), hydrocarbons (benzene, hexane), sulphoxides (dimethylsulphoxide).

The phase of pouring of the liquid polymerizable formulation in the mold comprises the complete filling of the microhydraulic system, i.e. of the microchannels and of the microcavities, of the mold.
To promote complete filling, the phase of pouring of the liquid polymerizable formulation is advantageously carried out at temperatures between 30°C and 120°C, preferably between 50°C and 100°C, more preferably between 60°C and 90°C. In this way the liquid polymerizable formulation is more fluid, i.e. has a lower viscosity, and the wettability of the walls of the mold is improved, also as a result of the previous surface treatment of the mold. In the case when the use of solvents is envisaged, for the purposes described above, the pouring phase is advantageously carried out at room temperature, to avoid excessive evaporation of said solvent.

In the pouring phase, normally the use of a slight excess of liquid polymerizable formulation is envisaged, which will then overflow from the edge of the mold so as to guarantee complete filling of the mold and perfect contact of the surface of the liquid polymerizable formulation with the substrate in the subsequent phase of positioning of the substrate.

The substrate, optionally treated as described previously, is positioned on the surface of the liquid polymerizable formulation in such a way that the surface of the substrate is completely in contact with the surface of the polymerizable formulation.

In this way, if the substrate is of plastic material and the formulation comprises an organic solvent, the solvent will start to attack the surface of the substrate, with consequent migration of the monomers of the polymerizable formulation within the substrate. To promote the action of the organic solvent, the mold-formulation-substrate system is heated, for example by means of a hot plate.

In general, the polymerization phase is carried out by exposure to UV-blue radiation or thermally.

Advantageously, the polymerization phase is carried out by exposure to radiation with a wavelength in the UV-blue range, i.e. from 200 to 500
nanometers. The energy of the UV-blue radiation is absorbed by a photoinitiator, which is able to convert light energy to free radicals. The presence of free radicals starts a chain reaction that converts the monomers to oligomers and finally to polymers.

In some cases, for example when the substrate is not transparent to UV-blue radiation, the polymerization phase can be carried out by thermal treatment. In this case a thermal initiator is preferably added to the polymerizable formulation. The polymerization temperature depends primarily on the decomposition temperature of the thermal initiator, but is preferably below 135°C, and in particular below 110°C.

It is also possible to use a combination of the two polymerization systems, photochemical and thermal. In this case the thermal phase is preferably carried out after the photochemical phase.

The resultant polymer will copy the surface of the mold, forming the negative of the structures made on said mold.

As mentioned previously, the use of acrylic and/or epoxy polymerizable material gives better wettability of the surface of the resultant patterned polymer layer.

The wettability of the surface of the patterned polymer layer of the present invention, when measured by the contact angle method using a water drop in contact with the surface, is less than or equal to 50°, preferably less than 40°, and more preferably less than 35°. The method comprises measurement of the angle formed between the surface of the patterned polymer layer and the tangent to the water drop at the point of contact. The water drop has a volume of about 5 ml and the measurement is carried out by measuring the width and the height of the projection of the drop enlarged on a screen, and applying the formula:

\[ a = 2 \arctan \left( \frac{2h}{D} \right) \]
where $a$ is the contact angle in degrees, $h$ is the height of the drop in mm, and $D$ is the diameter of contact of the drop in mm.

The separation phase comprises the removal of the mold from the patterned polymer layer of the present invention.

Mold removal is carried out mechanically, by applying a pulling force on the substrate, after carrying out some thermal cycles of heating/cooling which, exploiting the different thermal expansion of the materials constituting the mold and the substrate, cause and promote the process of detachment between the polymer layer and the mold.

Advantageously, to promote the process of separation between the polymer layer and the mold, at least one thermal cycle is carried out, preferably at least three, each cycle being carried out by first heating at a temperature above 50°C, preferably between 60°C and 100°C, more preferably about 80°C, and then cooling to a temperature below 30°C, preferably to room temperature.

For making the complete removal of the mold, it is possible to use separating systems of the mechanical type, where the two surfaces, initially joined together, can be separated without damaging the microstructure obtained. The patterned polymer layer, which will have a form that represents the negative of the structure produced in the mold, will remain on the substrate. Any residues of crosslinked polymer inside the mold can be removed with plasma treatments.

Advantageously, the patterned polymer layer of the present invention is thus obtained at very low cost.

Preferably, the patterned polymer layer of the present invention has an aspect ratio greater than five, preferably greater than ten, and more preferably greater than twenty. The aspect ratio is defined, as is known by a person skilled in the art, as the ratio of the height to the width of a structure. Referring to Fig. 3, in the patterned polymer layer 4 of the
present invention, the aspect ratio is defined as the ratio of the height of the groove 6 or of the wall 7 to their respective width.

The patterned polymer layer of the present invention comprises a microstructure having at least one dimension (height, width, or length) of micrometric dimensions, i.e., lower than 500 μm, preferably lower than 100 μm. Preferably, the patterned polymer layer of the present invention comprises a microstructure having at least two dimensions (height and width) of micrometric dimensions, i.e., lower than 500 μm, preferably lower than 100 μm. Referring to Fig. 3, in the patterned polymer layer 4 of the present invention, the height (h) of the groove 6 or of the wall 7 is lower than 500 μm, preferably lower than 200 μm, and more preferably between 1 and 100 μm; the width (w or w') of the groove 6 or of the wall 7 is lower than 500 μm, preferably lower than 100 μm, and more preferably between 1 and 50 μm.

The same degree of definition can only be obtained with techniques of dry etching on silicon or inorganic materials at high cost, where the final surface properties are closely linked to the type of process used for making the cavities, and require further treatments to make them wettable and biocompatible.

The definition achievable with the process of the present invention is closely correlated only with the limits of definition of the mold used. Advantageously, the process of the present invention makes it possible to produce a patterned polymer layer with a very high aspect ratio at low cost. This could not be achieved with the ordinary photolithographic techniques.

Moreover, the patterned polymer layer of the present invention possesses intrinsically, because of the nature of the material from which it is made, the surface characteristics of wettability, antifouling properties, and biocompatibility that are required for the biomedical
applications for which it is intended, without the need to submit it to further surface treatments, which would increase the cost.

Finally, the mold, made by rather expensive techniques, can be reused to make successive copies, which helps to keep down the costs of production of the patterned polymer layer of the present invention.

Furthermore, the mechanical properties of the patterned polymer layer of the present invention prove to be far better - since the polymer is less fragile than the inorganic materials (for example silicon) it is not susceptible to breakage or damage during any subsequent processes or during the service life of the article.

A preferred embodiment of the invention is described below with reference to Figs. 1-3.

Referring to Fig. 1(a), a mold 1 is shown, comprising a series of channel-patterned grooves 6 separated by walls 7. The mold 1 was preferably obtained by dry etching of silicon by techniques known by a person skilled in the art. The inside surface 8 of the mold has undergone low-power oxygen plasma treatment with formation of a layer of molecular oxygen physisorbed on said surface 8.

Referring to Fig. 1(b), the mold 1 is shown, in which the appropriate amount of liquid polymenzable formulation 2 was poured into the mold 1 so as to fill all the channel-patterned grooves 6 and the entire mold 1. The liquid polymerizable formulation 2 can have the composition described previously. In particular, the formulations shown in Table 1 below were used. Pouring was carried out at room temperature.

Next, referring to Fig. 1(c), a substrate 3 was positioned on the mold so as to match the edges of the mold 1 and the surface of the liquid polymerizable formulation 2. Advantageously, substrate 3 was made of PMMA, and the liquid polymerizable formulation 2 comprised dimethyl sulphoxide. The presence of dimethyl sulphoxide in the liquid polymerizable formulation 2 promoted the migration of the monomers
within the substrate, and the development of high strength of adhesion between polymer layer and substrate during the subsequent polymerization phase. The mold-substrate system is heated on a hot plate at temperatures between 40°C and 130°C, preferably between 80°C and 90°C. When using plastic material, such as, for example PMMA, the temperature is chosen to avoid any first order transition (fusion, crystallization) and/or any second order transition (glass transition). PMMA can be heated up to 120°C, beyond that the material start to soften and to warp.

Then the liquid polymerizable formulation 2 was polymerized by exposure to UV radiation with wavelength of about 320 nm, thus forming the patterned polymer layer 4 illustrated in Fig. 1(d). The device 5, constituted of the substrate 3 to which the patterned polymer layer 4 still adheres, was extracted from the mold by exerting a pulling force on the substrate. To promote the process of separation between the polymer layer and the mold, 3 thermal cycles of heating to about 80°C and then cooling to room temperature were carried out.

As shown in Fig. 2, using the procedure and materials similar to those described for Fig. 1, a cover 5' was made, constituted of a substrate 3' and a patterned polymer layer 4' obtained by polymerization of a liquid polymerizable formulation 2' suitably poured in a mold 1'.

The device 5 and the cover 5' are then assembled as shown in Fig. 3(a), thus obtaining the finished product 10 shown schematically in Fig. 3(b). Cover 5' was joined to device 5 by techniques known by a person skilled in the art, using adhesive or by means of ultrasound or techniques of solvent bonding, thermocompression, or laser welding.

Table 1 given below lists the acrylic liquid formulations used for production of the patterned polymer layer of the present invention. All of
the values are expressed in percentages by weight relative to the total weight of the formulation.

TABLE 1

<table>
<thead>
<tr>
<th>Components</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethylol propane ethoxylate triacrylate</td>
<td>65.7</td>
<td>65.7</td>
<td>60.7</td>
</tr>
<tr>
<td>PEG methacrylate</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Dimethyl sulphoxide</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Tego®Rad 2300N</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Esacure KIP 150</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Esacure KIP 100F</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Esacure KTO 46</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Tegorad 2300N: Silicone polyether acrylate, Evonik Tego Chemie GmbH
Esacure KIP 150: Polyfunctional oligomeric a-hydroxyketone, Lamberti S.p.A., Gallarate, Italy
Esacure KIP 100F: Mixture of oligo [2-hydroxy-2-methyl-1-[4-(1-methylvinyl)phenyl]propanone] and 2-hydroxy-2-methyl-1-phenyl propan-1-one, Lamberti S.p.A., Gallarate, Italy
Esacure KTO 46: Mixture of trimethylbenzophenone, polymeric hydroxy ketone, and trimethylbenzoyldiphenyl phosphine oxide, Lamberti S.p.A., Gallarate, Italy

The next Table 2 lists some epoxy liquid formulations used for production of the patterned polymer layer of the present invention. All of
the values are expressed in percentages by weight relative to the total weight of the formulation.

TABLE 2

<table>
<thead>
<tr>
<th>Components</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyracure 6105</td>
<td>0</td>
<td>0</td>
<td>88.09</td>
</tr>
<tr>
<td>EHPE 3150</td>
<td>71.41</td>
<td>71.41</td>
<td>0</td>
</tr>
<tr>
<td>Aldrich 654027</td>
<td>10</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Esacure 1064</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Cyclopentanone</td>
<td>18.55</td>
<td>18.55</td>
<td>9.22</td>
</tr>
<tr>
<td>Byk 310</td>
<td>0.035</td>
<td>0.035</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Cyracure 6105 3,4-Epoxy-cyclohexylmethyl-(3,4-epoxy) cyclohexane carboxylate, Dow Chemical

EHPE 3150 bis(3,4-Epoxy-cyclohexylmethyl) adipate, Daicel Chemical Industries

Aldrich 654027 Triarylsulphonium hexafluoroantimonate in propylene carbonate (1:1), Sigma-Aldrich

Esacure 1064 Arylsulphonium hexafluorophosphate, Lamberti, Italy

Byk 310 Dimethyl siloxane modified polyester, Byk Chemie
CLAIMS

1. A device (5, 5', 10) comprising at least one patterned polymer layer (4, 4') to be used in contact with biological solutions or fluids, wherein said patterned polymer layer (4, 4') has an aspect ratio greater than five and is made by polymerization of an acrylic and/or epoxy polymerizable material.

2. The device (5, 5', 10) according to Claim 1, wherein the surface of said patterned polymer layer has a wettability less than or equal to 50°, preferably less than 40°.

3. The device (5, 5', 10) according to Claim 1, wherein said patterned polymer layer (4, 4') has an aspect ratio greater than ten, preferably greater than twenty.

4. The device (5, 5', 10) according to Claim 1, wherein said acrylic polymerizable material comprises a mixture of at least one polyol tri(meth)acrylate monomer, and at least one polyalkylene glycol mono(meth)acrylate monomer.

5. The device (5, 5', 10) according to Claim 4, wherein said polyol tri(meth)acrylate monomer is selected from the group comprising polyol tri(meth)acrylate ethoxylate or propoxylate monomers.

6. The device (5, 5', 10) according to Claim 5, wherein said polyol tri(meth)acrylate monomer is selected from the group comprising trimethylolpropane triacrylate ethoxylate (TMPEOTA), trimethylolpropane triacrylate propoxylate (TMPPOTA), pentaerythritol triacrylate ethoxylate (PETEOIA), glyceryl triacrylate propoxylate
(GPTA), bisphenol A trimethacrylate ethoxylate, and trimethylolpropane trimethacrylate ethoxylate (TMPETMA).

7. The device (5, 5', 10) according to Claim 4, wherein said polyalkylene glycol mono(meth)acrylate monomer is selected from the group comprising polypropylene glycol monomethacrylate, polyethylene glycol monomethacrylate, polyethylene glycol-polypropylene glycol monomethacrylate, polypropylene glycol monoacrylate, polyethylene glycol-polytrimethylene monoacrylate, polyethylene glycol polytetramethylene glycol monomethacrylate, methoxypolyethylene glycol monomethacrylate, perfluoroalkylethyl polyoxyalkylene monomethacrylate, and combinations thereof.

8. The device (5, 5', 10) according to Claim 1, wherein said epoxy polymerizable material comprises (i) cycloaliphatic epoxy resins, (ii) epoxy resins of polyphenolic glycidyl ethers, epoxy resins of glycidyl esters, or mixtures thereof.

9. The device (5, 5', 10) according to Claim 8, wherein said cycloaliphatic epoxy resins are selected from the group comprising 2,4-epoxycyclohexylmethyl-(3,4-epoxy)cyclohexane carboxylate (ERL4221™, Union Carbide Plastics Company, or Araldite™CY1 79, Ciba Products Company), 3,4-epoxycyclohexylmethyl-(3,4-epoxy)cyclohexane carboxylate (Cyracure™UVR-61 05, Dow Chemical), bis(3,4-epoxy-6-methylcyclohexylmethyl) adipate (ERL4289™, Union Carbide Plastics Company, Araldite™CY1 78, Ciba Products Company), bis(3,4-epoxycyclohexylmethyl) adipate, (EHPE31 50, Daicel Chemical Industries), vinlycyclohexene dioxide (ERL4206™, Union Carbide Plastics Company), bis(2,3-epoxycyclopentyl) ether resin (ERL4205™, Union Carbide Plastics...
Company), 2-(3,4-epoxy)cyclohexyl-5,5-spiro(3,4-epoxy)-cyclohexane-
m-dioxane (Araldite™ CY1 75, Ciba Products Company), and mixtures thereof.

10. The device (5, 5', 10) according to Claim 1, wherein said acrylic and/or epoxy polymerizable material comprises from about 1% to about 25 wt.% relative to the total weight, of at least one thermal initiator or at least one initiator sensitive to UV and/or blue radiation (photoinitiator).

11. The device (5, 5', 10) according to Claim 10, wherein said acrylic polymerizable material comprises at least one multifunctional photoinitiator selected from the group comprising Esacure KIP 100, KIP 150, Esacure KTO-46, and Esacure ONE.

12. The device (5, 5', 10) according to Claim 10, wherein said epoxy polymerizable material comprises at least one cationic photoinitiator selected from the group comprising aromatic iodonium salts and aromatic sulphonium salts.

13. The device (5, 5', 10) according to Claim 1, wherein said patterned polymer layer adheres to a substrate, said substrate being made of at least one material selected from the group comprising silicon, polymers, photopolymers, and metals.

14. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3'), said method comprising the following phases:
   (a) surface treatment with a polymerization inhibitor of a mold (1, 1') previously formed,
   (b) pouring of an acrylic and/or epoxy liquid polymerizable formulation (2, 2') in said mold (1, 1'),
(c) positioning of said substrate (3, 3') on said liquid polymerizable formulation (2, 2'),
(d) polymerization of said liquid polymerizable formulation (2, 2'), and
(e) separation of said mold (1, 1') from said patterned polymer layer (4, 4').

15. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, said method comprising
(f) surface treatment of said substrate (3, 3').

16. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said liquid polymerizable formulation (2, 2') is acrylic, and in that said surface treatment of said mold (1, 1') comprises the formation of a layer of molecular oxygen physically absorbed on the surface of the mold.

17. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 16, wherein said surface treatment of said mold (1, 1') comprises a low-power oxygen plasma treatment.

18. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said liquid polymerizable formulation (2, 2') is epoxy, and in that said surface treatment of said mold (1, 1') comprises treatment with a plasma of Lewis acids, with aminosilanes, or with ammonia.

19. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said liquid polymerizable formulation (2, 2') is acrylic, and in that said surface
treatment of said substrate (3, 3') comprises a plasma treatment with nitrogen, forming gas, or rare gases.

20. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said liquid polymerizable formulation (2, 2') is epoxy, and in that said surface treatment of said substrate (3, 3') comprises treatment with a plasma of rare gases, oxygen, forming gas or nitrogen.

21. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said substrate (3, 3') is made of polymeric material.

22. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 21, wherein said liquid polymerizable formulation (2, 2') comprises at least one organic solvent selected from the group comprising ketones, alcohols, hydrocarbons, and sulphoxides.

23. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said pouring (b) is carried out at a temperature between 30°C and 120°C.

24. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 23, wherein said pouring (b) is carried out at a temperature between 50°C and 100°C.

25. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 24, wherein said pouring (b) is carried out at a temperature between 60°C and 90°C.
26. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 22, wherein said pouring (b) is carried out at room temperature.

27. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 26, wherein during said positioning (c), the system comprising said mold, said formulation and said substrate is heated to a temperature between 40°C and 130°C, preferably between 80°C and 90°C.

28. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said polymerization (d) is carried out by exposure to UV-blue radiation, by a thermal method, or by a combination thereof.

29. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 28, wherein said polymerization (d) is carried out by exposure to UV-blue radiation with a wavelength from 200 to 500 nanometers in the presence of a photoinitiator.

30. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 28, wherein said polymerization (d) is carried out by thermal treatment in the presence of a thermal initiator at a temperature below 135°C.

31. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 14, wherein said separation (d) is carried out mechanically after at least one thermal cycle.
32. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 31, wherein said thermal cycle is carried out first by heating to a temperature above 50°C, and then cooling to a temperature below 30°C.

33. Method of production of a patterned polymer layer (4, 4') adhering to a substrate (3, 3') according to Claim 32, wherein said thermal cycle is carried out first by heating to a temperature between 60°C and 100°C, and then cooling to room temperature.

34. A device (5, 5', 10) comprising at least one patterned polymer layer (4, 4') adhered to a substrate (3, 3'), wherein said patterned polymer layer (4, 4') has an aspect ratio greater than five and is made by polymerization of an acrylic and/or epoxy polymerizable material, and wherein said substrate is made of at least one material selected from the group comprising silicon, polymers, photopolymers, and metals.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. B81C99/00

ADD.

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)

B81C

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, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>sectl on 3. Substrate fabricatio</em>n</td>
<td>14</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. 

See patent family annex.

Date of the actual completion of the international search: 8 November 2010

Date of mailing of the international search report: 16/11/2010

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaal 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer: Mei ster, Martin
### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>