

(43) International Publication Date
22 September 2011 (22.09.2011)(10) International Publication Number
WO 2011/115580 A1

(51) International Patent Classification:

B81B 7/04 (2006.01) **B01L 3/00** (2006.01)
B29C 59/00 (2006.01) **B81C 3/00** (2006.01)
C12M 1/00 (2006.01) **G01N 33/48** (2006.01)

(21) International Application Number:

PCT/SG2011/000106

(22) International Filing Date:

17 March 2011 (17.03.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/314,871 17 March 2010 (17.03.2010) US

(71) Applicants (for all designated States except US): **AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH** [SG/SG]; 1 Fusionopolis Way, #20-10, Connexis, Singapore 138632 (SG). **NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LAW, Bee, Khuan, Jaslyn** [SG/SG]; c/o Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602 (SG). **LOW, Hong Yee** [MY/SG]; c/o Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602 (SG). **SURYANA, Mona** [ID/SG]; c/o Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602 (SG). **WEE, Kee, Fong** [SG/SG]; c/o

Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602 (SG). **SHEETZ, Michael, Patrick** [US/SG]; c/o National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **YIM, King, Fai, Evelyn** [CA/SG]; c/o National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **NG, Zheng, Jie** [SG/SG]; c/o National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **TEO, Kim, Kiat** [SG/SG]; c/o National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **KAM, Lance, Cameron** [US/SG]; c/o National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(74) Agent: **ELLA CHEONG SPRUSON & FERGUSON (SINGAPORE) PTE LTD**; Robinson Road Post Office, P.O. Box 1531, Singapore 903031 (SG).

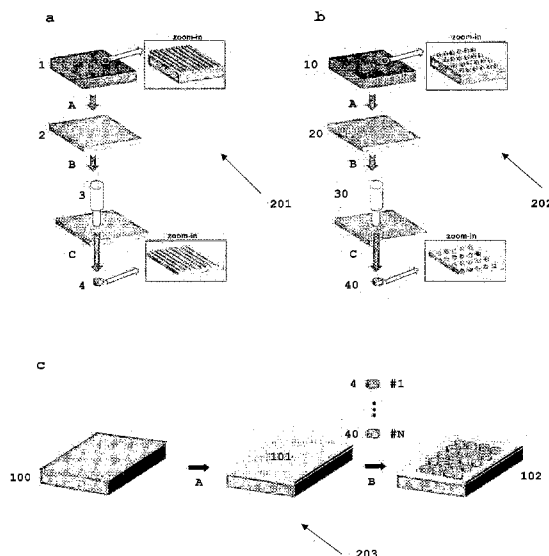
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG,

[Continued on next page]

(54) Title: A PROCESS FOR MAKING AN ARRAY

Figure 1



(57) Abstract: There is disclosed a method of making an array for cell assays comprising the step of providing an array of structures on a substrate, each of said structures having a pre-defined topography thereon, and wherein at least one structure has a different topography from at least one other structure.



ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

A Process for Making an Array

Technical Field

The present invention generally relates to a method
5 of making an array. The present invention also relates to
an array and uses thereof.

Background

Understanding how surface topography affects cellular
10 functions is integral in developing purpose-specific cell
regulating cues in various biomedical applications. For
example, the interaction of T-cells with antigen
presenting cells (APCs) is critical for the coordination
of the immune system and the activation of T-cells is
15 often studied using planar surfaces in place of APCs.

Presently, the main obstacle facing cell-
topographical studies is the lack of a single surface
possessing a large range of different topographies, such
as different heights, dimensions and feature shapes, in
20 order to facilitate fast microscopy-based screening and
subsequent analysis of the data generated.

The lack of such multi-architectural surfaces is due
to the fact that there is no one single patterning
technique that can be used to simultaneously fabricate
25 multiple surface topographical features onto a single
substrate. All existing lithography techniques are
limited to specific geometries and resolutions.
Therefore, different techniques have to be used for each
specific geometry, resolution and aspect ratio. For
30 example: optical lithography for micro-feature
resolutions; electron-beam lithography for nano-feature
resolutions; gray mask lithography for different aspect
ratio patterns; optical lithography in combination with
isotropic etching for curved features; and interference

lithography for three-dimensional structures. Thus, in order to create a multi-architectural topographical surface on a single substrate, each technique needs to be employed separately and it is also necessary to mask all the other patterns whilst each pattern is being fabricated. This task is clearly challenging and not practical if a large number of vastly different topographies are involved.

Accordingly, there is a need to provide method for making an array comprising multiple topographies on a single substrate that overcomes, or at least ameliorates, the disadvantages mentioned above.

Summary

According to a first aspect, there is provided a method of making an array for cell assays comprising the step of providing an array of structures on a substrate, each of said structures having a defined topography thereon, and wherein at least one structure has a different topography from at least one other structure.

It is an advantage of the method described herein that a plurality of different topographies can be presented on a single substrate to form a microarray, which would otherwise be difficult using existing single patterning techniques.

It is a further advantage of the method described herein that different topographies can be presented on a single substrate which permits customization of the array.

Advantageously, the method described herein provides a low cost fabrication process. In particular, nanoimprint technology, which is a cost-efficient, scalable, high throughput nanoimprinting technique, may be used to create the different topographies which are

otherwise prohibitively expensive if they are fabricated by conventional electron beam lithography.

It is a further advantage that the method as described herein may provide a biocompatible array.

5 It is a further advantage of the method as described herein that the array may be replicated using known casting methods, for example PDMS casting. This provides a cost-effective way of producing replicas of the original array.

10 Advantageously, the method as described herein may not encounter problems of resolution limit in providing the different topographies, as nanoimprinting may be used to form the topographies, where the limit of resolution is limited by the mold to be used for imprinting.

15 It is a further advantage of the method as described herein that the individual topographies of the array can be optimized as different topographies are imprinted separately.

It is a further advantage of the method as described
20 herein that a dry/etch-free method may be used to form the different topographies on a single substrate.

Advantageously, the method as described herein permits the use of different types of polymer (for example, PMMA, PC, ETFE and the like) to form the
25 structures having a defined topography thereon.

According to a second aspect there is provided an array comprising a substrate having an array of structures projecting therefrom, wherein each of said structures have a defined topography thereon, and wherein at least one
30 structure has a different topography from at least one other structure. In one embodiment, the array can be used as a master mold to make other array structures, such as by imprint lithography.

Definitions

While most of the terms used herein will be recognizable to those of skill in the art, the following definitions are nevertheless put forth to aid in the understanding of the present invention. It should be understood, however, that when not explicitly defined, terms should be interpreted as adopting a meaning presently accepted by those of skill in the art.

The term "micro-structure," as used herein relates to structures that have dimensions that are in the "microscale" size range.

The term "nano-structure," as used herein relates to structures that have dimensions that are in the "nanoscale" size range.

The term "structure density" in the context of this specification refers to the number of structures patterned or disposed within a defined area on a substrate.

The term "array" generally refers to multiple numbers of structures distributed within an area and spaced apart, unless otherwise indicated. Structures within an array all do not have to have the same orientation.

The term "ordered array" generally refers to the placement of elements in a specified or predetermined pattern where the elements have distinct spatial relationships to one another. Hence, the term "ordered array" generally refers to structures distributed within an area with distinct, specified or predetermined spatial relationships to one another. For example, the spatial relationships within an ordered array may be such that the structures are spaced apart from one another by generally equal distances. Other ordered arrays may use varying, but specified or predetermined, spacings.

The term "three-dimensional," abbreviated "3-D" and as defined herein, refers to structures or structural features that vary (structurally) with depth.

The term "spin-coating," as defined herein, generally refers to a process wherein a polymer solution is dispersed on a surface (e.g., a mold) and the surface is rapidly spun centrifugally to thereby force the solution to spread out on a surface and form a thin layer of desolvated polymer in the process.

10 The term "glass transition temperature" (T_g) is to be interpreted to include any temperature of a polymer at which the polymer lies between the rubbery and glass states. This means that above the T_g , the polymer becomes rubbery and can undergo elastic or plastic deformation without fracture. Above this temperature, such polymers can be induced to flow under pressure. When the temperature of the polymer falls below the T_g , generally, the polymer will become more inflexible but can be deformed when a stress is applied to the polymer. It should be noted that the T_g is not a sharp transition temperature but a gradual transition and is subject to some variation depending on the experimental conditions (e.g., film thickness, tacticity of the polymer, etc.). The actual T_g of a polymer film will vary as a function of film thickness. The T_g will be defined herein as being the bulk glass-transition temperature of the polymer substrate. The bulk glass transition temperature is a specific value that is widely agreed upon in the literature. Glass transition temperature values of polymers may be obtained from *PPP Handbook*TM software edited by Dr D. T. Wu, 2000.

The term "imprint" and grammatical variations thereof, in the context of this specification, is intended to cover any form of physical impression that has been

made in a pliable solid body, such as a thermoplastic polymer substrate. An imprint may form a structure on a substrate and typically, an imprint is a generally elongate structure that extends from the surface of a substrate along a longitudinal axis extending between a proximal end disposed on or adjacent to the substrate and a distal end opposite to the proximal end. Typically, the longitudinal axis is generally normal relative to a planar axis of the substrate but the longitudinal axis may be varied significantly such as at an angle of 45° from a planar axis of the substrate.

In an array of imprints that have been orderly formed as a series of rows and columns on a substrate, trenches may be formed between the adjacent rows. The imprint may be in the nanoscale or microscale size range both in their length dimension and thickness dimension, and hence the trenches may also be in the nanoscale or microscale size range.

The term "nanoimprinting lithography" is to be interpreted broadly to include any method for printing or creating a pattern or structure on the microscale and/or nanoscale size range on the surface of a substrate by contacting a mold with the defined pattern or structure on the surface at certain temperatures and pressures.

The term "thermoplastic" refers to a material that softens when sufficient heat and pressure is applied to the material and hardens as it cools down. When the term "thermoplastic" is used to refer to a polymer, the thermoplastic polymer softens at a temperature above the T_g and hardens when the temperature is decreased until below the T_g . As the thermoplastic polymer cools from the T_g , the thermoplastic polymer becomes less moldable or deformable as compared to the state of the thermoplastic polymer in

the first applying step (a) when the mold is applied to the polymer substrate.

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

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

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

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

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

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a method of making an array for cell assays will now be disclosed. The method comprises the step of providing an array of structures on a substrate, each of said structures having
15 a defined topography thereon, and wherein at least one structure has a different topography from at least one other structure.

In one embodiment, the array of structures may extend from a planar surface of the substrate. In one embodiment,
20 the structures may be generally elongate and have a proximal end that extends from the substrate and a distal end that is opposite to said proximal end. In another embodiment, the topography of said structures is provided on said proximal end.

In one embodiment the elongate structures have a longitudinal axis extending from their proximal end to their distal end and wherein the longitudinal axis is generally normal to a planar surface of said substrate. In one embodiment, the structures are in the form of
25 imprints. In another embodiment, the structures are generally rectangular, square, triangular, or cylindrical in shape when viewed in cross section in a plane normal to said longitudinal axis. In one embodiment, the structures are generally cylindrical in shape.
30

In another embodiment, the providing step comprises the step of adhering said structures to said substrate. The adhering step may comprise the step of providing an adhesive layer on said substrate before said adhering step.

5 In one embodiment, the adhesive layer may be selected from PDMS or optical adhesives that permit bonding of thermoplastic materials onto solid substrates such as silicon or glass.

In one embodiment, the adhesive layer is a polymeric
 10 solution. The polymer solution may be a silane-containing compound. The silane-containing compound may be selected from the group consisting of 2-mercaptoethyl trimethoxysilane, 3-mercaptopropyl trimethoxysilane, 2-mercaptopropyl triethoxysilane, 3-mercaptopropyl
 15 triethoxysilane, 2-mercaptoethyl tripropoxysilane, 2-mercaptoethyl tri sec-butoxysilane, 3-mercaptopropyl tri-t-butoxysilane, 3-mercaptopropyl triisopropoxysilane, 3-mercaptopropyl trioctoxysilane, 2-mercaptoethyl tri-2'-ethylhexoxysilane, 2-mercaptoethyl dimethoxyethoxysilane,
 20 3-mercaptopropyl methoxyethoxypropoxysilane, 3-mercaptopropyl dimethoxy methylsilane, 3-mercaptopropyl methoxy dimethylsilane, 3-mercaptopropyl ethoxy dimethylsilane, 3-mercaptopropyl diethoxy methylsilane, 3-mercaptopropyl cyclohexoxydimethyl silane, 4-mercaptopentyl
 25 trimethoxysilane, 3-mercapto-3-methylpropyltrimethoxysilane, 3-mercapto-3-methylpropyl-tripropoxysilane, S-mercapto-S-ethylpropyl-dimethoxy methylsilane, 3-mercapto-2-methylpropyl trimethoxysilane, 3-mercapto-2-methylpropyl dimethoxy phenylsilane, S-
 30 mercaptocyclohexyl-triethoxysilane, 12-mercaptododecyl trimethoxy silane, 12-mercaptododecyl triethoxy silane, 18-mercaptooctadecyl trimethoxysilane, 18-mercaptooctadecyl methoxydimethylsilane, 2-mercapto-2-methylethyl-tripropoxysilane, 2-mercapto-2-methylethyl-

triethoxysilane, 2-mercaptophenyl trimethoxysilane, 2-
 mercaptophenyl triethoxysilane, 2-mercaptotolyl
 trimethoxysilane, 2-mercaptotolyl triethoxysilane, 1-
 mercaptomethyltolyltrimethoxysilane, 1-mercaptomethyltolyl
 5 triethoxysilane, 2-mercaptoethylphenyl trimethoxysilane, 2-
 mercaptoethylphenyl triethoxysilane, 2-mercaptoethyltolyl
 trimethoxysilane, 2-mercaptoethyltolyl triethoxysilane, 3-
 mercaptopropylphenyl trimethoxysilane and, 3-
 mercaptopropylphenyl triethoxysilane, and the aminosilanes
 10 3-aminopropyltrimethoxysilane, 3-
 aminopropyltriethoxysilane, 4-aminobutyltriethoxy-silane, N-
 methyl-3-amino-2-methylpropyltrimethoxysilane, N-ethyl-3-
 amino-2-methylpropyltrimethoxysilane, N-ethyl-3-amino-2-
 methylpropyldiethoxymethylsilane, N-ethyl-3-amino-2-
 15 methylpropyltriethoxysilane, N-ethyl-3-amino-2-
 methylpropyl-methyldimethoxysilane, N-butyl-3-amino-2-
 methylpropyltrimethoxysilane, 3-(N-methyl-2-amino-1-methyl-
 1-ethoxy)-propyltrimethoxysilane, N-ethyl-4-amino-3, 3-
 dimethylbutyldimethoxymethylsilane, N-ethyl-4-amino-3, 3-
 20 dimethylbutyltrimethoxy-silane, N-(cyclohexyl) -3-
 aminopropyltrimethoxysilane, N-(2-aminoethyl)-3-
 aminopropyltrimethoxysilane, N-(2-aminoethyl)-3-
 aminopropyltriethoxy-silane, N-(2-aminoethyl)-3-
 aminopropylmethyldimethoxysilane,
 25 aminopropyltriethoxysilane, bis-(3-trimethoxysilyl-2-
 methylpropyl) amine, N-(3'-trimethoxysilylpropyl)-3-amino-
 2-methylpropyltrimethoxysilane and polydimethylsiloxane.

In one embodiment the silane-containing compound
 comprises polydimethylsiloxane (PDMS). In one embodiment,
 30 the polydimethylsiloxane solution is Sylgard®184. In an
 exemplary embodiment, the Sylgard®184 comprises an
 elastomer and curing agent. The PDMS solution may be
 formed by mixing the elastomer and the curing agent in a
 ratio from 10:1 to 3:1 (elastomer:curing agent). In one

embodiment the ratio is 3:1. Advantageously, different thicknesses of the adhesive layer during spin-coating onto the substrate can be obtained using different ratios of the elastomer and curing agent.

5 In one embodiment, the providing step comprises spin-coating the polymeric solution onto the substrate. The step of spin coating permits the PDMS polymer solution to be coated planarly onto the said substrate. The structures are then adhered on the said substrate via the PDMS
10 polymer. In one embodiment, the step of spin coating comprises removing the polymer solution while said array of structures are disposed in said polymer solution to form a polymer layer that adheres said structures to said substrate. In one embodiment, the step of removing the
15 solvent from the removing step comprises heating the polymeric solution.

In another embodiment, the array of structures adhered onto the said substrate via the PDMS polymer are heated to cure and harden the PDMS polymer to firmly attach
20 the said array of structures onto the said substrate. In one embodiment heating step is performed at a temperature range of from 30°C to 100°C. In another embodiment, the heating step is performed at a temperature of about 30°C, about 35°C about 40°C about 45°C about 50°C, about 55°C,
25 about 60°C, about 65°C, about 70°C, about 75°C, about 80°C, about 85°C, about 90°C, about 95°C or about 100°C. In one embodiment, the heating step is carried out at 70°C. The heating step may be carried out for a time selected from a range of from 30mins to 24 hours. In one embodiment, the
30 heating step is carried out for a time selected from about 30mins, about 45mins, about 1 hour, about 1 hour 30mins, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16

hours, about 18 hours, about 20 hours, about 22 hours or about 24 hours. In one embodiment, the heating step is carried out for 1 hour.

In one embodiment, the heating step is performed under vacuum conditions. Advantageously, this removes any residual bubbles under the structures when they are applied to the polymeric solution.

In another embodiment, the topography is formed by formations in the microscale or the nanoscale size range.

10 In another embodiment, the method as described herein comprises, before said providing step, the step of forming said topographic formations on a sacrificial substrate. In one embodiment, the sacrificial substrate is a polymer film. The polymer may be a thermoplastic polymer. The first and/or second substrates may be a polymer substrate such as a thermoplastic polymer substrate.

The sacrificial thermoplastic polymer substrate may comprise at least one monomer selected from the group consisting of acrylates, phthalamides, acrylonitriles, 20 cellulosics, styrenes, alkyls, alkyl methacrylates, alkenes, halogenated alkenes, amides, imides, aryletherketones, butadienes, ketones, esters, acetals, carbonates and combinations thereof. In one embodiment, the thermoplastic polymer is selected from poly carbonate 25 and poly methyl methacrylate.

During the forming step, the resultant formations on the sacrificial substrate are provided by the step of contacting the sacrificial substrate with a mold.

During the contacting step, the resultant topography 30 on the sacrificial substrate is of complementary configuration to that of the mold. For example, if the pattern on the mold comprises gratings, the gratings may result in channels of complementary configuration on the

sacrificial substrate when the mold is applied to the sacrificial substrate.

In one embodiment, during the step of contacting the mold to the sacrificial substrate, the method may comprise
5 the step of selecting a temperature that is above the glass transition temperature (T_g) of the sacrificial substrate. At this temperature, the polymer softens and may conform to the shape of the mold such that an imprint is created on the surface of the polymer whereby the pattern of the
10 imprint may be of complementary configuration to the pattern on the mold when the polymer is cooled and subsequently hardens. Furthermore, the mold may be preferably applied at a predetermined pressure for a certain period of time to form an imprint on the surface of
15 the sacrificial substrate. The temperature and pressure to be applied will be dependent on the polymer used and the topography of the mold.

The molds may be made of any suitable material that is chemically inert and may be harder than the softened
20 substrate when used at the temperature that is above the glass transition temperature of the substrate. The molds may be made of silicon, a metal, a glass, quartz, a ceramic or a combination thereof.

The temperature used when applying a substrate to a
25 mold is dependent on the type of thermoplastic polymer used. Typically, the temperature may be selected from the group consisting of about 120°C to about 200°C, about 140°C to about 200°C, about 160°C to about 200°C, about 180°C to about 200°C, about 120°C to about 140°C, about 120°C to about
30 160°C, about 120°C to about 180°C and about 150°C to about 160°C. The temperature selected is preferably a temperature greater than that of the glass transition temperature of the sacrificial substrate. Preferably, the temperature selected is approximately at least 20°C greater than the

glass transition temperature of the sacrificial substrate. The pressure used when contacting the mold to a surface of the sacrificial substrate may be independently selected from the group consisting of about 10 bar (1 MPa) to about 5 60 bar (6 MPa), 10 bar (1 MPa) to about 50 bar (5 MPa), about 10 bar (1 MPa) to about 40 bar (4 MPa), about 10 bar (1 MPa) to about 30 bar (3 MPa), about 10 bar (1 MPa) to about 20 bar (2 MPa).

The time period used when applying the sacrificial 10 substrate to the mold is dependent on the type of thermoplastic polymer used and the complexity of the imprint forming surface. Typically, the time period may be in the range of about 5 minutes to about 45 minutes, about 5 minutes to about 15 minutes, about 5 minutes to about 30 15 minutes, about 15 minutes to about 45 minutes, about 30 minutes to about 45 minutes and about 10 minutes to about 30 minutes.

It is an advantage of the method as described herein that as different topographies are imprinted separately, 20 imprinting conditions can be optimized with respect to specific designs. For example, in one embodiment, where imprints for topographies such as gratings, dimples, and curved lens structures a single imprinting condition is used comprising a temperature of 180°C at 60 Bar for 600 25 seconds. For more delicate structures such as pillars a preheating step may be performed, prior to imprinting, at 180°C for 120 seconds before imprinting at 180°C at 40 Bar for 300 seconds. For complex hierarchical structures a sequential imprinting process may be employed. For 30 example, a first imprinting process at 180°C at 60 Bar for 600 seconds and a second imprinting process at 140°C at 40 Bar for 300 seconds.

In one embodiment the step of forming said topographic formations comprises the step of providing the

sacrificial substrate with an array of the topographic structures in the microscale or nanoscale size range. In another embodiment, the array of microscale or nanoscale topographic structures on said sacrificial substrate are
5 selected from the group consisting of trenches, pillars, gratings, dimples, wells, curved lens structures, other 3-D structures and hierarchical 3-D structures.

The height of the topographic formations may be in the microscale. The height of the topographic formations
10 may be selected from the group consisting of about 1 micron to about 10 microns, about 1 micron to about 3 microns; about 1 micron to about 5 microns, about 1 micron to about 7 microns, about 1 micron to about 9 microns, about 3 microns to about 10 microns, about 5 microns to about 10
15 microns, about 7 microns to about 10 microns and about 9 microns to about 10 microns.

In another embodiment, the height of the topographic formations may be in the nanoscale. The height of the topographic formations may be selected from the group
20 consisting of about 10 nm to about 1000nm, about 10nm to about 500nm, about 10 nm to about 300 nm; about 10 nm micron to about 250 nm, about 10nm to about 200 nm, about 10 nm to about 150 nm, about 10 nm to about 100 nm.

In one embodiment, the topographical formations may
25 have an associated pitch. The pitch may be in the microscale and be selected from the group consisting of from about 0.5 microns to about 15 microns, about 1 micron to about 12 microns, about 2 microns to about 10 microns and about 5 microns to about 10 microns. Alternatively,
30 the pitch may be in the nanoscale and be selected from the group consisting of from about 100 nm to about 1000 nm, about 100 nm to about 500 nm, about 100 nm to about 250 nm, about 100 nm to about 200 nm and about 100 nm to about 150 nm.

In another embodiment, the width of the topographic formations may be in the nanoscale. The width of the topographic formations may be from 50 nm to about 1000 nm. In another embodiment, the width of the topographic formations may be selected from the group consisting of about 50 nm to about 1000 nm, about 50 nm to about 800 nm, about 50 nm to about 600 nm, about 50 nm to about 400 nm, about 50 to about 300, about 100 nm to about 260 nm, about 100 nm to about 140 nm, about 100 nm to about 180 nm, about 100 nm to about 220 nm, about 100 nm to about 260 nm, about 260 nm to about 300 nm, about 220 nm to about 300 nm, about 180 nm to about 300 nm, about 140 nm to about 300 nm and about 150 nm to about 250 nm. Where the topographic formation is cylindrical in shape, the width of the topographic formation refers to the diameter of the cylindrical-shaped topographic formation.

In another embodiment, the width of the topographic formations may be in the microscale. The width of the topographic formations may be selected from the group consisting of about 1 micron to about 30 microns, about 1 micron to about 20 microns, about 1 micron to about 15 microns, about 1 micron to about 10 microns, about 1 micron to about 5 microns; about 1 micron to about 4 microns, about 1 micron to about 3 microns, about 1 micron to about 2 microns.

The width of the gratings and/or trenches may be independently selected from the group consisting of about 1 microns to about 50 microns, about 2 microns to about 40 microns, about 5 microns to about 30 microns, about 5 microns to about 20 microns, about 5 microns to about 10 microns, about 10 microns to about 50 microns, about 20 microns to about 50 microns, about 30 microns to about 50 microns and about 40 microns to about 50 microns.

In another embodiment, the width of the gratings and/or trenches may be independently selected from the group consisting of about 100 nm to about 1000nm, about 200 nm to about 500 nm and about 250 nm to about 500 nm.

5 The grating formation may have a grating constant in the range of 100nm to 1000nm.

In one embodiment, the height of the grating formation may be in the range of about 10 to about 1000nm. The grating formation may have a shape, when viewed in
10 cross-section, selected from the group consisting of sinusoidal wave shape, square wave, trapezoidal shape, blazed shape and triangular shape.

The gratings and/or trenches may have an aspect ratio selected from the group consisting of about 0.1 to about
15 3.0, about 0.1 to about 2.5, about 0.1 to about 2.0, about 0.1 to about 1.5, about 0.1 to about 1.0, and about 0.1 to about 0.5. In one embodiment, the aspect ratio of the gratings and/or trenches may be about 0.5.

In a another embodiment the topographic formation may
20 comprise a line and space arrangement wherein the width of the line is selected from the group consisting of about 1 μ m, about 2 μ m, about 3 μ m, about 4 μ m and about 5 μ m. Alternatively, the width of the line is selected from the group consisting of about 100nm, about 150nm, about 200nm,
25 about 250nm about 300 about 350nm about 400nm and about 500nm. The width of the space is selected from the group consisting of about 1 μ m, about 2 μ m, about 3 μ m, about 4 μ m and about 5 μ m. Alternatively, the width of the space is independently selected from the group consisting of about
30 100nm, about 150nm, about 200nm, about 250nm, about 300 about 350nm, about 400nm and about 500nm.

In another embodiment, the topographic formations may comprise pillars and/or circular holes and/or wells. The diameter of the pillars and/or circular holes and/or wells

may be in the microscale. The topographic formations may also comprise pillars and/or dimples and/or lens structures. The diameter of the columns and/or circular holes and/or dimples and/or lens structures may be 5 independently selected from the group consisting of about 1 micron to about 10 microns, about 1 micron to about 8 microns, about 1 micron to about 6 microns, about 1 micron to about 4 microns, about 1 micron to about 2 microns, about 2 microns to about 10 microns, about 4 microns to 10 about 10 microns, about 6 microns to about 10 microns, about 8 microns to about 10 microns and about 4 microns to about 6 microns. In one embodiment, the diameter of the columns and/or circular holes may be about 5 microns.

The diameter of the pillars and/or circular holes 15 and/or wells may be in the nanoscale. The topographic formations may also comprise pillars and/or dimples and/or lens structures. The diameter of the columns and/or circular holes and/or dimples and/or lens structures may be independently selected from the group consisting of about 20 100 nm to about 1000nm, about 200 nm to about 500 nm and about 250 nm to about 500 nm.

The columns, pillars, dimples and/or circular holes and/or lens structures may have an aspect ratio selected from the group consisting of about 0.1 to about 2.0, about 25 0.1 to about 1.5, about 0.1 to about 1.0, and about 0.1 to about 0.5.

In one embodiment the topographical formations may have an aspect ratio selected from the group consisting of about 5 to about 10, about 5 to about 7, about 5 to about 30 9, about 7 to about 10, about 9 to about 10 and about 5 to about 7. In one embodiment the topographical formations may have an aspect ratio selected from the group consisting of about 0.1 to about 5, about 0.1 to about 4, about 0.1 to about 3, about 0.1 to about 2, about 0.1 to about 1, about

1 to about 3, about 1 to about 2, about 0.1 to about 1.5, about 0.1 to about 1.0, and about 0.1 to about 0.5.

In one embodiment, the method further comprises the step of removing a portion of said microscale or nanoscale topographic structures while attached to said sacrificial substrate. In one embodiment, the step of removing a portion of said microscale or nanoscale topographic structures while attached to said sacrificial substrate comprises cutting or punching.

10 In one embodiment, step of removing a portion of said microscale or nanoscale topographic structures while attached to said sacrificial substrate comprises driving a punch through said sacrificial substrate.

In another embodiment, the providing step comprises providing an ordered array of structures on said substrate. In one embodiment, said ordered array is in the form of a matrix, preferably consisting of 1 to "n" number of rows and 1 to "m" number of columns, wherein n and m are integers. In one embodiment, there n is a value from 2 to 10, from 2 to 10, from 2 to 20, from 2 to 30, from 2 to 40, from 2 to 50, from 2 to 60, from 2 to 70, from 2 to 80, from 2 to 90 or from 2 to 100. In another embodiment, there m from 2 to 10, from 2 to 20, from 2 to 30, from 2 to 40, from 2 to 50, from 2 to 60, from 2 to 70, from 2 to 80, from 2 to 90 or from 2 to 100.

In one embodiment, the array has a structure density of 2 to 10, from 2 to 20, from 2 to 30, from 2 to 40, from 2 to 50, from 2 to 60, from 2 to 70, from 2 to 80, from 2 to 90 or from 2 to 100 structures in a defined area of the array.

In one embodiment, the diameter of each structure is in the range of from 100 μ m to 5mm, from 500 μ m to 5mm, from 1mm to 5mm or from 1mm to 2mm. Advantageously, the the size of the structures can be customized to meet particular

requirements. In one embodiment, the structure may be a circle or a square. Alternatively, the structure may be of any arbitrary shape and size.

In one embodiment, the structures are formed from a thermoplastic polymer. The thermoplastic polymer may comprise monomers selected from the group consisting of acrylates, phthalamides, acrylonitriles, cellulose, styrenes, alkyls, alkyl methacrylates, alkenes, halogenated alkenes, amides, imides, aryletherketones, butadienes, ketones, esters, acetals, carbonates and co-monomers thereof. In one embodiment, the thermoplastic polymer is selected from polycarbonate or poly methyl methacrylate.

Exemplary monomers to form the thermoplastic polymer may be selected from the group consisting of methyls, ethylenes, propylenes, methyl methacrylates, methylpentenes, vinylidene, vinylidene chloride, etherimides, ethylenechlorinates, urethanes, ethylene vinyl alcohols, fluoroplastics, carbonates, acrylonitrile-butadiene-styrenes, etheretherketones, ionomers, butylenes, phenylene oxides, sulphones, ethersulphones, phenylene sulphides, elastomers, ethylene terephthalate, naphthalene terephthalate, ethylene naphthalene and combinations thereof.

The substrate may be comprised of an organic material, such as a polymer, or an inorganic material, such as metal. The substrate may be planar or non-planar. Exemplary substrate materials include silicon, glass, quartz, mica, ceramics, polymers such as polyethylene, polypropylene, polyesters such as polyethylene terephthalate, polybutylene terephthalate, polyamides, fluropolymers and polysulphone and metals such as gold, silver, copper. Preferably, the substrate is formed from silicon.

There is also provided an array comprising a substrate having an array of structures projecting therefrom, wherein each of said structures have a defined topography thereon, and wherein at least one structure has
5 a different topography from at least one other structure.

Advantageously, the array may be replicated using known casting methods, for example PDMS casting to form PDMS or hard PDMS replicas of the array made by the method as described herein. This provides a cost-effective way of
10 producing replicas of the original array.

There is also provided the use of an array according to the invention as a microarray for studying cells. Advantageously, the microarray may be used in high throughput screening for cell response studies (cell
15 adhesion, cell differentiation, cell proliferation, and gene expression), protein uptake experiments and bacterial adhesion studies and the like.

There is also provided an array according to the invention wherein said array is made in accordance with the
20 method as described herein.

There is also provided a microarray having a substrate body comprising a substrate having an array of structures projecting therefrom, wherein each of said structures have a defined topography thereon, and wherein
25 at least one structure has a different topography from at least one other structure.

In one embodiment, for easy reference to the locations of different topographies on the array, a document "map" that identifies the location of said
30 structures comprising different topographical formations on said array may be provided. In one embodiment, as a reference point for easy identification of the location of the different topographical formations, the first column of the said structures on the array may be displaced

further from an edge of the array than the last column of structures. This may then be correlated with the document map in order to locate particular topographical formations disposed on the array.

5

Brief Description Of Drawings

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

Figures 1a - 1c are schematic diagrams showing a process for producing an array as described herein.

Figure 2 is a photograph showing a working prototype of the array made according to an embodiment of the present disclosure.

Figure 3 (a) to (j) show Scanning Electron Microscopy (SEM) images of the different topographies of an array of the present disclosure.

Figure 4 (a) to (c) shows representative Atomic Force Microscopy (AFM) section analyses of different designs on the array, demonstrating some of the different co-existing height topographies achievable on the array.

Figure 5 is a photograph showing a Poly(dimethylsiloxane) (PDMS) replica of the design from an array according to the present disclosure.

Figure 6 is a graph showing the percentage of TuJ1 positive vs GFAP positive cells attached on various topographies of an array of the present disclosure.

Figure 7 is a graph showing the percentage of BrdU incorporation for Human Mesenchymal Stem cells attached on various topographies of an array of the present disclosure.

Figure 8a and 8b are graphs showing the amount of IL-2 secretion for T cells attached on various topographies of an array of the present disclosure.

Figure 9a and 9b are graphs showing the percentage of CD44⁺Cd24^{-low}ESA⁺ cancer cells attached on various topographies.

Detailed Description of Drawings

Figure 1a shows a schematic diagram of a method 201 for making a topographical structure in the form of a grating pattern onto a sacrificial substrate polymer film (steps (A) to (C)).

In Step (A) of Figure 1a, there is shown a Nano-Imprint Lithography (NIL) step to form a substrate having a topographical formation in the form of imprints as shown in the inset. NIL is a known technique to persons skilled in the art. In the NIL technique, the mold 1 is pressed into the surface of the substrate in the form of substrate 2, at a temperature of 180 °C and pressure of 60 Bars for 600 s to form a topographical structure on the substrate 2.

In Step (B) of Fig. 1a, the substrate 2 is cooled before demolding the substrate 2 from the mold 1.

In Step (C) of Fig. 1a, a portion of the topographical formation is removed from the substrate 2 using a tissue punch 3 to form a structure 4 having a topographical formation thereon for subsequent use in making an array.

Figure 1b shows a schematic diagram of a method 202 for fabricating a topographical formation in the form of a pillar pattern onto a sacrificial substrate polymer film (steps (A) to (C)).

In Step (A) there is shown a Nano-Imprint Lithography (NIL) step to form a substrate having a topographical formation in the form of an ordered array as shown in the inset. In the NIL method step A of Figure 1b, a preheating
5 step is first performed at 180 °C for 120 s. Then the mold **10** is pressed into the surface of the sacrificial substrate in the form of substrate **20**, at a temperature of 180 °C and a pressure of 40 Bars for 300 s to form a topographical formation on the substrate **20**.

10 In Step (B) of Figure 1b, the substrate **20** is cooled before demolding the substrate **20** from the mold **10**.

In Step (C) of Figure 1b, a portion of the topographical formation is removed from the substrate **20** using a tissue punch **30** to form a structure **40** for
15 subsequent use in making an array.

Figure 1c shows a schematic diagram of a method **203** for making an array **103** on a substrate **100**, a process whereby the structures produced by methods **201** and **202** are adhered to the substrate **100**.

20 In step (A) of Figure 1c, PDMS mixed in a ratio of 3:1 (elastomer:curing agent) is first degassed using a vacuum for 15 min to remove any bubbles in the mixture. The PDMS is then spin-coated onto the silicon substrate **100** at 3000 rpm for 30 seconds. The resultant layer of
25 PDMS **101** acts as a bonding material to adhere the structures **4**, **40** onto the substrate **100**.

In step (B) of Figure 1c, the structures **4**, **40** having topographical formations (1# to N#) produced by method **201** and **202** are adhered to the PDMS layer **101** in a matrix
30 arrangement. The PDMS is further degassed under vacuum conditions to remove any residual bubbles remaining in the

PDMS layer **101**. The PDMS layer **101** is then cured by heating the assembled substrate in a vacuum oven at 70 °C for 1 h. This adheres the structures **4**, **40** to the substrate firmly **100**, resulting in an array **102** according to the present disclosure.

Examples

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

Example 1

This example illustrates a process of the present disclosure for fabricating an array in accordance with the present disclosure using methods **201** - **203** as depicted in Figures 1a-c.

To demonstrate the wide range of different topographies which could be incorporated on a single substrate, a mixture of designs was used. The various topographies include, but are not limited to: gratings, pillars and dimples, curved lens structures as well as complex three-dimensional hierarchical structures, with different resolutions and aspect ratios.

Figures 1a and 1b respectively illustrate a process for producing a topographical formation with a grating pattern and a pillar pattern.

Thermal nanoimprint lithography was first used to fabricate various topographical formations separately onto individual substrates **2**, **20** (for example, Polycarbonate (PC) or Poly (methyl methacrylate film) (PMMA)). Referring

to Figure 1a for example, molds **1, 10** bearing the desired topography were pressed into the surface of the substrates **2, 20** at various optimized temperatures, pressures and durations depending on the desired topography, in order to imprint the desired pattern onto the sacrificial substrates **2, 20**. For gratings, dimples and curved lens structures, a single imprinting condition was used: 180°C, 60 Bars, 600s. For delicate structures such as pillar structures, a preheating step was performed at 180°C for 120s before the imprinting at 180°C, 40 Bars, 300s to allow high yield imprinting of such structures. For hierarchical structures, a sequential imprinting process was employed: 180°C, 60 Bars, 600s for the primary imprinting process; followed by 140°C, 40 Bars, 300s for the secondary imprinting process.

Next, the substrates **2, 20** were cooled before they were demolded from their respective molds **1, 10**. A Tissue puncher **3, 30** was then used to cut out a uniform 2mm diameter sized portion of the substrate **2, 20** resulting in the production of structures **4, 40** bearing the desired topographical formations.

The above process was repeated for different topographical formations, thereby producing "N" number of topographical formations (indicated as #1 - #N, in Figure 1c).

Figure 1c illustrates the process of making an array in accordance with the disclosure. Firstly, PDMS (Sylgard®184 mixed in a ratio of 3:1 (elastomer:curing agent)) was degassed in a vacuum for 15 minutes to remove any bubbles in the mixture. The PDMS was then spin-coated onto a silicon substrate **100** at 3000 rpm for 30s. The resultant layer of PDMS **101** acts as a bonding material for securing the structures to the substrate **100**.

Next, the structures with various topographical formations were assembled manually using tweezers onto the PDMS layer **101** in a matrix arrangement. The PDMS layer acts as a bonding layer to adhere the structures onto the substrate. Further degassing was then performed using under vacuum conditions to remove residual bubbles remaining beneath the PDMS layer **101**. The PDMS layer **101** was then cured by heating the assembled substrate in a vacuum oven at 70 °C for 1 h. This bonded the topographical structures (#1 - #N) firmly onto the substrate **100**, thereby producing an array **102** of the current invention.

Figure 2 shows a working prototype **300** of an array fabricated using the above process. The arrow points to an example of a 2mm topographical structure **301**. The prototype **300** consists of a matrix of 6 x 6 structures **301** having different topographical formations thereon.

SEM images of some of the different topographical formations that were fabricated are shown in Figure 3. The topographies shown are: (a) 2 μm line, 2 μm space; (b) 1 μm line, 2 μm space (c) 2 μm line, 1 μm space (d) 250 nm line 250 nm space (e) 1 μm dimple, 9 μm pitch (f) 2 μm pillars, 12 μm pitch (g) 500 nm pillars, 10 μm pitch (h) 250 nm pillars, 500 nm pitch (i) 1 μm pitch microlens (j) 2 μm line 2 μm space $\perp$ 250nm line 250nm space.

Figure 4 shows a range of topographical formations with different heights, clearly demonstrating the possibility of incorporating topographical formations of different aspect ratios onto a single array. The different topographies shown are: (a) 250 nm line 250 nm space gratings with 120 nm height; (b) 250 nm pillars, 500 nm pitch with 250 nm height; (c) 1 μm pitch microlens with 90 nm sag.

The array of the present disclosure can also be used to form replica copies of the design by using a conventional PDMS casting method for example. A PDMS replica copy **500** of the design from an array is shown in Figure 5. The replica array **500** has an array of structures **501** having different topographical formations thereon.

Example 2

In this example, an array as described herein was used to illustrate an application to facilitate fast microscopy based screening to investigate neural precursor cell differentiation enhancement with respect to various topographies.

Neural progenitor cells were isolated from the hippocampal region of a postnatal day 5 mouse brain. They were expanded on the tissue culture plate and coated with 8 µg/ml of natural mouse laminin (Invitrogen Pte. Ltd.) in a proliferation medium composed of DMEM/F12 (Biological Industries), N2 supplements (GIBCO, Invitrogen Pte Ltd) and Penicillin/Streptomycin. Growth factors, basic fibroblast growth factor, bFGF, (GIBCO, Invitrogen Pte. Ltd.), and epidermal growth factor, EGF, (Invitrogen Pte. Ltd.), were added freshly at a final concentration of 20 ng/ml to the expansion medium.

Polydimethyl siloxane (PDMS) substrates were replicated from an array master mold using a 10:1 ratio of elastomer and curing agent (SYLGARD®184 Elastomer Kit, Dow Corning). The elastomer and the curing agent were mixed thoroughly at a ratio of 10:1 and the mixture was degassed before pouring onto the microarray chip. Degassing was repeated subsequently for 45 minutes until there were no residual visible bubbles. The curing was completed by keeping the sample inside a curing oven at a temperature

of 65°C overnight. The replica array was peeled off from the PDMS substrate.

For neural differentiation, the PDMS replica substrates were plasma treated and coated consecutively with Poly-L-ornithine (Sigma Aldrich) and laminin at 20 µg/ml overnight. The cells (Passage 12 - 17) were detached from the tissue culture plate and seeded on the PDMS substrates at a density of 7,500 cells/cm². The differentiation medium during the first phase consisted of DMEM/F12, N2 supplements, Penicillin/Streptomycin and B27 supplements (GIBCO, Invitrogen Pte. Ltd.) with bFGF at a concentration of 5 ng/ml. After 7 days, the differentiation medium was changed to the second phase medium made up of neurobasal medium (Invitrogen Pte. Ltd.) supplemented with B27 and 0.25X N2 and mixed at a ratio of 1:1 with normal Neural Precursor Cell (NPC) expansion medium without N2 supplements and bFGF.

At the end of the time point, the cells were fixed in 4% paraformaldehyde and immunocytochemical staining was performed with the following primary antibodies with their respective optimized concentrations: anti-rabbit β-tubulin III or Tuj1 (Tuj1 = neuron-specific class III beta tubulin) antibody(1:600, Sigma Aldrich); anti-mouse glial fibrillary acidic protein, GFAP, antibody (1:600, Chemicon, Millipore Pte Ltd). The secondary antibodies were purchased from Invitrogen Pte Ltd: Alexor Fluor 546 Goat anti-mouse IgG (H+L) and Alexor Fluor 488 Goat anti-rabbit IgG (H+L) both at the concentration of 1:750. Nuclei were counterstained with 4',6-diamidino-2-phenylindole, DAPI, (Invitrogen).

Imaging was performed with a Leica fluorescent microscope equipped with Q imaging camera (Canada) and with the software, Q capture Pro. From each topographical formation, 7-10 images were captured and image analysis

was done using the software, Image J (NIH). Statistical analysis with one way ANOVA was done using Microsoft Excel after compiling the raw data. The percentage of neural precursor cells which differentiated into immature neurons (positive for Tuj1 staining) and astrocytes (positive for GFAP staining) was compared with respect to different topographical formations as well as coverslip control samples (CS). Figure 6 shows the proportion of immature neurons vs astrocytes attached to different topographical formations. The different topographies compared were: CS: control; I: 2 μm line, 2 μm space; II: 1 μm line, 2 μm space; III: 2 μm line, 1 μm space; IV: 250 nm space, 250 nm space; V: 1 μm diameter holes, 9 μm pitch; VI: 2 μm diameter pillars, 12 μm pitch; VII: 500 nm diameter pillars, 10 μm pitch; VIII: 130 nm diameter pillars, 400 nm pitch; IX: 2 μm line, 2 μm space $\perp$ 250 nm line, 250 nm space (hierarchy). The error bars in Figure 6 represent standard deviations from the mean.

As can be seen from Figure 6, a significant difference in the proportion of immature neurons vs astrocytes was observed for topographies I and IX.

Example 3

In this example, an array as described herein was used to illustrate an application to facilitate fast microscopy based screening to investigate the regulation of Human Mesenchymal Stem Cell proliferation with respect to various topographical formations.

BrdU incorporation was used as an indicator of DNA synthesis and therefore a proxy for measuring the extent of cell proliferation.

Polydimethyl siloxane (PDMS) substrates were replicated from an array in the form of a master mold using a 10:1 ratio of elastomer and curing agent.

(SYLGARD®184 Elastomer Kit, Dow Corning). The elastomer and the curing agent were mixed thoroughly at a ratio of 10:1 and the mixture was degassed before pouring onto the array chip. Degassing was repeated subsequently for 45 minutes until there was no residual visible bubble. The curing was completed by keeping the sample inside a curing oven at a temperature of 65°C overnight. The array was peeled off from the PDMS substrate. The PDMS replica array was then used in the cell assay. Human mesenchymal stem cells were cultured for 7 days on a PDMS stitch array replica and serum was withdrawn from serum-depleted cells for 6 hours on day 5.

On day 7, BrdU was added into culture medium (1:1000) and the samples were incubated for 4 hours. The cells were then fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X100, further incubated in 4N HCl for 10 minutes and then blocked with 10% goat serum and 1% BSA in PBS. BrdU was labeled with mouse anti-human BrdU diluted 1:50 as primary antibody, Alexa-Fluor 546 conjugated goat anti-mouse antibody diluted 1:1000 as secondary antibody, and the nuclei were counterstained with 4',6-Diamidino-2-phenylindole. Samples were then imaged with fluorescence microscope. Acquired fluorescence images were analyzed by Image J software. BrdU incorporation percentage was calculated as the percentage of BrdU positive-stained cells to the total number of cells on each of the different topographical formations of the replicated PDMS substrate.

Figure 7 shows the results obtained. The topographies compared were: A: 2 μm line, 2 μm space; B: 2 μm line, 1 μm space; C: 1 μm line, 2 μm space; D: 250 nm line, 250 nm space, 125nm height; E: 1 μm diameter pillars, 9 μm pitch; F: 2 μm diameter holes, 12 μm pitch; G: 500 nm diameter

holes, 10 μm pitch; H: 130 nm diameter holes, 400 nm pitch; I: 2 μm line, 2 μm space $\perp$ 250 nm line, 250nm space (hierarchy); J: 2 μm line, 2 μm space //250 nm line 250nm space (hierarchy); K: 2 μm line and 2 μm space + 250 nm diameter holes (hierarchy); L: 250 nm diameter holes, 500 nm pitch; M: 250 nm line, 250nm space, 250nm height; N: 1 μm diameter concave lens, 1 μm pitch; O: 1 μm diameter convex lens, 1 μm pitch; P: 1.8 μm diameter concave lens, 2 μm pitch; Q: 1.8 μm diameter convex lens, 2 μm pitch.

10 The percentage of BrdU incorporation indicates the extent of proliferation. In particular, the higher the percentage of BrdU incorporation, the greater the extent of Human Mesenchymal Stem Cell proliferation.

From the results it can be seen that narrower line widths of the topographical formation result in decreased stem cell proliferation. Secondly, topographies comprising pillars appear to significantly enhance proliferation. Lastly, the preliminary studies suggested that the line width may exert a more significant effect on the proliferation of Human Mesenchymal Stem Cells compared to the spacing between the lines.

Example 4

In this example, an array as described herein was used to illustrate an application to facilitate fast microscopy based screening to investigate T-cell activation with respect to various topographical formations.

An array in accordance with the disclosure was used as a master mold to form replica copies of the array using a material termed hard PDMS: 3.4 g of 7-8% vinylmethylsiloxane, 1 g of 25-30% methylhydrosiloxane, 18 microliters of Platinum(0)-2,4,6,8-tetramethyl-2,4,6,8-

tetravinylcyclotetrasiloxane), and 10 microliters of 2,4,6,8-Tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane were mixed and spin coated onto the array. Following hardening, a thick layer of PDMS (Sylgard®184, Dow
5 Corning) with a mixture of (elastomer:curing agent of 10:1) was poured in order to allow easy handling of the replica.

The replica arrays were coated with antibodies to TCR and CD28 (receptors on the T cell surface that upon this binding provide activating and co-stimulatory signals,
10 respectively). Naïve CD4⁺ T cells were isolated from mice and seeded onto these surfaces in RPMI media supplemented with 10% fetal bovine serum. T-cell activation was measured on the basis of secretion of IL-2, a cytokine indicative of T cell activation, over the course of six
15 hours. IL-2 secretion was assayed using a commercial, surface-capture method from Miltenyi Biotech (IL-2 secretion detection kit). Fluorescence associated with IL-2 secretion from this assay was measured on a cell-by-cell basis by microscopy, and compared using Kruskal-
20 Wallis non-parametric approaches.

Figure 8a and 8b illustrate the results obtained from the microscopy screening of a small subset of the topographical formations on the replica array. A - J in Figure 8a are topographies - A: 2 μm line, 2 μm space; B:
25 2 μm line, 1 μm space; C: 1 μm line, 2 μm space; D: 250 nm line, 250 nm space, 250 nm height; E: 250 nm line, 250 nm space, 150 nm height; F: 460 nm line, 70 nm space, G: 2 μm diameter holes, 12 μm pitch; H: 500 nm diameter holes, 10 μm pitch; I: 250 nm diameter holes, 500 nm pitch; J: 130
30 nm diameter holes, 400 nm pitch.

Figure 8b illustrates the results obtained using topographies of lens geometry consisting of :1 μm diameter, 1 μm pitch concave lens; 1.8 μm diameter, 2 μm pitch

concave lens; 1 μ m diameter, 1 μ m pitch convex lens; 1.8 μ m diameter, 2 μ m pitch convex lens.

Comparison of IL-2 secretion across the replica array revealed that T cells are able to recognize and respond differently to different topographies. Most prominently, these T cells showed higher levels of IL-2 secretion on surfaces presenting arrays of 1 μ m diameter, 1 μ m pitch lenses than those with 1.8 μ m diameter, 2 μ m pitch lens features. In addition, IL-2 secretion was higher on convex lenses rather than concave ones (such as those illustrated in Figure 8b).

A further potential extension of this application can be achieved by using this array for immunotherapy. T-cells can be extracted from patients, activated and expanded in cell culture for sufficient number of cells for therapeutic purposes with an array of specific topography. The activated T-cells are injected back to the patient for immunotherapy. As the substrate will not be transferred to the patients, there will be lower side-effects and toxicity. Thus this protocol/system will have limited regulatory barriers and can be brought to clinical trials very rapidly.

Example 5

This example was used to illustrate a potential application of using the microarray, as a unique, fast and low cost marker for cancer diagnostic applications, since different subpopulations of cancer cells display different adhesion responses to specific topographical formations.

This example shows that different topographical formations can influence the adhesion response of different subpopulations of cancer cells.

This example was not carried out on a replica array chip. This example has been included to illustrate that 1) different topographies can influence the response of cancer cells, and 2) since the microarray consists of a variety of different topographical formations, it can be potentially used as a cancer diagnostic tool.

Figure 9a shows the results obtained for the breast cancer cell line MCF7 and Figure 9b shows the results for human primary invasive ductal carcinoma (IDC) cells.

The results were analysed by ANOVA, which showed that there was a significant difference for all the topographies as compared to the control (flat surface/ no pattern surface). For MCF7 cells, t-tests showed a preferential isolation of $CD44^+CD24^{-/low}ESA^+$ MCF7 cells on the nano-scale features ($*p < 0.05$, mean $\pm$ SD, $n=3$) as compared to the micro-scale features (see Figure 9A).

In the IDC cell study, significantly more primary cells adhered onto the 250nm well ($\#p < 0.001$, mean $\pm$ SD, $n=3$) as compared to the control (see Figure 9B).

Culturing of MCF7 cells

The human breast cancer cell line, MCF7, was obtained from the American Type Culture Collection (ATCC) and maintained using Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS), 0.01 mg/ml bovine insulin, 1% penicillin streptomycin and 1% non-essential amino acids solution. The medium was changed every three days.

Culturing of Invasive Ductal Carcinoma (IDC) cells

IDC cells were obtained from the NUH-NUS Tissue Repository. These cells were derived from breast tumor tissue donated by patients under the approval of NUS Institutional Review Board. The cells were maintained in

culture using mammary epithelial growth medium (MEGM, Lonza) supplemented with 10% FBS, amphotericin B and gentamicin.

5 **Cell seeding on micro- or nano-topographical substrates**

Poly-L-lactic substrates with nanotopography patterns were cut to 1.0 cm by 1.0 cm dimensions. They were submerged in 70% ethanol solution and exposed to ultraviolet light simultaneously for 20 minutes for
10 sterilization.

Either MCF7 or IDC cells were seeded onto the PLLA films with a seeding density of about 10,000 cells/cm².

After 4 and 24 hours of culturing MCF7 cells on the PLLA films, the cells were fixed with 4% paraformaldehyde
15 and immuno-fluorescence stain for the stem cell markers with standard protocol, using primary antibodies specific for human cell surface markers CD44, CD24 and epithelial cell adhesion molecule (EpCAM or ESA). The primary antibodies used were: rat anti-CD44 FITC (Abcam), mouse
20 anti-CD24 PE (Abcam) and rabbit anti-ESA (Santa Cruz). The secondary antibodies used were: goat anti-mouse Alexa Fluor 546, goat anti-rabbit Alexa Fluor 647 and rabbit anti-rat FITC. The samples were then mounted on glass slides with fluoromount (Sigma) and imaged with a
25 fluorescence microscope at 20x, 40x and 63x magnification. Flow cytometry was also performed using the antibodies for CD44, CD24 and ESA. The breast cancer stem cells were identified as the CD44⁺/ CD24⁻/ ESA⁺ population.

30

Applications

The disclosed process may be applied in numerous industrial applications, not least in therapeutic applications, pharmaceutical compositions. The fabrication of different topographies on a single substrate surface

may be used for fast and efficient experimental studies investigating the effect of different surface topographies on a wide range of cell types. The array and its PDMS replica can also be used for fast microscopy screening for cell-topography interactions, for immunotherapy and cancer diagnostic applications and immunotherapy applications.

Advantageously, the disclosed process may be highly reproducible and may be used to simultaneously fabricate a large range of different topographies, such as different heights, dimensions and feature shapes onto a single substrate surface.

Advantageously, the method described herein provides a low cost fabrication process. In particular, nanoimprint technology, which is a cost-efficient, scalable, high throughput nanoimprinting technique, is used to create the different topographies which are otherwise prohibitively expensive if they are fabricated by conventional electron beam lithography.

It is a further advantage that the method as described herein provides a biocompatible array.

It is a further advantage of the method as described herein that the array may be replicated using known casting methods, for example PDMS casting. This provides a cost-effective way of producing replicas of the original array.

Advantageously, the method as described herein does not encounter problems of resolution limit in providing the different topographies, as nanoimprinting is used to form the topographies, where the limit of resolution is limited by the mold to be used for imprinting.

It is a further advantage of the method as described herein that the individual topographies of the array can be optimized as different topographies are imprinted separately.

It is a further advantage of the method as described herein that a dry/etch-free approach is used to form the different topographies on a single substrate.

Advantageously, the method as described herein
5 permits the use of different types of polymer (for example, PMMA, PC, ETFE and the like) to form the structures having a pre-defined topography thereon.

Advantageously, the array of the invention can also be used to form replica copies of the array design by
10 using various methods well known in the art, such as a conventional PDMS casting method.

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

Claims

1. A method of making an array for cell assays comprising the step of providing an array of structures on a substrate, each of said structures having a pre-defined topography thereon, and wherein at least one structure has a different topography from at least one other structure.
2. The method as claimed in claim 1, wherein the structures are generally elongate and have a proximal end that extends from the substrate and a distal end that is opposite to said proximal end.
3. The method as claimed in claim 2, wherein the topography of said structures is provided on said proximal end.
4. The method as claimed in claim 3, wherein the elongate structures have a longitudinal axis extending from their proximal end to their distal end and wherein said longitudinal axis is generally normal to a planar surface of said substrate.
5. The method as claimed in any one of the preceding claims, wherein the providing step comprises the step of adhering said structures to said substrate.
6. The method as claimed in claim 5, wherein said adhering step comprises the step of providing an adhesive layer on said substrate before said adhering step.

- 5 7. The method as claimed in claim 6, wherein said providing an adhesive layer on said substrate comprises the step of spin-coating a polymer solution onto said substrate.
- 10 8. The method as claimed in claim 7, removing the polymer solution while said array of structures are disposed in said polymer solution to form a polymer layer that adheres said structures to said substrate.
- 15 9. The method as claimed in any one of the preceding claims, wherein said topography is formed by formations in the microscale or the nanoscale size range.
- 20 10. The method as claimed in claim 9, comprising, before said providing step, the step of forming said topographic formations on a sacrificial substrate.
- 25 11. The method as claimed in claim 10, wherein the step of forming said topographic formations on a substrate comprises an imprint lithography step.
- 30 12. The method as claimed in claim 11, wherein the step of forming said topographic formations comprises the step of providing the sacrificial substrate with an array of the topographic structures in the microscale or nanoscale size range.

13. The method as claimed in claim 12, wherein the array of microscale or nanoscale topographic structures on said sacrificial substrate are selected from the group consisting of trenches, pillars, gratings, dimples, wells, and other 3-dimensional structures.

14. The method as claimed in claim 12 or 13, comprising the step of removing a portion of said microscale or nanoscale topographic structures while attached to said sacrificial substrate.

15. The method as claimed in any one of the preceding claims, wherein said providing step comprises providing an ordered array of structures on said substrate.

16. The method as claimed in any one of the preceding claims, wherein said structures are formed from a thermoplastic polymer.

17. The method as claimed in claim 16, wherein said thermoplastic polymer comprises monomers selected from the group consisting of acrylates, phthalamides, acrylonitriles, cellulose, styrenes, alkyls, alkyls methacrylates, alkenes, halogenated alkenes, amides, imides, aryletherketones, butadienes, ketones, esters, acetals, carbonates and co-monomers thereof.

18. An array comprising a substrate having an array of structures projecting therefrom, wherein each of said structures have a pre-defined topography thereon, and wherein at least one structure has a

different topography from at least one other structure.

5 19. The array as claimed in claim 18, wherein the structures are generally elongate and have a proximal end that extends from the substrate and a distal end that is opposite to said proximal end.

10 20. The array as claimed in claim 18 or claim 19, wherein said topography is on said proximal end.

15 21. The array as claimed in any one of claims 18 to 20, wherein the topography is dimensioned in at least one of the microscale or the nanoscale.

22. The array as claimed in any one of claims 1 to 4, wherein an adhesive layer is disposed between the structures and the substrate.

20 23. The array as claimed in any one of claims 18 to 22, wherein said structures are formed from a thermoplastic polymer.

25 24. The array as claimed in claim 23, wherein said thermoplastic polymer comprises monomers selected from the group consisting of acrylates, phthalamides, acrylonitriles, cellulose, styrenes, alkyls, alkyls methacrylates, alkenes, halogenated alkenes, amides, imides, 30 aryetherketones, butadienes, ketones, esters, acetals, carbonates and co-monomers thereof.

25. The array as claimed in any one of claims 18 to 24, wherein said structures are arranged in an ordered array.

5 26. The array as claimed in any one of claims 18 to 25, wherein said topography is selected from formations selected from the group consisting of trenches, gratings, columns, circular holes and other 3-dimensional structures.

10

27. The array as claimed in any one of the preceding claims, wherein the substrate is formed from silicon.

15

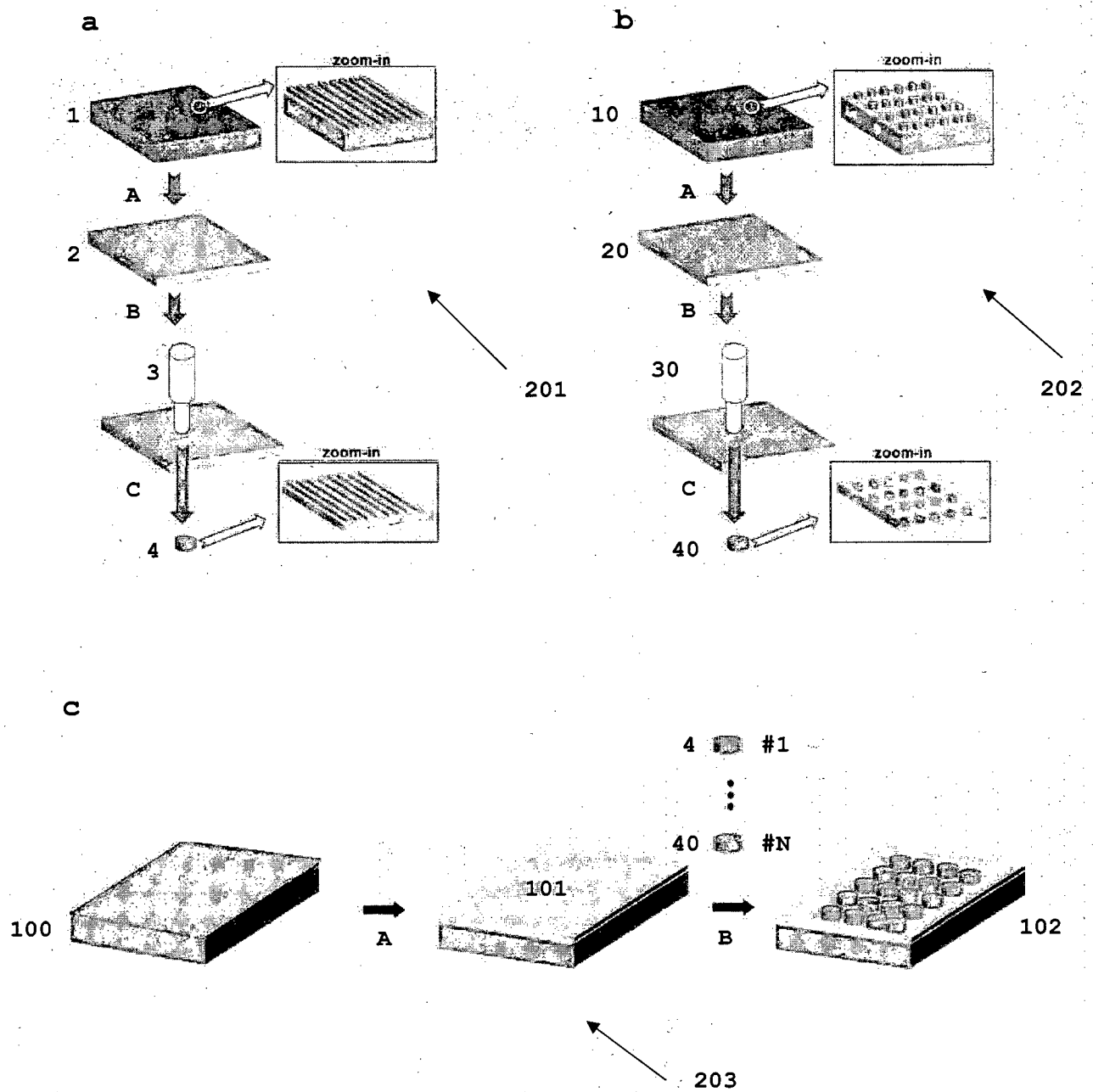


Figure 1

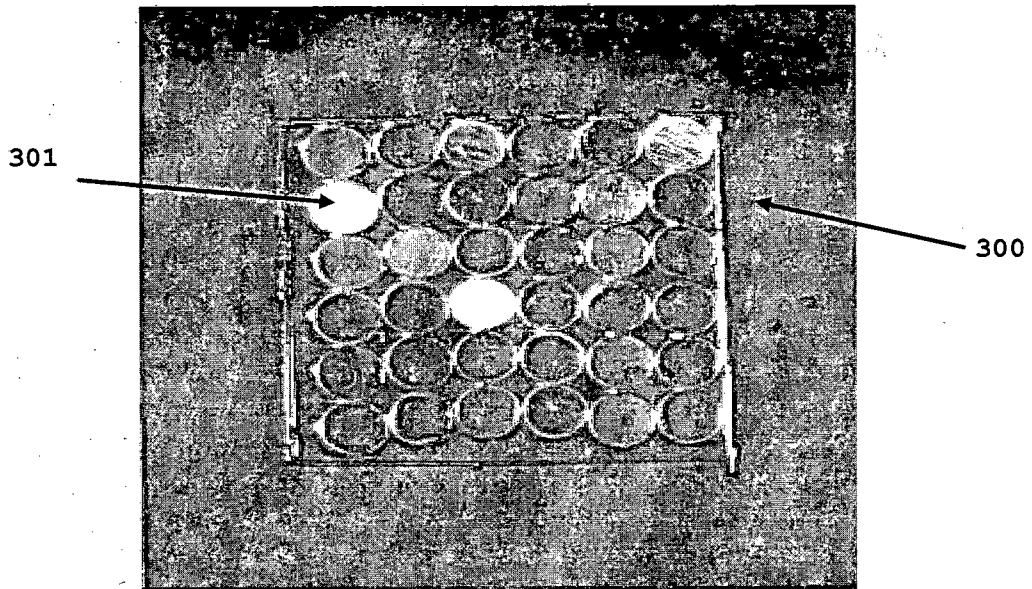


Figure 2

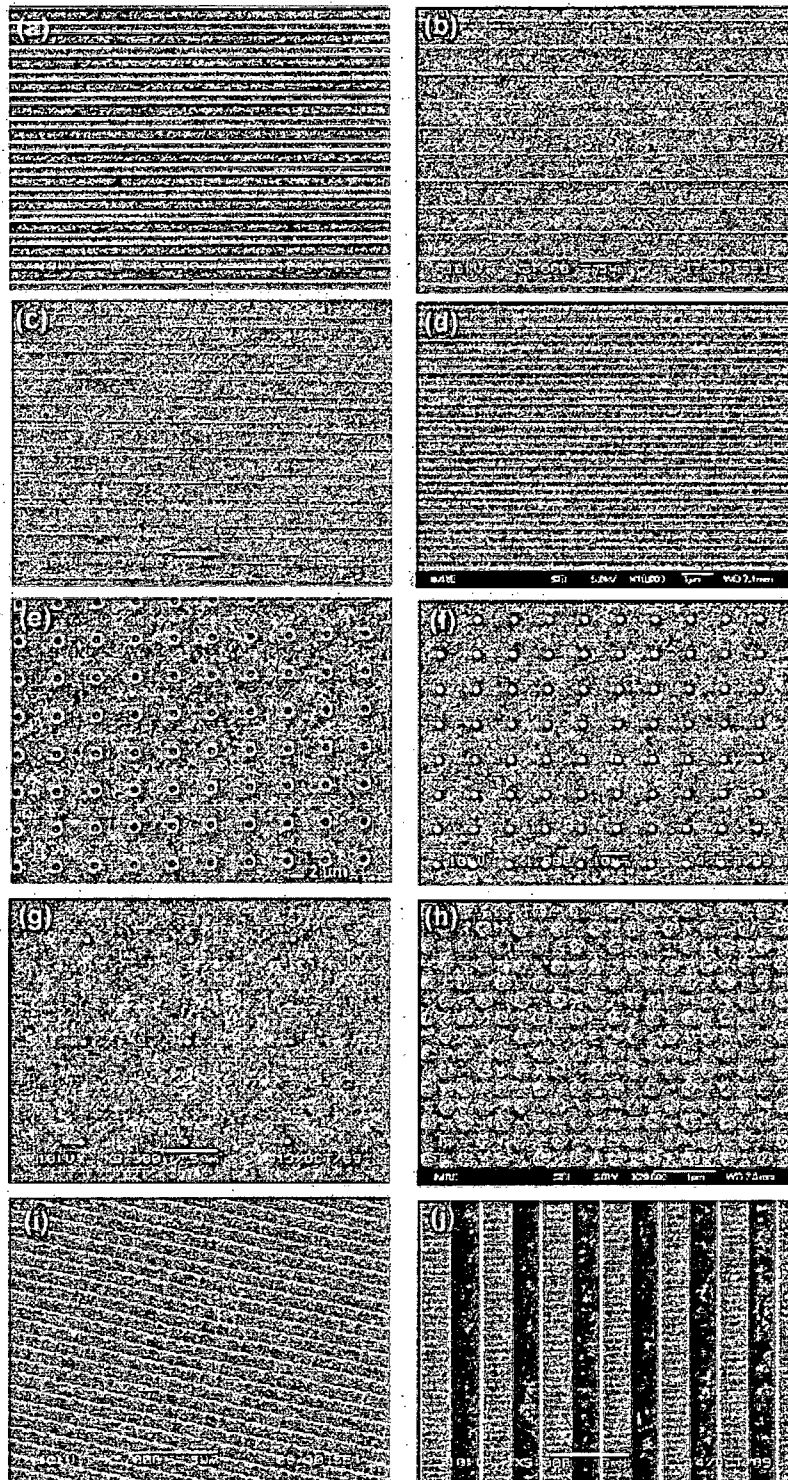


Figure 3

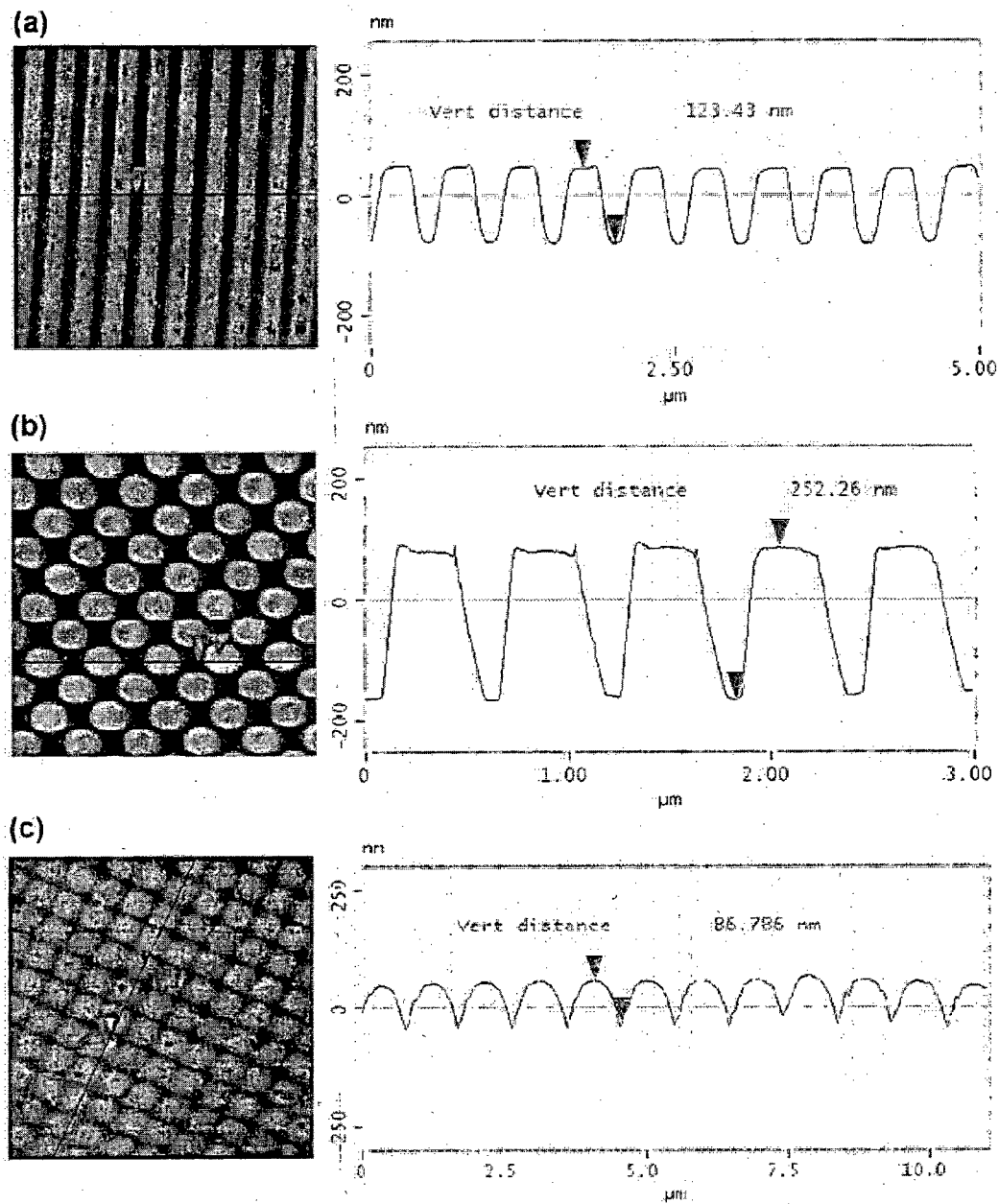
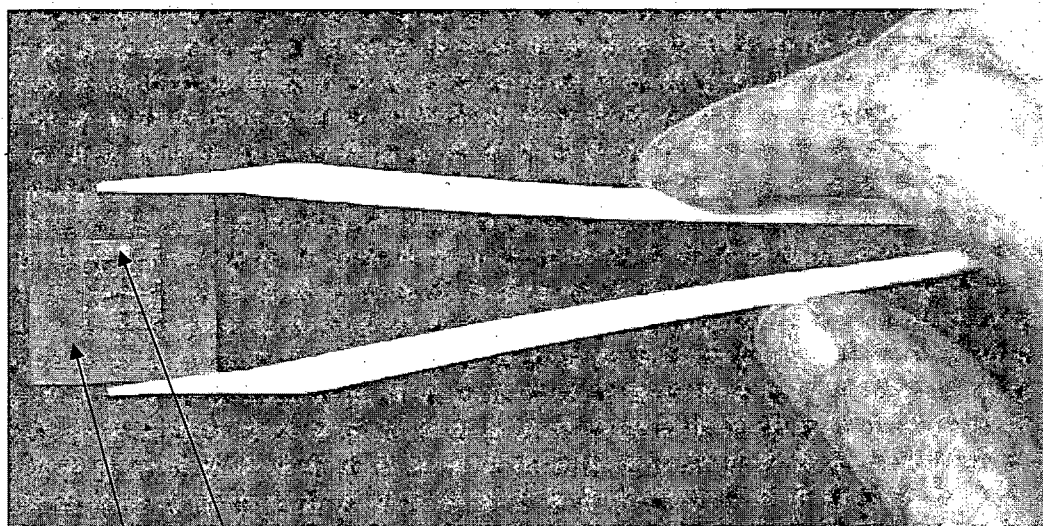


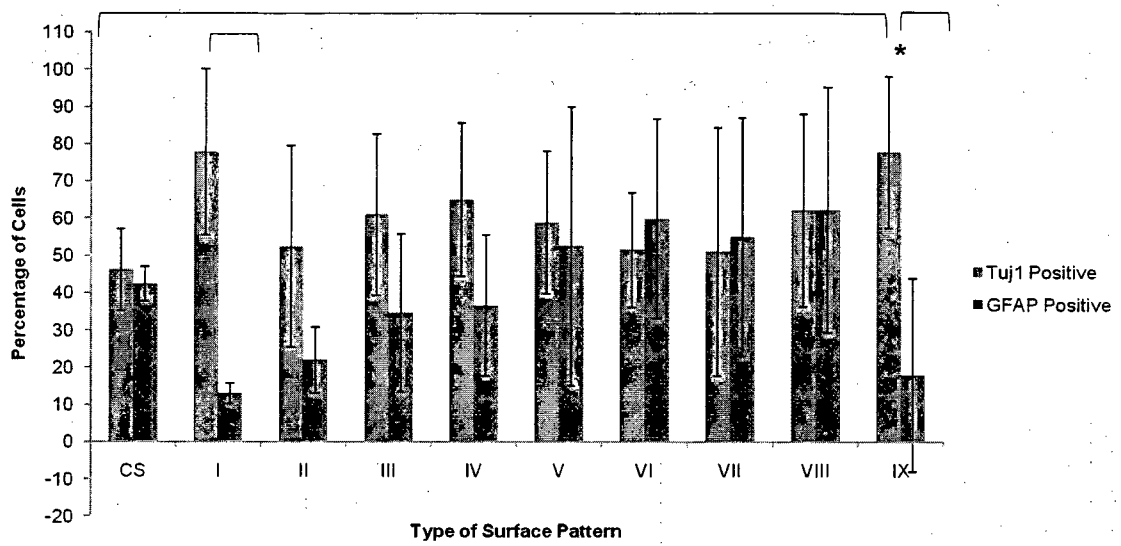
Figure 4



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501

Figure 5

**Figure 6**

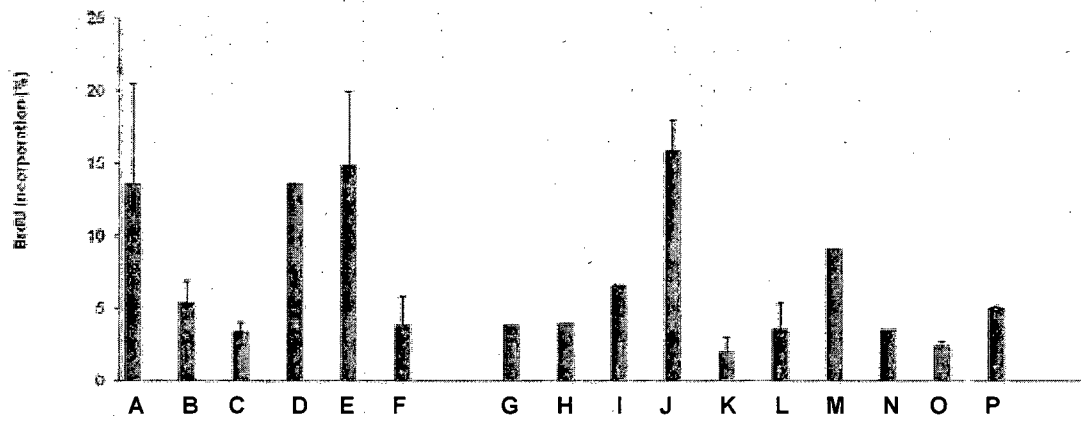
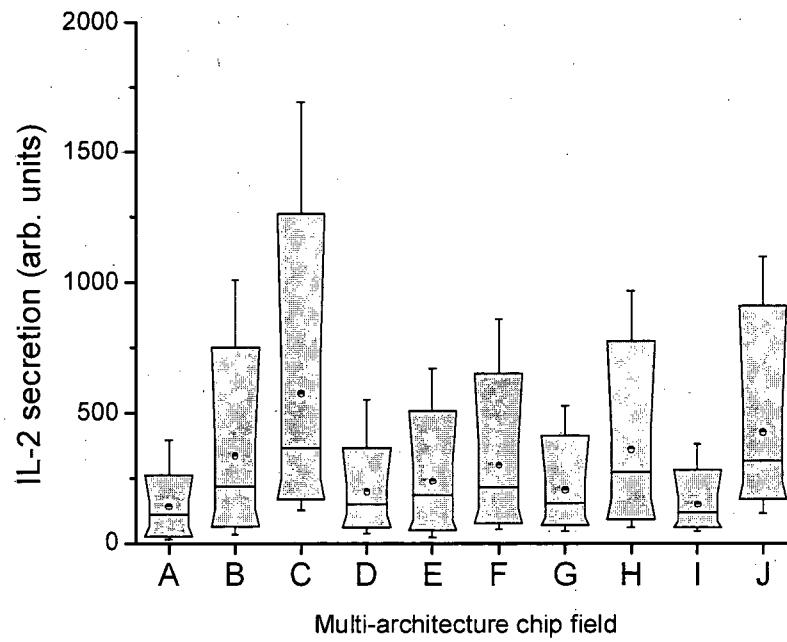
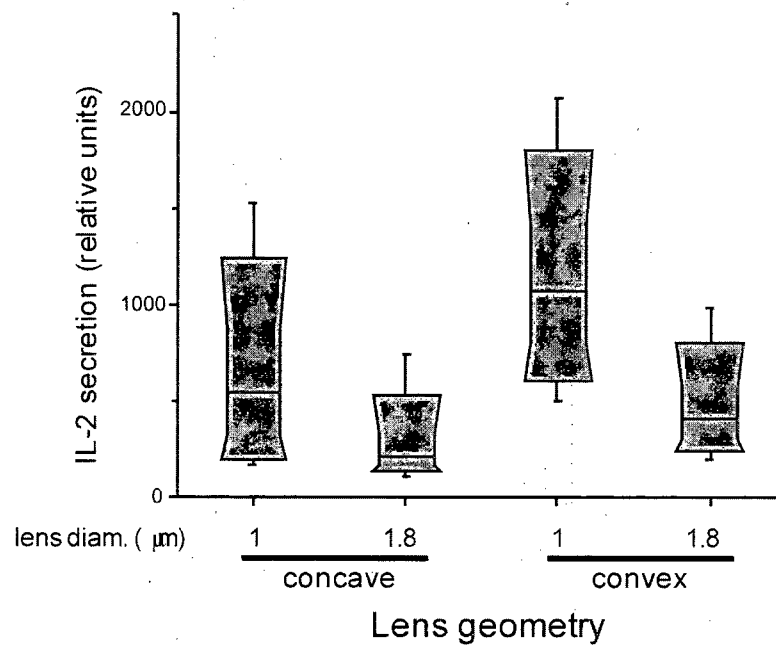


Figure 7

**Figure 8a**

**Figure 8b**

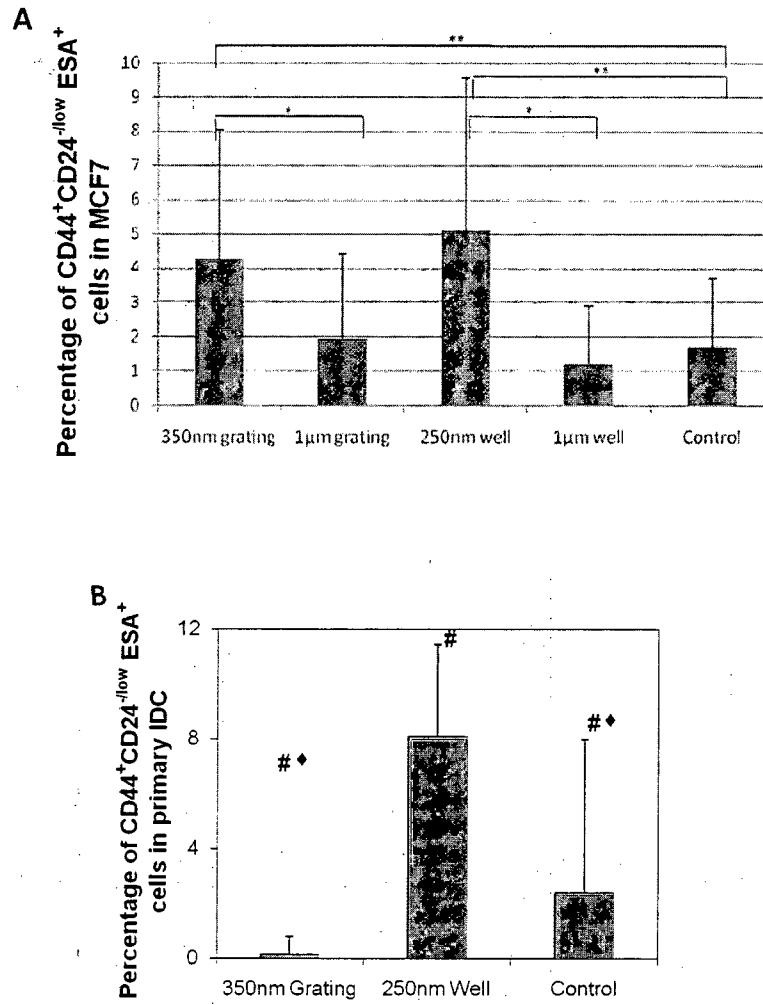


Figure 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2011/000106

A. CLASSIFICATION OF SUBJECT MATTER		
INT. CL.		
B81B 7/04 (2006.01)	B29C 59/00 (2006.01)	C12M 1/00 (2006.01)
B01L 3/00 (2006.01)	B81C 3/00 (2006.01)	G01N 33/48 (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPODOC, WPI: OR (THERMAL W NANO_IMPRINT+), (NANO_IMPRINT+ W LITHOGRAPH+), NIL, (NANO+ 2D PATTERN+), (MICRO+ 2D PATTERN+); +ARRAY+ OR ((CELL OR BIO+) W ASSAY?) OR LAB_ON_A_CHIP? OR MMCHIP?; (OR TOPOGRAPH+, TOPOLOG+, HEIGHT?, DIMENSION?, SHAPE?, SIZE, ARCHITECT+, STRUCTUR+, CONTOUR+, GEOMETR+, GRATING?, PILLAR?, DIMPLE?, (CURVED W LEN?), 3_D, THREE_DIMENTIONAL, TRENCH+, WELL?); (OR DIFFERENT, DIFFERENTIAL, DIFFER+, VARIABL+, VARY+, DISSIMILAR+, DISTINCT+, VARIOUS, UNLIKE, DISPARAT+, DISTINCT+, DIVERS+); and similar terms		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/066965 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 5 June 2008 Refer to the whole document and in particular to abstract, pg. 60, lines 3+, Fig. 33-37, pg 67 lines 15+; pg. 68, lines 1- 26, pg. 31 lines 9-26, pg. 29 lines 29 +, claims 45-47 & 58	1-4, 9-21, 23-27
X	US 2003/0030184 A1 (KIM <i>et. al.</i>) 13 February 2003 Refer to the whole document and in particular to abstract, Figs. 1-10, claims 13, 14, 17, 19, para [0024], [0138], [0140], [0142], [0147], [0152], [0181], [0185], [0192], [0194]-[0195], [0274]-[0275]	1-13, 15-27
☒ Further documents are listed in the continuation of Box C ☒ 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 application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" Document member of the same patent family	
Date of the actual completion of the international search 06 May 2011		Date of mailing of the international search report 11 MAY 2011
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999		Authorized officer FIROOZEH RABBANI AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2287

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2011/000106

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0106607 A1 (YIN <i>et. al.</i>) 19 May 2005 Refer to whole document	
A	US 2004/0121066 A1 (ANDERSON <i>et. al.</i>) 24 June 2004 Refer to whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2011/000106

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

Patent Document Cited in Search Report				Patent Family Member			
WO	2008066965	US	2010303722				
US	2003030184	AU	43397/02	AU	2002327385	AU	2002365249
		AU	2003213834	AU	2003223722	AU	2003282950
		AU	2003287170	CA	2429056	CA	2484058
		CA	2503186	CA	2503203	EP	1339838
		EP	1502108	EP	1558076	EP	1578194
		US	6699665	US	2003032076	US	6803205
		US	2003022269	US	6811968	US	2003022153
		US	6818403	US	2003032048	US	6844184
		US	2003040033	US	6864065	US	2003040087
		US	6893851	US	2003022362	US	6921660
		US	2005100974	US	6967074	US	2004002131
		US	6982171	US	2005250097	US	7001740
		US	2003040031	US	7033819	US	2003036188
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		US	2002168757	US	7166459	US	7211209
		US	2003017582	US	7326563	US	2003068637
		US	7351575	US	2003022197	US	7364899
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		US	7374906	US	2004142408	US	7419823
		US	2003073228	US	7439056	US	2006172280
		WO	0248676	WO	03012392	WO	03012726
		WO	03062920	WO	03078565	WO	03091730
		WO	2004038367	WO	2004038368		
US	2005106607	NONE					
US	2004121066	AU	63481/01	EP	1283748	US	6686184
		US	7267938	WO	0189788		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							