A DEVICE FOR TOPOGRAPHY STUDY, AND ASSOCIATED TEMPLATE MOULDS AND METHODS

TECHNICAL FIELD

The present disclosure relates broadly to a device for topography study (e.g. studying/evaluating biomaterials, extracellular matrix and/or topography related cell behaviour such as cell migration behaviour), and associated template moulds and methods.

BACKGROUND

Hundreds of different factors play a role in the collective stimulation of cell receptors, which in turn determine a plethora of responses, including cell migration in the early embryo, coordinated organogenesis, and wound repair throughout adult life. Cell migration is a critical process in organism morphogenesis throughout embryonic development, tissue repair, immune response, and pathological processes such as vascular disease, osteoporosis, and cancer metastasis.

Most of our understanding of the mechano-chemical guidance cues associated with cell migration comes from studies in which the cell substratum is defined to be flat and featureless. However, *in vivo*, the native cell adhesion surfaces are topographically more complex, primarily owing to a large diversity of extracellular matrix (ECM) features spanning multiple scales of size and organization.

Attempts to mimic cell migration, for instance, have employed quasi simple micro and nanopatterned surfaces like lines, pillars and craters which are either coated with an ECM component such as fibronectin. The development of numerous strategies to analyse the cellular response to substrate topography has provided limited insights into cell migration as mimicking complex topographies is difficult. Despite over a decade of intense research addressing the interaction between cells and surface topography, topographical interface design to control

cell migration has not been extensively studied, owing to, among others, the limitations of fabrication processes.

The limited number of methods/tools available for studying the role of surface topographies on cell migration suffers from several shortcomings. For example, some tools can only accommodate up to 10-15 cells per unit. Thus, such tools are not suitable for a number of mechno-biological studies including cell migration over the long term.

Thus, there is a need to provide a device for topography study (e.g. studying/evaluating biomaterials, extracellular matrix and/or topography related/mediated cell behaviour such as cell migration behaviour), and associated moulds and methods that address or at least ameliorate one or more of the above-mentioned problems.

SUMMARY

In one aspect, there is provided a device for topography study, the device comprising: a designated cell reservoir area for allowing cells to be seeded thereon; a designated cell migratory area for allowing cells to migrate from the designated cell reservoir area; an inner barrier surrounding the designated cell reservoir area and separating the designated cell reservoir area from the designated cell migratory area; and a plurality of separators segregating the designated cell migratory area into a plurality of smaller test areas having different surface topologies.

In one embodiment, the plurality of separators abut the inner barrier.

In one embodiment, the plurality of test areas comprise one or more test areas defined by the plurality of separators extending away from the inner barrier in a diverging fashion.

In one embodiment, the surface topology of a test area is defined by the absence, presence and/or types of feature providing structures thereon.

In one embodiment, the feature providing structures have an average size in the range of from 10 nm to 50 μm.

In one embodiment, the feature providing structures are built from one or more shapes selected from the group consisting of circles, triangles, rectangles and combinations thereof.

In one embodiment, the feature providing structures have heights that are lower than the height of the inner barrier and the separators.

In one embodiment, the device further comprises an outer barrier surrounding the plurality of test areas and defining the end of the test areas.

In one embodiment, the plurality of separators abut the outer barrier.

In one embodiment, the designated cell reservoir area is located substantially in the centre of the device.

In one embodiment, the designated cell reservoir area is substantially circular in shape.

In one embodiment, the substantially circular cell reservoir area has a diameter in the range of from 0.1 cm to 5 cm.

In one embodiment, the device is substantially circular in shape.

In one embodiment, the inner barrier and the separators have substantially the same height.

In one embodiment, the inner barrier and the outer barrier have substantially the same height.

In one embodiment, the height is in the range of from 10 nm to 100 µm.

In one embodiment, the inner barrier and the outer barrier each comprises substantially circular walls that are substantially concentric.

In one embodiment, the plurality of separators radiate from the inner barrier to the outer barrier.

In one embodiment, the plurality of test areas are substantially identical in shape with one another.

In one embodiment, the device is formed from a material selected from the group consisting of: an elastomer, a ceramic, a thermoplastic polymer, a UV curable polymer, a hydrogel, a metal and composites and combinations thereof.

In one aspect, there is provided a template mould for making the device, the template mould comprising: a surface pattern which is a negative image of the designated cell reservoir area; a surface pattern which is a negative image of

the designated cell migratory area; a surface pattern which is a negative image of the inner barrier; a surface pattern which is a negative image of the plurality of separators; and a surface pattern which is a negative image of the different topologies in the plurality of test areas.

In one embodiment, the template mould further comprises a negative image of an outer barrier of the device, the outer barrier surrounding the plurality of test areas and defining the end of the test areas on the device.

In one aspect, there is provided a method of making the device, the method comprising: applying a flowable material over the template mould; curing the flowable material over the template mould to harden the material; and removing the hardened material from the template mould to obtain the device.

In one aspect, there is provided a method of evaluating topography mediated cell migration behaviour, the method comprising: providing the device; seeding cells on the designated cell reservoir area of the device; allowing the cells to migrate from the designated cell reservoir area to the different test areas; and inspecting the cells.

In one embodiment, the method further comprises fixing the cells that have migrated to the different test areas.

DEFINITIONS

The term "micro" as used herein is to be interpreted broadly to include dimensions from about 1 micron to about 1000 microns.

The term "nano" as used herein is to be interpreted broadly to include dimensions less than about 1000 nm.

The term “particle” as used herein broadly refers to a discrete entity or a discrete body. The particle described herein can include an organic, an inorganic or a biological particle. The particle used described herein may also be a macro-particle that is formed by an aggregate of a plurality of sub-particles or a fragment of a small object. The particle of the present disclosure may be spherical, substantially spherical, or non-spherical, such as irregularly shaped particles or ellipsoidally shaped particles. The term “size” when used to refer to the particle

broadly refers to the largest dimension of the particle. For example, when the particle is substantially spherical, the term “size” can refer to the diameter of the particle; or when the particle is substantially non-spherical, the term “size” can refer to the largest length of the particle.

The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

The term "associated with" used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

The term “abut” used herein when referring to two elements indicate one element being in direct contact with another element, or one element being almost in direct contact with another element.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

Further, in the description herein, the word “substantially” whenever used is understood to include, but not restricted to, "entirely" or “completely” and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when “comprising” is used, reference to a “one” feature is also intended to be a reference to “at least one” of that feature. Terms such as “consisting”, “consist”, and the like, may in the appropriate context, be considered as a subset of terms such as "comprising",

"comprise", and the like. Therefore, in embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as “consisting”, “consist”, and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the individual numerical values within the range also include integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a description of a range of 1% to 5% is intended to have specifically disclosed the ranges 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%, 1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.,) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required,

a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

Exemplary, non-limiting embodiments of a device for topography study and associated template moulds and methods are disclosed hereinafter.

Topographic extracellular matrix (ECM) is known to influence cell behaviour. For example, ECM cues have been shown to alter cell adhesion, cell shape, and cell migration, and activate signal transduction pathways to influence gene expression, proliferation, and differentiation. In various embodiments, there is provided a device allowing for the controlled analysis of biomaterials, ECM features and how different topographic patterns can affect cell behaviour such as cell adhesion, cell shape, cell migration, cellular signal transduction, gene expression, proliferation and differentiation.

In various embodiments, there is provided a device for topography study, the device comprising: a designated cell reservoir area (e.g. on a substrate surface) for allowing cells to be seeded thereon; a designated cell migratory area (e.g. on a substrate surface) for allowing cells to migrate from the designated cell reservoir area; an inner barrier surrounding the designated cell reservoir area and separating the designated cell reservoir area from the designated cell migratory area; and a plurality of separators segregating the designated cell migratory area into a plurality of smaller test areas having different surface topologies.

Topography study may include studying/evaluating topography related/mediated cell migration behaviour. Studying/evaluating topography related/mediated cell migration behaviour may include studying/evaluating one or

more of measures/phenotypes/hallmarks associated with cell motility and/or adhesion such as presence/absence of translocation/displacement/migration, speed/velocity of translocation/displacement/migration, directionality of translocation/displacement/migration, linearity of translocation/displacement/migration, cell morphology, cell shape, cell polarization and actin organization in cell.

The device may also be used for studying/evaluating other cell behaviours (other than cell migration), or cell behaviour in general. For example, the device may be used for studying/evaluating cell division, cell growth, differentiation, transdifferentiation, cell reprogramming, direct reprogramming, apoptosis and so on.

The device may be used for evaluating/studying the cell behaviour of any types of cell. In some embodiments, the cell being evaluated comprises a cell involved in one or more of embryonic development, tissue repair, immune response, and pathological processes such as vascular disease, osteoporosis, and cancer metastasis. Examples of such cells include, but are not limited to, embryonic stem cells, precursor cells, progenitor cells, fibroblasts, immune cells, leukocytes, phagocytes (e.g. macrophages, neutrophils and dendritic cells), granulocytes, innate lymphoid cells, mast cells, eosinophils, basophils, natural killer cells, lymphocytes (e.g. T cells and B cells), endothelial cells or progenitors, osteoclasts, osteoblasts, cancer cells and the like. The device may also be used for evaluating/studying the behaviour of pathogens including, but not limited to, bacteria and fungi.

In some embodiments, the device may also be used for evaluating/studying biomaterials e.g. the features, properties, functions and/or behaviour associated with biomaterials. In some embodiments, the device may also be used for evaluating/studying extracellular matrix e.g. the features, properties, functions and/or behaviour associated with extracellular matrix.

In various embodiments, the device is substantially circular in shape. In various embodiments, the designated cell reservoir area is located substantially in the centre of the device. In various embodiments, the designated cell reservoir area is substantially circular in shape. In various embodiments, the designated

cell migratory area extends radially from the designated cell reservoir area and are separated radially into a plurality of smaller test areas (e.g. fan-shaped test areas) having different surface topologies. Advantageously, embodiments of the device have an efficient layout that minimises the footprint size while maximising the cell reservoir area and test areas.

In various embodiments, the device has a radius of from about 0.1 cm to about 5 cm, from about 0.5 cm to about 3 cm or from about 1 cm to about 2 cm. In one embodiment, the device has a radius of about 1.5 cm. The device may be scaled up or scaled down and hence, the radius may vary.

In various embodiments, the cell reservoir area is a defined area for loading/seeding/holding cells. Cells may be seeded/loaded to the full capacity in the cell reservoir area or they be seeded/loaded to partial capacity and then allowed to multiply to reach confluency. Cells may start migrating to the adjacent cell migratory area after reaching confluency in the cell reservoir area. The diameter of the substantially circular cell reservoir area may be in the range of from about 0.1 cm to about 5 cm, from about 0.1 cm to about 3 cm or from about 0.1 cm to about 1 cm. In one embodiment, the diameter is about 0.5 cm. The radius may be varied to vary the holding capacity of the cell reservoir area. In various embodiments, the designated cell reservoir area is surrounded by an inner barrier that separates the designated cell reservoir area from the designated cell migratory area. In various embodiments, the inner barrier substantially prevents the migration of cells until the cell reservoir area is confluent with cells.

In various embodiments, the cell migratory area is a defined area comprising a plurality of test areas having different surface topologies for evaluating topography mediated cell behaviour. The plurality of test areas may be segregated from each other by a plurality of separators. The plurality of separators may abut the inner barrier. In various embodiments, the plurality of test areas comprise one or more fan-shaped test areas defined by the plurality of separators extending away from the inner barrier in a diverging fashion. In various embodiments, the cell migratory area comprises at least about 10, at least about 50, at least about 100, at least about 150, at least about 200, at least about 250 or at least about 300 test areas. In various embodiments, the cell migratory area

comprises from about 100 to about 300 test areas. In one embodiment, the cell migratory area comprises about 160 test areas. Advantageously, embodiments of the device comprising a large number of test areas allow for a large number of different topographies to be screened/evaluated on how they influence/mediate cell behaviour in one batch. Advantageously, embodiments of the device allow for high throughput screening/evaluation on how different topographies influence/mediate cell behaviour. The plurality of the test areas may be substantially identical in size, or they may be non-identical in size. For example, each of the test areas may be substantially of the same size (e.g. the length of the inner and outer arcs defining each of test areas may be substantially the same). For example, one or more of the test areas may be of different sizes (e.g. the length of the inner and outer arcs defining one or more of the test areas may be different). In one embodiment, the test area is about 4×10^6 μm^2. In various embodiments, the plurality of test areas are substantially identical in shape with one another. It will be appreciated that the number of test areas can be varied. It will also be appreciated that the device may also be scaled up or down to accommodate a greater number or a smaller number of test areas. Accordingly, the size of the test area may also vary.

In various embodiments, the surface topology of a test area is defined by the absence, presence and types of feature providing structures thereon. For example, a test area may be devoid of any feature providing structures; the test area may be substantially flat and/or featureless. For example, a test area may comprise one or more feature providing structures; the test area is not flat and featureless. A feature providing structure may provide a micrometer or submicrometer scale feature such as a protrusion extending out of the surface of the test area or a pit extending into the surface. In various embodiments, the average size or height of the feature providing structure is in the range of from about 1 nm to about 1000 µm, from about 1 nm to about 1000 nm or from about 1 µm to about 1000 µm. In various embodiments, the feature providing structure is from about 1 µm to about 50 µm, from about 3 µm to about 70 µm or from about 5 µm to about 20 µm. In some embodiments, the feature providing structures have an average size or height in the range of from about 1 µm to about 50 µm,

from about 3 µm to about 70 µm, from about 5 µm to about 20 µm or from about 5 µm to about 15 µm. In some embodiments, the feature providing structures have an average size or height in the range of from about 10 nm to about 100 µm, from about 10 nm to about 50 µm or from about 10 nm to about 10 µm. In some embodiments, the feature providing structures have an average size or height of about 1 nm, about 10 nm or about 100 nm. In some embodiments, the feature providing structures have an average size or height of about 1 µm, about 10 µm or about 100 µm.

In some embodiments, the feature providing structure may provide a nanometer scale feature where suitable. For example, where the device is used for evaluating/studying extracellular matrix or a pathogen such as a bacteria cell or a fungi cell and/or the like, the feature providing structure may provide a nanometer scale feature. In such applications, the average size or height of the feature providing structure may be in the range of from about 1 nm to about 1000 nm, from about 1 nm to about 500 nm, from about 1 nm to about 100 nm, from about 1 nm to about 10 nm, from about 10 nm to about 100 nm or from about 100 nm to about 1000 nm,

A feature providing structure may be built from any two- or three-dimensional geometric shapes including but not limited to ellipses, circles, ovals, polygons, triangles, rectangles, squares, rhombuses, hexagons, trapezoids, stars, parallelograms, spheres, hemispheres, ellipsoids, cylinders, cubes, cuboids, cones, truncated cones, pyramids (e.g. triangular pyramids, square pyramids, polygonal pyramids, right pyramids, oblique pyramids etc.), truncated pyramids and the like and combinations thereof. In some embodiments, the feature providing structures are built from one or more shapes, optionally microscale and/or nanoscale shapes, selected from the group consisting of circles, triangles, rectangles and combinations thereof. Advantageously, a wide array of different topographic features/patterns may be achieved by just combining these three primitive shapes. For example, circles can create large smooth areas, triangles can generate angles, and rectangles can result in stretched elements. In various embodiments, a feature providing structure is built from one or more shapes providing a smooth edge/area, an angle, a corner

and/or a stretched element/edge/area. In various embodiments, the triangles comprise isosceles triangles. In some embodiments, the isosceles triangles comprise one angle that is about 20° to about 50° and two other angles that are about 65° to about 80° each. In some embodiments, the isosceles triangles comprise one angle that is about 30° to about 40° and two other angles that are about 70° to about 75° each. In one embodiment, the isosceles triangles comprise one angle of about 36° and two angles of about 75°. In various embodiments, the rectangles comprise thin rectangles. In various embodiments, the rectangles/thin rectangles have a width of from about 1 μm to about 5 μm. In one embodiment, the rectangles/thin rectangles have a width of about 3 μm. In various embodiments, the rectangles/thin rectangles have a length of from about 1 nm to about 100 μm, from about 10 nm to about 100 μm, from about 10 nm to about 10 μm, from about 10 nm to about 1 μm, from about 1 nm to about 100 nm, from about 10 nm to about 100 nm, from 1 μm to about 100 μm, from about 1 μm to about 20 μm, from about 1 μm to about 15 μm, from about 1 μm to about 10 μm, from about 3 μm to about 20 μm, from about 3 μm to about 15 μm, from about 3 μm to about 10 μm, from about 5 μm to about 20 μm, from about 5 μm to about 15 μm, from about 5 μm to about 10 μm, from about 3 μm to about 8 μm or from about 5 μm to about 8 μm. In some embodiments, the rectangles/thin rectangles have a length of from about 5.4 μm to about 7.9 μm, optionally from about 5.42 μm to about 7.89 μm. In various embodiments, the rectangles/thin rectangles have a length of at least about 3 μm, at least about 3.5 μm, at least about 4 μm, at least about 4.5 μm or at least about 5 μm. In various embodiments, the rectangles/thin rectangles have a length of no more than about 10 μm, no more than about 9.5 μm, no more than about 9 μm, no more than about 8.5 μm or no more than about 8 μm.

In various embodiments, the feature providing structure is built from at least about one triangle, at least about two triangles, at least about three triangles, at least about four triangles, at least about five triangles, at least about six triangles, at least about seven triangles, at least about eight triangles, at least about nine triangles or at least about ten triangles. In various embodiments, the feature providing structures comprise at least about one triangle/angular

projection, at least about two triangles/angular projections, at least about three triangles/angular projections, at least about four triangles/angular projections, at least about five triangles/angular projections, at least about six triangles/angular projections, at least about seven triangles/angular projections, at least about eight triangles/angular projections, at least about nine triangles/angular projections or at least about ten triangles/angular projections.

A test area may comprise one type of feature providing structure or two or more different types of feature providing structures (e.g. two or more different types of feature providing structures built from a different combination of shapes or two or more different types of feature providing structures built from the same combinations of shapes in different arrangements). Each of the plurality of test areas may have a distinct topography profile. For example, each of the plurality of test areas may comprise different feature providing structures. For example, where a test area comprises more than one type of feature providing structures, each of the plurality of test areas may comprise different feature providing structures or different combinations/permutations/arrangements of the two or more feature providing structures. In some embodiments, each of the plurality of test areas comprises a single type of feature providing structure. In some embodiments, each of the plurality of test areas comprises a different type of feature providing structure. In various embodiments, the feature providing structures are regularly distributed over the test area. The distance/spacing between the feature providing structures may vary. In various embodiments, from about 1 nm to about 100 μm, from about 10 nm to about 100 μm, from about 10 nm to about 10 μm, from about 10 nm to about 1 μm, from about 1 nm to about 100 nm, from about 10 nm to about 100 nm, from 1 μm to about 100 μm, from about 1 μm to about 20 μm or from about 1 μm to about 10 μm. In various embodiments, the distance/spacing between two feature providing structures (i.e. the inter-feature distance) is at least about 10 nm, at least about 20 nm, at least about 30 nm, at least about 40 nm, at least about 50 nm, at least about 60 nm, at least about 70 nm, at least about 80 nm, at least about 90 nm or at least about 100 nm. In various embodiments, the distance/spacing between two feature providing structures (i.e. the inter-feature distance) is at least about 1 μm, at least

about 2 μm, at least about 3 μm, at least about 4 μm, at least about 5 μm, at least about 6 μm, at least about 7 μm, at least about 8 μm, at least about 9 μm or at least about 10 μm. In various embodiments, the distance/spacing between two feature providing structures (i.e. the inter-feature distance) is more than about 5 μm, more than about 5.5 μm, more than about 6 μm, more than about 6.5 μm, more than about 7 μm or more than about 7.5 μm. In one embodiment, the distance/spacing between two feature providing structures (i.e. the inter-feature distance) is more than about 7.57 μm.

The one or more type of feature providing structures in a test area may mimic a surface architecture observed in nature e.g. a ECM architecture or they may provide a surface architecture that is artificial. For example, the one or more type of feature providing structures in a test area may mimic the interlinking of collagen fibrils and fibres in ECM.

In various embodiments, the one or more type of feature providing structures in a test area may mimic/produce similar/identical function and/or structure associated with a surface architecture observed in nature e.g. a ECM architecture. In some embodiments, the one or more type of feature providing structures in a test area is structurally biomimetic. In some embodiments, the one or more type of feature providing structures in a test area is functionally biomimetic. In some embodiments, the one or more type of feature providing structures in a test area is both structurally biomimetic and functionally biomimetic.

In various embodiments, the test area is devoid of or lacks ECM molecules contributing to a surface architecture.

In various embodiments, one or more of the plurality of test areas have an identical topography profile i.e. one or more of the plurality of test areas comprise the same feature providing structure(s) with the same inter-feature distance. In various embodiments, one or more of the plurality of test areas have different/non-identical topography profiles i.e. one or more of the plurality of test areas comprise different feature providing structure(s) and/or different inter-feature distance. In some embodiments, each or all of the plurality of test areas have different/non-identical topography profiles. In various embodiments, one or

more of the plurality areas have different/non-identical topography profiles that induce or promote different rates of cell migration. In various embodiments, each or all of the plurality areas have different/non-identical topography profiles that induce or promote different rates of cell migration.

In various embodiments, at least one of the plurality of test areas comprise a feature providing structure built from at least a rectangle/thin rectangle having a designated length. In various embodiments, the designated length of the rectangle/thin rectangle is from about 1 nm to about 100 μm, from about 10 nm to about 100 μm, from about 10 nm to about 10 μm, from about 10 nm to about 1 μm, from about 1 nm to about 100 nm, from about 10 nm to about 100 nm, from 1 μm to about 100 μm, from about 1 μm to about 20 μm, from about 1 μm to about 15 μm, from about 1 μm to about 10 μm, from about 3 μm to about 20 μm, from about 3 μm to about 15 μm, from about 3 μm to about 10 μm, from about 5 μm to about 20 μm, from about 5 μm to about 15 μm, from about 5 μm to about 10 μm, from about 3 μm to about 8 μm or from about 5 μm to about 8 μm. In some embodiments, the designated length of the rectangle/thin rectangle is from about 5.4 μm to about 7.9 μm, optionally from about 5.42 μm to about 7.89 μm. In various embodiments, the designated length of the rectangle/thin rectangle is at least about 3 μm, at least about 3.5 μm, at least about 4 μm, at least about 4.5 μm or at least about 5 μm. In various embodiments, the designated length of the rectangle/thin rectangle is no more than about 10 μm, no more than about 9.5 μm, no more than about 9 μm, no more than about 8.5 μm or no more than about 8 μm.

In various embodiments, at least one of the plurality of test areas comprise a feature providing structure built from at least a designated number of triangles and/or angular projections. In various embodiments, the designated number of triangles and/or angular projections is at least about one triangle/angular projection, at least about two triangles/angular projections, at least about three triangles/angular projections, at least about four triangles/angular projections, at least about five triangles/angular projections, at least about six triangles/angular projections, at least about seven triangles/angular projections, at least about

eight triangles/angular projections, at least about nine triangles/angular projections or at least about ten triangles/angular projections.

In various embodiments, at least one of the plurality of test areas comprise a designated inter-feature distance. The designated inter-feature distance is from about 1 nm to about 100 μm, from about 10 nm to about 100 μm, from about 10 nm to about 10 μm, from about 10 nm to about 1 μm, from about 1 nm to about 100 nm, from about 10 nm to about 100 nm, from 1 μm to about 100 μm, from about 1 μm to about 20 μm or from about 1 μm to about 10 μm or at least about 10 nm, at least about 20 nm, at least about 30 nm, at least about 40 nm, at least about 50 nm, at least about 60 nm, at least about 70 nm, at least about 80 nm, at least about 90 nm, at least about 100 nm, at least about 1 μm, at least about 2 μm, at least about 3 μm, at least about 4 μm, at least about 5 μm, at least about 6 μm, at least about 7 μm, at least about 8 μm, at least about 9 μm or at least about 10 μm. In various embodiments, the designated inter-feature distance is more than about 5 μm, more than about 5.5 μm, more than about 6 μm, more than about 6.5 μm, more than about 7 μm or more than about 7.5 μm. In one embodiment, the designated inter-feature distance is more than about 7.57 μm.

In various embodiments, the feature providing structures have heights that are lower than the height of the inner barrier and/or the separators. The height of the inner barrier may be substantially greater than the heights of the feature providing structures such that cells in the test areas are substantially prevented from translocating back to the cell reservoir area after crossing the inner barrier area to reach the test area. The heights of the separators may be substantially greater than the heights of the feature providing structures such that cells in the test areas are substantially prevented from crossing/translocating across the separators defining a boundary of each test area. In other words, cells in a designated test area are substantially prevented from translocating to another test area.

In various embodiments, the device further comprises an outer barrier surrounding the plurality of test areas and defining the boundary/end of the test areas. In various embodiments, cells may not cross/translocate across the outer barrier. In various embodiments, the feature providing structures have heights

that are lower than the height of the outer barrier. The height of the outer barrier may be substantially greater than the heights of the feature providing structures such that cells in the test areas are substantially prevented from crossing/translocating across the outer barriers defining a boundary/end of the test area. In other words, cells in a test area are substantially prevented from leaving the test area or the device.

In various embodiments, the plurality of separators abut the outer barrier. In various embodiments, the plurality of separators radiate from the inner barrier to the outer barrier. In various embodiments, the inner barrier and the outer barrier each comprises walls that at least have a portion that is curved or shaped to resemble at least part of the arc of a circle (e.g. when viewed from the top). The curvature of the walls may be such that the concave portion of the inner barrier faces the interior of the designated cell seeding area and/or the concave portion of the outer barrier faces the cell migratory area. In various embodiments, the inner barrier and the outer barrier each comprises substantially circular walls that are substantially concentric.

In various embodiments, the inner barrier, the separators and/or the outer barrier define the boundary of a designated test area. In various embodiments, the heights of the inner barrier, the separators and/or the outer barrier are substantially greater than the heights of the feature providing structures such that cells in a designated test area are substantially prevented from leaving a designated test area.

In various embodiments, the inner barrier, the separators and/or the outer barrier have substantially the same height. In various embodiments, the height is in the range of from about 1 nm to about 1000 µm, from about 10 nm to about 100 µm, from about 1 µm to about 100 µm, from about 10 µm to about 100 µm, from about 20 µm to about 80 µm, from about 30 µm to about 70 µm or from about 40 µm to about 60 µm. In various embodiments, the height is in the range of from about 1 nm to about 1000 nm, from about 1 nm to about 100 nm or from about 10 nm to about 100 nm. In various embodiments, the height is about 1 nm, about 5 nm, about 10 nm, about 50 nm or about 100 nm. In various embodiments, the height is about 1 µm, about 5 µm, about 10 µm, about 50 µm or about 100

μm. In various embodiments, the inner barrier, the separators and/or the outer barrier have substantially the same width/thickness. In various embodiments, the width/thickness of each of the inner barrier, the separators and/or the outer barrier is from about 5 μm to about 20 μm or from about 5 μm to about 15 μm. In one embodiment, the width/thickness is about 10 μm. In various embodiments, the width/thickness of each of the inner barrier, the separators and/or the outer barrier is from about 1 nm to about 100 nm or from about 10 nm to about 100 nm. In various embodiments, the width/thickness is about 1 nm, about 10 nm or about 100 nm. In various embodiments, the width/thickness of each of the inner barrier, the separators and/or the outer barrier is from about 1 nm to about 100 μm, from about 10 nm to about 100 μm, from about 10 nm to about 10 μm, from about 10 nm to about 1 μm or from about 100 nm to about 1 μm.

In various embodiments, the device is formed from a material that is non-toxic, biocompatible, has good tensile strength, has elastic properties and/or is compatible with photolithography (e.g. soft lithography) and/or microfabrication production techniques. In various embodiments, the device is formed from a biomaterial/ biopolymer. In various embodiments, the device is formed from a thermoresponsive polymer, a UV responsive polymer, a UV curable polymer and/or an elastomer. In various embodiments, the device is formed from a gel. In various embodiments, the device is formed from a metal. In various embodiments, the device is formed from a ceramic. Examples of polymers include, but are not limited to, poly(dimethylsiloxane), methylchlorosilanes, ethylchlorosilanes, phenylchlorosilane, polyethylene, polycarbonate, polystyrene, polybutadiene, polyurethane, polyisoprene, polyacrylic rubber, fluorosilicone rubber, polyimide, Novolac polymers, fluoroelastomers, block copolymer thermoplastic elastomers, polyolefin, perfluoropolyethers, polylactide (PLA), poly-L-lactide (PLLA), poly-D-lactide (PDLA), the like and combinations thereof. Examples of metals include, but are not limited to, gold, silver, copper, cadmium, zinc, palladium, platinum, mercury, lead, iron, chromium, manganese, tungsten, alloys thereof, the like and combinations thereof. Examples of gels include, but are not limited to, hydrogel, Matrigel, collagen (e.g. collagen I), the like and combinations thereof. Examples of ceramic materials include, but are not

limited to, zirconia, alumina, titania, silica, magnesia, yttria, ceria and mixtures thereof. In various embodiments, the device is formed from a material selected from the group consisting of: an elastomer, a ceramic, a thermoplastic polymer, a UV curable polymer, a hydrogel, a metal and composites and combinations thereof. In some embodiments, the perfluoropolyether's device is formed from a silicone elastomer. In some embodiments, the silicone elastomer comprises poly(dimethylsiloxane) (PDMS). The materials can be further processed e.g. to modify its properties including surface properties. In some examples, the materials can be further processed to deposit a layer of titanium, ceramic and/or the like thereon.

In various embodiments, there is provided a method of evaluating topography related/mediated cell behaviour (e.g. cell migration behaviour), the method comprising: providing the device, seeding/loading cells into the designated cell reservoir area of the device optionally until confluency, allowing the cells to migrate from the designated cell reservoir area to the different test areas and inspecting the cells. Inspecting the cells may comprise visually inspecting the cells and/or inspecting the cells through the use of an instrumentation such as a microscope or a camera and/or the use of a staining agent such as an actin stain. In some embodiments, inspecting the cells comprises performing live cell imaging and/or time lapse imaging of the cells. In some embodiments, inspecting the cells comprises fixing the cells and optionally examining their morphology with or without staining the actin cytoskeletons. In some embodiments, inspecting the cells comprises imaging the cells and analysing the images with an image analysis software. An example of an image analysis software is CellProfiler. It will be appreciated that other suitable image analysis software may also be used. Inspecting the cells may comprise examining one or more of measures/phenotypes/hallmarks associated with cell motility and/or adhesion such as presence/absence of translocation/displacement/migration, speed/velocity of translocation/displacement/migration, directionality of translocation/displacement/migration, linearity of translocation/displacement/migration, cell morphology, cell shape, cell

polarization and actin organization in cell. Inspecting the cells may also comprise analysing other cell behaviours (other than cell migration of moving cells), or cell behaviour in general. For example, inspecting the cells may also comprise analysing cell division, cell growth, differentiation, transdifferentiation, cell reprogramming, direct reprogramming, apoptosis and so on (e.g. through one or more of measures/phenotypes/hallmarks associated with such processes).

In various embodiments, the method further comprises identifying one or more topographies (or hit topographies) on which the cells exhibit particularly high and/or low migration. For example, the method may comprise identifying the topography on which the cells exhibit the highest migration and the topography on which the cells exhibit the lower migration. In various embodiments, the method further comprises harvesting/collecting/isolating the cells from the hit topographies and determining/evaluating/comparing their gene expression profiles (e.g. through transcriptomic and/or genomic analysis) to determine the effect of topology on the behaviour of cells e.g. the speed/velocity of migration.

In various embodiments, the method further comprises identifying one or more topographies (or hit topographies) on which the cells exhibit linear migration and one or more topographies (or hit topographies) on which the cells exhibit non-linear migration. In various embodiments, the method further comprises harvesting/collecting/isolating the cells from the hit topographies and determining/evaluating/comparing their gene expression profiles (e.g. through transcriptomic and/or genomic analysis) to determine the effect of topology on the behaviour of cells e.g. the linearity of migration.

In various embodiments, there is provided a template mould for making the device. In various embodiments, the template mould comprises a surface pattern which is a negative image of the designated cell reservoir area; a surface pattern which is a negative image of the designated cell migratory area; a surface pattern which is a negative image of the inner barrier; a surface pattern which is a negative image of the plurality of separators; and a surface pattern which is a negative image of the different topologies in the plurality of test areas. In various embodiments, the template mould further comprises surface pattern which is a negative image of an outer barrier of the device, the outer barrier surrounding the

plurality of test areas and defining the end of the test areas on the device. The template mould may be made using a photolithography process. For example, a template mould may be made using the following steps. A negative photoresist is applied/coated e.g. spin coated on a substrate (e.g. bare silicon substrate) with a layer of mask film. The substrate is then heated (e.g. soft baked) to drive off solvents and to solidify the photoresist film. One or more photomask comprising a pattern corresponding to the designated cell reservoir area, the designated cell migratory area, the inner barrier, the plurality of separators, the different topologies in the plurality of test areas and/or the outer barrier is aligned with the substrate. The substrate is then exposed to ultraviolet (UV) radiation. During UV radiation, parts of the negative photoresist that are not covered by the photomask and therefore exposed to the UV radiation undergo a chemical change that makes them insoluble to a photoresist developer. The substrate is then subjected to a post exposure heating (e.g. bake) to stabilise the photoresist patterns and then bathed in a photoresist developer solution to remove the unexposed photoresist. Etching is performed and the photoresist is stripped away to obtain a substrate (e.g. a silicon) comprising a surface pattern which is a negative image of the designated cell reservoir area, a surface pattern which is a negative image of the designated cell migratory area, a surface pattern which is a negative image of the inner barrier, a surface pattern which is a negative image of the plurality of separators, a surface pattern which is a negative image of the different topologies in the plurality of test areas and/or a surface pattern which is a negative image of an outer barrier of the device. It will be appreciated that a positive photoresist may also be used in place of a negative photoresist to make the template mould. In various embodiments, the template mould is made of silicon. It will also be appreciated that besides a silicon substrate, other suitable substrates may also be used. It is also possible to modify, add or remove one or more processing steps described for the making of the template mould. Other suitable methods such as additive manufacturing methods (e.g. 3D printing techniques including but not limited to stereolithography selective laser sintering, fused deposition modeling, digital light process, multi jet fusion, polyJet, direct metal laser sintering, electron beam melting or the like), micromachining, thermoforming and

the like may also be employed in making the template mould. Depending on the specific materials used (e.g. the substrate, the photoresist etc.), the processing parameters and conditions may vary. The selection of suitable processing parameters and conditions is within the purview of a person skilled in the art.

In various embodiments, there is provided a method of making the device, the method comprising: applying a flowable material over the template mould, curing the flowable material over the template mould to harden the material; and removing the hardened material from the template mould to obtain the device. In various embodiments, the flowable material is non-toxic, biocompatible, has good tensile strength, has elastic properties and/or is compatible with photolithography (e.g. soft lithography) and/or microfabrication production techniques. In various embodiments, the flowable material comprises a biomaterial/biopolymer. In various embodiments, the flowable material comprises a thermoresponsive polymer, a UV responsive polymer, a UV curable polymer and/or an elastomer. In various embodiments, the flowable material comprises a gel. In various embodiments, the flowable material comprises a ceramic. In various embodiments, the flowable material comprises an elastomer. Examples of flowable materials include, but are not limited to, poly(dimethylsiloxane), methylchlorosilanes, ethylchlorosilanes, phenylchlorosilane, polyethylene, polycarbonate, polystyrene, polybutadiene, polyurethane, polyisoprene, polyacrylic rubber, fluorosilicone rubber, polyimide, Novolac polymers, fluoroelastomers, block copolymer thermoplastic elastomers, polyolefin, perfluoropolyethers, polylactide (PLA), poly-L-lactide (PLLA), poly-D-lactide (PDLA), hydrogel, Matrigel, collagen (e.g. collagen I), zirconia, alumina, titania, silica, magnesia, yttria, ceria, the like and mixtures, composites and combinations thereof. In various embodiments, the flowable material is selected from the group consisting of: an elastomer, a ceramic a thermoplastic polymer, a UV curable polymer, a hydrogel and composites and combinations thereof. In various embodiments, the flowable material comprises a silicone elastomer. In various embodiments, the flowable material comprises poly(dimethylsiloxane) (PDMS). Depending on the specific flowable material employed, the processing parameters and conditions (e.g. curing temperature, curing time, concentration

of the flowable material etc.) may vary. The selection of suitable processing parameters is within the purview of a person skilled in the art. The method may further comprise one or more material processing steps. For example, in some embodiments, the method further comprises depositing a layer of titanium, ceramic or the like on the hardened material before removing the hardened material from the template mould to obtain the device

In various embodiments, the material or flowable material for making the device comprises a material that is suitable for cell culture ie. a material that is suitable as a cell culture substrate. Examples of such materials include, but are not limited to, polylactide (PLA), poly-L-lactide (PLLA), poly-D-lactide (PDLA), hydrogel, Matrigel, collagen (e.g. collagen I), the like and combinations thereof. In some embodiments therefore, cells may be seeded and/or cultured on the test areas with topographies for topography study. In some embodiments, there is provided a cell culture substrate in association with the device. In some embodiments, there is provided a cell culture substrate in the form of the device or comprising one or more features of the device. In various embodiments, the device is suitable for culturing, maintaining, proliferating and/or growing cells.

In various embodiments, the method has high reproducibility. In various embodiments, the devices produced by the method are similar. In various embodiments, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 5% ± 0.3%, no more than about 4% ± 0.3%, no more than about 3% ± 0.3%, no more than about 2% ± 0.3%or no more than about 1% ± 0.3%. In one embodiment, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 1.2% ± 0.3%. In various embodiments, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 0.5% ± 0.05%, no more than about 0.4% ± 0.05%, no more than about 0.3% ± 0.05%, no more than about 0.2% ± 0.05% or no more than about 0.1% ± 0.05%. In one embodiment, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 0.24%

± 0.05%. In various embodiments, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 200 nm ± 30 nm, no more than about 180 nm ± 30 nm, no more than about 150 nm ± 30 nm, or no more than about perfluoropolyether's 120 nm ± 30 nm. In one embodiment, the average difference in the height profile measurements of the feature providing structures of the devices produced by the method is not more than about 120 nm ± 22 nm.

In various embodiments, there is provided a product or a method as described herein.

BRIEF DESCRIPTION OF FIGURES

FIG. 1 is a top-down view of a device (named cellompics chip) for evaluating topography related cell behaviour in accordance with an embodiment disclosed herein.

FIGs. 2A-2C show schematic illustrations of the current challenges in understanding the role of topographies in dictating cellular behavior, the cellompics chip screening technology in accordance with an embodiment disclosed herein and a method of making the cellompics chip in accordance with an embodiment disclosed herein.

FIGs. 3A-3C show the properties of the cellompics chip and cells disposed on the cellompics chip in accordance with embodiments disclosed herein.

FIGs. 4A-4H show analysis of the distance travelled by cells on different topographies in the cellompics chip in accordance with an embodiment disclosed herein.

FIGs. 5A-5F show analysis of the linearity of migrating cells on different topographies in the cellompics chip in accordance with an embodiment disclosed herein.

FIG. 6 illustrates four exemplary topographic features in the cellompics chip in accordance with an embodiment disclosed herein.

FIG. 7 is a heatmap showing some of the differentially expressed genes between cells showing a high speed of migration and cells showing a low speed

of migration in the cellompics chip in accordance with an embodiment disclosed herein.

FIG.8 is a heatmap showing a subset of differentially expressed genes between cells showing a high speed of migration and cells showing a low speed of migration identified from network analysis in the cellompics chip in accordance with an embodiment disclosed herein.

EXAMPLES

Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications related to structural, chemical and biological changes may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments. The example embodiments should not be construed as limiting the scope of the disclosure.

The following examples pertain to an exemplary embodiment of presently disclosed device that is based on what the inventors term as a Cellompics chip technology with algorithmically designed complex surface topographies arranged in radial race track fashion. This exemplary device provides a convenient method/tool to investigate the role of surface topographies on cell migration. The fabrication of the Cellompic chip represents a breakthrough in micro, nanotechnologies and materiomics. In one example, the Cellompic chip has elucidated the role of topographic design factors on fibroblast migration. The disclosure has also helped unravel an interesting array of material surface properties for modulating cell growth and differentiation. The disclosure also systematically dissects the role of topographic design parameters and its effect on the genetic repertoire in response to surface topography mediated migration.

FIG. 1 is a top-down view of a Cellompic chip device for topography study in accordance with an embodiment disclosed herein. The device 100 is circular in shape and comprises a circular cell reservoir area 102 located in the centre of

the device 100, a cell migratory area 104, an inner barrier 106 surrounding the cell reservoir area 102 and separating the cell reservoir area 102 from the cell migratory area 104, a plurality of separators 108 extending away from the inner barrier 106 in a diverging fashion and segregating the cell migratory area 104 into a plurality of smaller test areas 110A, 110B and 110C having different surface topologies and an outer barrier 112 surrounding the plurality of test areas 110 and defining the end of the test areas. The test areas 110 comprise feature providing structures 114 giving rise to/defining the surface topologies of the test areas 110. The plurality of separators 108 radiate from the inner barrier 106 to the outer barrier 112, the inner barrier 106 and outer barrier 112 being concentric. The device can be scaled up or down to accommodate a larger or smaller number of test areas. In use, cells are seeded on the circular cell reservoir area 102 of the device 100 and allowed to migrate to the different test areas 110 comprising the different topographies (e.g. 110A, 110B and 110C).

FIGs. 2A-2C show schematic illustrations of the current challenges in understanding the role of topographies in dictating cellular behavior, the cellompics chip screening technology in accordance with an embodiment disclosed herein and a method of making the cellompics chip in accordance with an embodiment disclosed herein.

FIG. 2A illustrates the current challenges in understanding the role of topographies in dictating cellular behavior. Numerous strategies that have been developed to analyse cellular response to substrate topography, including employing quasi simple micro and nanopatterned surfaces like lines, pillars and craters and coating with ECM component(s), have provided only limited insights into cell migration as mimicking complex topographies is difficult and the strategies are low-throughput.

FIG. 2B illustrates a cellompics chip screening technology in accordance with an embodiment as disclosed herein. A cellompics chip in accordance with an embodiment disclosed herein can comprise as many as 160 different topographies (can be potentially scaled up or down to accommodate a larger or smaller number of different topographies) in designated test areas for high content and high throughput screening of cellular response, such as cell

migration, to different topographies. In one embodiment of the cellompics chip screening technology, cells are seeded on the designated cell reservoir area of the chip and allowed to migrate to the different test areas comprising the different topographies. The cells are inspected and the topographies that are associated with a higher speed/velocity of migration and the topographies that are associated with a lower speed/velocity of migration are identified. The cells on these topographies are then subjected to transcriptomic and genomic analysis using microarray to elucidate how topologies affect the mechanobiology of cells.

FIG. 2C illustrates a microfabrication process of the chip involving Si wafer fabrication using photolithography and subsequent PDMS soft lithography in accordance with an embodiment disclosed herein. In Step I, a layer of masking film is applied to a bare silicon substrate. A negative photoresist is then spin coated on the substrate in Step II to form a layer of photoresist film. The substrate can be soft baked in Step I and/or Step II to enhance adhesion of the layers, drive off solvents and/or to solidify the films. In Step III, one or more photomask comprising a pattern corresponding to the features of the chip (e.g. one or more photomasks making up a pattern corresponding to a cell reservoir area, a cell migratory area, an inner barrier, a plurality of separators, the different topologies in the plurality of test areas and/or an outer barrier) is aligned with the substrate. The substrate is then exposed to ultraviolet (UV) radiation in Step III. During UV radiation, parts of the negative photoresist that are not covered by the photomask and therefore exposed to the UV radiation undergo a chemical change that makes them insoluble to a photoresist developer. The substrate is then subjected to post-exposure bake and then bathed in a photoresist developer solution to remove the unexposed photoresist to obtain a silicon substrate with developed photoresist in Step IV. Etching is performed and the photoresist is stripped away to obtain a patterned silicon substrate comprising a surface pattern which is a negative image of the features of the chip in Step V. The patterned silicon substrate can then be used as a template mould for making the chip. PDMS is poured onto the substrate and cured in Step VI and the cured PDMS is then peeled from the substrate to obtain a PDMS replicate of the chip in the final step VII.

FIGs. 3A-3C show the properties of the cellompics chip and cells disposed on the cellompics chip in accordance with embodiments disclosed herein. FIG. 3A shows scanning electron micrographs of cellompics chip in PDMS showing efficient lateral replication of micro topographies (scale bar: 100 μm). FIG. 3B shows the height profiles of topographies measured using confocal laser scanning microscopy. FIG. 3C shows the morphology of human foreskin fibroblasts (HFF) on different topographies in cellompics chip. Fluorescent microscopic images of HFF show different alignment on topographic features (actin stained with Alexa Fluor 488 phalloidin; nucleus stained with DAPI; scale bar: 100 μm).

FIGs. 4A-4H show analysis of the distance travelled by cells on different topographies in the cellompics chip in accordance with an embodiment disclosed herein. FIG. 4A is a heat map showing integrated distance travelled by cells on each topography. FIG. 4B and FIG. 4C are receiver operating characteristic (ROC) curves obtained by correlating the integrated distance travelled with topographical design parameters without feature selection (FIG 4B) and with feature selection (FIG. 4C). FIG. 4D is an SEM image of a topography showing high migration. Scale bar: 50 μm. FIG. 4E shows fluorescent microscopic images showing HFF topography leading to higher migration. FIG. 4F is a SEM image of a topography showing low migration. Scale bar: 50 μm, FIG. 4G shows fluorescent microscopic images showing HFF alignment on topography leading to lower migration (actin stained with Alexa Fluor 488 phalloidin and nucleus stained with DAPI; scale bar: 100 μm). FIG. 4H is a Local Interpretable Model-Agnostic Explanations (LIMES) plot showing the contribution of topographic parameters to high or low cell migration

FIGs. 5A-5F show analysis of the linearity of migrating cells on different topographies in the cellompics chip in accordance with an embodiment disclosed herein. FIG. 5A is a heat map showing the linearity of migrating cells on each topography. FIG. 5B and FIG. 5C are ROC curves obtained by correlating the linearity with topographical design parameters without feature selection (FIG. 5B) and with feature selection (FIG. 5C). FIG. 5D and IFG. 5E are fluorescent microscopic images show HFF alignment on different topography leading to

alignment in X direction (FIG. 5D) or random alignment (FIG. 5E) (actin stained with Alexa Fluor 488 phalloidin and nucleus stained with DAPI; scale bar: 100 μm). FIG. 5F is a Local Interpretable Model-Agnostic Explanations (LIMES) plot showing the contribution of topographic parameters to linear or non-linear cell migration.

FIG. 6 illustrates four exemplary topographic features in the cellompics chip in accordance with an embodiment disclosed herein. A wide variety of topographic features may be obtained by overlapping one or more primitive shapes selected from circles, triangles and rectangles.

FIG. 7 is a heatmap showing some of the differentially expressed genes between cells showing a high speed of migration and cells showing a low speed of migration in the cellompics chip in accordance with an embodiment disclosed herein. TRIM55, SFRP1, MGP, CYP1B1 and TMOD1 were the top five differentially expressed genes.

FIG.8 is a heatmap showing a subset of differentially expressed genes between cells showing a high speed of migration and cells showing a low speed of migration identified from network analysis in the cellompics chip in accordance with an embodiment disclosed herein. RHOA, ROCK1, CDC42, CDK, DIAPH1, ARHGEF7 are all genes that are responsible for actin cytoskeleton dynamics and are differentially regulated between high and low migratory cells.

It is envisaged that the Cellompics chip would be an invaluable tool for identification of surface topographies for various biological interactions with cells including cell migration, identification of topographies that promote or inhibit cancer metastasis and for surface engineering of biomaterials for clinical use such as but not limited to wound healing applications.

Design and Fabrication of Cellompic chip

A library of 160 randomly designed surface topographies was designed using mathematical algorithms. They were reproduced on a circular area of 1.5 cm radius which the inventors call a Cellompic chip. Each Cellompic chip consists of an innermost circular area of 0.5 cm diameter surrounded by a circular wall,

this area is designated as cell reservoir. The surface topographies are arranged in radial areas originating from cell reservoir and extending to the outermost wall of the chip. Adjacent distinct surface topographies are separated from each other with a wall of 10 µm thickness and 50 µm in height such that each individual area (test unit) consists of an area of 4 x 10^6 µm^2 containing a unique topography.

Topographic features are built up using three types of microscale and/or nanoscale primitive shapes: circles, isosceles triangles (with one angle of 36° and two angles of 72°), and thin rectangles (3 µm width). These shapes were chosen because by combining these primitives, different types of patterns can be generated —e.g., circles can create large smooth areas, triangles can generate angles, and thin rectangles can result in stretched elements. A feature is generated by first selecting uniformly at random one of the three possible feature sizes - 10x10 µm^2, 20x20 µm^2, or 28x28 µm^2. Next, parameter values are selected for the number of primitives used and the distribution over the different primitive types, the size of the primitives, and the standard deviation for the rotation of individual primitives. The distribution over the primitive types is selected such that all seven possible combinations of primitive types used (only circle primitives, only triangle primitives, only rectangle primitives, the three combinations of two primitive types, and the combination of all three primitive types) is as likely to be selected, and within a combination of primitive types used, each possible division is as likely to be selected. Selections for all other parameter value are made uniformly at random from a range of possible values. Ranges for the parameter values depend on the size of the feature. See Table 1 for the parameter ranges for a feature of 20x20 µm^2.

Table 1: Ranges for parameter values used for generation of a 20x20µm^2 feature.

Parameter	Range
Number of primitives	3 – 12
Circle primitive diameter	3 – 10 µm
Triangle primitive shortest side length	3 - 8 µm
Rectangle primitive length	3 -16 µm
Standard deviation for primitive rotation	0 - 180°

The orientation of a triangle or rectangle primitive is determined as follows: a triangle primitive is positioned with its sharp corner pointing to the right and a rectangle primitive is positioned horizontally, then the primitive is rotated by a number of degrees drawn from a normal distribution with mean 0° and standard deviation as determined during selection of the parameter values. The primitive is placed with the chosen orientation at a position where it is completely inside the feature, selected uniformly at random. Overlapping of primitives is allowed. See FIG. 6 for exemplary features.

With this design, a silicon mould was fabricated using conventional photolithography and etching which was then used for Cellompic chip in Polydimethylsiloxane (PDMS) using soft lithography. Fabricated chips were then characterized by scanning electron microscopy for lateral measurements and confocal laser scanning microscope for height profile measurements. The average difference in height profile measurements was 120 nm with a standard deviation of 22 nm. The average feature height was found to be 10 µm and wall height was 50 µm.

Analysis of the effect of topographies on cell behaviour using the Cellompic chip

To analyse the effect of topographies on cell behaviour, human foreskin fibroblasts (HFF) were serum starved for 24 hours and seeded into the cell reservoir of Cellompic chips. The cells were allowed to attach on the non-patterned cell reservoir for 3 hours prior to replacing the media. Live cell imaging was started after 48 hours of cell attachment when the cells reached confluency in the cell reservoir. Cells start migrating into the radially arranged test units 48 hours after seeding and reaching confluency in the cell reservoir.

To confirm that the topographic features affect HFF behaviour, the cells were fixed 36 hours after seeding and their actin cytoskeleton were stained. Visual inspection of the chip confirmed that a multitude of different cellular morphologies were induced by surface topographies. For instance, test units were found in which HFFs adopted an elongated shape, or in which cells spread

extensively, but also test units were seen in which the HFFs remained mostly rounded. In many test units, cells exhibited extensive filopodia, and many cells were observed that clearly followed the outlines of the features. Also, cells could be found to align either parallel or perpendicular to some topographies.

Deconvolution of topographies and design parameters influencing the extent of cell migration

To understand if topographies and their designs affect the migratory behaviour of cells, time lapse imaging of 7 chips was performed on cells stained with CM-Dii for 50 hours. This time lapse experiment resulted in a data comprising 112,000 images. The images were corrected using a customised Matlab code and analysed by an automated analysis pipeline using Cellprofiler (Marc Hulsman, Frits Hulshof, Hemant Unadkat, Bernke J. Papenburg, Dimitrios F. Stamatialis, Roman Truckenmüller, Clemens van Blitterswijk, Jan de Boer, Marcel J.T. Reinders (2015). Analysis of high-throughput screening reveals the effect of surface topographies on cellular morphology. *Acta biomaterialia, 15*, 29–38. https://doi.org/10.1016/j.actbio.2014.12.019).

Briefly, a Matlab code was designed which corrected the background and signal intensity distribution. The subsequent Cellprofiler pipeline consisted of primary object identification and object tracking modules. The Cellprofiler output resulted in at least 9 object tracking parameters which were subsequently used for classifier and machine learning analysis. Since all cells on the Cellompic chip share the same 5 ml of medium, it is likely that the biological response is not a direct result of the surface topography but rather mediated through short-range signals produced by cells in adjacent test units. Under these circumstances, a “colony” of positive test units would be expected, which was not observed in the heat maps. The Cellprofiler output yielded us 5 “hit” topographies which promoted maximum cell migration. It was found that topography number 75 and 104 yielded the highest extent of cell migration while topography number 22 yielded the lowest extent of cell migration.

Furthermore, to characterise the extent to which the surface topography is predictive of class fate (migrating or non-migrating), Decision Tree, Gradient

Boosting Machine (simple GBM), and K-Nearest Neighbors (KNN) was used to distinguish between high- and low-scoring test units based on surface topography parameters (design and Fourier). The classifier was used to perform a 10-fold cross-validation, in which it was trained with a subset of the test unit measurements, and afterwards used to predict the measurements of another subset of test units. The predicted measurements were then compared with the actual measurements, and an area under curve score (AUC) was plotted from the receiver operating characteristics (ROC) curve. This resulted in an AUC value of 0.73 (FIG. 4), confirming that the design of surface topographies correlates to the migration of HFFs, and thus migration of HFF's depends on surface topography design.

A forward feature selection helped in the understanding of the effect of topographic design parameters on cell migration and it was discovered that the number of triangles in a feature determined the extent of cell migration. The analysis showed that if the number of triangles in a feature are more than 3, then the extent of migration is significantly enhanced. Similarly, if there are no triangles in the feature then the feature would most likely be a bottom hit. Also, if the spacing between the feature is more than 7.57 μm then the feature is most likely to be a top hit. In other words, the number of angular projections in a feature and inter-feature distance determine the extent to which cells can migrate on them.

Identification of topographic design parameters influencing the linearity of cell migration

Directional cell migration is an important component of cell behaviour which arises from inherent ability of cells or due to external regulatory factors. The mechanisms that drive this process need to be decrypted by identifying the specific factors that promote random versus directionally persistent cell migration. Topography of the extracellular matrix provides a valuable physical cue that can promote directionally persistent migration, possibly by promoting front–rear polarity with geometrically constrained adhesion formation. However, *in vivo* extracellular matrix is topographically very complex, mainly due to a large diversity of ECM features spanning multiple scales of size and organization. For

instance, collagen fibrils and fibres which are interlinked within complex matrices are best examples of this three-dimensional (3D) topographic complexity.

Embodiments of the Cellompic chip presents a simple lucrative synthetic avenue which does not require the use of ECM molecules to study this complex interaction. It was postulated that the topographic design dictates the directionality or linearity of migration. The Cellprofiler pipeline allowed for the measurement of the directionality of migration. It could rapidly be identified the topographic designs and design parameters which determine if the cells will migrate randomly or in a directionally persistent manner. It was observed that cells on topographies that favor a linear mode of migration are polarized compared to cells on substrates favoring random migration. It was also observed that cell bodies of cells displaying linear migration appear elongated while cells choosing a random migration showed spread out cells (FIG. 4). These unique microscale or nanoscale topographic designs have an identical effect as that of quasi-3D, nanopatterned surfaces, wherein a linear migration of cells with response to a topographic gradient was observed. Here, it was observed that linearity of migration is significantly enhanced if the distance between the features is more than 7.57 μm and when the rectangular feature length is more than 5.42 μm but less than 7.89 μm. Furthermore, when the length of rectangular primitive in the feature exceeded 14.5 μm, the features were most likely to induce random or non-linear cell migration.

Changes in the genetic repertoire of the cells with response to surface topographies leading to high Vs. low cell migration

To validate the results, two topographies that showed high speed of migration and two topographies that showed low speed of cell migration were selected. These selected topographies were fabricated on larger areas so as to fit a well of 6 well plate. HFFs were seeded on these chips followed by a transcriptomic and genomic analysis using microarray. To identify differentially expressed genes (DEG) between high and low migrating samples, linear modelling approach with empirical Bayesian method was used. Further, the p-values were adjusted for multiple test correction. A threshold of adjusted p-value

0.035 led to the identification of 1567 differentially expressed genes. The selected DEG's are shown FIG. 7.

TRIM55, SFRP1, MGP, CYP1B1 and TMOD1 were the top five differentially expressed genes. Of these, TRIM55 or MuRF2 is from the family of Muscle ring finger (MuRF) proteins which are known for transmitting mechanical forces to cell signaling pathways through their interactions with the giant protein titin. There is evidence to link mechanically-induced stimuli with the control of serum response factor (SRF) activity through MuRF2. SRF is considered central to mechanotransduction and plays a vital role in processes directly related to cytoskeleton dynamics and cell migration. MuRF2 is known to be downregulated in highly migrating cancer cells which is consistent with the observation from the experiment.

SFRP1 or Secreted frizzled-related protein 1 is known to modulate the WnT signaling pathway. It was observed that SFRP1 is significantly downregulated in cells which showed high migration compared to cells that showed low migration. This finding is consistent with data which shows that SFRP1 inhibits cell proliferation, migration and invasion, and promotes apoptosis.

Matrix Gla Protein (MGP) is significantly upregulated in cells on hit topographies. It is known that MGP binds to fibronectin resulting in enhanced cell attachment, spreading and migration, thus supporting the results.

Additionally, CYP1B1 which is significantly upregulated on the positive hits is also well known to promote cell proliferation and migration through activation of Wnt/β-Catenin signaling pathways.

Tropomodulin 1 (TMOD1) caps the pointed ends of actin filaments of stress fibers in fibroblasts to stabilize focal adhesions, thereby modulating cell migration rates. TMOD1 also binds to tropomyosin, which greatly enhances the actin filament pointed-end capping activity. TMOD1 overexpression leads to the translocation of β-catenin to nucleus via the NF-κB pathways, leading to activation of MMPs resulting in enhanced cell migration. Thus, it is reasonable to say that surface topographies modulate the genetic repertoire of fibroblasts via conventional pathways which are known to play a role in cell migration.

To understand the interaction patterns of the DEG (source genes), Network analysis was then performed with the selected genes of interest (target genes) which are known to play a vital role in cell migration. The top 50 genes were identified from the set of all DEG genes for the source gene set based on *q-value*. Such network analysis enables for the discovery of new connections which are not yet recorded in established pathways, due to continuously updated databases. Using the pathlinker in Cytoscape, paths between genes of the two sets were identified. Hundreds of such paths were found. However, to condense the network to a visually more manageable size, in the subsequent analysis, only paths of length three were considered. This led to the discovery of 25 unique paths.

Five source genes (CDC25C, AURKA, FOXM1, FANCD2 and EPB41L3) with very high connectedness to that of the target genes were identified. CDC25C has a comprehensive function in the cell cycle, regulating the G1/S transition, S phase and the G2/M transition. Cell migration is known to occur during the G1/S phase. The data suggests that regulation of CDC25C by means of surface topographies results in downstream regulation of TP53 and thereby activating the RhoA-CDC42 mediated cell migration. Similarly, FOXM1 is known to cause ROCK activation by directly interacting with coiled-coil region of ROCK2 thereby increasing cell migration. Aurora kinase A (AURKA) is known to play an important role for centrosome formation and the progression of mitosis but recently it has been attributed as a direct activator of cell migration upon interaction with PLD, FAK and Src. EPB41L3 was over expressed in highly migratory cells suggesting its potential role via the YWHAZ-ROCK1 signalling mechanism. Thus, this network analysis allowed for the decipherment of potentially significant roles of various genes and pathways involved in topography mediated cell migration.

To understand the functions of these genes and confirm their roles in various pathways, pathway over-representation analysis was done. Pathway representation analysis revealed a prominent role of the regulation of actin cytoskeleton pathway in topography induced cell migration. RHOA, ROCK1, CDC42, CDK, DIAPH1, ARHGEF7 are all genes that are responsible for actin cytoskeleton dynamics and are differentially regulated between high and low

migratory cells. Next, these genes were visualized using heatmap which are shown in FIG. 8.

Conclusion and future outlook

This disclosure serves to illustrate the usefulness of Cellompic chip technology for biomaterials research. Cell–material interactions has already been proven critical for optimizing biomaterials performance and high throughput screening technologies are proving a boon for this understanding. Using primitive design parameters, this disclosure systematically describes the topographic properties for optimal cell migration by means of extent as well as linearity.

Gene expression profiles highlighted the dramatic effects of surface topographies on various cellular signaling pathways, indicating the added value of genome-wide screening. Moreover, the analysis indicated the roles of chemokine signaling, hippo signaling, focal adhesion and cancer signaling pathways in topography mediated cell migration. Additionally, by coupling expression profiles to surface topographic designs, the quintessential role of actin cytoskeleton signaling for topography mediated cell migration was confirmed. The Cellompic chip technology aids researchers in the design of a new generation of biomaterials with right bioactive topographies to influence stem cell homing, migration and attachment. It is also useful for the understanding of cancer cell invasion and metastasis, topography induced migration of cell in embryonic and organ development, thereby allowing for the development of informed approaches in regenerative medicine.

Materials and Methods

Chip Design and Fabrication

The Cellompics chip is a circular chip with radially arranged arrays of surface topographies. The topographies were designed using three types of primitive shapes—i.e., triangles, circles, and rectangles. 160 different topographies were generated by varying the size, number, orientation and combination of these 3 primitives which are arranged as a radial array. The chip

has an outer diameter of 3 cm and an inner diameter of 1 cm. The central portion of the chip is called the cell reservoir as seen in FIG. 1.

After designing the chip, a chromium mask was made and used for photolithography. The micro patterns were fabricated using photolithography and two steps etching on a silicon wafer to generate a silicon master. Using this silicon master as a mould, PDMS (Sylgard 184, Dow Corning, MI, USA) replicates of the chips were made by soft lithography. 1:10 ratio of PDMS to crosslinker was used. These chips were cleaned with Nitrogen gas and were plasma treated for 15 minutes to make the surface hydrophilic. These chips were then disinfected with 70% Isopropanol for 15 minutes inside biosafety cabinet and were subsequently sterilized with UV Light for 15 minutes and kept equilibrated in cell culture media overnight.

Generation of topographical parameters

Three categories of parameters to describe the topographies were generated. First were the design parameters which included the fraction and dimensions of primitive shapes used to design topographies. The second set of parameters were the Fourier parameters as described earlier under “Design and Fabrication of Cellompic chip”. The third set of parameters were mechanical parameters obtained by mechanical modelling of topographies. Table 1 earlier lists the parameters used in this study.

Culturing Fibroblasts on the chips

HFFs were grown till 80% confluence in a T25 flask and were reseeded into cell reservoir. Before reseeding, cells were labelled with Cell trackerTM CM-DiI as per manufacturer protocol. Cells were allowed to attach for 2 hours at 37 °C and then 3 mL media was added into each well containing chip. Cells achieved confluence within the cell reservoir between 2-3 days, following which the cells were imaged for 63 hours in the Bio station CT. Images of all the Chips in 6 well plate were captured at an interval of one hour. These images were stitched and individual radial topographies were cropped and migratory pattern over each topography was analysed using cell profiler.

Immunofluorescence Staining

After cell culture, the chips were washed with phosphate buffered saline and cells were fixed with 4% (w/v) paraformaldehyde for 10 min and permeabilized with 0.01% Triton X-100. Chips were stained with Alexa fluor phalloidin 488 (Life Technologies) and DAPI and imaged using confocal microscope Nikon A1R.

RNA extraction

Five hit topographies (showing highest and lowest migration) were selected and scaled up. Silicon wafer was created for individual hit topographies. HFF were cultured for 3 days on these chips containing individual topography and RNA was extracted using TRIZOL RNA Isolation Protocol. Microarray was performed using Illumina HT12.

Data description:

For HFF, the inventors have integrated distance/linearity values across 7 different chips for 160 topographies. The object is to estimate the hit probability (bottom hit - 0, top hit - 1) based on the median integrated distance/linearity across all the chips. To this end, the significance in the difference between the medians of a. median integrated distance/linearity across all the chips for a particular topography and b. median integrated distance/linearity on control topographies were checked using the Kruskal-Wallis test, particularly because it's a non-parametric test. With a significance level of 0.1 (kept higher than the general standard of 0.05 to avoid label imbalance), if the median (across 7 chips) of the median integrated distance/linearity for a topography is significantly different (p-value lower than 0.1) and lower than that of the control topographies, it is labelled as a bottom hit and vice versa. As a result, for integrated distance, 84 bottom and 76 top hits were obtained, whereas for linearity, 113 bottom hits and 47 top hits were obtained.

The objective is to train machine learning algorithms based on this newly obtained target to classify an unseen topography as a top hit or a bottom hit using

its morphological, fourier, design, and mechanical features, and subsequently obtain feature importance as well as feature contribution in the probability of a particular topography being a top hit. The number of features sums up to 50 for each of integrated distance and linearity.

Since the inventors have a high number of features and only 160 topographies to build the model on, the inventors chose 2 efficient gradient boosting algorithms called Extreme Gradient Boosting (XGBoost) and Light Gradient Boosting Machine (lightGBM). These methods make use of gradient boosting trees to create a classifier. The feature selection in these algorithms is 2-fold:

1. They employ L1 and L2 regularization - Regularization controls the coefficients of features while estimating the target function in logistic regression. L2 regularization reduces the coefficients to very small values in order to avoid overfitting, whereas L1 regularization reduces the coefficients of unimportant features to zero, thereby effectively acting as a feature selector. In boosting trees however, the regularization penalty is levied on leaf scores instead of feature coefficients, which in turn controls the depth of the trees and hence the number of features being used.
2. Boosting trees carry out classification by consecutively splitting all the samples in the training data based on the features in the training data. If a feature cannot effectively split the data based on the target (top hit, bottom hit), it's automatically removed from the classification in consideration of appropriate hyperparameter setting. Tree complexity can be controlled by maximum depth, or maximum number of leaves, or minimum sample (count or weight) per leaf, or minimum criterion gain.

Data division:

Training set - 75%

Validation - 10-fold cross validation

Test (hold out) set - 25%

The test (hold out) set is never shown to the model, and it's used to determine how the model performs on unseen data. A stratified split was performed so as to keep the proportion of top hits with respect to bottom hits constant across all sets.

LIME (Local Interpretable Model-Agnostic Explanations)

LIME is a model explanation technique that takes a machine learning model along with its predictions on multiple samples as input, and gives local feature contributions. The contributions can be understood as the amount by which a certain feature contributes to the target (hit probability in this case). The contribution could either be positive or negative, but all the individual contributions sum up to the predicted probability minus some randomness which LIME couldn't explain. LIME only gives local contributions, i.e. it calculates the contributions separately for all the samples. Global contributions can be obtained by averaging these local contributions. The amount of contribution largely depends on the AUC of the model, higher AUC would mean that there's a strong association between the input features and the target and the contributions would be higher.

In order to understand if the hit probability prediction is not a result of a chance event but the result of a good association between the input features and the target, the inventors make use of machine learning algorithms that do not employ feature selection and observe the AUC upon using these models instead. These include Decision Tree, Gradient Boosting Machine (simple GBM), and K-Nearest Neighbors (KNN).

It will be appreciated by a person skilled in the art that other variations and/or modifications may be made to the embodiments disclosed herein without departing from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like across different exemplary embodiments. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.