Title: DETECTION OR ISOLATION OF TARGET MOLECULES USING A MICROCHANNEL APPARATUS

Abstract: A microflow apparatus for separating or isolating cells from a bodily fluid or other liquid sample uses a flow path where straight-line flow is interrupted by a pattern of transverse posts. The posts are spaced across the width of a collection region in the flow path, extending between the upper and lower surfaces thereof; they have rectilinear surfaces, have arcuate cross-sections, and are randomly arranged so as to disrupt streamlined flow. Sequestering agents, such as Abs, are attached to all surfaces in the collection region via a hydrophilic coating, preferably a hydrogel containing isocyanate moieties or a PEG or polyglycine of substantial length, and are highly effective in capturing cells or other targeted biomolecules as a result of such streamlined flow disruption.

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DETECTION OR ISOLATION OF TARGET MOLECULES USING A MICROCHANNEL APPARATUS

FIELD OF THE INVENTION

[0001] This invention relates in general to detection or isolation of target molecules and more particularly to apparatuses or methods useful for detecting or isolating desired target cells or biological molecules.

BACKGROUND OF THE INVENTION

[0002] Effective isolation and collection of rare cells from a heterogeneous cell population remains of high interest, due to the increasing demand for isolated cell populations for use in disease diagnosis and treatment, e.g. gene therapy, as well as for basic scientific research. For example, pathologically changed cells, such as cancerous cells, can be separated from a larger normal cell population, and the cleaned cell populations may then be transplanted back into the patient.

[0003] One prominent demand is for the isolation of fetal cells to permit early fetus diagnosis, such as early screening of potential chromosomal disorders during pregnancy; fetal cells have been obtained by methods such as amniocentesis or chorionic villus sampling. Although one using such methods can obtain a significant amount of fetal cells such as to permit reliable diagnosis, such methods pose risks, especially to the fetus, because they require invasion into the uterus and typically can only be done after the first trimester.

[0004] Fetal cells are also present in circulating maternal blood as these cells pass from fetus to the maternal bloodstream in very low numbers; however, this results in ratios of fetal cells
to maternal cells on the order of only a few ppm. In principle, a sample of the maternal blood obtained by venipuncture may be used for fetal diagnosis; however, there are some significant challenges associated with such a method to isolate and collect the rare fetal cells from the major population of maternity cells. These challenges also exist in separating fetal cells from cervical mucosa, and they may also be common to other rare cell recoveries from bodily fluids or the like, as well as to the detection and isolation of other biomolecules present in only minute quantities.

[0005] Cell detection and separation is a rapidly growing area of biomedical and clinical development, and improved methods of separating a desired cell subset from a complex population will permit a broader study and use of cells that have relatively uniform and defined characteristics. Cell separation is also widely used in research, e.g. to determine the effect of a drug or treatment on a targeted cell population, to investigate biological pathways, to isolate and study transformed or otherwise modified cell populations; etc. Present clinical uses include, for example, the isolation of hematopoietic stem cells for reconstitution of blood cells, particularly in combination with ablative chemo- and radiation therapy.

[0006] Cell separation is often achieved by targeting molecules on the cell surface with specific affinity ligands in order to achieve selective, reversible attachment of a target cell population to a solid phase. In a subsequent step, nonspecifically adsorbed cells are removed by washing, followed by the release of target cells. Such specific affinity ligands may be antibodies, lectins, receptor ligands, or other ligands that bind proteins, hormones, carbohydrates, or other molecules with biological activity.

[0007] One of the current methods used to recover rare cells from a major bystander population is to use polystyrene macrobeads coated with an antibody selective for the target
cell population. Such macrobeads coated with specific antibodies are often allowed to settle by gravity down through a suspension of a heterogeneous cell population so that macrobeads capture target cells by interception.

[0008] There are also other substrates used with columns to separate the target cells from a sample fluid, and generally, the type of substrate selected for performing the separation will determine how the targets are ultimately separated from the sample fluid. A substrate is generally provided with characteristics such that the desired targets will have substantially different binding propensities to the substrate than will the remaining components of the sample. An example of a column-type apparatus for cell separation is found in U.S. Patent No. 5,240,856, issued August 31, 1993, where the cells bind to a matrix within the column. In U.S. Patent No. 5,695,989, issued December 9, 1997, a column is designed to serve as a pliable vessel which can be squeezed to facilitate removal of bound cells. U.S. Patent No. 5,672,481, issued September 30, 1997 describes an apparatus for separation in a closed sterile field, where a single rigid vessel is used for collection, concentration and transfer. U.S. Patent No. 5,763,194 describes a cell separation device comprised of an array of semi-permeable hollow fibers, where ligands are attached to the inner surface.

[0009] A number of problems and technical difficulties are associated with the above-mentioned approaches and also with the fairly widely used, macro bead-capture approach, which is frequently referred to as the column method as it basically utilizes macrobeads settling through a column. One major hurdle yet to be cleared to make the column method widely practical is the difficulty with non-specific binding. Moreover, using macrobeads to capture target cells in such a column separation method requires the recovery of the beads from the fluid stream and then cleaning, the usual procedure for which is washing. Beads
may be subject to loss, collapse or aggregation during washing, leading to a less efficient collection of the target cells.

[0010] Capture agents, often antibodies selective for the particular target cell population, are generally physically or chemically attached to the bead surface. Capture agents which are physically attached to beads may fall off or be displaced as a result of transfer and handling. However, if they are attached strongly through covalent bonds so that the captured cells will definitely stay on the beads during washing, they may not thereafter be released and recovered during collection.

[0011] In addition to the column separation method, other methods have now been developed for separating target cells from a diverse population of cells such as may be found in bodily fluid or the like. For example, U.S. Patent Publication No. 2004/0142463 teaches systems for separating fetal cells from maternal blood or the like where undesired components from the blood sample are first separated using surfaces of plates or other solid supports, including columns, which carry specific binding members; then electrophoresis or the like is used to complete the separation and analysis of the target cells.

[0012] Published U.S. Patent Application No. 2004/038315 attaches releasable linkers to the interior luminal surfaces of capillary tubing, with the desired bound cells subsequently being released via a cleavage reagent and recovered. U.S. Published Patent Application No. 2002/132316 uses microchannel devices to separate cell populations through the use of a moving optical gradient field. U.S. Patent No. 6,074,827 discloses the use of microfluidic devices that are constructed to have "enrichment channels" wherein electrophoresis is used to separate and identify particular nucleic acids from samples. Also mentioned is the optional use of antibodies or other binding fragments to retain a desired target biomaterial. U.S.
Patent No. 6,432,630 discloses a microflow system for guiding the flow of a fluid containing bioparticles through channels where selective deflection is employed, and it indicates that such systems may be used to separate fetal cells from maternal blood samples.

[0013] K. Takahashi et al., J. Nanobiotechnology, 2, 5 (13 June 2004) (6 pp) disclose on-chip cell sorting systems wherein multiple microfluidic inlet passageways lead to a central cell-sorting region fashioned in a PDMS plate (made in a master mold created in photoresist epoxy resin) that is closed by a glass plate. Agar gel electrodes are provided in the PDMS plate which facilitate the separation of undesired cells by the application of electrostatic forces that direct these cells into a parallel, continuous stream of buffer during their flow through a short, cell-sorting region of confluence. Also disclosed is a post-type filter arrangement for physically trapping large size dust particles.

[0014] U.S. Patent No. 6,454,924 discloses microfluidic devices wherein analyte-containing liquids are caused to flow generally downward past sample surfaces disposed atop upstanding pillars on which capture agents are attached, with the side surfaces of such pillars having been rendered hydrophobic so as to facilitate flow in channels that they define.

[0015] Published International Application WO 2004/029221 discloses microfluidic devices that can be used for cell separation, such as separating fetal red blood cells from maternal blood. A sample including the cells is introduced into a microfluidic channel which contains a plurality of obstacles, with the surfaces of the obstacles having binding moieties, e.g., antibodies, suitably coupled thereto, which moieties will bind to cells in the sample. U.S. Patent No. 6,344,326 discloses microfluidic devices having a plurality of enrichment channels wherein binding elements, such as antibodies, are coupled to crosslinked glass filaments or the like to capture biomolecules of interest. U.S. Patent No. 5,147,607 teaches
the use of devices for carrying out immunoassays, such as sandwich assays, where antibodies are mobilized in microchannels. A recessed area can be provided in the microchannel that contains a group of protrusions which extend upward from the bottom surface of the channel and to which the antibodies are immobilized.

[0016] The foregoing, briefly described references provide evidence that there is continuing searching for improved methods for isolating or detecting cells or other biomaterials from bodily fluids or the like.

SUMMARY OF THE INVENTION

[0017] It is the discovery of the present invention that a randomized flow pattern, especially a randomized flow pattern provided by a randomized fluidic multi-channel pattern can be used for isolating or detecting target molecules or entities. Accordingly the present invention provides apparatuses and methods useful for isolating or detecting target molecules, especially target cells or biological molecules.

[0018] In one embodiment, the invention provides a microflow apparatus which comprises a body having a randomized flow path and comprising an inlet means, an outlet means, and a microchannel arrangement extending between said inlet and outlet means, wherein the microchannel arrangement includes a plurality of transverse separator posts being integral with a base surface of the microchannel and projecting therefrom, wherein the posts are arranged in a pattern capable of providing said randomized flow path.

[0019] In another embodiment, the invention provides a kit comprising the apparatus of the invention and an instruction for coating the surface of the microchannel of the apparatus with a sequestering agent.
In yet another embodiment, the invention provides a kit comprising the apparatus of the invention wherein the surface of the microchannel of the apparatus is coated with a sequestering agent.

In still another embodiment, the invention provides a method of capturing a target molecule in a sample comprising causing a body of liquid containing the sample to flow through the microchannel of the apparatus of the invention, wherein the surface of the microchannel is coated with a sequestering agent capable of binding to the target molecule.

In still yet another embodiment, the invention provides a method of detecting a target molecule in a sample comprising causing a body of liquid containing the sample to flow through the microchannel of the apparatus of the invention, wherein the surface of the microchannel is coated with a sequestering agent capable of binding to the target molecule and detecting the target molecule.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1 is a perspective view of a substrate for a microflow apparatus wherein there is fabricated a simplified post-containing collection region in a microchannel.

FIGURE 2 is an enlarged fragmentary view showing a portion of the collection region of FIGURE 1 where the patterned posts are located.

FIGURE 3 is a front cross-sectional view of the substrate of FIGURE 1 taken along the line 3-3 with a cover plate attached to its bottom surface.

FIGURE 4 is a schematic perspective view of an apparatus that incorporates two valves with a substrate as generally shown in FIGURE 1 through the inclusion of an intermediate plate.
FIGURE 5 is a cross-sectional view taken along line 5-5 of FIGURE 4.

FIGURE 6 is a schematic plan view showing a substrate of the type shown in FIGURE 1, wherein pumps are fabricated as part of the microflow apparatus.

FIGURE 7 is a schematic view of a portion of a substrate in which a micro-mixer is incorporated into the supply region.

FIGURE 8 is a schematic representation of antibodies attached throughout a collection region via the application of a hydrophilic coating.

FIGURES 9 and 10 are schematic representations of chemistry that may be used to covalently attach sequestering agents, i.e. the antibodies of choice, throughout a collection region using a hydrophilic coating, along with depiction of capture of desired target cells.

FIGURE 11 is a flow sheet illustrating the steps of a cell recovery operation utilizing such a patterned post, cell separation device.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is the discovery of the invention that a randomized flow path, especially a randomized flow path provided by a randomized fluidic multi-channel pattern can be used for isolating or detecting target molecules or entities. Accordingly the invention provides apparatuses and methods useful for isolating or detecting target molecules, especially target cells or biological molecules.

According to one aspect of the invention, it provides an apparatus which comprises an inlet means, an outlet means, and a microchannel arrangement extending between the inlet and outlet means. The microchannel arrangement of the invention can be any microchannel
pattern capable of providing a randomized flow path. According to the invention, a randomized flow path can be any flow path that contains minimum, e.g., insubstantial amount or none streamlined flow or repeating flow pattern. In one embodiment, the randomized flow path of the invention is a flow path that has none streamlined flow or repeating flow pattern. In another embodiment, the randomized flow path of the invention is a flow path that interrupts or inhibits straight-line flow. In yet another embodiment, the randomized flow path of the invention is a flow path corresponding to a pattern that is mathematically random as known to one skilled in the art.

[0035] The randomized flow path of the invention can be provided using any suitable means known or later discovered in the field. For example, the randomized flow path of the invention can be generated using a microchannel arrangement with a plurality of transverse separator posts being integral with the base surface of the microchannel and projecting therefrom and arranged in a pattern capable of providing the randomized flow path of the invention. In general, the posts within a microchannel arrangement, e.g., a single unit or region can vary in size, e.g., cross sectional size and shape. For example, a microchannel arrangement of the invention can include posts with at least two or three different cross sectional sizes, e.g., big, small, and medium. A microchannel arrangement of the invention can also include posts with a single shape or more than one shape. Normally the posts of the invention with different sizes and/or shapes are distributed continuously or uniformly according to the random pattern of the invention.

[0036] In one embodiment, the mean cross sectional size of the posts is related to the size of a target molecule to be flown through the microchannel arrangement. Usually the relative ratio between the mean cross sectional size of the posts and the size of a target molecule is about 0.5 :5, 0.5:8, 1:5, 1:8, 2:5, 2:9, 3:5, or 3:8. In another embodiment, the cross sections
of the posts occupy between about 20% to about 75% of the cross section of the microchannel's base surface containing the posts therein. In yet another embodiment, the total volume of said posts (e.g., solid volume fraction) is about 15% to about 25% of the total volume of said microchannel (e.g., void volume fraction). In still yet another embodiment, the minimum distance between two posts is related to the smallest cross sectional size of the posts, e.g., equals to the smallest cross sectional size of the posts.

[0037] According to one embodiment of the invention, the posts of the invention can be arranged in a pattern corresponding to a random pattern generated by a mathematical algorithm, e.g., a computer program. For example, one can use a mathematical algorithm that generates random patterns based on certain pre-determined parameters, e.g., the total number of posts and minimum distance between two posts. In particular, one can obtain a random pattern generated by a mathematical algorithm by identifying the total number of posts in each size group and the minimum distance between two posts.

[0038] According to another embodiment of the invention, the surface of the microchannel arrangement of the invention can be optionally coated, partially or entirely with at least one sequestering agent. Usually a sequestering agent can be any entity capable of interacting in a specific fashion with a target molecule, e.g., biological molecule to physically sequester the target.

[0039] The sequestering agent of the invention can include nucleic acids, e.g., DNA, RNA, PNA, or oligonucleotide, ligands, proteins, e.g., receptors, peptides, enzymes, enzyme inhibitors, enzyme substrates, immunoglobulins (particularly antibodies or fragments thereof), antigens, lectins, modified proteins, modified peptides, biogenic amines and complex carbohydrates. Synthetic molecules can also be used, e.g., drugs and synthetic
ligands designed to have certain specific binding activity. By "modified" proteins or polypeptides is meant those proteins or peptides having one or more amino acids within the molecule altered by the addition of new chemical moieties, by the removal of existing chemical moieties or by some combination of both removal and addition. This alteration may include both natural and synthetic modifications. Natural modifications may include, but are not limited to, phosphorylation, sulfation, glycosylation, nucleotide addition, and lipidation. Synthetic modifications may include, but are not limited to, chemical linkers to facilitate binding to hydrogel, microstructures, nanostructures, e.g. quantum dots, or other synthetic materials. In addition, modification may include the removal of existing functional moieties, e.g. hydroxyl, sulfhydryl or phenyl groups, or the removal or alteration of native side chains or the polypeptide amide backbone.

[0040] Examples of complex carbohydrates include, but are not limited to, natural and synthetic linear and branched oligosaccharides, modified polysaccharides, e.g. glycolipids, peptidoglycans, glycosaminoglycans or acetylated species, as well as heterologous oligosaccharides, e.g. N-acetylglucosamine or sulfated species. Examples of naturally-occurring complex carbohydrates are chitin, hyaluronic acid, keratin sulfate, chondroitan sulfate, heparin, cellulose and carbohydrate moieties found on modified protein such as albumin and IgG.

[0041] According to the invention, the surface of the microchannel of the invention can be coated, e.g., directly or indirectly linked or coupled to at least one or two or more sequestering agents. In one embodiment, combinations of two or more of such agents are immobilized upon the surface of the microchannel of the invention, e.g., the base surface and/or the surface of the posts, and such combinations can be added as a mixture of two entities or can be added serially. In another embodiment, one or more sequestering agents are
coupled to the surface of the microchannel of the invention that is treated with one or more blocking agents. For example, the surface of the microchannel of the invention can be treated with excess Ficoll or any other suitable blocking agent to reduce the background signal of the microchannel of the invention.

[0042] According to another aspect of the invention, it provides kits comprising the apparatus of the invention, and optionally an instruction. In one embodiment, the kit of the invention comprises the apparatus of the invention wherein the surface of the microchannel of the apparatus is not coated with a sequestering agent and the kit optionally includes an instruction for coating the surface of the microchannel with a sequestering agent. In another embodiment, the kit of the invention comprises the apparatus of the invention wherein the surface of the microchannel of the apparatus is coated with a sequestering agent and optionally a blocking agent, and the kit optionally includes an instruction for using such apparatus.

[0043] According to yet another aspect of the invention, it provides methods of using the apparatus of the invention for capturing or detecting target molecules, e.g., target biological molecules. These target biological molecules may be any of a wide variety of cells, as well as nucleic acids, proteins, peptides, viruses, carbohydrates and the like. However, without being limited in any scope the invention is believed to exhibit particular efficiencies and has particular advantages in cell separation and detection. Although the term "cell" is used throughout this application, it should be understood to include cell fragments and/or remnants that would likewise carry the surface ligands specific to the sequestering agents. In one embodiment, the target cell of the invention is a neoplastic, e.g., a cancer or tumor cell. In another embodiment, the target cell of the invention is a fetal cell, e.g., in a blood or cervical mucous sample from the subject carrying the fetus. In yet another embodiment, the target
cell has a very low presence in a sample, e.g., the ratio of target cell vs. total cell population in a sample is less than about 1:10⁷, 1:10⁸, or 1:10⁹.

[0044] The target molecules captured by the apparatus of the invention can be detected or analyzed either in situ or after releasing from the surface of the microchannel. For example, cells can be detected directly in situ via FISH or any other suitable methods. Nucleotides and proteins can also be analyzed directly in situ either before or after releasing from the surface of the microchannel of the invention.

[0045] According to one particular embodiment of the invention, it provides an apparatus which includes a substrate 11 that has a flow path defined therein that includes at least one microchannel 13 having a collection region 17, which flow path is linked to a sample inlet 15 and a liquid outlet 19. As mentioned hereinafter, the flow path may include several microchannels, arranged in series, each of which has one such collection region. Alternatively, a microchannel may have more than one collection region, arranged in series, and there may also be more than one inlet and more than one outlet, all as well known in this art. Moreover it can be a part of an integrated microfluidic apparatus constructed on a chip, a disk or the like; in such an apparatus, substantially all of the MEMS (micro-electro-mechanical systems) or components needed to carry out cell recovery and/or diagnosis of biomolecules isolated from a sample may be incorporated as part of a single, compact, easily handled unit.

[0046] FIGURE 1 is a perspective view of a substrate 11 which is formed with a flow path that includes a microchannel 13 to which sample liquid is to be supplied through an opening or well 15 that serves as an entrance or inlet and an opening 19 that serves as an outlet. The cross-section of the collection region 17 is greater than that of an inlet section 18 that leads
thereinto from the inlet opening 15. The inlet section contains a pair of axially aligned divider/supports 21 just upstream of where it widens at the end of the region 18 to enter the collection region 17. These central dividers break the flow into two paths and serve to distribute the flow of liquid more evenly as it is delivered to the entrance end of the collection region 17. The collection region contains a plurality of upstanding posts 23 that are aligned transverse to the liquid flow path and arranged in an irregular, generally random pattern across the entire width of the collection region portion of the flow channel. The pattern of the posts is such that there can be only minimum or none straight-line flow through the collection region and that streamlined flow streams are disrupted or inhibited, assuring there is good contact between the liquid being caused to flow along the flow path and the surfaces of the posts. The posts are integral with the flat base 22 of the collection region 17 and extend perpendicular thereto, presenting surfaces that are vertical relative to a horizontal path of liquid being caused to flow through the flow channel of the substrate 11. Preferably they extend to and are affixed at their free end surfaces as by bonding to the surface of a facing flat closure plate 27 which is parallel to the base surface 22 and which closes the flow channel, as is described in detail hereinafter. Inlet and outlet holes 24a and 24b may be drilled through such a closure plate, but they are preferably provided in the substrate 11. Another flow divider/support 21a is located at the exit from the collection region.

[0047] As is well known in this art, a substrate may be formed with a flow path that includes a pair of parallel microchannels, each of which has a collection region. Such could be used in a series flow arrangement, or they could be used in parallel flow operation. Flow may be achieved by pumping, e.g. using a syringe pump or the like, or by vacuum that would draw liquid through from a reservoir at an inlet well provided by a large diameter inlet hole 24a.
Preferably such a well is included which has a capacity to hold about 50 µl to about 500 µl of liquid sample.

[0048] The design of the flow channels is such that, at flow rates through the apparatus within a reasonable range, e.g. injection of maternal blood using a standard Harvard Apparatus infusion syringe pump to create a flow in the collection region at a rate of about 0.01 to 100 mm per second, there is substantial disruption of streamlined flow through the region without creating turbulence; this results from the random arrangement of posts of different sizes and the relative spacing of the posts throughout the collection region. Relatively smooth, non-streamlined flow without dead spots is achieved at a preferred liquid flow rate of between about 0.3 to 10 mm/sec, and more preferably the flow rate is maintained between about 0.5 and 5 mm/sec and is achieved by suction from an inlet well of defined size.

[0049] Generally the substrate 11 can be made from any suitable laboratory-acceptable material, such as silicon, fused silica, glass and polymeric materials. It may be desirable to use a material that is optically transparent, particularly when a diagnosis function is desired to be optionally employed. In its simplest embodiment, the substrate carrying the fabricated microchannel is sealed with a plate 27 having a flat surface that will abut the facing surface of the substrate 11 as depicted in FIGURE 3. Such plate may be fabricated from the same material or may simply be a cover plate made of glass; however, an intermediate flow regulation plate 25 may be included as explained hereinafter. Suitable plastics which may be used include polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), polycarbonate, polystyrene, polyethylene terephthalate, as well as other polymeric resins well known for acceptable laboratory material usage. Such patterned substrates may be fabricated
using any convenient method such as those selected from among conventional molding and casting techniques.

[0050] Substrates may be conveniently fabricated from polymeric materials using a master or negative mold structure, which can be created in a thick negative photoresist, using optical lithography, as well known in this art and described in the J. Nanobiotechnology article, the disclosure of which is incorporated herein by reference. For example, the construction layer can be formed from a mixture of commercially available, standard grade epoxy resin (EPON SU-8) photoresist and hardener (SU-8 2025), which may be spun onto silicon wafer substrates at 2000 rpm to provide, for example, a 40 or 50 μm thick film of such photoresist. The thickness determines the height of the flow path in the collection region. The film is subjected to pre-exposure baking for 3 minutes at 60°C and then 7 minutes at 95°C on a precisely level hot plate to assure even thickness throughout, and the resultant samples are cooled to room temperature. A Karl Suss Contact Mask Aligner is used to expose a film with the desired pattern for the flow path in the ultimate device. The film is then post-baked at 65°C for 2 minutes and then at 95°C for 5 minutes before it is developed in a commercial SU-8 developer for 5 minutes, with light stirring being applied during developing. This creates a negative pattern mold in the epoxy resin photoresist that is then used as a molding master for replication of patterned post substrates in PDMS or other suitable polymeric resin.

[0051] As one example, a PDMS composition is prepared from a mixture of a PDMS prepolymer and a curing agent (Sylgard 184 kit, Dow Corning) at a 10:1 ratio by weight. The mixture is subjected to vacuum to evacuate bubbles that may be formed during mixing, before being poured over the epoxy resin master mold, which is located in a cavity of desired depth to create a substrate of desired thickness. The master mold may be optionally pre-coated with a thin layer (~50 nm) of a suitable metal (e.g. gold) to improve the release of the
PDMS replica after curing. Curing of PDMS substrate may be carried out at 80°C for 90 minutes; however, by initially undercuring the PDMS, it may be possible to facilitate subsequent functionalization of the collection region including the post surfaces as discussed hereinafter.

[0052] The layout and the dimensions of the microchannel 13 and of patterned posts 23 in the collection region 17 are determined by the mask used in exposure step of the fabrication of the master mold. The depth of the microchannel 13 is controlled by the thickness of the SU-8 layer of the master mold, which is determined by spin-coating conditions. FIGURE 2 provides a top view of the microchannel 13 showing an enlargement of the posts 23 in the collection region 17 in a preferred generally random arrangement.

[0053] In alternative embodiments, holes 24 could be drilled into or otherwise created in the flat, unbroken surface of a released PDMS replica substrate or in the cover plate to provide for inlet and outlet connections. In the former instance, it could be mated with a simple microscope cover slip or other suitable flat plate, such as a thin flat piece of PDMS, that would provide an imperforate cover or base plate for the substrate. After subjecting the two components to plasma-cleaning for two minutes, the two cleaned surfaces are immediately placed in surface contact, without touching the facing surfaces, which then become sealed by surface reaction as well known in this art, forming a permanent seal and closing the microfluidic flow path.

[0054] Should it be desired to integrate on-chip flow management into such an apparatus, a separate SU-8 molding master incorporating cavities for flow regulation features, such as pneumatic valves and the like, may be similarly fabricated. A flow regulation plate or layer 25 produced from such a master mold would first be laminated to the microchannel substrate
(see FIGURES 4 and 5), and it would in turn be laminated to a flat closure plate 27. The employment of such flow-regulating components and other MEMS in a microflow apparatus is shown in U.S. Patent Nos. 6,074,827 and 6,454,924, the disclosures of which are incorporated herein by reference. By carefully aligning such a flow regulation plate 25 with a microchannel carrying substrate 11 and then annealing overnight at 80°C, a composite structure is fabricated. Thereafter, cavities in the flow regulation plate 25 are closed by a flat plate or glass slide 27 using the same technique described earlier. As a further option, a second flow regulation plate might be laminated to the first plate 25, employing the same technique, should it be desired to incorporate still more sophisticated controls and optional processing.

[0055] For example, on-chip flow regulation mechanisms could be provided in a multichannel system formed in a substrate 11 by disposing them in a flow regulation layer 25 that would be sealed to the substrate. A simple system is illustrated in FIGURES 4 and 5 where passageways 24a and 24b lead to the inlet and exit. Air supply to pneumatic valves 29 may be via drilled or otherwise suitably formed holes 30 that extend through the substrate 11 into the plate 25. The flow regulation plate 25 or the substrate 11 could optionally contain alternative supply passageways that could deliver liquid to the inlet 15 and also might include an alternative exit or removal passageway as well known in this art.

[0056] As mentioned an arrangement wherein two series-connected collection regions are provided, lends itself to different methods of operation and use. For example, when a sample liquid is to be treated that potentially contains two different subpopulations of target biomolecules or cells of interest, one type of sequestering agent can be attached to the posts in one collection region or chamber, and a different type of sequestering agent can be attached to posts in a downstream collection chamber. Alternatively, in an instance where the
target cells are extremely rare, it might be desirable to attach the same sequestering agents to the posts in both collection chambers so as to enhance the likelihood of being able to capture nearly 100% of the cells in the liquid sample.

[0057] The polymeric surface of the patterned post region can be derivatized in various ways to enable the attachment onto all the surfaces of sequestering agents that are specific to the desired target cells or other biomolecules. For example, after plasma treatment and closure of the microchannel-carrying substrate, a 1 to 50 volume % solution of an amino-functional silane (e.g. a 10% solution of Dow Corning Z-6020), or a thio-functional silane, in ethanol may be injected into the microchannel to fill the region 17 between the openings 15 and 19, and the flooded microchannel 13 may then be left to incubate for 30 minutes at room temperature. Derivitization can be performed on a non-fully cured polymer, such as PDMS, before the closure of the microchannel region with the plate. In such case, as earlier mentioned, an alternative is to slightly undercure the PDMS substrate and then complete the curing after affixing the seal plate and treating with the substituted silane or other functionalizing reagent. For example, a final heating step of about 90 minutes at about 50 to 90°C might be used to complete the curing after treating with the Z-6020. Alternatively one or two days at room temperature would also complete the curing. Such derivatization treatment may also be performed before the closure of the microchannel region because derivatization of the facing flat surface is no real consequence. The flow path is then purged with ethanol, and the microchannel is ready for attachment of biomolecule sequestering agents.

[0058] Sequestering agents can be directly or indirectly immobilized upon the posts, and the posts may be pre-treated and/or coated to facilitate attachment. Indirect immobilization is clearly preferred and contemplates the employment of an intermediate agent or substance that
is first linked to the post, and moreover, it may be desired to use coupling pairs to link to the
intermediate agent. For example, streptavidin, or an antibody directed against another
species antibody, might be attached to the intermediate agent, which would thereafter couple
to a biotinylated Ab or to an Ab of such other species.

[0059] The use of antibodies as sequestering agents may be preferred for cell separation, and
their attachment is discussed in U.S. Patent Nos. 5,646,404 and 4,675,286 and throughout the
prior art. For example, procedures for non-covalent bonding are described in US Patent
4,528,267. Procedures for covalently bonding antibodies to solid supports are also described
by Ichiro Chibata in IMMOBILIZED ENZYMES; Halstead Press: New York (1978) and in
A. Cuatrecasas, J. Bio. Chem. 245:3059 (1970), the contents of both of which are hereby
incorporated by reference. The antibody is preferably bound to the solid post surfaces
indirectly, such as through the use of a surface layer or a coating of long linkers to which the
Abs are then attached. For example, the surface can be first coated with a bifunctional or
polyfunctional agent, such as a protein; the agent is then coupled with the antibody using a
coupling agent, e.g., glutaraldehyde. The antibody can also be effectively bound by applying
the antibody in aqueous solution to a surface that has been coated with a layer having free
isocyanate or equivalent groups, such as a polyether isocyanate, or the antibody might be
coupled to a hydroxylated material by cyanogen bromide. Particularly preferred is the use of
a hydrophilic polyurethane-based hydrogel layer having free isocyanate groups, which is
disclosed in a copending patent application and described in an example hereinafter, or the
use of a hydrophilic linker of substantial length, such as one of PEG, polyglycine.

[0060] When antibodies (Abs) are used, they are suitably attached, preferably through such
intermediate agents, using any mechanisms well known in this art. For example, Abs may be
treated with 2-aminothiolane to thiolate them, and the resulting thiolated Abs conjugated with
posts that have been treated with PEG-maleimide; alternatively, the Abs may be directly covalently bonded to an appropriate hydrophilic coating having reactive isocyanate groups or thiocyanate groups.

[0061] With the antibodies or other sequestering agents in place throughout the patterned post collection region, the microchannel device is ready for use. A bodily fluid, such as a blood or urine sample, or some other pretreated liquid containing or being suspected of containing the target cell population, is caused to flow along a flow path through the collection region 17, as by being discharged carefully from a standard syringe pump into an inlet passageway 24a leading to the inlet 15 for such a microchannel device or drawn by a vacuum pump or the like therethrough from a sample reservoir provided by a relatively large diameter inlet passageway 24a which serves as well to hold the desired volume of sample for a test. The opening 24a may contain a fitting (not shown) for mating with tubing connected to such a syringe pump when such is used. The pump may be operated to effect a flow of about 0.5-10 µl/min. through the apparatus. Depending upon the bodily fluid, or other cell-containing liquid that is to be treated and/or analyzed, a pretreatment step may be used to reduce its volume and/or to deplete it of undesired biomolecules, as is known in this art.

[0062] To potentially increase the overall efficiency of a cell separation method, it may be desirable to collect the sample exiting the outlet 19 and cause it to flow through the microchannel device more than once; such repeat treatment may be particularly useful when the cells are particularly rare and thus are likely very few in number in the sample. However, because of the high efficiency of capture achieved by the apparatus, it is expected that such repeat flow will seldom be needed. Alternatively, two collection chambers linked in series might be used as earlier mentioned. Moreover, if somewhat larger volumes of bodily fluid
samples are being processed, two or more microchannels could be used in parallel on a substrate.

[0063] Sequestering agents (e.g. Abs) are attached to the base, the facing surface, the posts and the sidewalls of the collection regions in the microchannels; however, such sidewall surfaces are not particularly effective in capturing cells as are the base, facing surface and the posts which disrupt the flow. It has been determined that flow of liquid containing cells or other biomolecules through even a confined lumen results in the cells being primarily present in the central flow stream region where flow shear is the least; as a result, capture upon sidewalls that carry sequestering agents is quite sparse in comparison to the capture upon surfaces in the immediate regions where the transverse posts have disrupted streamlined flow. In these regions, sequestering agents that can assume their native 3-dimensional configurations as a result of properly coupling are surprisingly effective.

[0064] Following the completion of flow of the liquid sample through the apparatus, the targeted cells would, if present, have been captured within the collection region, and purging is first carried out with buffers so as to remove all of the extraneous biomaterial that had been part of the sample and that has not been strongly captured by the antibodies or other sequestering agents in the collection region. Such purging with effective buffers is expected to leave only the target cells attached in the collection region in the microchannel apparatus, having removed all nonspecifically bound material.

[0065] Once purging with buffer has been completed, if the objective of the treatment method is cell collection alone, the captured cells are then suitably released. As mentioned hereinafter, in some instances, it may be desired that some analysis be carried out in situ. For example, the cells may be counted while attached, or they may be lysed and then subjected to
PCR either in the collection chamber or downstream. Alternatively the cells can be observed or detected directly while they are captured or attached, e.g., via FISH or any other suitable detection methods.

[0066] When release is to be effected, any method known in this art may be used, such as mechanical (e.g. high fluid flow), chemical (e.g. change in pH), or through the use of enzymatic cleavage agents or the like. For example, a reagent may be applied to cleave the sequestering agent or to cleave the bond between the agent and the cells in order to release the target cells from the collection region. For instance, trypsin or a specifically focused enzyme may be used to degrade the Abs and/or the cell surface antigens. Specific methods for both attaching Abs or the like and then effectively removing captured ligands are discussed in U.S. Patent No. 5,378,624. For example, if the cells have been sequestered through the use of antibodies that are specific to surface characteristics of the rare cells, release may be effected by treating with a solution containing trypsin or another suitable protease, such as Proteinase K. Alternatively, a collagenase may be used to effect release from other sequestering agents, or a specifically cleavable linker may be used to attach the sequestering agent. During such cleavage, the outlet from the microchannel is connected to a reservoir or other collector, and the discharge stream carrying the released rare cells is collected for further analysis. The microchannel device may be fabricated with more than one exit passageway at the outlet and with valves for regulating which exit is open; such allows one exit passageway to be used for the waste discharge during the preliminary steps and then a different exit passageway to direct the target cell stream to a collection container.

[0067] It has been found that the placement and shape of the posts 23 in the patterned post collection region 17 can be engineered for optimal fluid dynamics and enhancement of capture of target cells through their specific surface characteristics. Very generally, in most
instances, the preferred shape of the horizontal cross-section of the transverse fixed posts 23 avoids sharp angles which might promote nonspecific binding to the transverse surfaces of the posts. The posts 23 have rectilinear exterior surfaces and preferably have either a generally circular cross sectional shape or regular polygonal of 6 or more sides. Alternative shapes that might be used are tear-drop shape where the tip is at the downstream end and shallowly curved, or oval shape; however, should more impact be desired, a square shape might be used. The pattern of the posts should create a flow pattern in the liquid stream which enhances the capture of target cells by the sequestering agents attached to the surfaces of the posts, the base and the facing surface. To achieve this end, it has been found that the posts should be of different sizes and be arranged in a set random pattern. Surprisingly, a random pattern of posts 23 of different cross sectional sizes, e.g. circular cross section posts of at least about 3 or 4 different sizes, about 70 to about 130 microns in diameter, in a collection region about 100 microns high and about 2 to 4 mm wide, appears to promote a particularly effective capture of cells from the flow of a liquid sample, when the minimum separation spacing between posts is 50 to 70 µm and preferably about 60 µm.

[0068] It is particularly preferred that the cross sectional area of the posts, which all have sidewalls formed by parallel lines which are perpendicular to the base, is such that they occupy between and about 15 to 25% of the volume of the collection region. Preferably the post pattern will be such that they occupy about 20% of the volume of the collection region, leaving a void volume for liquid flow of about 80%. The particular random pattern of post locations shown in FIGURE 2 appears to particularly enhance the tendency of the cells to be captured by sequestering agents in these regions where streamlined flow has been effectively disrupted. The posts 23 are substantially spaced apart from one another, e.g. by at least about
60 microns, and posts of different sizes are preferably located upstream and downstream of one another.

[0069] Smaller posts may create eddy regions downstream of larger posts, and as a result of the flow pattern that is generated, the surfaces in the vicinity may show particular effectiveness in capturing target cells. As shown in FIGURE 2, any straight line extending longitudinally of the flowpath at a location more than about 100 microns from a sidewall will intersect a plurality of posts. As previously mentioned, the posts are integral with the base surface of the substrate and are preferably affixed at their opposite or free ends to the facing surface, i.e. either a flow-regulation plate 25 or a flat closure plate 27.

[0070] As indicated before, attachment of the sequestering agents, such as antibodies, throughout the collection region may be facilitated and the sequestering agents made to perform more efficiently by coating the surfaces therein with a thin layer of a particular hydrophilic hydrogel substance or of a hydrophilic linker, such as PEG, polyglycine or the like of a molecular weight of at least about 1,000 daltons, preferably having a MW of about 2,000 to 100,000 daltons, and more preferably between about 3,000 and 50,000 daltons. Particularly preferred is the employment of a hydrophilic hydrogel coating which is an isocyanate-functional polymer containing PEG, PPG or a copolymer thereof that is polymerized by urethane bonds and that contains reactive isocyanate groups. Details of the formulation of such coating material are disclosed in a co-pending U.S. patent application Serial No. 11/021,304, filed December 23, 2004, which is assigned to the assignee of this application. Schematically shown in FIGURE 8 is a representation of a collection region within a microchannel wherein there are a plurality of posts 61 of varying diameter that are randomly arranged to disrupt streamlined flow through the chamber, wherein each of the posts 61 and the facing flat surfaces carry an exterior coating 63. Also depicted are
sequestering agents 65 in the form of antibodies that are attached to the hydrophilic hydrogel coatings on the posts and which, as a result, retain their native three-dimensional confirmation, unaltered by attachment to the hydrogel which is primarily water.

[0071] FIGURE 9 is provided as a schematic representation of chemistry that may be employed when a hydrogel of the preferred character is used, as represented in FIGURE 8. Shown are representative sequences of attaching sequestering agents, such as antibodies, to all the surfaces throughout the collection region 17”, where a hydrophilic hydrogel polymer coating 49 is applied to the surfaces. Point 1 of FIGURE 9 shows a surface following amino-derivatization by treatment with an aminosilane or the like. This step is followed by using non-fat milk to casein-coat the surfaces, see Point 2. Point 3 represents the coated surface after coating has been carried out using a prepolymer containing PEG of a molecular weight of about 3400 that is end-capped with toluenediisocyanate. Such prepolymer may be dissolved in a water-miscible, organic solvent, such as a mixture of NMP and CH₂CN. The hydrogel formulation preferably contains tri- or higher functional polyols, e.g. PEGs and PPGs, and may contain trifunctional isocyanate. An aqueous solution is prepared containing about 98.5 weight percent water, which solution is pumped through the microchannel so that the surfaces of the posts and the facing surfaces of the collection region become coated with this hydrophilic hydrogel coating, as a result of reaction of the end-capped isocyanate groups at the amine-derivatized surfaces. The end result is represented at Point 3 in FIGURE 9.

[0072] Point 4 represents the addition of antibodies which will have surface amino groups. They can be attached directly to such hydrophilic hydrogel coatings of the posts, as shown in Point 5, by covalent bonding of the Abs amines to either isocyanate or thiocyanate groups carried by the hydrophilic coating. Alternatively, the antibodies may first be thiolated as depicted at Point 6 of FIGURE 9, and these thiolated antibodies then supplied in aqueous
solution to the collection chambers, where they will in turn readily covalently bond to the isocyanate groups of the coated polymers, see Point 7.

[0073] As depicted at Points 8 and 9 in FIGURE 92, when cells in a liquid sample that is being caused to flow through the collection chamber, as a result of the disrupted streamlined flow, come in contact with the posts and/or the facing surfaces, antigens on the cell surfaces specific to the antibodies become conjugated thereto, effectively capturing the cells.

[0074] FIGURE 10 is provided as a schematic representation of chemistry that may be employed when an elongated PEG or PPG linear polymer is used to tether a sequestering agent, particularly an antibody, to the surfaces in a collection region. The linear polymer is selected so as to be of such length that the antibody will be able to assume its native three dimensional configuration in an aqueous environment where capture is being carried out. Point 1 of FIGURE 10 shows the surface following amino-derivatization by treatment with an aminosilene or the like. This step is again followed by using non-fat milk solids to casein-coat the surfaces as described above. Following washing, all the surfaces are treated with a linear PEG or PPG having a molecular weight of at least about 2000, and preferably at least about 3000, which has a NHS moiety at one end and maleimidyl moiety at the opposite end. The N-hydroxy-succinimidyl ester moiety reacts readily with the amino groups on the surfaces to provide a coating at least about 1 micron thick. After suitable incubation, the microchannel is drained and washed with a suitable buffer, leaving the maleimido-PEG-coated surfaces as represented by point 3 of FIGURE 10. Point 4 represents antibodies which are specific for trophoblasts and which inherently have surface amino groups. The antibodies are preferably thiolated using a suitable reagent, such as Traut's reagent, to reach the point depicted as point 5 in FIGURE 10. The thiolated antibody is then conjugated with the maleimido-PEG-coated posts by introduction of the purified thiolated antibody into the
microchannel in a buffered solution and allowing it to appropriately incubate. The
microchannel is then washed with a suitable buffer, and the conjugated arrangement depicted
in FIGURE 6 is obtained.

[0075] Point 7 of the schematic representation of FIGURE 10 shows the capture of a
trophoblast by an antibody that is tethered to a surface by the linear PEG coupling agent.

[0076] The following examples illustrate effective use of prototype microchannel devices of
this type to sequester trophoblast cells from an extract of cervical mucosa. They should, of
course, be understood to be merely illustrative of only certain embodiments of the invention
and not to constitute limitations upon the scope of the invention which is defined by the
claims which are appended at the end of this description.

Example 1

[0077] A microflow apparatus for separating biomolecules is constructed using a prototype
substrate as in Example 1. The substrate is formed from PDMS and is bonded to a flat glass
plate to close the flow channel. The interior surfaces throughout the collection region are
derivatized by incubating for 30 minutes at room temperature with a 10 volume % solution of
Dow Corning Z-6020. After washing with ethanol, they are treated with nonfat milk at room
temperature for about one hour to produce a thin casein coating. Following washing with
10% ethanol in water, a treatment is effected using a hydrogel based on isocyanate-capped
PEG triols, average MW of 6000. The formulation used consists of about 3% polymer. A
hydrogel prepolymer is made using 1 part by weight polymer to 6 parts of organic solvent,
i.e. Acetonitrile and DMF, was mixed with an lmg/ml antibody solution in 100 mM Sodium
Borate pH 8.0 containing BSA. The specific formulation comprises 100 mg Prepolymer in
Acn/DMF; 350 µL of 0.25 mg/ml Antibody Mix in borate buffer; and 350 µL of 1mg/ml BSA in borate buffer, and it contains about 2% Polymer by weight.

[0078] For this test, it is desired to isolate trophoblasts from a sample of cervical mucous, and Kawata et al., J. Exp. Med., 160:653 (1984) discloses a method for isolating placental cell populations by detecting target cells using cell-specific Abs, e.g. monoclonal antibodies against human trophoblasts (anti-Trop-1 and anti-Trop-2). The '596 patent teaches the use of other such Abs for the same purpose, and U.S. Patent No. 5,503,981 identifies three other monoclonal Abs which can be used for this purpose. Antibodies to Trop-1 and Trop-2 are specific to ligands carried by the exterior surfaces of trophoblasts which are of fetal origin.

The buffer of choice for these antibodies was first changed to a buffer more compatible with the planned modification and stability of the antibodies, by repeated concentrations on Amicon's Centricon-30™ membrane-based micron concentrator. Antibody (0.1 mg) was then dissolved in 100 µl of 0.2 M sodium borate/0.15 M NaCl containing 5 mM EDTA (pH 8.3) and reacted with 5 µl of 40 mM Traut's reagent at RT for one hour to effect thiolation. The excess Traut's reagent is reacted with 10 µl of 100 mM glycine followed by purification of the thiolated antibody on the Centricon-30™. Thiolation was confirmed by standard laboratory procedures.

[0079] About 5 micrograms total of the thiolated anti-Trop-1 and 2 in aqueous solution, at a concentration of about 0.5 mg/ml, are supplied to the pretreated microflow apparatus, and the solution is left to incubate for 2 hours at 25°C. Following this incubation period, the flow channel is flushed with a 1% PBS/BSA to give antibody-coated surfaces which were then used to try to isolate fetal trophoblast cells.
Cervical mucous from expectant mothers (8-12 weeks gestation) was diluted to 10 ml with HAM's media (Invitrogen) and treated with DNAse (120 units) at 37°C for 30 minutes. After filtering through a 100 µm cell strainer, the cells were spun at 1500 RPM for 30 minutes. The cell pellet was resuspended in HAM's media (100 µl) and passed through the Trop-1 and Trop-2 coated microchannel by hooking the microflow separation apparatus up to outlet tubing from a Harvard Apparatus syringe pump which is filled with about 50 microliters of this cell suspension of cervical mucosa extract. The syringe pump is operated to produce a slow continuous flow of the sample liquid through the microflow apparatus at room temperature and a rate of about 10 µl/min. During this period, the Trop-1 and Trop-2 Abs, that have been attached to the surfaces in the collection region where the random pattern of transverse posts is located, capture trophoblasts that are present in the sample. After the entire sample has been delivered by the syringe pump, a slow flushing is carried out with a 1% PBS/BSA aqueous buffer. About 100 µl of this aqueous buffer is fed through the apparatus over a period of about 10 minutes, which effectively removes all non-specifically bound biomaterial from the flow channel in the device. Two additional washings are then carried out, each with about 100 µl of 1% PBS plus 1% BSA over a period of about 10 minutes.

At this time, inasmuch as the apparatus is made of optically clear material, microscopic examination can be made of the effects of the capture, as by using photomicroscopy. The bound cells were stained with cytokeratin 7 and cytokeratin 17 that are specific to captured cells which are of trophoblast origin. By counting cells in such photomicrographs, it is estimated that substantially 97% of the trophoblasts estimated to be present in the sample have been captured in the patterned post collection region, which is considered to be a very excellent result.
In a repeat of this procedure through the capture and washing steps, the captured trophoblasts are released by causing a solution of 100 µl of a 0.25% solution of trypsin to slowly flow through the flow channel at 27°C over a period of 20 minutes. This reagent causes digestion of the Abs, releasing the trophoblasts into the aqueous flow where they pass through the outlet and are collected. Analysis of the collected cells by PCR and FISH based technologies shows that they are indeed the trophoblasts that were targeted by the Abs that were employed.

Example 2

A microflow apparatus for separating biomolecules is constructed using a prototype substrate as in Example 1. The substrate is formed from PDMS and is bonded to a flat glass plate to close the flow channel. The interior surfaces throughout the collection region are derivatized by incubating for 30 minutes at room temperature with a 10 volume % solution of Dow Corning Z-6020. After washing with ethanol, they are treated with nonfat milk at room temperature for about one hour to produce a thin casein coating. Following washing with 10% ethanol in water, a treatment is effected using a hydrogel based on isocyanate-capped PEG triols, average MW of 6000. The formulation that is used consists of about 3% polymer. A hydrogel prepolymer is made using 1 part by weight polymer to 6 parts of organic solvent, i.e. acetonitrile and DMF, and is mixed with an 1 mg/ml antibody solution in 100 mM sodium borate, pH 8.0, containing BSA. The resultant specific formulation comprises 100 mg prepolymer in Acn/DMF; 350 µL of 0.25 mg/ml antibody mix in borate buffer; and 350 µL of 1mg/ml BSA in borate buffer, and it contains about 2% polymer by weight. Antibodies Trop-1 and Trop-2, which are specific to ligands carried by the exterior surfaces of trophoblasts, are again used.
[0084] About 5 microliters total of the Trop-1 and 2 hydrogel aqueous solution are supplied to the pretreated microflow apparatus, and the solution is left to incubate for about 30 minutes at 25°C. Following this incubation period, the flow channel is flushed with mineral oil which is slowly pushed into the flow channel to displace and push out excess hydrogel. This results with an oil-filled flow channel which has a thin layer of hydrogel coating separating oil from PDMS material. After 3 hours, hydrogel is fully cured, and oil is flushed out with a 1x PBS / 0.1% Tween solution. The device is then filled with 1x PBS solution to preserve the Abs.

[0085] Cervical mucous from expectant mothers (8-12 weeks gestation) is diluted to 10 ml with HAM’s media (InVitrogen) and treated with DNAse (120 units) at 37°C for 30 minutes. After filtering through a 100 μm cell strainer, the cells are spun at 1500 RPM for 30 minutes. The cell pellet is resuspended in HAM’s media (100 μl) and passed through the Trop-1 and Trop-2 coated microchannel by hooking the microflow separation apparatus up to outlet tubing from a Harvard Apparatus syringe pump, which is filled with about 50 microliters of this cell suspension of cervical mucosa extract. The syringe pump is operated to produce a slow continuous flow of the sample liquid through the microflow apparatus at room temperature and a rate of about 10 μl/min, during which period the Trop-1 and Trop-2 Abs, which are attached to the surfaces in the collection region, capture trophoblasts that are present in the sample. After the entire sample is delivered by the syringe pump, a slow flushing is carried out with a 1% PBS/BSA aqueous buffer. About 100 μl of this aqueous buffer is fed through the apparatus over a period of about 10 minutes, which effectively removes all non-specifically bound biomaterial from the flow channel in the device. Two additional washings are then carried out, each with about 100 μl of 1% PBS plus 1% BSA over a period of about 10 minutes.
Microscopic examination is again made of the effects of the capture by using photomicroscopy, after staining the bound cells with cytokeratin 7 and cytokeratin 17. By counting cells in such photomicrographs, it is determined that excellent capture of the trophoblasts estimated to be present in the sample is achieved.

Example 3

Another microflow apparatus for separating biomolecules is constructed using a prototype substrate as in Example 1. The substrate is formed from PDMS and is bonded to a flat glass plate to close the flow channel. The interior surfaces throughout the collection region are derivatized by incubating for 30 minutes at room temperature with a 10 volume % solution of Dow Corning Z-6020. After washing with ethanol, they are treated with nonfat milk at room temperature for about one hour to produce a thin casein coating.

Following washing with 10% ethanol in water, a treatment is effected using 10\mu l of 2.5 mM NHS-polyglycine (ave. MW about 4500) in 0.2 MOPS/0.5M NaCl, pH 7.0, by incubating at RT for 2 hours with gentle pumping of the solution back and forth in the channel to provide agitation. The microchannel is washed three times with 500\mu l of pH 7.0 MOPS buffer to obtain maleimido-polyGly-coated channels.

Antibodies Trop-1 and Trop-2, which are specific to ligands carried by the exterior surfaces of trophoblasts are treated as in Example 1 to thiolate them.

About 5 micrograms total of thiolated anti-Trop-1 and 2 in aqueous solution, at a concentration of about 0.25 mg/ml, are supplied to the pretreated microflow apparatus, and the solution is left to incubate for 2 hours at 25\degree C. Following this incubation period, the flow
channel is flushed (3 times) with a 1% PBS/BSA to provide the antibody-coated surfaces which are then used to try to isolate fetal trophoblast cells.

[0091] Cervical mucous from expectant mothers (8-12 weeks gestation) was diluted to 10 ml with HAM's media (InVitrogen) and treated with DNAse (120 units) at 37°C for 30 minutes. After filtering through a 100 µm cell strainer, the cells were spun at 1500 RPM for 30 minutes. The cell pellet was resuspended in HAM's media (100 µl) and passed through the Trop-1 and Trop-2 coated microchannel by hooking the microflow separation apparatus up to outlet tubing from a Harvard Apparatus syringe pump which is filled with about 50 microliters of this cell suspension of cervical mucosa extract. The syringe pump is operated to produce a slow continuous flow of the sample liquid through the microflow apparatus at room temperature and a rate of about 10 µl/min, during which period the Trop-1 and Trop-2 Abs, which are attached to the surfaces in the collection region, capture trophoblasts that are present in the sample. After the entire sample is delivered by the syringe pump, a slow flushing is carried out with a 1% PBS/BSA aqueous buffer. About 100 µl of this aqueous buffer is fed through the apparatus over a period of about 10 minutes, which effectively removes all non-specifically bound biomaterial from the flow channel in the device. Two additional washings are then carried out, each with about 100 µl of 1% PBS plus 1% BSA over a period of about 10 minutes.

[0092] Microscopic examination is again made of the effects of the capture by using photomicroscopy after staining the bound cells with cytokeratin 7 and cytokeratin 17. By counting cells in such photomicrographs, it is determined that excellent capture of the trophoblasts estimated to be present in the sample is achieved.
Although the invention has been described with regard to certain preferred
embodiments which constitute the best mode presently known to the inventor for carrying out
this invention, it should be understood that various changes and modifications as would be
obvious to one having ordinary skill in this art may be made without departing from the scope
of the invention which is defined in the claims which follow. For example, although certain
preferred materials have been described for the fabrication of the substrate in which the
microchannels are defined, there is a broad range of structural materials that may be
employed as are well known in this art as being suitable for laboratory devices such as this.
Although the emphasis has generally been upon the separation of fetal cells from a maternal
blood sample or trophoblasts from a cervical mucosa extract, it should be understood that the
invention is useful for separating a wide variety of blood cells, e.g. nucleated erythrocytes,
lymphocytes and the like, metastatic cancer cells, stem cells, etc.; moreover, other biological
materials, e.g. proteins, carbohydrates, viruses, etc., might also be separated from a liquid
sample. When the sample contains specific subpopulations of cells, the target cells to be
captured may be a group of unwanted cells to be separated from rare cells or the like.
Moreover, once targeted cells have been collected, they may also be lysed in situ to provide
the cell DNA, which may be collected for analysis downstream or alternatively subjected to
PCR within the collection chamber. U.S. Published Application 2003/0153028 teaches
lysing such bound cells to obtain the nucleic acid that is released. If there are two different
subpopulations of target cells in a sample, different sequestering agents may be attached to
the posts in the upstream and downstream collection chambers. In another situation, one
genus of cells may be first collected in an upstream collection chamber, released, and then
screened again in a downstream chamber to isolate a subgenus of cells.
[0094] From a constructional standpoint, some additional components that might optionally be incorporated into such an apparatus are illustrated in FIGURES 6 and 7. FIGURE 6 shows a microchannel similar to that depicted in FIGURE 1 in which peristaltic-type pump arrangements are incorporated into an inlet passageway region and an outlet passageway region flanking the collection chamber. Illustrated is a microchannel arrangement 13' that includes an inlet 15', a collection chamber 17' and an outlet 19' wherein an integrated pumping arrangement 41 is constructed by the incorporation of three specially designed membrane valves located in an entrance passageway 18' leading to the collection chamber. The schematic representation is of an arrangement similar to that shown in FIGURES 4 and 5 where the application of air or other high pressure gas to a passageway 30' leading to the pressure side of each valve membrane in a flow regulation layer or plate causes that membrane to expand, squeezing the liquid in the adjacent region of the microchannel with which it is associated. By programming a control unit so as to operate the three valves in sequence, from left to right, a wave movement is set up whereby the liquid in the entrance region 18' of the microchannel is pumped to the right and through the collection device 17'. If desired, a similar peristaltic-type pumping arrangement 43 is also incorporated into the exit passageway region 45 leading downstream from the collection chamber 17'.

[0095] As another potential alternative, a micromixing arrangement is illustrated in FIGURE 7. A micromixer 51 is illustrated that includes a circular pathway 53 that leads to a supply passageway 55, which could be an entrance passageway leading to a collection chamber in a substrate such as earlier described. A pair of inlet channels 57a and 57b are provided to supply liquids to the circular pathway 53, and liquid flow through the pathways 55, 57a and 57b are controlled via pneumatic valves 59. Three additional pneumatic valves 61 are positioned in the passageway itself and constitute a peristaltic-type pump 63 of the type
previously described. The arrangement provides an efficient way of micro-mixing two liquids in the substrate itself prior to delivery to a collection chamber or the like. For example, by filling the circular pathway 53 with some liquid from one inlet channel 57a and with some buffer from the inlet channel 57b, mixing can then be effected by operating the three valves 61 in sequence to pump the liquid around the ring provided in the circular pathway; thus, the liquid can be thoroughly mixed it before its discharge through a delivery passageway 55.

Example 4: Separation of Fetal Cells in Blood Samples - MACS vs. CEE

[0096] Basic experimental approach:

• Collect 6x10 ml blood per donor

• Prepare Ficoll cell pellet from each 10 ml (contains primarily the nucleated white blood cell component of blood)

• Spike ~50 JEG cells into each cell pellet

• Run 3 on MACS; 3 on CEE; i.e. triplicate runs from same donor

• Both MACS and CEE are coated with EpCAM antibody, commonly used in MACS to capture epithelial cells in blood

• Count recovery of JEG cells from MACS and CEE

• Count background DAPI-positive cells on MACS and CEE

• Repeat with 6 different donors (18 total samples per device)
Results:

Performance

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Conclusions:

- Cumulative CEE capture was ~2X higher than MACS (p<0.0001)
- Overall MACS non-specific capture was ~13X higher than CEE (p<0.0001)
- Specific and non-specific capture variability from Ficoll is high on both procedures, and appears to be largely due to sample handling and not sample variability

The disclosures of all US patents and applications specifically identified herein are expressly incorporated herein by reference.

Particular features of the invention are emphasized in the claims which follow.
WHAT IS CLAIMED IS:

1. A microflow apparatus comprising:
   a body having a randomized flow path which comprises an inlet means, an outlet means, and a microchannel arrangement extending between said inlet and outlet means, wherein said microchannel arrangement includes a plurality of transverse separator posts being integral with a base surface of said microchannel and projecting therefrom, wherein said posts are arranged in a pattern capable of providing said randomized flow path.

2. The apparatus of claim 1, wherein said posts are aligned substantially perpendicular to said base surface.

3. The apparatus of claim 1, wherein said posts are arranged in a randomized pattern generated by a mathematical algorithm.

4. The apparatus of claim 1, wherein said posts are arranged in a randomized pattern generated by a mathematical algorithm using the total number of said posts and the minimum distance between two of said posts.

5. The apparatus of claim 1, wherein said posts have at least two different cross sectional sizes.

6. The apparatus of claim 1, wherein the mean cross sectional size of said posts is related to the size of a target molecule to be flown through said microchannel.

7. The apparatus of claim 1, wherein the cross sections of said posts occupy between about 20% to about 75% of the cross section of said base surface of said microchannel.

8. The apparatus of claim 1, wherein the total volume of said posts is about 15% to about 25% of the total volume of said microchannel.
9. The apparatus of claim 1, wherein the minimum distance between two said posts is related to the smallest cross sectional size of said posts.

10. The apparatus of claim 1, wherein the surface of said microchannel is coated with a hydrophilic layer.

11. The apparatus of claim 1, wherein the surface of said microchannel is coated with a hydrophilic layer of at least about 1 micron thick comprising isocyanate-functional polymer of PEG, PPG, or a copolymer thereof.

12. The apparatus of claim 1, wherein the surface of said microchannel is coated with a sequestering agent.

13. The apparatus of claim 1, wherein the surface of said microchannel is coated with a sequestering agent selected from the group consisting of antibody, antigen, receptor, ligand, oligonucleotide, and peptide.

14. The apparatus of claim 1, wherein a sequestering agent is coupled to the surface of said microchannel by a linker.

15. The apparatus of claim 1, wherein a sequestering agent is coupled to the surface of said microchannel by a hydrophilic linker or a layer of hydrogel.

16. The apparatus of claim 1 wherein said inlet means includes a well capable of holding a liquid sample.

17. The apparatus of claim 1 wherein said microchannel is sealed with a plate affixed to the free ends of said posts.

18. The apparatus of claim 1, wherein said microchannel comprises an optically transparent base surface and can be viewed by optical detection means.

19. The apparatus of claim 1, wherein said microchannel is sealed with a plate affixed to the free ends of said posts and wherein said base surface of said microchannel and said plate are optically transparent.
20. A kit comprising the apparatus of claim 1 and an instruction for coating the surface of said microchannel with a sequestering agent.

21. A kit comprising the apparatus of claim 1, wherein the surface of said microchannel is coated with a sequestering agent.

22. A method of capturing a target molecule in a sample comprising causing a body of liquid containing said sample to flow through said microchannel of the apparatus of claim 1, wherein the surface of said microchannel is coated with a sequestering agent capable of binding to the target molecule.

23. A method of detecting a target molecule in a sample comprising:
   causing a body of liquid containing said sample to flow through said microchannel of the apparatus of claim 1, wherein the surface of said microchannel is coated with a sequestering agent capable of binding to the target molecule and
   detecting the target molecule.

24. The method of claim 23, wherein the target molecule is a cell associated with a condition.

25. The method of claim 23, wherein the target molecule is a cancer or tumor cell.

26. The method of claim 23, wherein the target molecule is a fetal cell.

27. The method of claim 23, wherein the target molecule is a target cell and the ratio of number of target cells and number of total cells in the sample is at least 1:107, 1:108, or 1:109.

28. The method of claim 23, wherein the body of liquid containing said sample flows through said microchannel at a speed of about 0.5 mm to about 5 mm per second.
X = Y = NCO, NCS
R = (CH₂CH₂O)ₙ
where n = 2 - 200
R = Three Dimensional Polymer, including but not limited to Hydrogel, Nucleic Acids, Peptides, Proteins and Enzymes
X = COOH, CHO, NHS, ICH₂CO-
Y = Maleimido, SH, Halo-CH₂CO-

FIG. 9
Schematics of Coating of Channels with Antibodies

FIG. 10

SUBSTITUTE SHEET (RULE 26)
ATTACH ABS TO POSTS
IN CHANNELS

FLOW MATERNAL BLOOD
THROUGH CHANNEL

WASH TO REMOVE
NON-SPECIFICALLY BOUND CELLS

RELEASE TARGET CELLS
FROM ABS ON POSTS

COLLECT RELEASED
TARGET CELLS

FIG. 11