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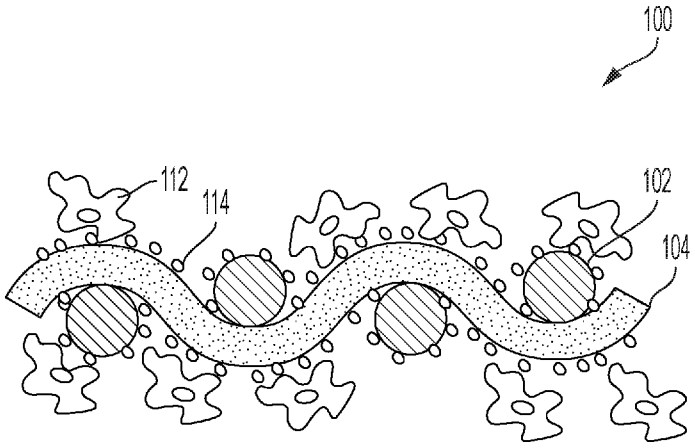


FIG. 2

(57) Abstract: A bioreactor and bioreactor system for mass production of engineered T-cells is provided. The bioreactor has a matrix comprising either a single type of mesh or more types of mesh, and may be packed in the bioreactor in a stacked or rolled configuration. The bioreactor allows for the binding, activation, and transduction of patient-derived T-cells, and then expansion of the resultant engineered T-cells, all in a single bioreactor. The bioreactor system includes the bioreactor and further has a cell culture conditioning vessel having conditioned cell culture media and may further comprise a cell culture media feed vessel having fresh cell culture media. The configuration of the bioreactor and the bioreactor system solves the problem of efficient large-scale and commercial production of engineered T-cells, reducing the time, cost, and contamination risks associated with current engineered T-cell production technologies.

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PACKED BED REACTORS FOR ENGINEERED T-CELL PRODUCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119 of U.S. Provisional Application Serial No. 63/601,910 filed on November 22, 2023, the content of which is relied upon and incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure relates to cell culture systems for engineered T-cell manufacturing, including therapeutic lines such as high efficiency chimeric antigen receptor-modified T-cells (CAR-T cells) and engineered T-cell receptor cells (TCR-T cells).

BACKGROUND

[0003] For the past decade, immune cell therapy has emerged as a new promising treatment option for cancer patients. In 2017, the Food and Drug Administration (FDA) made historical approvals of two chimeric antigen receptor-modified T cells (CAR-T cells) therapies for blood cancers. Currently, tests are also being run with engineered T-cell receptor cells (TCR-T cells) that target certain solid tumors. Both types of engineered T-cell therapies use T-cells that are genetically altered in a lab to enable them in locating in destroying cancer cells more effectively.

[0004] Currently, there are over 300 CAR-T clinical trials running around the world. Many clinical trials have shown very encouraging results. Although engineered T-cell technology is very promising, the cost of therapy for patients remains cost prohibitive. The cost is estimated to be about $350K for a single treatment, not including hospital stays and other related expenses. The primary driver of the high cost for these life-saving treatments is primarily due to inefficiency and costly manufacturing processes experienced with current technologies for engineered T-cell therapies. The present disclosure provides a solution to the current inefficiencies in the manufacturing process for engineered T-cells by providing a novel fixed-bed bioreactor system to make engineered T-cell manufacturing more efficient and more cost effective.

SUMMARY

[0005] According to some aspects of the present disclosure, a bioreactor is provided that comprises an inlet and an outlet and a housing vessel comprising a matrix. The matrix comprises a mesh substrate having individual fibers or fiber bundles configured for binding, activating, transducing, or culturing T-cells. The bioreactor may do one or more of binding, activating, transducing, and culturing T-cells, and it may do all of these actions. In some embodiments, these steps all occur in the same bioreactor.

[0006] According to some aspects of the present disclosure, the matrix comprises mesh substrate packed in a stacked layer and in other aspects, the matrix comprises mesh substrate packed in a roll. In some aspects, the matrix comprises at least two types of mesh substrate, where the first mesh substrate is layered with the second mesh substrate. In some aspects, the different types of mesh substrate are packed together in a stack and in other aspects, the different types of mesh substrate are packed together in a roll.

[0007] According to some aspects of the present disclosure, the mesh substrate is comprised of individual fibers and the individual fibers have a fiber thickness from about 5 μm to about 200 μm. In other aspects, the mesh substrate is comprised of fiber bundles that create each weave of the mesh and the individual fiber bundles each have a thickness from about 5 μm to about 200 μm.

[0008] According to some aspects where at least two different types of mesh substrate are used, the individual fibers of the first mesh substrate each have a thickness of about 5 μm to about 30 μm, and the individual fibers of the second mesh substrate each have a thickness of about 50 μm to about 200 μm. In some embodiments, the individual fibers of the first mesh substrate each have a thickness of about 5 μm to about 20 μm, and the individual fibers of the second mesh substrate each have a thickness of about 100 μm to about 160 μm.

[0009] According to some aspects of the present disclosure, the bioreactor further comprises a central support member. In some embodiments, the mesh substrate is attached to the central support member.

[0010] According to some aspects of the present disclosure, the mesh substrate further comprises a coating that binds and activates CD3+ T-cells. In some embodiments, the coating comprises anti-CD3 antibody, anti-CD28 antibody, anti-CD19 antibody, or a combination thereof. In some embodiments, when this type of coating is on the mesh substrate, the mesh substrate reactivates T-cells during proliferation of engineered T-cells.

[0011] According to some aspects of the present disclosure, the mesh substrate further comprises a coating that improves the efficiency of transduction of viral vectors into the T-cells. In some embodiments, the coating comprises a recombinant fibronectin fragment. Recombinant fibronectin fragments include ones such as a 63 kDa recombinant fragment from human fibronectin, including commercially available options such as RetroNectin® from Taka Bio USA, Inc.

[0012] According to some aspects of the present disclosure, a bioreactor system is provided that comprises a bioreactor and a cell culture media conditioning vessel. The bioreactor comprises a housing vessel, an inlet, an outlet, and a matrix. The matrix comprises a first mesh substrate that binds CD3+ T-cells. In some aspects, the bioreactor system comprises a pump between the bioreactor and the cell culture media conditioning vessel. In some aspects, the bioreactor system comprises a media refeed vessel.

[0013] In some aspects, the matrix further comprises a second mesh substrate that binds CD3+ T-cells. When the matrix has at least two types of mesh substrates, in some embodiments, the first and second mesh substrates are packed as layers alternating between the first mesh and the second mesh. In some aspects, the first mesh substrate has an individual fiber thickness from about 5 μm to about 20 μm. In some aspects, the first mesh substrate has an individual fiber thickness from about 5 μm to about 30 μm and the second mesh substrate has an individual fiber thickness from about 50 μm to about 200 μm. In some embodiments of these aspects, the first and second mesh have a coating comprising antibodies chosen from anti-CD3, anti-CD28, and anti-CD-19, or a combination thereof. In some embodiments, the first mesh substrate has a coating that binds CD3+ T-cells, and the second mesh substrate has a coating different from the coating on the first mesh that binds CD3+ T-cells.

[0014] According to some aspects of the present disclosure, a method of producing engineered T-cells is provided that comprises the steps of (a) providing the bioreactor of claim 1; (b) providing CD3+ T-cells to the bioreactor; (c) binding the CD3+ T-cells to the mesh substrate; (d) activating the CD3+ T-cells in the bioreactor; (e) transducing the activated CD3+ T-cells in the bioreactor; (f) culturing engineered T-cells from the transduction in the bioreactor; and (g) harvesting the cultured engineered T-cells from the bioreactor. The method of producing engineered T-cells generates viable engineered T-cells from at least 70% of the engineered T-cells that are generated from the bioreactor. In some aspects, the step of providing CD3+ T-cells to the bioreactor comprises providing blood having CD3+ T-cells in the blood. In some embodiments, the blood is patient-derived blood.

[0015] Additional features and advantages will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments as described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0016] It is to be understood that both the foregoing general description and the following detailed description are merely exemplary and are intended to provide an overview or framework to understanding the nature and character of the claims. The accompanying drawings are included to provide a further understanding and are incorporated in and constitute a part of this specification. The drawings illustrate one or more embodiment(s), and together with the description serve to explain principles and operation of the various embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The following is a description of the figures in the accompanying drawings, given purely by way of non-limiting example. The figures are not necessarily to scale, and certain features and certain views of the figures may be shown exaggerated in scale or in schematic in the interest of clarity and conciseness.

[0018] FIG. 1A shows a perspective view of a three-dimensional model of a mesh substrate, according to embodiments.

[0019] FIG. 1B is a two-dimensional plan view of the substrate of FIG. 1A, according to embodiments.

[0020] FIG. 1C is a cross-sectional view of the substrate in FIG. 1A. according to embodiments.

[0021] FIG. 1D a cross-sectional view of the substrate in FIG. 1A indicating a height h_1 and widths w_1 and w_2 between peaks and troughs of the substrate, according to embodiments.

[0022] FIG. 2 is a schematic showing a cross-sectional view of a surface-functionalized substrate that assists in T-cell binding to the substrate, according to embodiments.

[0023] FIG. 3 is a perspective view of an exemplary bioreactor with a matrix having stacked substrate, according to embodiments.

[0024] FIG. 4 is an exploded view of an exemplary bioreactor with a matrix having a stacked substrate, according to embodiments.

[0025] FIG. 5 is an exploded view of a matrix having a rolled substrate according to embodiments.

[0026] FIG. 6 is a cross-sectional view of a bioreactor with a matrix having a rolled substrate and a support member, according to embodiments.

[0027] FIG. 7 is a schematic of a bioreactor system for engineered T-cell production, according to embodiments.

[0028] FIG. 8 is a schematic of a bioreactor system for engineered T-cell production, according to embodiments.

DETAILED DESCRIPTION

[0029] The various aspects and embodiments will now be fully described herein. These aspects and embodiments may, however, be embodied in many different forms and should not be construed as limiting; rather, these embodiments are provided so the disclosure will be thorough and complete, and will fully convey the scope of the present subject matter to those skilled in the

art. All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

[0030] Modifications of the disclosure will occur to those skilled in the art and to those who make or use the disclosure. Therefore, it is understood that the embodiments shown in the drawings and described above are merely for illustrative purposes and not intended to limit the scope of the disclosure, which is defined by the following claims, as interpreted according to the principles of patent law, including the doctrine of equivalents.

A. Definitions

[0031] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, particular methods and materials are now described.

[0032] As used herein the terms "the," "a," or "an," mean "at least one," and should not be limited to "only one" unless explicitly indicated to the contrary. Thus, for example, reference to "a component" includes embodiments having two or more such components unless the context clearly indicates otherwise.

[0033] Unless otherwise stated, the use of individual numerical values are stated as approximations as though the values were preceded by the word "about" or "approximately." Similarly, the numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word "about" or "approximately." In this manner, variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. As used herein, the terms "about" and "approximately" when referring to a numerical value shall have their plain and ordinary meanings to a person of ordinary skill in the art to which the disclosed subject matter is most closely related or the art relevant to the range or element at issue. The amount of broadening from the strict numerical boundary depends upon many factors. For example, some of the factors which may be considered include

the criticality of the element and/or the effect a given amount of variation will have on the performance of the claimed subject matter, as well as other considerations known to those of skill in the art. As used herein, the use of differing amounts of significant digits for different numerical values is not meant to limit how the use of the words "about" or "approximately" will serve to broaden a particular numerical value or range. Thus, as a general matter, "about" or "approximately" broaden the numerical value. Also, the disclosure of ranges is intended as a continuous range including every value between the minimum and maximum values plus the broadening of the range afforded by the use of the term "about" or "approximately." Consequently, recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, and each separate value is incorporated into the specification as if it were individually recited herein.

[0034] As used herein, the term "and/or," when used in a list of two or more items, means that any one of the listed items can be employed by itself, or any combination of two or more of the listed items can be employed. For example, if a composition is described as containing components A, B, and/or C, the composition can contain A alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

[0035] As used herein, "have," "having," "include," "including," "comprise," "comprising" or the like are used in their open ended sense, and generally mean "including, but not limited to."

[0036] "Optional" or "optionally" means that the subsequently described element, component or circumstance may or may not occur, so that the description includes instances where the element, component, or circumstance occurs and instances where it does not.

[0037] In this document, relational terms, such as first and second, top and bottom, and the like, are used solely to distinguish one entity or action from another entity or action, without necessarily requiring or implying any actual such relationship or order between such entities or actions.

[0038] All scientific and technical terms used herein have meanings commonly used in the art unless otherwise specified. The definitions provided herein are to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

B. Introduction

[0039] The present disclosure provides a more efficient and cost-effective manufacturing process for mass production of engineered T-cells than currently exists. Engineered T-cell manufacturing generally includes the following four steps: (a) CD3+ T-cell isolation, (b) T-cell gene viral transduction (*e.g.*, with CAR or TCR genes), (c) T-cell activation, and (d) engineered T-cell culturing (proliferation). CD3+ T-cells are T-cells that have a CD3+ surface marker on the outside of the T-cell. CD3+ T-cell complexes are lymphocytes that assist in targeting and destroying foreign material that harms a living body. To be used for engineered T-cell manufacturing, CD3+ T-cells are first extracted from a patient through blood draws that are then purified to isolate the CD3+ T-cells. This relates to step (a) in the manufacturing process. To become engineered T-cells, the CD3+ T-cells are then genetically engineered to incorporate genes such as chimeric antigen receptor (CAR) or T-cell receptor (TCR) so the engineered T-cells can make those receptors on their surfaces. This process is accomplished by transduction of the activated cells with a viral vector that comprises the desired gene to be expressed (*e.g.*, CAR, TCR) for the specific type of cancer, which relates to step (b) in the manufacturing process. The engineered T-cells are then activated by anti-CD3 and anti-CD28 monoclonal antibodies. This relates to step (c) in the manufacturing process. The activated, engineered T-cells can then recognize and bind to cancer cells of a specific type, and they can also proliferate. Finally, to create a therapeutic amount of engineered T-cells, the engineered T-cells must be multiplied in large quantities (relates to step (d) in the manufacturing process) for a single treatment that goes back into the patient whose blood gave the original CD3+ T-cells.

[0040] The current technologies for engineered T-cell production are inefficient for manufacturing large quantities of engineered T-cells and result in extremely high treatment costs as a result. For example, the most recent FDA approved CAR-T cell treatment costs upwards of $450K per treatment. The present disclosure solves the inefficiency problem by providing a novel packed bed reactor system that allows each of the four identified manufacturing steps to be

performed in a single device. The packed bed reactor system of the present disclosure further provides a fixed bed with a large surface area and uniform flow to provide efficient isolation of T-cells. It further has unique surface functionality to increase antibody adsorption and preserve the antibody configuration and activity for effective activation of T-cells. In conventional engineered T-cell production systems, T-cell activation is done with beads or particle-based activation. These beads and particles can be internalized into the cells, and can end up in drug products, which are problematic for drug safety. The present invention's use of mesh for T-cell activation instead of beads and particles prevents the problems of internalization experienced by activation with beads and particles. Further yet, the present disclosure improves T-cell activation by providing an improved surface curvature in the packed bed reactor system. The uniform flow for the fixed bed reactor provided by the system. The invention of the present disclosure further eliminates contamination and operator error issues that plague current manufacturing processes. It also provides a perfusion system that provides better T-cell expansion and easy harvesting at the end of the culturing process.

[0041] Additional features and advantages will be set forth in the detailed description which follows and will be apparent to those skilled in the art from the description or recognized by practicing the embodiments as described in the following description, together with the claims and appended drawings.

C. Substrates for the Reactors

[0042] The bioreactor for the system comprises a substrate that captures CD3+ T-cells for further processing, and that assists during the steps of activating CD3+ T-cells, transduction of activated T-cells into engineered T-cells, and culturing (proliferating) of engineered T-cells. The substrate can be fabricated from monofilament or multifilament fibers of polymeric materials that are compatible in cell culture applications, including, for example, polystyrene, polyethylene terephthalate, polycarbonate, polyvinylpyrrolidone, polybutadiene, polyvinylchloride, polyethylene oxide, polypyrroles, and polypropylene oxide. In one specific embodiment, the material for the substrate comprises polyethylene terephthalate (PET). In some embodiments, the substrate is a polymer-based material, and can be formed as a molded polymer sheet; a polymer

sheet with openings punched through the thickness; a number of filaments that are fused into a mesh-like layer; or a 3D-printed substrate.

[0043] Embodiments of the present disclosure comprise a substrate having defined and ordered structure. This is in contrast to substrates used in cell culture bioreactors with an undefined or unordered structure (*i.e.*, non-woven substrates of randomly ordered fibers), which are typically used in cell culture bioreactors. The defined and ordered structure allows for consistent and predictable cell culture results. In addition, the substrate has an open porous structure that prevents cell entrapment and enables uniform flow through the packed bed. This construction enables improved activation of CD3+ T-cells, genetic engineering of CD3+ T-cells with genes for selected receptors nutrient delivery through, genetically engineered T-cell growth, and cell harvesting. The substrate has a plurality of holes or openings. In some embodiments, the substrate may be arranged in a thin, sheet-like construction having first and second sides separated by a relatively small thickness, such that the thickness of the sheet is small relative to the width and/or length of the first and second sides of the substrate. The substrate material is of a size and geometry that allows T-cells to adhere to antibodies on the substrate material or to be trapped by the substrate material, while also allowing adequate fluid flow around the substrate material and through the openings. The physical structure of the substrate generates a high surface-to-volume ratio for culturing T-cells. According to various embodiments, the substrate can be arranged or packed in a bioreactor in certain ways discussed in the present disclosure for uniform seeding, and improved activation, growth and harvesting.

[0044] In some embodiments, the substrate may comprise mesh. The mesh substrate may have patterns or weaves, including, for example knitted, warp-knitted, or woven. In some embodiments, mesh may be woven. Examples of weave types include plain woven, plain dutch, twill, dutch twill, and five needle weave. In some embodiments, the mesh may instead be extruded, oriented, expanded or tubular. In other embodiments, the mesh may be knitted, welded, expanded, wintered, photochemically etched, coiled, or electroformed.

[0045] For embodiments comprising a mesh substrate, the mesh substrate may comprise a mesh size, a linear fiber density and an opening area size. The mesh size (also called mesh count) is the number of openings per linear inch, as measured from the center of one fiber to the center of

an adjacent fiber. The opening area is the space between parallel fibers that are adjacent to each other. The linear fiber density is the density of the fiber per length of mesh. When a single type of mesh is employed, the mesh substrate comprises a plurality of fibers having individual fiber thicknesses of between about 5 μm and about 200 μm. In one embodiment, a single type of mesh is employed, and the mesh substrate comprises a plurality of fibers having individual fiber thicknesses of between 5 μm and 50 μm.

[0046] Two or more different types of meshes may be used as substrates in the matrix, which is a mixed mesh matrix. The types of mesh used for a mixed mesh matrix may be of different materials, different surface functionalities, different fiber diameters or opening sizes, different mesh sizes, different linear fiber densities, different curvatures for strands of fibers or bundles, or any combination thereof. In some embodiments, a matrix comprises two mesh types, wherein a first mesh substrate is layered onto a second mesh substrate and wherein the first mesh substrate is not the same as the second mesh substrate. In one embodiment, a first mesh substrate has individual fiber thicknesses and/or opening sizes (distance between two parallel fibers in a mesh) that are different from a second mesh substrate. The difference in sizes of the individual fibers of the two mesh substrates allows T-cell binding and activation to occur while simultaneously providing enough space for cells and media to pass through the substrate.

[0047] FIGS. 1A and 1B show a three-dimensional (3D) perspective view and a two-dimensional (2D) plan view, respectively, of an exemplary single mesh substrate 100, according to an example of one or more embodiments of this disclosure. The mesh substrate 100 is a woven mesh layer made of a first plurality of fibers 102 running in a first direction and a second plurality of fibers 104 running in a second direction. The woven fibers of the mesh substrate 100 form a plurality of openings 106, which can be defined by one or more widths or diameters (*e.g.*, D_1, D_2). The size and shape of the openings can vary based on the type of weave (*e.g.*, number, shape and size of filaments; angle between intersecting filaments, etc.). A woven mesh may be characterized as, on a macro-scale, a two-dimensional sheet or layer. However, a close inspection of a woven mesh reveals a three-dimensional structure due to the rising and falling of intersecting fibers of the mesh. Thus, as shown in FIG. 1C, a thickness T of the woven mesh 100 may be thicker than the thickness of a single fiber (*e.g.*, t_1). As used herein, the thickness T is the maximum thickness between a first side 108 and a second side 110 of the woven mesh. Without wishing to be bound

by theory, it is believed that the three-dimensional structure of the substrate 100 is advantageous as it provides a large surface area for culturing T-cells, and the structural rigidity of the mesh can provide a consistent and predictable cell culture matrix structure that enables uniform fluid flow.

[0048] In FIG. 1B, the openings 106 have a distance D_1, defined as a distance between opposite fibers 102, and a distance D_2, defined as a distance between opposite fibers 104. D_1 and D_2 can be equal or unequal, depending on the weave geometry. Where D_1 and D_2 are unequal, the larger can be referred to as the major distance, and the smaller as the minor distance. In some embodiments, the distance across an opening may refer to the widest part of the opening. Unless otherwise specified, the opening distance, as used herein, will refer to a distance between parallel fibers on opposite sides of an opening.

[0049] A given fiber of the plurality of fibers 102 has a thickness t_1, and a given fiber of the plurality of fibers 104 has a thickness t_2. In the case of fibers of round cross-section, as shown in FIG. 1C, or other three-dimensional cross-sections, the thicknesses t_1 and t_2 are the maximum diameters or thicknesses of the fiber cross-section. According to some embodiments, the plurality of fibers 102 all have the same thickness t_1, and the plurality of fiber 104 all have the same thickness t_2. In addition, t_1 and t_2 may be equal. However, in one or more embodiments, t_1 and t_2 are not equal such as when the plurality of fibers 102 are different from the plurality of fiber 104. In addition, each of the plurality of fibers 102 and plurality of fibers 104 may contain fibers of two or more different thicknesses (*e.g.*, t_{1a}, t_{1b}, etc., and t_{2a}, t_{2b}, etc.). According to embodiments, the thicknesses t_1 and t_2 are relative to the size of the cells cultured thereon, so that the fibers provide an approximation of a curved surface from the perspective of the cell, which can enable better cell attachment and growth as compared to some other solutions in which the fiber size is large (*e.g.*, much larger than the cell diameter). Due to three-dimensional nature of woven mesh, as shown in FIGS. 1A-1C, the 2D surface area of the fibers available for cell attachment and proliferation exceeds the surface area for attachment on an equivalent planar 2D surface.

[0050] In some embodiments, when a single mesh is used as a substrate, an individual fiber may have a thickness (t_1 or t_2) in a range of about 5 μm to about 200 μm; about 5 μm to about 160 μm ; about 5 μm to about 125 μm ; about 5 μm to about 100 μm; about 5 μm to about 50 μm ; about 5 μm to about 30 μm ; or about 5 μm to about 15 μm. These thicknesses and thickness

ranges are exemplary. Other thicknesses or ranges may be used, but preferably the thickness of the fiber creates a curvature that approximates a cell size that T-cells will bind to. In some other embodiments, when a mixed mesh is used as a substrate, a first mesh substrate may comprise a first plurality of fibers having individual fiber thicknesses (t_{1a} or t_{2a}) of between about 5 μm and about 30 μm. In one specific embodiment, a first mesh substrate comprises a first plurality of fibers having individual fiber thicknesses of between about 5 μm and about 20 μm, or between about 5 μm and about 15 μm. In some embodiments using a mixed mesh, a second mesh substrate comprises a second plurality of fibers having individual fiber thicknesses (t_{1b} or t_2) of between about 50 μm and about 200 μm. In one specific embodiment, a second mesh substrate comprising a second plurality of fibers having individual fiber thicknesses of between about 100 μm and about 160 μm.

[0051] Mesh openings (with either distance D_1 or D_2) may range from about 15 μm to about 400 μm. In some embodiments, the mesh openings may be between about 30 μm to about 200 μm, between about 30 μm to about 100 μm, between about 20 μm to about 75 μm, from about 50 μm to about 150 μm, or any other range between 15 μm to 400 μm. When a mixed mesh is employed, mesh opening sizes in a first mesh may be different than mesh opening sizes in a second mesh. For example, a first mesh substrate may have mesh openings between about 30 μm and 100 μm, while a second mesh substrate may have mesh openings between about 150 μm to 350 μm. In some embodiments, when a mixed mesh is used, a first mesh will have smaller mesh openings and a second mesh will have mesh openings larger than the first mesh.

[0052] The provided ranges of the fiber thickness and opening sizes are examples of some embodiments but are not intended to limit the possible feature sizes of the mesh according to all embodiments. The combination of fiber thickness and opening sizes is chosen based on the desired size of surface area for T-cell binding, activation, and the amount of proliferation anticipated compared to the unit volume of reactor space. For example, when a smaller thickness is employed for individual fibers (*e.g.*, 10 μm for a single type of mesh), the opening size may be one the smaller side (*e.g.*, between 25 μm and 50 μm). As another example, when a larger thickness is employed for the individual fibers (*e.g.*, 100 μm), the opening size may be on the larger side (*e.g.*, between 150 μm to 300 μm).

[0053] In embodiments where the mesh substrate is comprised of fiber bundles, the thickness of each bundle may be the thickness as those described for an individual fiber above. The size of the individual fibers in each bundle may vary so long as the total thickness of the bundle remains the same as those used for the individual fibers above.

[0054] A given individual fiber also has a curvature κ in the mesh along the length of the fiber (in embodiments with a fiber bundle, it is the fiber bundle that has the curvature). The amount of curvature κ at a point in the fiber is affected by the height h between the peaks and troughs of a fiber of the mesh substrate 100, the width w_1 between two adjacent peaks of a fiber of the mesh substrate 100, and the width w_2 between two adjacent troughs of a fiber of the mesh substrate 100, shown in the cross-sectional mesh schematic in FIG. 1D. According to some embodiments, the plurality of fibers 102 all have the same width w_1 and height h_1, and the plurality of fibers 104 all have the same width w_2 and height h_2. In addition, w_1 and w_2 may be equal or h_1 and h_2 may be equal, or both w_1 and w_2 may be equal and h_1 and h_2 may be equal. However, in one or more embodiments, w_1 and w_2, or h_1 and h_2 are not equal such as when the plurality of fibers 102 are different from the plurality of fibers 104. In addition, each of the plurality of fibers 102 and plurality of fibers 104 may contain fibers of two or more different curvatures due to different heights and widths of the peaks and troughs between the fibers (*e.g.*, w_{1a}, w_{1b}, etc., and w_{2a}, w_{2b}, etc. and h_{1a}, h_{1b}, etc. and h_{2a}, h_{2b}, etc.).

[0055] In one specific embodiment, width w_1 is the approximately same as width w_2, and is between 20 μm and 800 μm, between 20 μm and 700 μm, between 20 μm and 600 μm, between 20 μm and 500 μm, between 20 μm and 400 μm, between 20 μm and 300 μm, between 20 μm and 200 μm, or between 20 μm and 100 μm. In another specific embodiment, width w_1 is the approximately the same as width w_2, and is between 200 μm and 800 μm, or between 400 μm and 640 μm. In yet another specific embodiment, width w_1 is the approximately same as width w_2, and is between 20 μm and 90 μm, between 20 μm and 80 μm, between 20 μm and 70 μm, between 20 μm and 60 μm, between 20 μm and 50 μm, or between 20 μm and 40 μm.

[0056] In one specific embodiment, height h_1 is between 7 μm and 400 μm, between 7 μm and 300 μm, between 7 μm and 200 μm, between 7 μm and 100 μm, between 7 μm and 50 μm, or

between 7 μm and 20 μm. In another specific embodiment, height h_1 is between 60 μm and 400 μm, between 60 μm and 350 μm, between 100 μm and 350 μm, or between 120 μm and 320 μm.

[0057] In some embodiments the surface of the substrate is functionalized to help improve interactions between the substrate and either antibodies, cells or both cells and antibodies. Such modifications can be made through the chemical treatment of the polymer material of the mesh substrate or by grafting antibodies or other proteins or protein complexes that promote T-cell binding to the fiber surface. Alternatively, mesh substrate can be coated with a thin layer antibodies that promote T-cell binding including, for example, anti-CD3, anti-CD28, Concanavalin A, or a combination thereof. Alternatively, surfaces of the fibers of the mesh substrate can be rendered with T-cell adhesive properties through the treatment processes with various types of plasmas, process gases, and/or chemicals known in the industry. FIG. 2 shows one embodiment of a surface-functionalized mesh substrate in a cross-sectional view of mesh substrate 100 with a plurality of fibers 102 approximately perpendicular to plurality of fibers 104 that has been surface functionalized with antibodies 114, that help recruit and bind T-cells 112.

D. Engineered T-Cell Reactors

[0058] An engineered T-cell reactor of the present disclosure is a bioreactor comprising a housing vessel, an inlet, an outlet, and matrix having substrate. In certain embodiments, the matrix comprises stacks of layered substrate. In some embodiments, the matrix comprises rolled substrate. The matrix is contained inside the housing vessel. The inlet provides liquid feed into the housing vessel. The outlet disposes of liquid from the housing vessel.

[0059] In some embodiments, the engineered T-cell reactors of the present disclosure can capture and activate CD3+ T-cells with the mesh substrate of the bioreactor. In one embodiment, this is accomplished with monoclonal antibodies attached to the mesh substrate (through coating, covalent attachment, or other technique) which bind to the protein complex CD3 on the surface of CD3+ T-cells (cell attachment) and concurrently activate the CD3+ T-cells. In some embodiments, the activated CD3+ T-cells in the bioreactor are then transduced in the bioreactor. Transduction of the activated T-cells may be accomplished by providing to the bioreactor a plurality of viral vectors containing the gene(s) of proteins or protein complexes desired to be expressed by the activated T-cells. Examples of such genes include, but are not limited to, a CAR gene or a TCR gene. The

transduced T-cells in the bioreactor are considered engineered T-cells and can now target cancer cells in the body of the patient. In some embodiments, engineered T-cells are proliferated in the bioreactor with cell culture media for growing T-cells. Once the desired level of engineered T-cells has been reached, the cells can then be harvested from the bioreactor.

[0060] It should be understood that the steps of purification, activation, transduction, and expansion can all be performed in the bioreactors of the present disclosure, but it is not required. In some embodiments, fewer than all steps may occur with a bioreactor. For example, in some embodiments, purification of T-cells from blood is performed separately from the bioreactor, but the steps of activation, transduction, and expansion occur within the bioreactor. As another example, purification, activation, and expansion may occur within a bioreactor, but transduction occurs separately. In yet another example, purification is followed by transduction, separate from the bioreactor, followed by activation and expansion in the bioreactor. These examples are merely exemplary combinations of steps that can occur in the bioreactor, other combinations of steps may also occur, as understood by those in the art. For example, only activation and transduction may occur in the bioreactor.

[0061] Over time, some activated T-cells may dissociate from the substrate and float or circulate in the bioreactor, or even re-circulate through the bioreactor, while other steps (for example, proliferation) are still being performed. Dissociated T-cells may experience attenuation of at least some of the activities that were activated. In some embodiments, T-cells with attenuated activity are re-activated to their activated state when they re-bind to the substrate in the bioreactor. Conventional bioreactor system for engineered T-cell production are unable to re-activate T-cells because the activation materials (*e.g.*, beads and particles) have to be removed after the activation step to prevent contamination of the drug product (the proliferated engineered T-cells). This is a significant advantage of the present disclosure.

[0062] In general, the flow direction of fluid in the bioreactor may be configured in different orientations, including flow that is approximately perpendicular to substrate layers, or flow that is approximately parallel to substrate layers. In an exemplary embodiment of a bioreactor 300 having housing vessel 302, shown in FIG. 3, the bulk flow direction is in a direction from an inlet 310 to an outlet 312, and, in this example, the first and second sides of layers of substrates

308 in a stacked configuration are perpendicular to the bulk flow direction. In contrast, the example shown in FIG. 4 is of an embodiment having a stacked substrate in which a bioreactor 320 includes a housing vessel 322 and a stack of substrates 328 in layers within a cell culture chamber 324, the substrates having first and second sides that are parallel to a bulk flow direction, which corresponds to a direction shown by the flow lines into inlets 330 and out of outlets 332. Thus, the matrices of embodiments of this disclosure can be employed in either configuration. As depicted in FIG. 3, a matrix 306 comprises stacked substrate 308 and the interior space defined by the cell culture chamber 304. In each of bioreactors 300 and 320, the mesh substrates 308, 328 are sized and shaped to fill the interior space defined by the cell culture chamber 304, 324 so that the culture spaces in each housing vessel are filled for cell growth surfaces to maximize efficiency in terms of cells per unit volume. Although FIG. 4 shows multiple inlets 330 and multiple outlets 332, it is contemplated that the bioreactor 320 may be fed by a single inlet and have a single outlet. Further, although FIGS. 3-4 are examples of a bioreactor having stacked substrate, the flow direction configurations are also applicable to bioreactors having rolled substrate.

[0063] According to various embodiments herein, distribution plates can be used to help distribute the media, cells, or nutrients across a cross-section of the packed bed (either stacked or rolled mesh substrates) and thus improve uniformity of fluid flow through the packed bed. When multiple inlets for a housing vessel are present, the multiple inlets represent how a distribution plate can be provided with a plurality of holes across the packed-bed cross-section for creating more uniform flow.

[0064] Factors such as the fiber thickness, opening size, and weave type/pattern will determine the surface area available in the bioreactor for T-cell attachment and growth of engineered T-cells. In addition, when the matrix for the reactor includes a stack, roll, or other arrangement of overlapping substrate, the packing density of the matrix will impact the surface area of the packed bed matrix. Packing density can vary with the packing thickness (*e.g.*, the space needed for a layer of the substrate) of the substrate material. For example, if a stack of substrate has a certain height, each layer of the stack can be said to have a packing thickness determined by dividing the total height of the stack by the number of layers in the stack. The packing thickness will vary based on fiber thickness and weave but can also vary based the alignment of adjacent layers in the stack. For instance, due to the three-dimensional nature of a woven layer, there is a

certain amount of interlocking or overlapping that adjacent layers can accommodate based on their alignment with one another. In a first alignment, the adjacent layers can be tightly nestled together, but in a second alignment, the adjacent layers can have zero overlap, such as when the lower-most point of the upper layer is in direct contact with the upper-most point of the lower layer. It may be desirable for certain applications to provide a matrix with a lower density packing of layers (*e.g.*, when higher permeability is a priority) or a higher density of packing (*e.g.*, when maximizing substrate surface area is a priority). According to one or more embodiments, the packing thickness can be from about 20 μm to about 1000 μm; about 100 μm to about 750 μm; about 125 μm to about 600 μm; about 150 μm to about 500 μm; about 200 μm to about 400 μm; about 200 μm to about 300 μm. In another embodiment, the packing thickness can be from about 20 μm to about 200 μm, from about 20 μm to about 150 μm, from about 20 μm to about 100 μm, or from about 20 μm to about 50 μm.

[0065] The above structural factors can determine the surface area of a matrix of the bioreactor, whether of a single layer of the substrate or of a matrix having multiple layers of substrate. For example, in one specific embodiment, a single layer of woven mesh substrate having a circular shape and diameter of 6 cm can have an effective surface area of about 68 cm^2. The "effective surface area," as used herein, is the total surface area of fibers in a portion of substrate material that is available for cell attachment and growth. Unless stated otherwise, references to "surface area" refer to this effective surface area. According to one or more embodiments, a single woven mesh substrate layer with a diameter of 6 cm may have an effective surface area of about 50 cm^2 to about 90 cm^2; about 53 cm^2 to about 81 cm^2; about 68 cm^2; about 75 cm^2; or about 81 cm^2. These ranges of effective surface area are provided for example only, and some embodiments may have different effective surface areas.

Stacked Substrate Bioreactors

[0066] A bioreactor is provided, according to one or more embodiments, in which a matrix is used within a culture chamber inside a housing vessel of a bioreactor. In one embodiment, the matrix comprises a stack of layers of mesh substrate. The stack of layers of mesh substrate may comprise a single type of mesh or may comprise two or more types of mesh. FIG. 3 shows an example of a bioreactor 300 that includes a housing vessel 302 having a cell culture chamber 304

in the interior of the housing vessel 302. Within the cell culture chamber 304 is a matrix 306 that comprises a stack of substrate layers 308. The substrate layers 308 are stacked with the first or second side of a substrate layer facing a first or second side of an adjacent substrate layer. The bioreactor 300 has an inlet 310 at one end for the input of media, cells, and/or nutrients into the culture chamber 304, and an outlet 312 at the opposite end for removing media, cells, or cell products from the culture chamber 304. By allowing stacking of substrate layers in this way, the system can be easily scaled up without negative impacts on cell attachment and proliferation, due to the defined structure and efficient fluid flow through the stacked substrates. While the bioreactor may generally be described as having an inlet and an outlet, some embodiments may use one or both of the inlet and outlet for flowing media, cells, or other contents both into and out of the culture chamber. For example, using the embodiment shown in FIG. 3, inlet 310 may be used for flowing media or cells into the culture chamber 304 during cell seeding, activation, perfusion, or culturing phases, but may also be used for removing one or more of media, cells, or cell products through the inlet 310 in a harvesting phase. Thus, the terms "inlet" and "outlet" are not intended to restrict the function of those openings.

[0067] In one or more embodiments, flow resistance and volumetric density of the packed bed can be controlled by interleaving substrate layers of different types, sizes, and/or geometries. In particular, mesh size and geometry (*e.g.*, fiber diameter, opening diameter, and/or opening geometry) define the fluid flow resistance in packed bed format. By interlaying meshes of different types, sizes and/or geometries, flow resistance can be controlled or varied in one or more specific portions of the bioreactor. This will enable better uniformity of liquid perfusion in the packed bed. In one example, a matrix of a bioreactor may comprise a first layer having a first type of mesh below a second layer having a second type of mesh. This layering pattern is continued until the desired matrix height or density has been achieved. When more than one type of mesh is used, the meshes are different from each other. As another example, a matrix of a bioreactor may comprise two layers of a first type of mesh, followed by five layers of a second type of mesh, followed by three layers of a third type of mesh. This layering pattern is continued until the desired matrix height or density has been achieved.

[0068] In some embodiments, mesh substrate (with either a single type of mesh substrate or multiple types of mesh substrates) may be rotated between layers to achieve different fluid flow

resistance and different substrate accessibility patterns than are achieved with a single direction of layering. For example, the matrix may comprise a first layer of a first type of mesh followed by a second layer of the first type of mesh that is rotated 45 degrees relative to the first layer, followed by adding a third layer of the first type of mesh that rotated 45 degrees relative to the second layer, followed by adding a fourth layer of the first type of mesh rotated 45 degrees relative to the third layer. This pattern is continued until the desired packing height or density is achieved for the matrix. In another example, a first layer of a first type of mesh is layered on a second layer of a second type of mesh that is rotated 30 degrees relative to the first layer. This layering pattern is then repeated until the desired packing height or density is achieved for the matrix. Rotation between layers may also prevent nesting, a phenomenon where at least part of one mesh substrate layer sits down into a second mesh layer, causing uneven fluid flow and non-uniform binding sites for T-cells.

[0069] The above examples of layering and rotation are examples only and are used for illustrative purposes without intending to be limiting on the possible combinations. Indeed, various combinations of meshes of different types or adjacent meshes of different orientations are possible to obtain different profiles of volumetric density of cells growth surface and flow resistance. For example, a packed bed column with zones of varying volumetric cells densities or areas of substrate accessibility (*e.g.*, a series of zones creating a pattern of low/high/low/high, etc. densities or substrate accessibility) can be assembled by interleaving meshes of different sizes, types, and/or geometries.

Rolled Substrate Reactor

[0070] A bioreactor is provided, according to one or more embodiments, in which a matrix is used within a culture chamber inside a housing vessel of a bioreactor. In one embodiment, the substrate is packed as roll. Substrate can be packed in a cylindrical roll format within the housing vessel of the bioreactor (see FIGS. 5 and 6). In such an embodiment, scalability of the packed bed bioreactor can be achieved by increasing the overall length of the mesh substrate and its height. The amount of mesh used in the cylindrical roll configuration can vary based on the desired packing density of the packed bed. For example, the cylindrical rolls can be densely packed in a tight roll or loosely packed in a loose roll. The density of packing will often be determined by the

required substrate surface area required for a given application or scale. In one embodiment, the required length of the mesh can be calculated from the packed bed bioreactor diameter by using following formula:

$$L = \frac{\pi\left(R^2 - r^2\right)}{t} \qquad \text{Equation 1}$$

where L is the total length of mesh required to pack the bioreactor, R is the internal radius of packed bed culture chamber, r is the radius of an inner support (support 366 in FIG. 6) around which mesh is rolled, and t is the thickness of one layer of the mesh. In such a configuration, scalability of the bioreactor can be achieved by increasing diameter or width (*i.e.*, W in FIG. 5) of the packed bed cylindrical roll and/or increasing the height (*i.e.*, H in FIG. 5) of the packed bed cylindrical roll, thus providing more substrate surface area for seeding and growing adherent cells.

[0071] When the matrix comprises more than one type of mesh, the mesh may be layered with the desired number of mesh layers before the cylindrical roll configuration is formed. For example, if two mesh types are desired, mesh of one type may be layered onto mesh of a different type and then the stacked layers can be rolled into a matrix having a cylindrical roll configuration. Similarly, if a single type of mesh is desired, but with layers having the mesh oriented differently between the layers, mesh of one type may be layered onto a mesh of the same type but oriented differently, then the stacked layers can be rolled into a matrix having a cylindrical roll. These types of exemplary configuration lessen or remove any nesting effect created by adjacent mesh of the same type. These are examples only and are used for illustrative purposes without intending to be limiting on the possible combinations. Indeed, various combinations of meshes of different sizes are possible to obtain different profiles of volumetric density of cells growth surface and flow resistance.

[0072] FIG. 5 shows an embodiment of a matrix in which the substrate is formed into a cylindrical roll 350. For example, a sheet of a matrix material that includes a mesh substrate 352 is rolled into a cylinder about a central longitudinal axis y. The cylindrical roll 350 has a width W along a dimension perpendicular to the central longitudinal axis y and a height H along a direction perpendicular to the central longitudinal axis y. In one or more preferred embodiments, a matrix comprising a cylindrical roll 350 is designed to be within a housing vessel such that the central

longitudinal axis y is parallel to a direction of bulk flow F of fluid through the bioreactor or culture chamber that houses the cylindrical roll. FIG. 6 shows a cross-section of a bioreactor 360 having a housing vessel 362 that houses a matrix 364 in such a cylindrical roll configuration. Like the cylindrical roll 350 in FIG. 5, the matrix 364 has a central longitudinal axis, which, in FIG. 6, extends into the page. The bioreactor 360 further includes a central support member 366 around which the matrix 364 is positioned. The central support member 366 can be provided purely for physical support and/or alignment of the matrix 364, but can also provide other functions, according to some embodiments. For example, the central support member 366 can be provided with one or more openings for supplying media to the matrix 364 along the length H of the matrix. In other embodiments, the central support member 366 may include one or more attachment sites for holding one or more portions of the matrix 364 at the inner part of the cylindrical roll. These attachment sites may be hooks, clasps, posts, clamps, or other means of attaching the substrate to the central support member 366.

E. Bioreactor System

[0073] The bioreactor system of the present disclosure is designed to manufacture large quantities of engineered T-cells in less steps and less time than conventional systems. In one embodiment, a matrix is provided with at least one substrate having surface area for T-cells to bind, become activated, transduced, and/or proliferate that has good mechanical strength and forms a highly uniform multiplicity of interconnected fluidic networks when assembled in a packed bed or other bioreactor. In particular embodiments, a mechanically stable, non-degradable woven mesh can be used as the substrate to support engineered T-cell production from CD3+ T-cells. The matrix disclosed herein supports attachment, activation, transduction, and/or proliferation of T-cells in a high volumetric density format. When the CD3+ T-cells are seeded directly from blood, the bioreactor system of the present disclosure can purify large quantities of blood due to the large surface areas for binding available in the bioreactors. For example, depending on the concentration of CD3+ T-cells in the blood, even 1L to 5L of blood may be perfused through the bioreactor, and in some embodiments, the blood may be recirculated back through the bioreactor to more effectively capture and seed the bioreactor. Uniform cell seeding of the matricies of the present disclosure is achievable, as well as efficient harvesting of cells or other products of the bioreactor. In addition, the embodiments of this disclosure support cell culturing to provide uniform cell

distribution during the inoculation step and allow multiple steps of the engineered T-cell production to occur in a single vessel, something not possible with current systems for engineered T-cell production. Thus, the matrix also eliminates diffusional limitations during operation of the bioreactor. Further, the matrix enables easy and efficient CAR-T cell production as it allows all steps of the CAR-T production to occur in a single vessel, minimizing loss of cells due to contamination (a problem in current processes requiring transfer of cells between steps of the production) and allowing efficient, large-scale production of CAR-T cells. The structurally defined matrix of one or more embodiments enables complete cell recovery and consistent cell harvesting from the packed bed of the bioreactor.

[0074] FIG. 7 shows an exemplary bioreactor system 400 according to one or more embodiments. The system 400 includes a bioreactor 402 housing the matrix and substrate of one or more embodiments disclosed herein. The bioreactor 402 can be fluidly connected to a media conditioning vessel 404, and the system is capable of supplying a cell culture media 406 within the conditioning vessel 404 to the bioreactor 402. The media conditioning vessel 404 can include sensors and control components found in typical bioreactors used in the bioprocessing industry for a suspension batch, fed-batch or perfusion culture. These include but are not limited to DO oxygen sensors, pH sensors, oxygenator/gas sparging unit, temperature probes, and nutrient addition and base addition ports. A gas mixture supplied to sparging unit can be controlled by a gas flow controller for N_2, O_2, and CO_2 gasses. The media conditioning vessel 404 may also contain an impeller for media mixing. All media parameters measured by sensors listed above can be controlled by a media conditioning control unit 418 in communication with the media conditioning vessel 404, and capable of measuring and/or adjusting the conditions of the cell culture media 406 to the desired levels. As shown in FIG. 7, the media conditioning vessel 404 is provided as a vessel that is separate from the bioreactor 402. This can have advantages in terms of being able to condition the media separate from where the cells are cultured, and then supplying the conditioned media to the area surrounding the substrate. However, in some embodiments, media conditioning can be performed within the bioreactor vessel 402.

[0075] The media 406 from the media conditioning vessel 404 is delivered to the bioreactor 402 via an inlet 408, which may also include an injection port for cell inoculum to seed and begin culturing of cells. The bioreactor 402 may also include one or more outlets 410 through which the

cell culture media 406 exits the bioreactor 402. In addition, cells or cell products may be output through the outlet 410. To analyze the contents of the outflow from the bioreactor 402, one or more sensors 412 may be provided in the line. In some embodiments, the bioreactor system 400 includes a flow control unit 414 for controlling the flow into the bioreactor 402. For example, the flow control unit 414 may receive a signal from the one or more sensors 412 (*e.g.*, an O_2 sensor) and, based on the signal, adjust the flow into the bioreactor 402 by sending a signal to a pump 416 (*e.g.*, peristaltic pump) upstream of the inlet 408 to the bioreactor 402. Thus, based on one or a combination of factors measured by the sensors 412, the pump 416 can control the flow into the bioreactor 402 to obtain the desired cell culturing conditions.

[0076] The media perfusion rate is controlled by the signal processing unit 414 that collects and compares sensors signals from media conditioning vessel 404 and sensors located at the bioreactor outlet 410. Because of the pack flow nature of media perfusion through the bioreactor 402, nutrients, pH and oxygen gradients are developed along the packed bed. The perfusion flow rate of the bioreactor can be automatically controlled by the flow control unit 414 operably connected to the peristaltic pump 416.

[0077] The bioreactor systems of the present disclosure may have other configurations and other set-ups. For example, in one embodiment, a bioreactor system comprises a bioreactor, a matrix housed within the bioreactor, an outlet, an inlet, a media conditioning vessel, and a media refeed vessel. FIG. 8 depicts one example of this embodiment. Bioreactor system 400 has a bioreactor 402 with an inlet 408, an outlet 410, a media conditioning vessel 404, a media refeed vessel 405, and a matrix 450 housed within the bioreactor 402. In FIG. 8, the matrix 402 comprises a rolled substrate. However, the matrix 402 may instead comprise a stacked layer substrate as described earlier above. CD3+ T-cells that have been isolated from a patient may enter the bioreactor through inlet 408. The cells may either enter bioreactor 402 directly through a port (not shown) on the inlet 408, or, as shown in FIG. 8, the cells may be added to a line connected to the inlet through a port (not shown) on the line with an insertion device, such as the syringe 440 shown in FIG. 8. The port may be any type known to those of ordinary skill in the art. Cell culture media 406 may flow through a line connecting media conditioning vessel 404 to inlet 408 on the bioreactor 402. Used media flows out of the outlet 410 and may either be sent back to the media conditioning vessel 404 or optionally may be discarded as media waste (shown as waste bin 411

in FIG. 8). A pump 416 may control the flow of cell culture media 406 from the media conditioning vessel 404, controlled as described earlier. Flow control valves 415 (415*a*, 415*b*, 415*c*) may be employed to control flow to the vessels in the bioreactor system. For example, flow control valve 415*b* may be employed to control the flow between the media refeed vessel 405 and the media conditioning vessel 404. As another example, flow control valve 415*c* may be employed to control the flow from the outlet of the bioreactor 402 back into the media refeed vessel 404. As yet another example, flow control valve 415*a* may be employed to control the flow from the outlet of the bioreactor 402 to the waste bin 411. The flow control valves may operate simultaneously or some may be employed while others are not, and which ones are actively working may vary throughout the manufacturing process.

[0078] In running a production for engineered T-cells, CD3+ T-cells that have entered the bioreactor can disperse throughout the cell culture chamber of the bioreactor and bind to monoclonal antibodies (or other CD3+ T-cell binders) that have been coated onto the substrate in the bioreactor. This binding of the CD3+ T-cells helps purify the CD3+ T-cells when blood is flowed directly into the bioreactor. When activator antibodies such as anti-CD3, anti-CD28, or a combination thereof are used as the monoclonal antibody bound to the substrate, the CD3+ T-cells will bind to the monoclonal antibodies and will also become activated by the antibodies. It should be understood that other activator antibodies such as anti-CD19 and other known to those of ordinary skill in the art may be used as well. If a non-activator-type binder is instead used as an attachment between the CD3+ T-cells and the substrate, the activator may instead be added in a similar manner as the CD3+ T-cells were added after the CD3+ T-cells have bound to the non-activator-type binder, and the activator can then be dispensed in the bioreactor to activate the bound CD3+ T-cells.

[0079] After activation, the activated CD3+ T-cells in the bioreactor may be transduced in the bioreactor. Transduction of the activated T-cells may be accomplished by any transduction method known to those of ordinary skill in the art. For example, viral vectors originating from lentiviruses comprising a gene of desired interest (*e.g.*, CAR, TCR, etc.) may be perfused into the bioreactor. Perfusion flow rates for transduction may be from about 0.1 mL/min to 100 mL/min depending on the size of the bioreactor and the efficiency of the transduction. In one embodiment, the flow rate may be from 0.1 mL/min to about 75 mL/min, from about 0.1 mL/min to about 50

mL/ min, from about 0.1 mL/min to about 25 mL/min, or any other value or range between 0.1 mL/min to 100 mL/min. In some embodiments, the perfusion flow rate for the transduction stage may be set low to assist in transduction of the viral vectors into the CD3+ T-cells. In one embodiment, the transduction perfusion flow rate may be between 0.1 mL/min and 0.5 mL/min. In some embodiments, the transduction perfusion flow rate is between 0.1 mL/min and 1 mL/min, or 0.1 mL/min and 5 mL/min, or 0.1 mL/min and 10 mL/min. Further, in some embodiments, compounds that improve transduction efficiency may be either perfused into the bioreactor or may also be coated on the substrate. For example, compounds such as recombinant fibronectin fragments, including from humans, as known to those skilled in the art to improve viral transfection and transduction may be used. One commercially available example is RetroNectin® (Taka Bio USA, Inc.), a 63 kDa recombinant fibronectin polypeptide from human genes, may be used to improve the efficiency of viral transfection of the T-cells.

[0080] After transduction is complete, the engineered T-cells are proliferated in the bioreactor by adjusting the cell culture media to be media that grows the engineered T-cells, as understood by those of ordinary skill in the art. The engineered T-cells are proliferated through perfusion until the desired level of cells is reached. As described earlier, cells that lose their activation activities (prevalent in conventional methods) can be reactivated during this stage by re-binding to the antibodies coated onto the substrate in the bioreactor. Accordingly, the bioreactor and bioreactor system creates a reactivation mechanism during proliferation using substrate of the present disclosure. In some embodiments, at least 5%, 10%, 20%, 30%, 40%, 50% or more of the engineered T-cells are reactivated during the proliferation stage.

[0081] Embodiments of the present disclosure achieve a viable harvest of cultured cells, as measured by Trypan blue exclusion. In one aspect of the present disclosure, it is possible to harvest viable engineered T-cells from the bioreactor system, including between 70% to 100% viable engineered T-cells, or from about 80% to about 100% viable engineered T-cells, or from about 90% to about 100% viable engineered T-cells, as measured by Trypan blue exclusion. For example, of the engineered T-cells that are harvested, at least 70% are viable, at least 80% are viable, at least 80% are viable, at least 85% are viable, at least 90% are viable, at least 91% are viable, at least 92% are viable, at least 93% are viable, at least 94% are viable, at least 95% are

viable, at least 96% are viable, at least 97% are viable, at least 98% are viable, or at least 99% are viable, as measured by Trypan blue exclusion.

[0082] While the present disclosure includes a limited number of embodiments, those skilled in the art, having benefit of this disclosure, will appreciate that other embodiments can be devised which do not depart from the scope of the present disclosure.

CLAIMS

What Is Claimed Is:

1. A bioreactor, comprising:

an inlet and an outlet; and

a housing vessel comprising a matrix;

wherein the matrix comprises a mesh substrate having individual fibers or fiber bundles configured for binding, activating, transducing, or culturing T-cells.

2. The bioreactor of claim 1, wherein the mesh substrate is configured for binding, activating, transducing, and culturing T-cells in the same bioreactor.

3. The bioreactor of claim 1, wherein the matrix further comprises mesh substrate packed in a layered stack.

4. The bioreactor of claim 1, wherein the matrix further comprises mesh substrate packed in a roll.

5. The bioreactor of claim 1, wherein the matrix further comprises at least two types of mesh substrate, and wherein a first type of mesh substrate of the at least two types of mesh substrate is layered with a second type of mesh substrate.

6. The bioreactor of claim 5, wherein the at least two types of mesh substrate are packed in a stack.

7. The bioreactor of claim 5, wherein the at least two types of mesh substrate are packed in a roll.

8. The bioreactor of claim 1, wherein the individual fibers of the mesh substrate have a fiber thickness from about 5 μm to about 200 μm.

9. The bioreactor of claim 1, wherein the fiber bundles of the mesh substrate have a thickness from about 5 μm to about 200 μm.

10. The bioreactor of claim 5 wherein individual fibers of the first mesh substrate each have a thickness of about 5 μm to about 30 μm and wherein individual fibers of the second mesh substrate each have a thickness of about 50 μm to about 200 μm.

11. The bioreactor system of claim 5, wherein individual fibers of the first mesh substrate each have a thickness of about 5 μm to about 20 μm and wherein individual fibers of the second mesh substrate each have a thickness of about 100 μm to about 160 μm.

12. The bioreactor of claim 7, further comprising a central support member.

13. The bioreactor of claim 12, wherein the mesh substrate is attached to the central support member.

14. The bioreactor of claim 1, wherein the mesh substrate further comprises a coating that binds and activates CD3+ T-cells.

15. The bioreactor of claim 14, wherein the coating comprises anti-CD3 antibody, anti-CD28 antibody, anti-CD19 antibody, or a combination thereof.

16. The bioreactor of claim 15, wherein the mesh substrate reactivates T-cells during proliferation of engineered T-cells.

17. The bioreactor of claim 1, wherein the mesh substrate further comprises a coating that improves the efficiency of transduction of viral vectors into T-cells.

18. The bioreactor of claim 17, wherein the coating comprises a recombinant fibronectin fragment.

19. A bioreactor system, comprising:

a bioreactor comprising a housing vessel, an inlet, an outlet, and a matrix; and

a cell culture media conditioning vessel;

wherein the matrix comprises a first mesh substrate that binds CD3+ T-cells.

20. The bioreactor system of claim 19, further comprising a pump between the bioreactor and the cell culture media conditioning vessel.

21. The bioreactor system of claim 19, wherein the matrix comprises a second mesh substrate that binds CD3+ T-cells.

22. The bioreactor system of claim 20 further comprising a media refeed vessel.

23. The bioreactor system of claim 19, wherein the first and second mesh substrates are packed as layers alternating between the first mesh and the second mesh.

24. The bioreactor system of claim 19, wherein the first mesh substrate has individual fiber thicknesses from about 5 μm to about 20 μm.

25. The bioreactor system of claim 21, wherein the first mesh substrate has a mesh size from about 5 μm to about 30 μm and wherein the second mesh substrate has a mesh size from about 50 μm to about 200 μm.

26. The bioreactor system of claim 19, wherein first and second mesh substrate have a coating comprising antibodies chosen from anti-CD3, anti-CD28, and anti-CD19, or a combination thereof.

27. The bioreactor system of claim 21, wherein the first mesh substrate has a coating that binds CD3+ T-cells, and wherein the second mesh substrate has a coating different from the coating on the first mesh that binds CD3+ T-cells.

28. A method of producing engineered T-cells comprising the steps of:

(a) providing the bioreactor of claim 1;

(b) providing CD3+ T-cells to the bioreactor;

(c) binding the CD3+ T-cells to the mesh substrate;

(d) activating the CD3+ T-cells in the bioreactor;

(e) transducing the activated CD3+ T-cells in the bioreactor;

(f) culturing engineered T-cells from the transduction in the bioreactor; and

(g) harvesting the cultured engineered T-cells from the bioreactor;

wherein at least 70% of the engineered T-cells from the bioreactor are viable.

29. The method of claim 28, wherein the step of providing CD3+ T-cells to the bioreactor comprises providing blood having CD3+ T-cells in the blood.

30. The method of claim 29, wherein the blood is patient-derived blood.

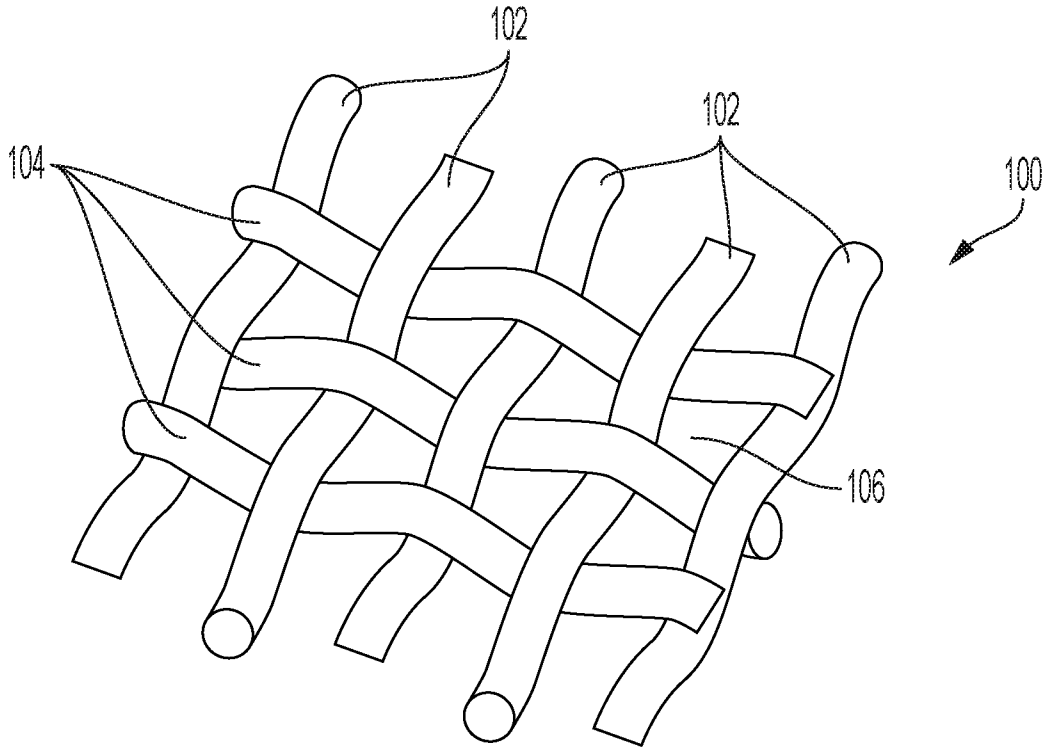


FIG. 1A

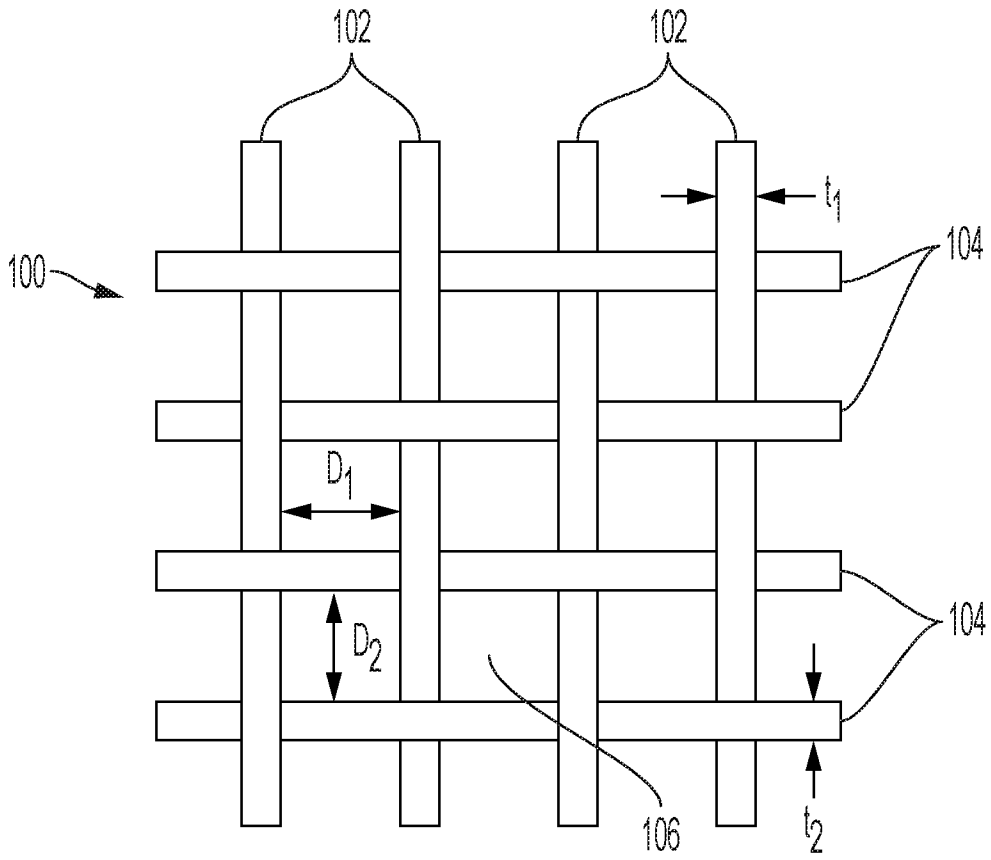


FIG. 1B

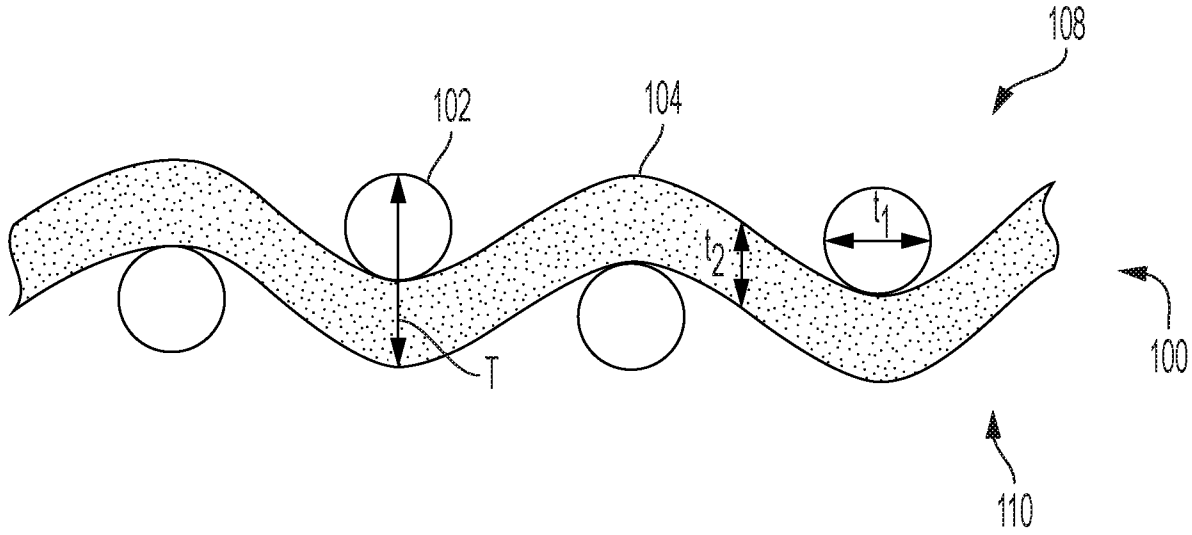


FIG. 1C

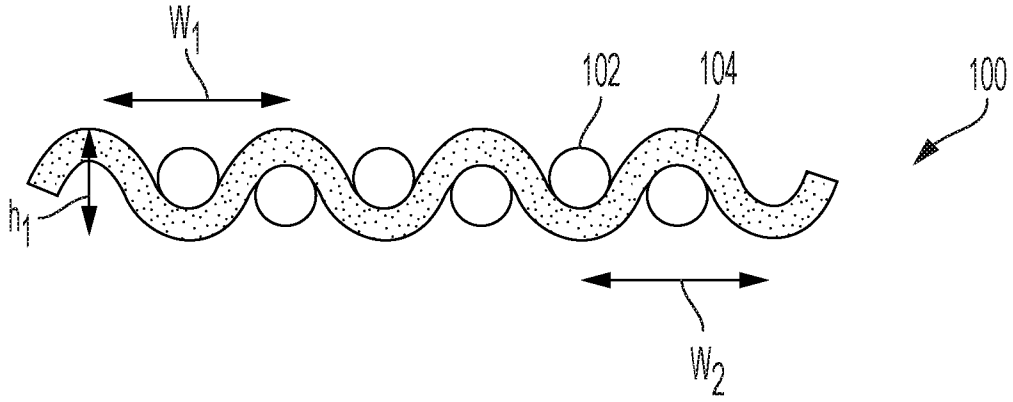


FIG. 1D

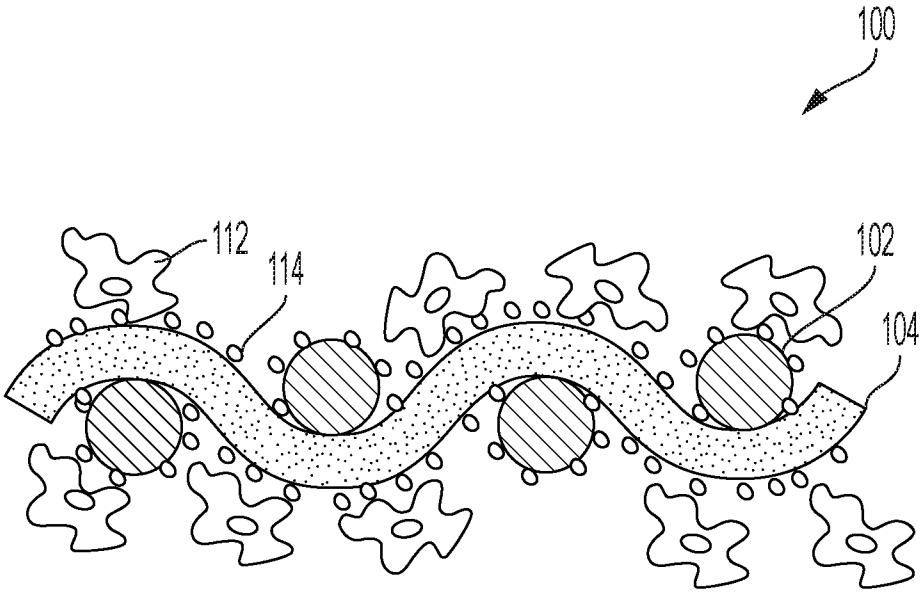


FIG. 2

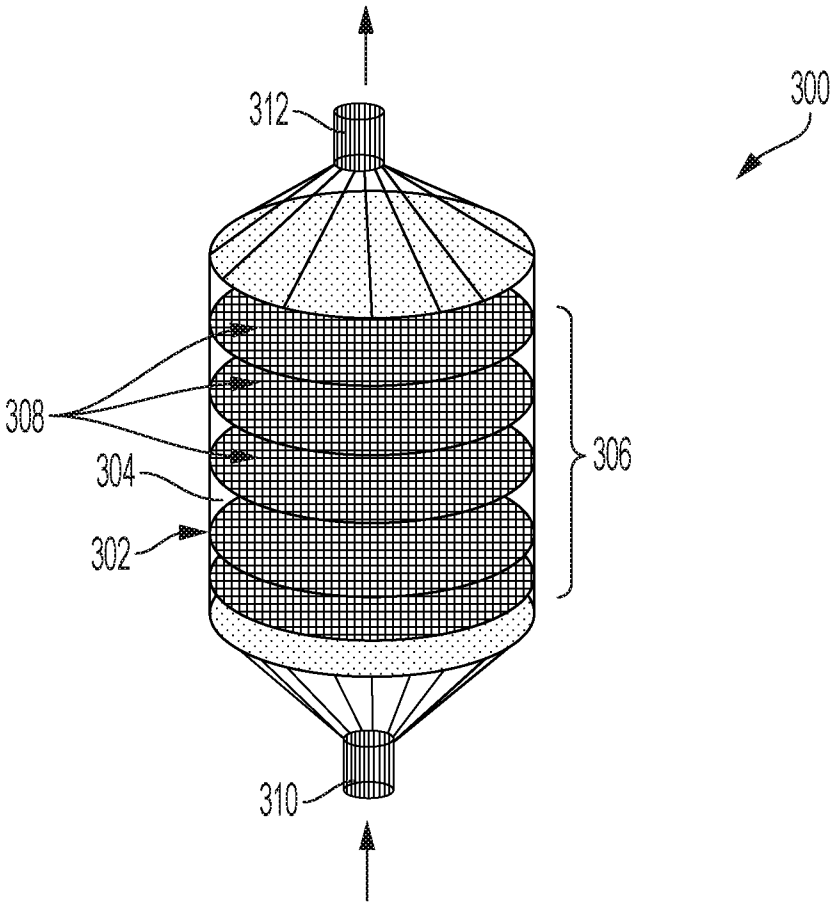


FIG. 3

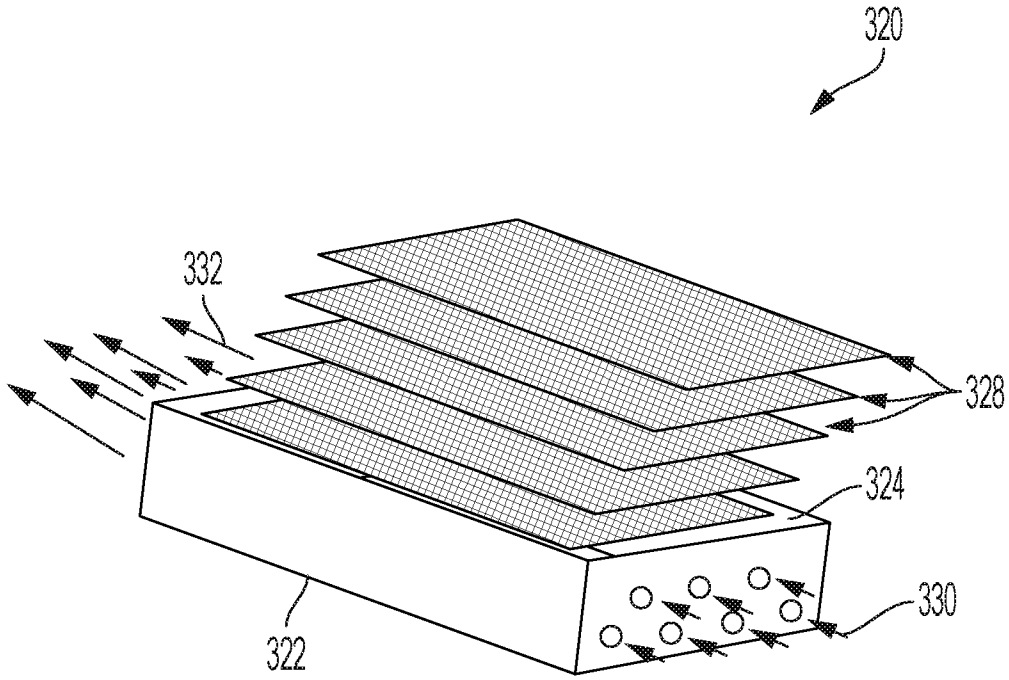


FIG. 4

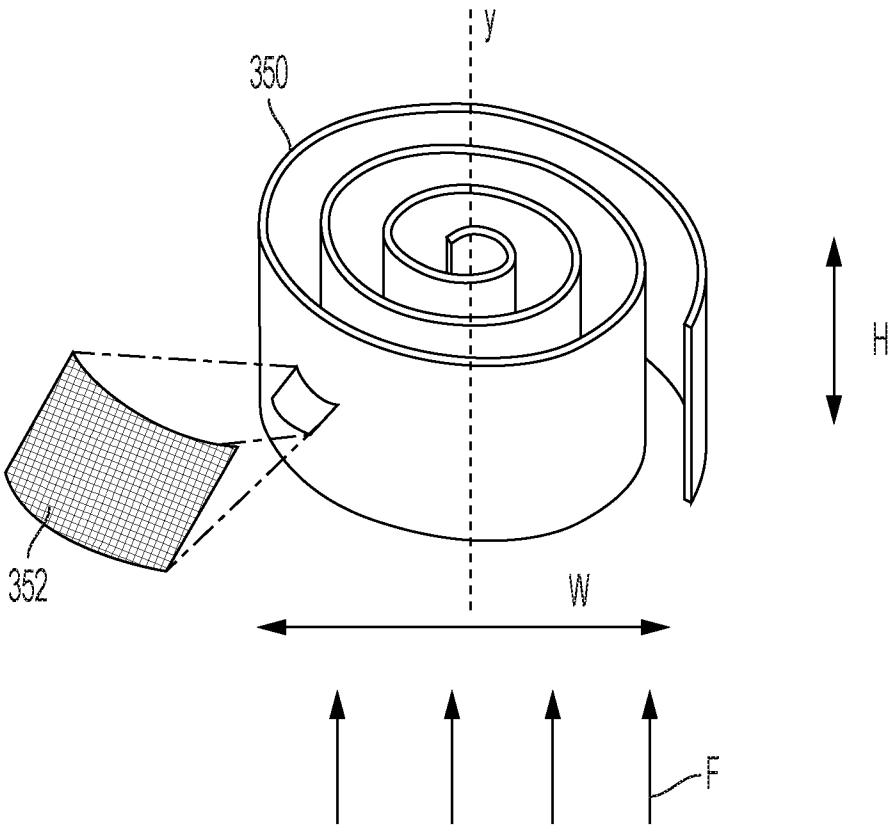


FIG. 5

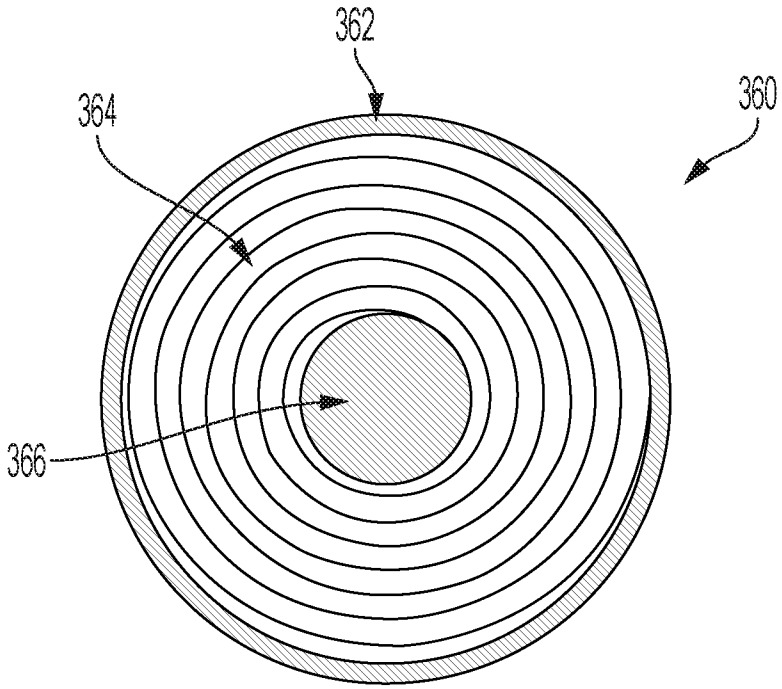


FIG. 6

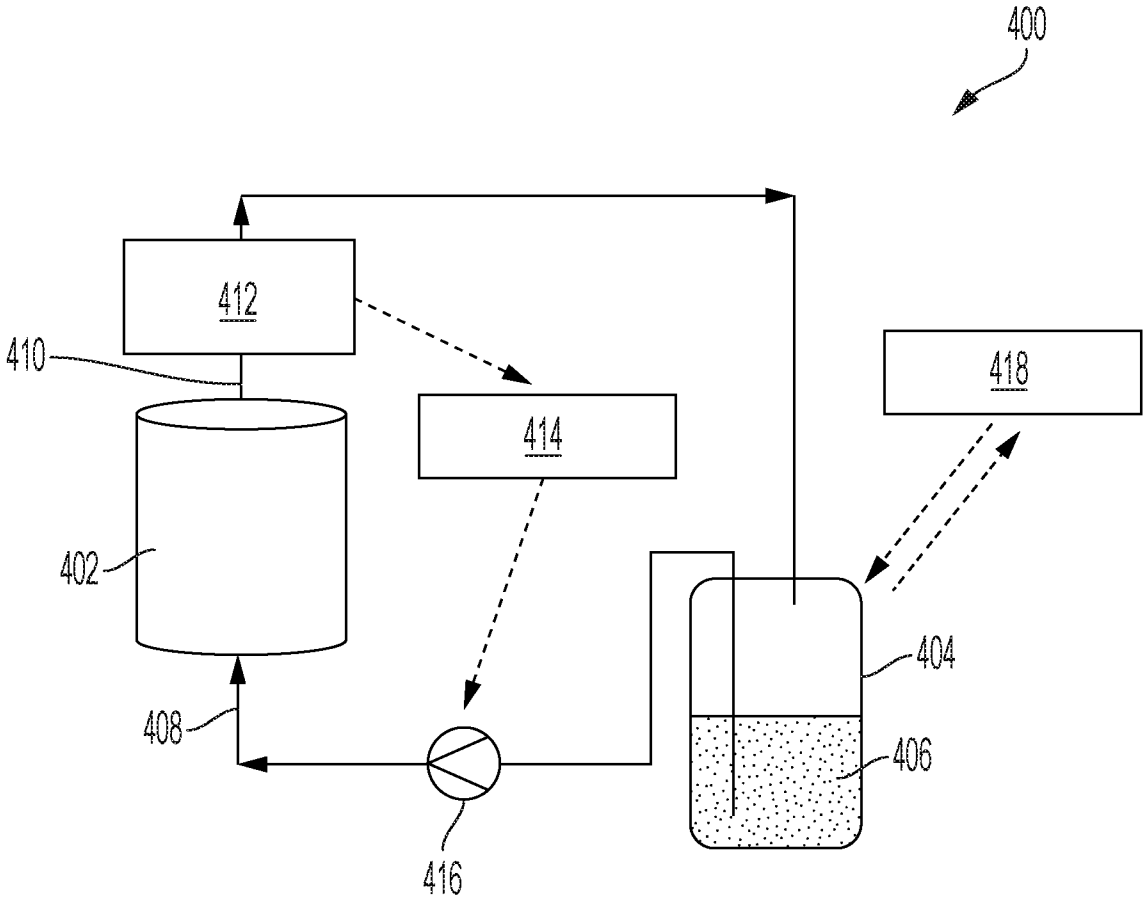


FIG. 7

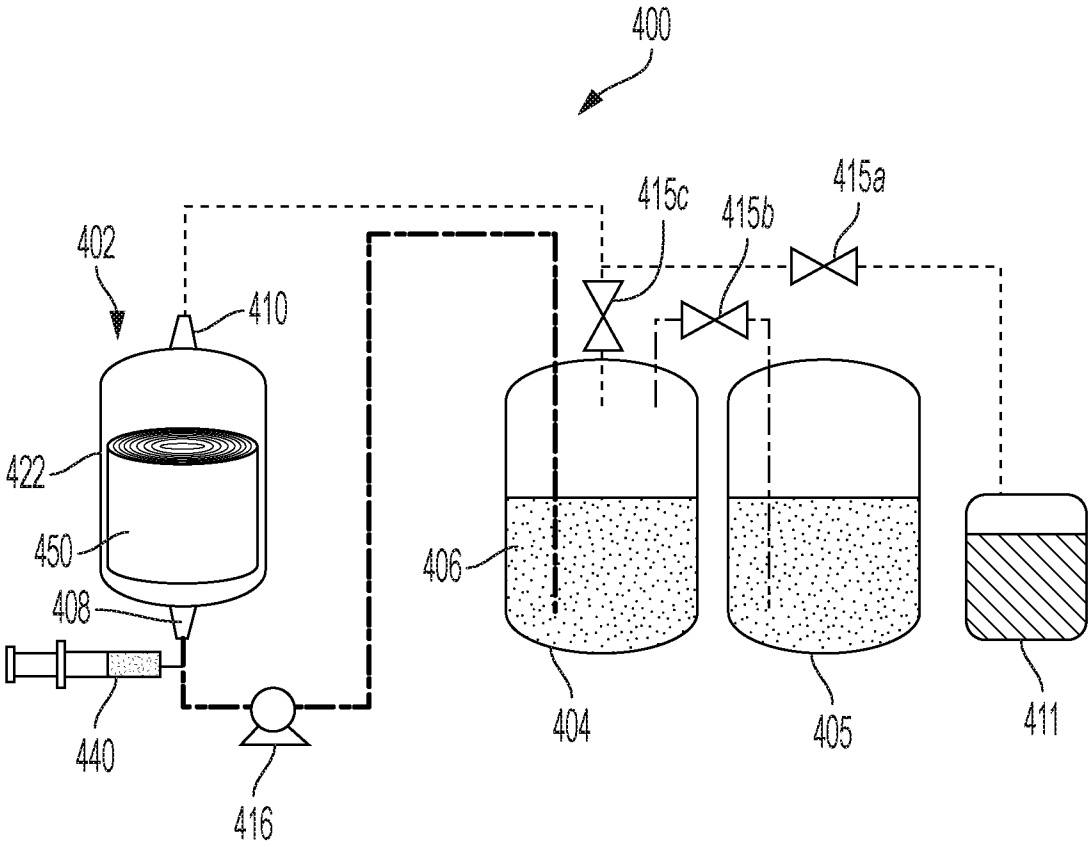


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/055646

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12M1/12 C12M1/42 C12N5/0783
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2023/073620 A1 (AZIZGOLSHANI HESHAM [US] ET AL) 9 March 2023 (2023-03-09) paragraphs [0010], [0077], [0147], [0153], [0156] paragraphs [0166], [0170], [0180], [0181], [0194], [0199] paragraphs [0212], [0213], [0328] figures 1,2,5	1-30
X	CN 113 373 025 A (JIANGSU HOLOSENSOR PHARMACEUTICAL TECH CO LTD) 10 September 2021 (2021-09-10) the whole document	1,2,14, 15,19

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

[x] See patent family annex.

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"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2025	17/03/2025

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

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/055646

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2022/076639 A2 (CORNING INC [US]) 14 April 2022 (2022-04-14) paragraph [0013] paragraph [0067] - paragraph [0072] paragraphs [0075], [0080], [0082] figures 1A,3A,3B,5	5-13, 23-25

A	US 2021/355420 A1 (LING JIAN [US] ET AL) 18 November 2021 (2021-11-18) paragraphs [0004], [0008], [0012], [0022], [0027], [0045], [0046]	1-30

2

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Information on patent family members

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PCT/US2024/055646

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		WO 2021231222 A1	18-11-2021