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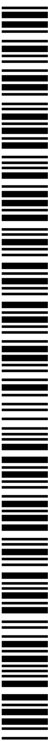

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**(54) Title**: EX VIVO METHODS FOR MINIMIZING RISKS AND MAXIMIZING BENEFITS OF ALLOGENEIC BLOOD AND MARROW TRANSPLANTATION

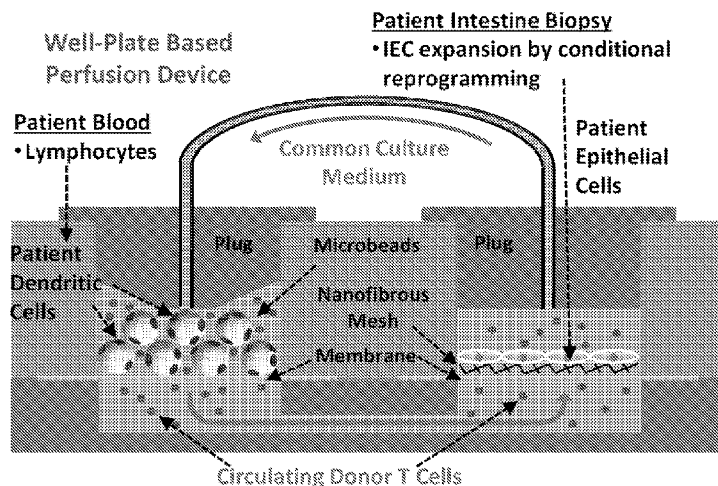


Figure 1

**(57) Abstract**: The described invention provides an ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo; a method for optimizing donor selection for allogeneic blood and marrow transplantation (BMT) therapy; and a method for minimizing risks and maximizing benefits of performing allogeneic blood and marrow transplantation (BMT) therapy in a recipient subject, wherein the recipient subject has a hematologic malignancy.

# EX VIVO METHODS FOR MINIMIZING RISKS AND MAXIMIZING BENEFITS OF ALLOGENEIC BLOOD AND MARROW TRANSPLANTATION

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. provisional patent application serial number 62/101,181, filed January 8, 2015, entitiled "EX VIVO METHODS FOR MINIMIZING RISKS AND MAXIMIZING BENEFITS OF ALLOGENEIC BLOOD AND MARROW TRANSPLANTATION", the content of which is incorporated by reference herein in its entirety.

## FIELD OF INVENTION

[0002] The described invention relates to allogeneic blood and bone marrow transplantation (BMT), ex vivo approaches to minimizing gastrointestinal graft versus host disease prior to BMT, and ex vivo approaches to maximizing graft versus tumor effects.

## BACKGROUND OF THE INVENTION

### Graft-versus-Host Disease (GVHD) Screening and Prevention

[0003] Allogeneic blood and marrow transplantation (allo-BMT) is an effective immunotherapeutic treatment that can provide partial or complete remission for patients with drug-resistant hematological malignancies, including acute myeloid leukemia, lymphoma, and multiple myeloma.

[0004] According to the Center for International Blood & Marrow Transplant Research, 7,000 allo-BMT procedures were performed in the U.S. in 2011 (Pasquini MC, Current use and outcome of hematopoietic stem cell transplantation: CIBMTR Summary Slides, <http://www .cibmtr.org> (2013)). In this procedure, mature donor T cells in the donor inoculum play a central role in mediating graft-versus-tumor (GVT) responses by destroying residual tumor cells that persist after conditioning regimens. However, the full exploitation of this clinical intervention has been greatly limited by the development of graft-versus-host disease (GVHD), a complication that can occur after a stem cell or bone marrow transplant that is caused by donor T cell recognition of alloantigens expressed on the patient's tissue cells, particularly in the lymphoid compartment, intestine, skin, and liver (Ferrara, et al., Lancet (2009) 373, 1550-1561; Sung, et al., Clinical Haematology (2013) 26, 285-292;

Sung, et al., Stem Cells Translational Medicine (2013) 2, 25-32). Even in fully human leukocyte antigen (HLA)-matched transplant settings, alloreactivity towards minor histocompatibility antigens (miHA), which are self peptides presented by HLA molecules, drives both the development of GVT and GVHD effects. However, clinical dissociation and regulation of these two responses to improve transplant outcomes have not yet been achieved. As a result, the global incidence of acute GVHD ranges from 26-34% in fully HLA-matched sibling recipients and 42-52% in HLA-matched, unrelated recipients (Sung, et al., Clinical haematology (2013) 26, 285-292; Jacobs, et al., Bone marrow transplantation (2012) 47, 1470-1473; Jagasia, et al., Blood (2012) 119, 296-307). GVHD of the gastrointestinal (GI) tract is closely associated with non-relapse mortality following allo-BMT (Harris, et al., Clinical Haematology (2012) 25, 473-478).

[0005] Consistent with a 2005 National Institutes of Health (NIH) Consensus Conference, classification of GVHD is based on clinical presentation rather than time of onset. (Pidala, J. et al., "Overlap subtype of chronic graft-versus-host disease is associated with an adverse prognosis, functional impairment, and inferior patient-reported outcomes: a Chronic Graft-versus-host Disease Consortium Study, Haematologica 97(3): 451-458 (2012)).

[0006] Acute GVHD manifestations include erythematosus or maculopapular rash, nausea and vomiting or diarrhea and cholestatic hepatitis, and historically was limited to within 100 days following HCT. Grading for acute GVHD divides acute GVHD into four stages based on the extent of involvement of the skin, liver and gastrointestinal tract. In stage I, there is a skin rash over <25% of the body, bilirubin is measured at 26-60 μmol/L, with a gut fluid loss 500-1000mL/day. In stage II, a skin rash covers 25-50% of the body, the bilirubin is measured at 61-137 μmol/L, and the gut loses from 1000-1500 mL/day. Stage III is characterized by involving >50% of the skin, the bilirubin is measured at 138-257μmol/L, and the gut has lost more than 1500 mL/day. Stage IV is characterized by bullae desquamation (blisters with shedding of epidermal cells) of skin, the bilirubin exceeds >257μmol/L, and the gut fluid loss is >2500 mL/day or ileus (disruption of the normal propulsive ability of the gastrointestinal tract; bowel obstruction).

[0007] In acute GVHD, histological tissue damage can be observed as early as 8 days in HLA-matched recipients and sometimes as late as one to two months after BMT. (MacMillan, M.L. et al., "What Predicts high risk acute graft-versus host disease (GVHD) at onset?: identification of those at highest risk by a novel acute GVHD risk score. Br. J.

Haematol. 157: 732-41, doi: 10.111/j.1365-2141.2012.09114.x (2012); Kambham, N. et al., "Hematopoietic stem cell tyransplantation: graft versus host disease and pathology of gastrointestinal tract, liver, and lung," Adv. Anat. Pathol. 21: 301-320, doi: 10.1097/PAP.0000000000000032 (2014)). The relatively slow progression of GVHD is due to the presence of low frequency responses against minor histocompatibility antigen (miHA) differences that are present in HLA-matched settings and drive the alloreactive response. Zilberberg, J. et al, "Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-versus-host disease as determined by Vbeta spectratype analysis," J. Immunol. 180: 5352-59 (2008); Berger, M. et al, T cell subsets involved in lethal graft-versus host disease directed to immunodominant minor histocompatibility antigens," Transplantation 557: 1095-1102 (1994); Korngold, R. & Sprent, J., "Lethal graft-versus host disease across minor histocompatibility barriers in mice," Clin. Haematol. 12: 681-693 (1983)). In vivo, T-cell frequencies to miHA have been observed on the order of 1 in 300,000 cells per target, but it can be increased to 1 in 5,000-8,000 upon activation and expansion of the specific T cell clones. The, H.S. et al., Selection of the T cell repertoire during ontogeny: limiting dilution aanalysis," Eur. J. Immunol. 12: 887-892, doi: 10.1002/eji.1830121016 (1982); Simon M. M. & Eichmann, K., "Limiting dilution analysis of alloreactive T helper cells: precursor frequencies similar to that of alloreactive cytotoxic T cells," Immunobiol. 164: 78-89, doi: 10.1016/S0171-2985(83)80020-5 (1983)). In a graft-versus leukemia murine model, exposure to allogeneic tumor miHA significantly increased tumor-specific T-cell frequency and significantly decreased the number of T cells required to abrogate tumor burden. (Fanning, S.L., et al., "Unraveling graft-versus-host disease and graft-versus-leukemia responses using TCR Vbeta spectratype analysis in a murine bone marrow transplantation model." J. Immunol. 190: 447-57, doi: 10.4049/jimmunol.1201641 (2013)). However, conventional in vitro static activation of primary human T cells lacks the capability of providing these persistent stimulatory events found in vivo, because APCs are often killed by donor T cells or are otherwise exhausted. In contrast, lymph nodes act in vivo as a site for continued activation, proliferation, and differentiation for effector T cells that can then migrate to target tissues.

[0008] Acute GVHD manifestations occurring more than 100 days after hematopoietic cell transplantation are classified as "persistent", "recurrent", or "late onset" acute GVHD, depending on the antecedent history of acute GVHD and absence of other chronic GVHD manifestations. Id.

[0009] Classic chronic GVHD, which can result in multiple clinical features involving multiple sites (eyes, gastrointestinal tract, liver, lungs, heart, bone marrow and kidneys), is defined by diagnostic manifestations of chronic GVHD without characteristic features of acute GVHD, with extensive skin involvement, elevated bilirubin, gastrointestinal tract involvement and progressive onset from acute GVHD as poor prognostic findings. (Pidala, J., "Overlap subtype of chronic graft-versus-host disease is associated with an adverse prognosis, functional impairment, and inferior patient-reported outcomes: a Chronic Graft-versus-Host Disease Consortium study," Haematologica 97(3): 451-458 (2012)).

[0010] An overlap subtype of GVHD, which displays features of both chronic and acute GVHD, is a condition with an adverse prognosis, functional impairment, and significantly higher symptom burden. Patients with acute features have significantly higher non-relapse mortality and lower overall survival. These patients suffer significant and diverse functional impairments compared to those with classic chronic GVHD, suggesting a systemic functional impairment beyond the more direct ramifications of concurrent acute GVHD manifestations. Id.

[0011] There is a very strong correlation between GI GVHD severity with patients as well as with experimental outcomes. (Harris, A.C., Levine, J.E., & Ferrara, J.L. "Have we made progress in the treatment of GVHD? Best Pract. Res. Clin. Haematol. 25: 473-78, doi: 10.1016/j.beha.2012.10.010 (2012); Fanning, S. L. et al., "Unraveling graft-versus-host disease and graft-versus-leukemia responses using TCR Vbeta spectratype analysis in a murine bone marrow transplantation model." J. Immunol. 190: 447-57, doi: 10.4049/jimmunol.1201641 (2013); Zilberberg, et al., Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-versus host disease as determined by Vbeta spectratype analysis. J. Immunol. 180: 5352-59 (2008). Two independent investigations on risk factors for developing acute GVHD by sampling cohorts of more than 5,000 (6) and 864 (19) patients, found that (1) only 1-3% experienced liver GVHD of any grade; (2) 17-21% had skin + gastrointestinal (GI) GVHD; (3) 56% developed skin GVHD grades II-1V (apparent without biopsy); and (4) 17% had GI GVHD grades II-1V.

[0012] One of the major determinants for development and severity of acute GVHD in human transplantation is disparity in major and minor histocompatibility antigens, with an increasing number of mismatched antigens predicting greater risk of acute GVHD and nonrelapse mortality. (Goulmy, E et al, "Mismatches of minor histocompatibility

antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation," N. Eng. J. Med. (1996) 334 (5): 281-285; Lee, SJ et al., "High resolution donor recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood (2007) 110 (13): 4576-83). Polymorphism in non-HLA genes, including cytokines such as tumor necrosis factor (TNF), interleukin 10 (IL-10), interferon gamma, KIR polymorphism, and NOD2/CARD15 gene polymorphism, also may contribute to the development and severity of acute GVHD. (J. Pidala, "Graft-vs-Host Disease Following Allogeneic Hematopoietic Cell Transplantation" (2011) Cancer Control; 18(4): 268-276, at 269).

[0013] There are several hypotheses as to mechanisms of chronic GVHD pathogenesis: (1) thymic damage, in part mediated by prior acute GVHD, may impair the process of negative selection by thymic medullary epithelial cells that eliminate pathogenic T cells responsible for immunity; (2) the potential role of transforming growth factor-beta has been supported by amelioration of chronic GVHD manifestations after neutralization of this cytokine in murine models, and the clinical observation of an inverse relationship between transforming growth factor-beta signaling in CD4 and CD8 cells and the risk of chronic GVHD; and (3) B cells may play a role in chronic GVHD pathogenesis. (Id. at 271).

[0014] A conceptual model for GVHD suggests that GVHD is composed of phases that include tissue damage from conditioning therapy and activation of antigen-presenting cells, activation of donor T cells resulting in differentiation and migration, and finally an effector phase in which host tissue damage is mediated by inflammatory cytokines, such as TNF-α and IL-1, and effector cells, most notably cytotoxic T cells. Pidala, J., "Graft-vs-Host Disease Following Allogeneic Hematopoietic Cell Transplantation," Cancer Control 18(4): 268-276 (2011). It is additionally complicated by disturbances in pathways of immunological reconstitution and failure to acquire immunological tolerance, thereby resulting in both alloimmune and autoimmune attacks on multiple host tissues. (S.Z. Pavletic and D.H. Fowler, "Are we making progress in GVHD prophylaxis and treatment?" Hematology: Am. Soc. Hematol. Educ. Program (2012); 2012: 251-264.)

[0015] The process of donor alloreactive T cell migration and infiltration in the body starts with activation of these cells in secondary lymphoid tissues, where they encounter antigen presenting cells (APCs). During the first 2-3 days post BMT, alloreactive T cells recognize their cognitive alloantigens on APCs and both cell types produce and respond to proinflammatory cytokines, such as IFN-γ and TNF-α. A complex cascade of events enables

the activated/effector T cells, which have upregulated surface expression of chemokine receptors and integrins, to migrate to the inflamed tissue sites where they continue to see alloantigen and induce reactivity against targeted epithelial tissues. Clark, R.A. "Skin-resident T cells: the ups and downs of on site immunity. J. Invest. Dermatol. 130: 362-370, doi: 10.1038/jid.2009.247 (2010); Farber, D.L., et al, "Human memory T Cells: generation, compartmentalization and homeostasis," Nature Rev. Immunol. 14: 24-35, doi: 10.1038/nri3567 (2014); Wysocki, C.A. et al, Leukocyte migration and graft-versus-host disease," Blood 105: 4191-99, doi: 10.1182/blood-2004-12-4726 (2005). The activated/effector T cells then re-enter the circulatory system and via upregulated adhesion molecules migrate to target organs where they mediate the pathologic damage to tissues resulting in GVHD. Ferrara, J. L. et al, "Graft versus host disease," Lancet 373: 1550-61, doi: 10.1016/S0140-6736 (009)60237-3 (2009); Wysocki, C.A. et al., "Leukocyte migration and graft versus host disease," Blood 105: 4191-99, doi: 10.1182/blood-2004-12-4726 (2005); Korngold, R. and Antin, JH, "Biology and management of acute graft versus host disease," Cancer Treat. Res. 144: 257-75, doi: 10.1007/978-0-387-78580-6_11 (2009).

[0016] Approaches to decrease GVHD incidence after allo-BMT have focused mainly on suppression or deletion of donor T cells by broadly acting agents or via cell separation techniques. These methods lack specificity and increase the risk of complications, including: (1) slow immune reconstitution and subsequent opportunistic infections; and (2) more critically, tumor relapse.

[0017] Although fatal GVHD, manifesting as acute or chronic inflammatory destruction of the gut, lungs, skin, and other organs, can be completely abrogated in animals and humans by careful depletion of mature lymphocytes from the donor bone marrow graft, when this approach has been taken in patients being treated for various cancers, the incidence of tumor relapse is greatly increased, due to the loss of a graft vs. tumor (GVT) effect, which is characterized by an immune response to a graft recipient's tumor cells by a donor's transplanted immune cells in the bone marrow or peripheral blood. Donor immune cells that have been implicated in the GVT effect include CD4+ T cells, CD8+ T cells and natural killer (NK) cells. These cells are believed to use Fas-dependent killing and perforin degranulation to eradicate malignant cells. In addition to immune cells, cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) have been shown to potentiate the GVT effect. (Ringden, O. et al., "The allogenic graft-versus-cancer effect", Brit.Haematol. (2009); doi:10.1111/j.1365-2141.2009.07866.x).

[0018] Currently, there is no clinically viable means of assessing which HLA-matched patients will develop GVHD, and particularly in the GI tract, prior to BMT. Significant technical challenges associated with expanding primary human IECs ex vivo and with finding an IEC-T cell co-culture system that enables alloreactive responses has made such assessment difficult, if not impossible.

[0019] Although microphysiologically relevant human 3D tissue and tumor models cannot replicate the biological and physiological complexity associated with homeostatic and disease progressions that occur over a long period of time, such models may provide "snapshot" ex vivo reproductions of authentic phenotypic cell functions and interactions relating to specific persons and disease states.

[0020] It is well recognized that serially cultured human diploid cells have a finite lifetime in vitro. Hayflick, L. Exptl Cell Res. 37: 614-636 (1965). After a period of active multiplication, generally less than one year, these cells demonstrate an increased generation time, gradual cessation of mitotic activity, accumulation of cellular debris, and, ultimately, total degeneration of the culture. Id. However, conventional practices of immortalizing human cells into cell lines by gene transfection perturbs the cells' gene expression profiles, cellular physiology and physical integrity of their genome. Even if primary cells can be grown and maintained, gene expression and cellular physiology of such cells can be fundamentally different in 2D versus 3D culture environments.

[0021] The described invention provides a multiwell plate-based perfusion tissue cell culture device that is engineered to mimic the persistent stimulatory events encountered by circulating T cells at the lymph node and tissue levels much like it occurs in a patient's body.

[0022] This biomimetic approach is a departure from current in vitro cultures in which donor T cells are activated solely with patient-derived peripheral blood lymphocytes (Friedman, et al. Blood (2008) 112, 3517- 3525; Jarvis, et al. J.Clin. Path. (2002) 55, 127-132).

[0023] The described invention provides an ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo; methods for optimizing donor selection for allo-BMT, methods for assessing a patient's risk of developing GVHD before allo-BMT, which can help in optimizing donor selection, methods for measuring the potential killing of patient-derived intestinal epithelial cells (IECs) by donor T cells, where IECs are the primary population

targeted in GI GVHD (Hanash, et al., Immunity (2012) 37, 339-350; El-Asady, et al., J. Exp. Med. (2005) 201, 1647-1657), and methods to minimize risk of GVHD before allo-BMT and to maximize GVT effects in allo-BMT.

## SUMMARY OF THE INVENTION

[0024] According to one aspect, the described invention provides an ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo comprising (a) a multiwell plate-based perfusion culture device, comprising, from top to bottom: a bottomless multi-well plate comprising a plurality of bottomless wells; a first micropatterned polymer layer attached to a bottom surface of the bottomless multi-well plate to form a plurality of adjacent wells, one or more of each pair of adjacent wells comprising a transparent polymer membrane placed within the one ore mover of each pair of adjacent wells; a second micropatterned polymer layer comprising two or more holes that correspond to two or more adjacent wells, the second micropatterned polymer layer being attached to a bottom surface of the first micropatterned polymer layer, such that each hole of the second micropatterned polymer layer is aligned with the two or more adjacent wells in the first micropatterned polymer layer, one or more of each pair of adjacent wells comprising the transparent polymer membrane; a microfluidic channel formed between the two adjacent wells that allows internal fluidic communication between the two adjacent wells; one or more removable polymer plugs, each located at a top surface of each of the plurality of wells, and one or more tubes, each connected to the one or more polymer plugs; a pump connected to a reservoir that removably connects to the tubes; a transparent, optical grade glass layer attached to the bottom surface of the second micropatterned polymer layer that forms a bottom surface for the plurality of wells and that seals the multi-well plate perfusion culture device; wherein one or more of the two adjacent wells is a culture chamber for culturing a population of cells; (b) an expanded population of cells derived from a recipient subject comprising a cell-specific antigen in the first adjacent well of the device in (a); (c) an expanded population of T lymphocytes derived from a potential donor of a BMT graft in the second adjacent well of the device in (a), wherein the potential donor is allogeneic to the recipient subject; (d) a liquid culture medium that is flowable between the first adjacent well and the second adjacent well; the model being characterized by: circulation of the liquid medium from the first well into the second well and back to the first well through the microfluidic channel; an interaction between the population of cells comprising the cell antigen derived from the recipient subject in the first well and the population of T lymphocytes is effective to generate alloreactive effector T lymphocytes; alloreactive effector T lymphocyte-induced quantifiable damage to the

population of cells comprising the cell antigen derived from the recipient by the population of alloreactive effector T lymphocytes from the donor allogeneic to the recipient.

[0025] According to one embodiment, the population of T lymphocytes in (c) is derived from peripheral blood lymphocytes of the potential donor. According to another embodiment, the population of T lymphocytes comprises a suspension of nonadherent cells.

[0026] According to one embodiment, the first micropatterned polymer layer and the second micropatterned polymer layer comprises an organic polymer.

[0027] According to one embodiment, the organic polymer is polydimethyl siloxane (PMDS) or polystyrene.

[0028] According to one embodiment, the transparent polymer membrane comprises a nanofibrous mesh. According to one embodiment, the nanofibrous mesh is placed on a top surface of the transparent polymer membrane to coat the top surface of the transparent polymer membrane. According to another embodiment, the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass. According to another embodiment, the transparent polymer membrane comprises a plurality of microbeads preconditioned with an adhesion-promoting agent in an amount effective to promote adhesion of a population of cells to a surface of the microbeads. According to another embodiment, the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of a subpopulation of the population of T lymphocytes to the microbead surface. According to another embodiment, the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

[0029] According to one embodiment, the suspension of nonadherent cells contains T lymphocytes derived from the allogeneic donor.

[0030] According to one embodiment, the population of alloreactive effector T lymphocytes comprises alloreactive activated antigen presenting cells. According to another embodiment, the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

[0031] According to one embodiment, the quantifiable damage to the population of cells comprising the cell-specific antigen derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

[0032] According to one embodiment, the population of cells comprising the cell-specific antigen in (b) are a population of intestinal epithelial cells derived from the recipient subject, and the alloreactive effector T lymphocyte-induced quantifiable damage to the population of intestinal epithelial cells of the recipient subject is an ex vivo measure of risk of graft vs. host disease.

[0033] According to another aspect, the described invention provides a method for optimizing donor selection for allogeneic blood and marrow transplantation (BMT) therapy comprising, in order: (a) acquiring a tissue sample from a recipient subject allogeneic to a potential donor of a BMT graft, the tissue sample comprising a population of primary intestinal epithelial cells comprising an intestinal epithelial cell-specific antigen; (b) seeding the population of primary intestinal epithelial cells of (a) in a first adjacent well of a multiwall plate-based perfusion culture device, the first adjacent well comprising a transparent polymer membrane, expanding the population in a first liquid medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer and generating a population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising the intestinal cell-specific antigen derived from the recipient subject; (c) acquiring a population of T lymphocytes from the potential donor allogeneic to the recipient; (d) seeding and expanding in a second adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes derived from the potential donor of (c), (e) co-culturing in a second liquid medium the CRIECs derived from the recipient subject in the first adjacent well and the T lymphocytes derived from the potential donor allogeneic to the recipient subject in the second adjacent well, the co-culturing being characterized by: the first adjacent well being fluidly connected to the second adjacent well so that the second liquid medium is flowable between the first adjacent well and the second adjacent well; an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes that is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor; (f) measuring damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of a risk of intestinal graft versus host disease in the recipient subject; (g) ranking a plurality of potential donors by the measure of the risk of intestinal graft versus host disease; and (h) treating the recipient subject with a BMT graft derived from a selected

donor allogeneic to the recipient subject whose T lymphocytes are characterized by a reduced risk of intestinal graft-versus-host disease.

[0034] According to one embodiment, the potential donor of the BMT graft is haploidentical to the recipient subject.

[0035] According to one embodiment, the tissue sample comprising a population of primary intestinal epithelial cells is derived from small intestine, large intestine or colon of the recipient subject.

[0036] According to one embodiment, the transparent polymer membrane comprises a nanofibrous mesh to which the population of CRIECs is adherent. According to another embodiment, the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass. According to another embodiment, the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads. According to another embodiment, the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

[0037] According to one embodiment, the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject. According to another embodiment, the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

[0038] According to one embodiment, the expanded population of T lymphocytes derived from the donor allogeneic to the recipient subject in (d) comprise a suspension of nonadherent cells.

[0039] According to one embodiment, the population of alloreactive effector T lymphocytes comprises a population of alloreactive activated antigen presenting cells. According to another embodiment, the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

[0040] According to one embodiment, the quantifiable damage to the population of CRIECs derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

[0041] According to one embodiment, the method further comprising (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of

CRIECs derived from the recipient subject; and (ii) selectively deleting the T lymphocyte clones from the population of T lymphocytes derived from the donor.

[0042] According to another aspect, the described invention provides, a method for minimizing risks and maximizing benefits of performing allogeneic blood and marrow transplantation (BMT) therapy in a recipient subject, wherein the recipient subject has a hematologic malignancy comprising, in order: (A) evaluating a population of T lymphocytes derived from a potential donor allogeneic to the recipient subject for a potential to damage intestinal epithelial cells of the recipient subject according to the method of claim 17 steps (a) through (g); (B) evaluating the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject for an effective graft versus tumor response against the tumor-specific antigens by: (i) acquiring a specimen comprising a population of tumor cells derived from the recipient subject, the population of tumor cells comprising one or more tumor specific antigens; (ii) seeding and expanding the population of tumor cells in the second liquid medium in a third adjacent well of the multiwell plate-based perfusion culture device; the third adjacent well comprising a transparent polymer membrane; (iii) acquiring a population of T lymphocytes derived from the potential donor allogeneic to the recipient subject; (iv) seeding and expanding in a fourth adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes of (iii), (v) co-culturing in the second liquid medium the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject in the third adjacent well and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject in the fourth adjacent well, the co-culturing being characterized by: the third adjacent well being fluidly connected to the fourth adjacent well so that the second liquid medium is flowable between the third adjacent well and the fourth adjacent well; an interaction between the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject that is effective to generate alloreactive effector T lymphocytes derived from the potential donor; (vi) measuring damage to the population of tumor cells derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of an effective graft versus tumor (GVT) response against the tumor-specific antigens; (vii) ranking a plurality of potential donors by the measure of the effective GVT response against the tumor-specific antigens; and (C) treating the recipient subject with

a BMT graft derived from a selected donor allogeneic to the recipient, the selected donor being characterized by a reduced risk of intestinal graft versus host disease, and an effective GVT response against the tumor-specific antigens.

[0043] According to one embodiment, the potential donor of the BMT graft is haploidentical to the recipient subject.

[0044] According to one embodiment, the specimen comprising the population of tumor cells derived from the recipient subject in (i) is a blood sample, a bone marrow sample, or a leukapheresis sample.

[0045] According to one embodiment, the transparent polymer membrane comprises a nanofibrous mesh to which the population of tumor cells is adherent. According to another embodiment, the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass. According to another embodiment, the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads. According to another embodiment, the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

[0046] According to one embodiment, the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject. According to another embodiment, the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

[0047] According to one embodiment, the expanded population of T lymphocytes derived from the allogeneic donor in (iv) comprise a suspension of nonadherent cells.

[0048] According to another embodiment, the population of alloreactive effector T lymphocytes in (v) comprises a population of alloreactive activated antigen presenting cells. According to another embodiment, the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

[0049] According to one embodiment, the quantifiable damage to the population of tumor cells derived from the recipient subject induced by the population of nonadherent alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient comprises cell death.

[0050] According to one embodiment, the method further comprising enriching the population of T lymphocytes derived from the potential donor for an effective GVT therapeutic effect by (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of tumor cells derived from the recipient subject; and (ii) selecting the T lymphocyte clones from the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject; and (iii) expanding the T lymphocyte clones to obtain a therapeutic amount of the T cell clones effective to mediate a GVT response against the tumor-specific antigens.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0051] For a more complete understanding of the described invention, reference is made to the following detailed description of an exemplary embodiment considered in conjunction with the accompanying drawings, in which:

[0052] FIG. 1 shows a drawing of an embodiment of the described multiwell plate-based perfusion culture device for co-cultivating recipient subject intestinal epithelial cells and an allogeneic donor's T lymphocytes.

[0053] FIG. 2(a) and 2(b) show immunofluorescence analysis of m-CRIECs. Staining was performed using a directly conjugated anti-pan cytokertin antibody on mCRIEC cells. 50 px scale-bar equals 16.1 μm (20x) and 8.05 μm (40x). Figure 2(c) shows flow cytometric analysis of extracellular (pan cytokeratin, EpCAM, CD24, and CD44) and intracellular (Lgr5) IEC cell markers.

[0054] FIG. 3 shows upregulation of MHC I and II in m-CRIEC. M-CRIEC were cultured in different cell culture media for 72 h in the presence of 20 ng/ml TNF-α and 10 U/ml IFN-γ to induce the upregulation of MHC class 1 (lab) and class II (H2kb) molecules on the surface of m-CRIEC. Maximal MHC II expression was obtained when using cRPMI medium or cRPMI plus a nanofibrous mesh.

[0055] FIG. 4 shows morphology of m-CRIECs cultured in CRC medium, cRPMI and cRPMI on nanofibrous mesh for 7 days to evaluate morphology and viability. Loss of cobble stone morphology (black arrow) was observed without nanofibrous mesh.

[0056] FIG. 5 shows a killing assay of B10.BR T cells against B6 m-CRIEC. B6 m-CRIEC cultured with nanofibrous mesh and cRPMI were stimulated with 20 ng/ml TNF-α and 10 U/ml IFN-γ for 48-72 h (panel I) and cocultured with MLC-stimulated B10.BR T cells at an E:T ratio of 5:1 (panel II) and 10:1 (panel III). On day 6, m-CRIEC at E:T ratio 10:1

were visibly compromised as determined by trypan blue staining (panel III). Arrows indicate T cells.

[0057] FIG. 6 shows a well plate-based perfusion device: (a) – (c) schematic illustrations of the device and fabrication; (d) actual device used for preliminary results; (e) SEM image of nanofibrous mesh coated onto a PC membrane.

[0058] FIG. 7 (a) m-CRIEC from B6 mice were cultured for 7 days in the IEC culture chamber; (b) and (c) followed by the introduction of circulating MHC-mismatched B10.BR m-T cells for 5 more days. (b) SEM and (c) fluorescence images showing m-CRIECs and m-T cells. Scale bar: 50 μm.

[0059] FIG. 8 shows flow cytometric analysis of T cell proliferation upon DC stimulation in 2D vs. 3D culture. eGFP B6 T cells were labeled with eFluor 670 and cultured for 4 days with BALB.B DCs to assess proliferation. In comparison to 2D, the total percent (%) of live T cells [undivided cells (U) + proliferating (P)] was greater in 3D, indicating more T cells remained viable in 3D perfusion culture. This enhanced viability was also reflected by the increased R:S ratio (10:1 vs. 2:1). Furthermore, the percentage of proliferating T cells (P) was greater in 3D than in 2D (40% vs. 33%).

[0060] FIG. 9 shows circulation of primary murine T cells through primary murine IECs: (a) schematic illustration of the device configuration and use; (b) SEM image showing collagen/PCL nanofiber mesh on PC membrane with 10 μm pores; (c) merged fluorescence image showing IEC cytoskeleton (red, ActinRed) and nucleus (blue, DAPI) after day 7 on collagen/PCL nanofiber mesh; (d) effect of IEC presence on T cell viability; and (e) bright field image showing IECs and T cells cultured for 6 h on nanofiber mesh.

## DETAILED DESRIPTION OF THE INVENTION

### Glossary

[0061] Various terms used throughout this specification shall have the definitions set out herein.

[0062] The term "activation" or "lymphocyte activation" refers to stimulation of lymphocytes by specific antigens, nonspecific mitogens, or allogeneic cells resulting in synthesis of RNA, protein and DNA and production of lymphokines; it is followed by proliferation and differentiation of various effector and memory cells. For example, a mature B cell can be activated by an encounter with an antigen that expresses epitopes that are

recognized by its cell surface immunoglobulin (Ig). The activation process may be a direct one, dependent on cross-linkage of membrane Ig molecules by the antigen (cross-linkage-dependent B cell activation) or an indirect one, occurring most efficiently in the context of an intimate interaction with a helper T cell ("cognate help process"). T-cell activation is dependent on the interaction of the TCR/CD3 complex with its cognate ligand, a peptide bound in the groove of a class I or class II MHC molecule. The molecular events set in motion by receptor engagement are complex. Among the earliest steps appears to be the activation of tyrosine kinases leading to the tyrosine phosphorylation of a set of substrates that control several signaling pathways. These include a set of adapter proteins that link the TCR to the ras pathway, phospholipase Cγ1, the tyrosine phosphorylation of which increases its catalytic activity and engages the inositol phospholipid metabolic pathway, leading to elevation of intracellular free calcium concentration and activation of protein kinase C, and a series of other enzymes that control cellular growth and differentiation. Full responsiveness of a T cell requires, in addition to receptor engagement, an accessory cell-delivered costimulatory activity, e.g., engagement of CD28 on the T cell by CD80 and/or CD86 on the antigen presenting cell (APC). The soluble product of an activated B lymphocyte is immmunoglobulins (antibodies). The soluble product of an activated T lymphocyte is lymphokines.

[0063] The term "administering" as used herein includes in vivo administration, as well as administration directly to tissue ex vivo. Generally, compositions can be administered systemically either orally, buccally, parenterally, topically, by inhalation or insufflation (i.e., through the mouth or through the nose), or rectally in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired, or can be locally administered by means such as, but not limited to, injection, implantation, grafting, topical application, or parenterally.

[0064] The term "alloantigen" as used herein refers to a genetically determined antigen present in some but not all individuals of a species (as those of a particular blood group) and capable of inducing the production of an alloantibody by individuals which lack it—called also isoantigen.

[0065] The term "allogeneic" as used herein refers to taken from different individuals of the same species.

[0066] The term “allogeneic bone marrow transplantation”(allo-BMT) as used herein refers to a procedure in which a recipient subject receives cells from a genetically similar, but not identical, donor.

[0067] The term “alloreactivity” refers to a strong primary T cell response against allelic variants of major histocompatibility complex (MHC) molecules in a species. Alloreactivity is manifested in the rejection of tissue grafts between individuals of the same species.

[0068] The term “antigen presenting cell” or APC as used herein refers to a cell that displays foreign antigen complexed with MHC molecules on its surface.

[0069] The terms “apoptosis” or “programmed cell death” refer to a highly regulated and active process that contributes to biologic homeostasis comprised of a series of biochemical events that lead to a variety of morphological changes, including blebbing, changes to the cell membrane, such as loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation, without damaging the organism.

[0070] Apoptotic cell death is induced by many different factors and involves numerous signaling pathways, some dependent on caspase proteases (a class of cysteine proteases) and others that are caspase independent. It can be triggered by many different cellular stimuli, including cell surface receptors, mitochondrial response to stress, and cytotoxic T cells, resulting in activation of apoptotic signaling pathways

[0071] The caspases involved in apoptosis convey the apoptotic signal in a proteolytic cascade, with caspases cleaving and activating other caspases that then degrade other cellular targets that lead to cell death. The caspases at the upper end of the cascade include caspase-8 and caspase-9. Caspase-8 is the initial caspase involved in response to receptors with a death domain (DD) like Fas.

[0072] Receptors in the TNF receptor family are associated with the induction of apoptosis, as well as inflammatory signaling. The Fas receptor (CD95) mediates apoptotic signaling by Fas-ligand expressed on the surface of other cells. The Fas-FasL interaction plays an important role in the immune system and lack of this system leads to autoimmunity, indicating that Fas-mediated apoptosis removes self-reactive lymphocytes. Fas signaling also is involved in immune surveillance to remove transformed cells and virus infected cells. Binding of Fas to oligimerized FasL on another cell activates apoptotic signaling through a cytoplasmic domain termed the death domain (DD) that interacts with signaling adaptors

including FAF, FADD and DAX to activate the caspase proteolytic cascade. Caspase-8 and caspase-10 first are activated to then cleave and activate downstream caspases and a variety of cellular substrates that lead to cell death.

[0073] Mitochondria participate in apoptotic signaling pathways through the release of mitochondrial proteins into the cytoplasm. Cytochrome c, a key protein in electron transport, is released from mitochondria in response to apoptotic signals, and activates Apaf-1, a protease released from mitochondria. Activated Apaf-1 activates caspase-9 and the rest of the caspase pathway. Smac/DIABLO is released from mitochondria and inhibits IAP proteins that normally interact with caspase-9 to inhibit apoptosis. Apoptosis regulation by Bcl-2 family proteins occurs as family members form complexes that enter the mitochondrial membrane, regulating the release of cytochrome c and other proteins. TNF family receptors that cause apoptosis directly activate the caspase cascade, but can also activate Bid, a Bcl-2 family member, which activates mitochondria-mediated apoptosis. Bax, another Bcl-2 family member, is activated by this pathway to localize to the mitochondrial membrane and increase its permeability, releasing cytochrome c and other mitochondrial proteins. Bcl-2 and Bcl-xL prevent pore formation, blocking apoptosis. Like cytochrome c, AIF (apoptosis-inducing factor) is a protein found in mitochondria that is released from mitochondria by apoptotic stimuli. While cytochrome C is linked to caspase-dependent apoptotic signaling, AIF release stimulates caspase-independent apoptosis, moving into the nucleus where it binds DNA. DNA binding by AIF stimulates chromatin condensation, and DNA fragmentation, perhaps through recruitment of nucleases.

[0074] The mitochondrial stress pathway begins with the release of cytochrome c from mitochondria, which then interacts with Apaf-1, causing self-cleavage and activation of caspase-9. Caspase-3, -6 and-7 are downstream caspases that are activated by the upstream proteases and act themselves to cleave cellular targets.

[0075] Granzyme B and perforin proteins released by cytotoxic T cells induce apoptosis in target cells, forming transmembrane pores, and triggering apoptosis, perhaps through cleavage of caspases, although caspase-independent mechanisms of Granzyme B mediated apoptosis have been suggested.

[0076] Fragmentation of the nuclear genome by multiple nucleases activated by apoptotic signaling pathways to create a nucleosomal ladder is a cellular response characteristic of apoptosis. One nuclease involved in apoptosis is DNA fragmentation factor (DFF), a caspase-activated DNAse (CAD). DFF/CAD is activated through cleavage of its

associated inhibitor ICAD by caspases proteases during apoptosis. DFF/CAD interacts with chromatin components such as topoisomerase II and histone H1 to condense chromatin structure and perhaps recruit CAD to chromatin. Another apoptosis activated protease is endonuclease G (EndoG). EndoG is encoded in the nuclear genome but is localized to mitochondria in normal cells. EndoG may play a role in the replication of the mitochondrial genome, as well as in apoptosis. Apoptotic signaling causes the release of EndoG from mitochondria. The EndoG and DFF/CAD pathways are independent since the EndoG pathway still occurs in cells lacking DFF.

[0077] Hypoxia, as well as hypoxia followed by reoxygenation can trigger cytochrome c release and apoptosis. Glycogen synthase kinase (GSK-3) a serine-threonine kinase ubiquitously expressed in most cell types, appears to mediate or potentiate apoptosis due to many stimuli that activate the mitochondrial cell death pathway. Loberg, RD, et al., J. Biol. Chem. 277 (44): 41667-673 (2002). It has been demonstrated to induce caspase 3 activation and to activate the proapoptotic tumor suppressor gene p53. It also has been suggested that GSK-3 promotes activation and translocation of the proapoptotic Bcl-2 family member, Bax, which, upon agregation and mitochondrial localization, induces cytochrome c release. Akt is a critical regulator of GSK-3, and phosphorylation and inactivation of GSK-3 may mediate some of the antiapoptotic effects of Akt.

[0078] The term "chemokine" as used herein refers to a class of chemotactic cytokines that signal leukocytes to move in a specific direction. The terms "chemotaxis" or "chemotactic" refer to the directed motion of a motile cell or part along a chemical concentration gradient towards environmental conditions it deems attractive and/or away from surroundings it finds repellent.

[0079] The term “conditioning regimens” as used herein refers to treatments used to prepare a patient for stem cell transplantation (a procedure in which a person receives blood stem cells, which make any type of blood cell). A conditioning regimen may include chemotherapy, monoclonal antibody therapy, and radiation to the entire body. It helps make room in the patient’s bone marrow for new blood stem cells to grow, helps prevent the patient's body from rejecting the transplanted cells, and helps kill any cancer cells that are in the body.

[0080] The term “conditional reprogramming cells (CRC)” as used herein refers to epithelial cells cultured on irradiated fibroblast feeders in the presence of the Rho kinase inhibitor Y-27632. Saenz, F.R. et al., “Conditionally reprogrammed normal and transformed

mouse mammary epithelial cells display a progenitor-cell-like phenotype," PloS One 9, e9766, doi: 10.1371/journal.pone.0097666 (2014); Palechor-Ceron, N. et al, "Radiation induces diffusible feeder cell factor(s) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells," Am. J. Pathol. 183: 1862-70, doi: 10.1016/j.ajpath.2013.08.009 (2013); Liu, X. et al, "ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells," Am. J. Pathol. 180: 599-607, doi: 10.1016/j.ajpath.2011.10.036 (2012). The inductive conditions consist of F medium containing the ROCK inhibitor Y-27632 and irradiated Swiss 3T3-J2 mouse fibroblasts. When the feeder cells and Y-27632 were removed, the CRCs exhibited normal differentiation. The CRCs are genetically stable, can be cultured indefinitely, and can bypass senescence.

[0081] The term "condition", as used herein, refers to a variety of health states and is meant to include disorders or diseases caused by any underlying mechanism or injury.

[0082] The term "culture" as used herein refers to the cultivation of cells in or on a controlled or defined medium. The terms "culture-expanded" or "expanded" are used interchangeably to refer to an increase in the number of cells by cultivation of the cells in or on a controlled or defined medium.

[0083] The term "cytokine" as used herein refers to small soluble protein substances secreted by cells, which have a variety of effects on other cells. Cytokines mediate many important physiological functions, including growth, development, wound healing, and the immune response. They act by binding to their cell-specific receptors located in the cell membrane, which allows a distinct signal transduction cascade to start in the cell, which eventually will lead to biochemical and phenotypic changes in target cells. Generally, cytokines act locally. They include type I cytokines, which encompass many of the interleukins including interleukin 2 (IL-2), as well as several hematopoietic growth factors; type II cytokines, including the interferons and interleukin-10; tumor necrosis factor ("TNF")-related molecules, including TNFα and lymphotoxin; immunoglobulin super-family members, including interleukin 1 ("IL-1"); and the chemokines, a family of molecules that play a critical role in a wide variety of immune and inflammatory functions. The same cytokine can have different effects on a cell depending on the state of the cell. Cytokines often regulate the expression of, and trigger cascades of, other cytokines.

[0084] The term "dendritic cells" (DCs) as used herein, refers to professional APCs capable of presenting both MHC-I and MHC-II antigens.

[0085] The term “derived from” as used herein is used to refer to originating, sourced, or coming from.

[0086] The term “disease” or “disorder,” as used herein, refers to an impairment of health or a condition of abnormal functioning.

[0087] The term “drug” as used herein refers to a therapeutic agent or any substance used in the prevention, diagnosis, alleviation, treatment, or cure of disease.

[0088] The term “graft” as used herein, refers to any tissue or organ for transplantation. It includes, but is not limited to, a self-tissue transferred from one body site to another in the same individual (“autologous graft”), a tissue transferred between genetically identical individuals or sufficiently immunologically compatible to allow tissue transplant (“syngeneic graft”), a tissue transferred between genetically different members of the same species (“allogeneic graft” or “allograft”), and a tissue transferred between different species (“xenograft”).

[0089] The term “graft versus host” as used herein, refers to a systemic autoimmune syndrome resulting from cells of an engrafted hematopoietic-cell transplant mounting an immune response against the host. In human recipients of bone marrow, chronic GVHD syndrome is a major clinical problem, leading to fibrosis, pathology and autoantibodies, which can result in immune dysfunction, increased risk of infection, potentially serious impaired organ function, and poor quality of life. The syndrome occurs even in recipients of autologous marrow, although in a milder form. (See, e.g. Kennedy, Autologous graft versus host disease. Med. Oncol. 12: 149-15;1995). Acute GVHD is a clinical syndrome caused by T cell-mediated recognition of minor histocompatibility antigens followed by organ-specific vascular adhesion, migration, proliferation, cytokine release, and direct cell-mediated attack on normal tissues. Chronic GVHD is more complex, incorporating both conventional T-cell effector functions, as well as humoral and antigen-presenting effects of B cells. (Joseph Antin, “T-cell depletion in GVHD: less is more?” Blood (2011) 117(23): 6061-6063)

[0090] The term “graft-versus-tumor (GVT)” as used herein refers to an immune response to a graft recipient’s tumor cells by immune cells present in a donor's transplanted tissue, such as bone marrow or peripheral blood.

[0091] The term “haploidentical“ as used herein refers to sharing a haplotype, meaning having the same alleles at a set of closely linked genes on one chromosome.

[0092] The term “human leukocyte antigen matching” as used herein refers to a process in which blood or tissue samples are tested for human leukocyte antigens (HLAs). HLAs are molecules found on the surface of most cells in the body. They make up a person’s tissue type, which varies from person to person. They play an important part in the body's immune response to foreign substances. Human leukocyte antigen matching is done before a donor stem cell or organ transplant to find out if tissues match between the donor and the person receiving the transplant. Also called HLA matching.

[0093] The term “hematopoietic-cell transplantation” (HCT) is used herein to refer to blood and marrow transplantation (BMT), a procedure that involves infusion of cells (hematopoietic stem cells; also called hematopoietic progenitor cells) to reconstitute the hematopoietic system of a patient.

[0094] The term “inhibit” and its various grammatical forms, including, but not limited to, “inhibiting” or “inhibition”, are used herein to refer to reducing the amount or rate of a process, to stopping the process entirely, or to decreasing, limiting, or blocking the action or function thereof. Inhibition can include a reduction or decrease of the amount, rate, action function, or process of a substance by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%.

[0095] The term "inhibitor" as used herein refers to a second molecule that binds to a first molecule thereby decreasing the first molecule's activity. Enzyme inhibitors are molecules that bind to enzymes thereby decreasing enzyme activity. The binding of an inhibitor can stop a substrate from entering the active site of the enzyme and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically, for example, by modifying key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and produce different types of inhibition depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both. Enzyme inhibitors often are evaluated by their specificity and potency.

[0096] The term “injury,” as used herein, refers to damage or harm to a structure or function of the body caused by an outside agent or force, which can be physical or chemical.

[0097] The term “immunomodulatory cell(s)” as used herein refer(s) to cell(s) that are capable of augmenting or diminishing immune responses by expressing chemokines, cytokines and other mediators of immune responses.

[0098] The term “inflammatory cytokines” or “inflammatory mediators” as used herein refers to the molecular mediators of the inflammatory process, which may modulate being either pro- or anti-inflamatory in their effect. These soluble, diffusible molecules act both locally at the site of tissue damage and infection and at more distant sites. Some inflammatory mediators are activated by the inflammatory process, while others are synthesized and/or released from cellular sources in response to acute inflammation or by other soluble inflammatory mediators. Examples of inflammatory mediators of the inflammatory response include, but are not limited to, plasma proteases, complement, kinins, clotting and fibrinolytic proteins, lipid mediators, prostaglandins, leukotrienes, platelet-activating factor (PAF), peptides and amines, including, but not limited to, histamine, serotonin, and neuropeptides, pro-inflammatory cytokines, including, but not limited to, interleukin-1-beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IF-γ), and interleukin-12 (IL-12).

[0099] The term “interleukin (IL)” as used herein refers to a cytokine secreted by, and acting on, leukocytes. Interleukins regulate cell growth, differentiation, and motility, and stimulates immune responses, such as inflammation. Examples of interleukins include interleukin-1 (IL-1), interleukin 2 (IL-2), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-12 (IL-12).

[00100] The term “lymphocyte” refers to a small white blood cell formed in lymphatic tissue throughout the body and in normal adults making up about 22-28% of the total number of leukocytes in the circulating blood that plays a large role in defending the body against disease. Individual lymphocytes are specialized in that they are committed to respond to a limited set of structurally related antigens. This commitment, which exists before the first contact of the immune system with a given antigen, is expressed by the presence on the lymphocyte’s surface membrane of receptors specific for determinants (epitopes) on the antigen. Each lymphocyte possesses a population of receptors, all of which have identical combining sites. One set, or clone, of lymphocytes differs from another clone in the structure of the combining region of its receptors and thus differs in the epitopes that it can recognize. Lymphocytes differ from each other not only in the specificity of their receptors, but also in their functions.

[00101] Two broad classes of lymphocytes are recognized: the B-lymphocytes (B-cells), which are precursors of antibody-secreting cells, and T-lymphocytes (T-cells),

***B-lymphocytes***

[00102] B-lymphocytes are derived from hematopoietic cells of the bone marrow. A mature B-cell can be activated with an antigen that expresses epitopes that are recognized by its cell surface. The activation process may be direct, dependent on cross-linkage of membrane Ig molecules by the antigen (cross-linkage-dependent B-cell activation), or indirect, via interaction with a helper T-cell, in a process referred to as cognate help. In many physiological situations, receptor cross-linkage stimuli and cognate help synergize to yield more vigorous B-cell responses. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00103] Cross-linkage dependent B-cell activation requires that the antigen express multiple copies of the epitope complementary to the binding site of the cell surface receptors because each B-cell expresses Ig molecules with identical variable regions. Such a requirement is fulfilled by other antigens with repetitive epitopes, such as capsular polysaccharides of microorganisms or viral envelope proteins. Cross-linkage-dependent B-cell activation is a major protective immune response mounted against these microbes. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00104] Cognate help allows B-cells to mount responses against antigens that cannot cross-link receptors and, at the same time, provides costimulatory signals that rescue B cells from inactivation when they are stimulated by weak cross-linkage events. Cognate help is dependent on the binding of antigen by the B-cell's membrane immunoglobulin (Ig), the endocytosis of the antigen, and its fragmentation into peptides within the endosomal/lysosomal compartment of the cell. Some of the resultant peptides are loaded into a groove in a specialized set of cell surface proteins known as class II major histocompatibility complex (MHC) molecules. The resultant class II/peptide complexes are expressed on the cell surface and act as ligands for the antigen-specific receptors of a set of T-cells designated as CD4+ T-cells. The CD4+ T-cells bear receptors on their surface specific for the B-cell's class II/peptide complex. B-cell activation depends not only on the binding of the T cell through its T cell receptor (TCR), but this interaction also allows an activation ligand on the T-cell (CD40 ligand) to bind to its receptor on the B-cell (CD40)

signaling B-cell activation. In addition, T helper cells secrete several cytokines that regulate the growth and differentiation of the stimulated B-cell by binding to cytokine receptors on the B cell. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00105] During cognate help for antibody production, the CD40 ligand is transiently expressed on activated CD4+ T helper cells, and it binds to CD40 on the antigen-specific B cells, thereby tranducing a second costimulatory signal. The latter signal is essential for B cell growth and differentiation and for the generation of memory B cells by preventing apoptosis of germinal center B cells that have encountered antigen. Hyperexpression of the CD40 ligand in both B and T cells is implicated in the pathogenic autoantibody production in human SLE patients. (Desai-Mehta, A. et al., "Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production," J. Clin. Invest. 97(9): 2063-2073 (1996)).

***T-lymphocytes***

[00106] T-lymphocytes derive from precursors in hematopoietic tissue, undergo differentiation in the thymus, and are then seeded to peripheral lymphoid tissue and to the recirculating pool of lymphocytes. T-lymphocytes or T cells mediate a wide range of immunologic functions. These include the capacity to help B cells develop into antibody-producing cells, the capacity to increase the microbicidal action of monocytes/macrophages, the inhibition of certain types of immune responses, direct killing of target cells, and mobilization of the inflammatory response. These effects depend on their expression of specific cell surface molecules and the secretion of cytokines. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00107] T cells differ from B cells in their mechanism of antigen recognition. Immunoglobulin, the B cell's receptor, binds to individual epitopes on soluble molecules or on particulate surfaces. B-cell receptors see epitopes expressed on the surface of native molecules. Antibody and B-cell receptors evolved to bind to and to protect against microorganisms in extracellular fluids. In contrast, T cells recognize antigens on the surface of other cells and mediate their functions by interacting with, and altering, the behavior of these antigen-presenting cells (APCs). There are three main types of antigen-presenting cells in peripheral lymphoid organs that can activate T cells: dendritic cells, macrophages and B cells. The most potent of these are the dendritic cells, whose only function is to present

foreign antigens to T cells. Immature dendritic cells are located in tissues throughout the body, including the skin, gut, and respiratory tract. When they encounter invading microbes at these sites, they endocytose the pathogens and their products, and carry them via the lymph to local lymph nodes or gut associated lymphoid organs. The encounter with a pathogen induces the dendritic cell to mature from an antigen-capturing cell to an antigen-presenting cell (APC) that can activate T cells. APCs display three types of protein molecules on their surface that have a role in activating a T cell to become an effector cell: (1) MHC proteins, which present foreign antigen to the T cell receptor; (2) costimulatory proteins which bind to complementary receptors on the T cell surface; and (3) cell-cell adhesion molecules, which enable a T cell to bind to the antigen-presenting cell (APC) for long enough to become activated. ("Chapter 24: The adaptive immune system," Molecular Biology of the Cell, Alberts, B. et al., Garland Science, NY, 2002).

[00108] T-cells are subdivided into two distinct classes based on the cell surface receptors they express. The majority of T cells express T cell receptors (TCR) consisting of α and β chains. A small group of T cells express receptors made of γ and δ chains. Among the α/β T cells are two important sublineages: those that express the coreceptor molecule CD4 (CD4+ T cells); and those that express CD8 (CD8+ T cells). These cells differ in how they recognize antigen and in their effector and regulatory functions.

[00109] CD4+ T cells are the major regulatory cells of the immune system. Their regulatory function depends both on the expression of their cell-surface molecules, such as CD40 ligand whose expression is induced when the T cells are activated, and the wide array of cytokines they secrete when activated.

[00110] T cells also mediate important effector functions, some of which are determined by the patterns of cytokines they secrete. The cytokines can be directly toxic to target cells and can mobilize potent inflammatory mechanisms.

[00111] In addition, T cells particularly CD8+ T cells, can develop into cytotoxic T-lymphocytes (CTLs) capable of efficiently lysing target cells that express antigens recognized by the CTLs. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00112] T cell receptors (TCRs) recognize a complex consisting of a peptide derived by proteolysis of the antigen bound to a specialized groove of a class II or class I MHC protein. The CD4+ T cells recognize only peptide/class II complexes while the CD8+

T cells recognize peptide/class I complexes. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00113] The TCR's ligand (i.e., the peptide/MHC protein complex) is created within antigen-presenting cells (APCs). In general, class II MHC molecules bind peptides derived from proteins that have been taken up by the APC through an endocytic process. These peptide-loaded class II molecules are then expressed on the surface of the cell, where they are available to be bound by CD4+ T cells with TCRs capable of recognizing the expressed cell surface complex. Thus, CD4+ T cells are specialized to react with antigens derived from extracellular sources. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00114] In contrast, class I MHC molecules are mainly loaded with peptides derived from internally synthesized proteins, such as viral proteins. These peptides are produced from cytosolic proteins by proteolysis by the proteosome and are translocated into the rough endoplasmic reticulum. Such peptides, generally nine amino acids in length, are bound into the class I MHC molecules and are brought to the cell surface, where they can be recognized by CD8+ T cells expressing appropriate receptors. This gives the T cell system, particularly CD8+ T cells, the ability to detect cells expressing proteins that are different from, or produced in much larger amounts than, those of cells of the remainder of the organism (e.g., vial antigens) or mutant antigens (such as active oncogene products), even if these proteins in their intact form are neither expressed on the cell surface nor secreted. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00115] T cells can also be classified based on their function as helper T cells; T cells involved in inducing cellular immunity; suppressor T cells; and cytotoxic T cells.

***Helper T cells***

[00116] Helper T cells are T cells that stimulate B cells to make antibody responses to proteins and other T cell-dependent antigens. T cell-dependent antigens are immunogens in which individual epitopes appear only once or a limited number of times such that they are unable to cross-link the membrane immunoglobulin (Ig) of B cells or do so inefficiently. B cells bind the antigen through their membrane Ig, and the complex undergoes endocytosis. Within the endosomal and lysosomal compartments, the antigen is fragmented into peptides

by proteolytic enzymes and one or more of the generated peptides are loaded into class II MHC molecules, which traffic through this vesicular compartment. The resulting peptide/class II MHC complex is then exported to the B-cell surface membrane. T cells with receptors specific for the peptide/class II molecular complex recognize this complex on the B-cell surface. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00117] B-cell activation depends both on the binding of the T cell through its TCR and on the interaction of the T-cell CD40 ligand (CD40L) with CD40 on the B cell. T cells do not constitutively express CD40L. Rather, CD40L expression is induced as a result of an interaction with an APC that expresses both a cognate antigen recognized by the TCR of the T cell and CD80 or CD86. CD80/CD86 is generally expressed by activated, but not resting, B cells so that the helper interaction involving an activated B cell and a T cell can lead to efficient antibody production. In many cases, however, the initial induction of CD40L on T cells is dependent on their recognition of antigen on the surface of APCs that constitutively express CD80/86, such as dendritic cells. Such activated helper T cells can then efficiently interact with and help B cells. Cross-linkage of membrane Ig on the B cell, even if inefficient, may synergize with the CD40L/CD40 interaction to yield vigorous B-cell activation. The subsequent events in the B-cell response, including proliferation, Ig secretion, and class switching (of the Ig class being expressed) either depend or are enhanced by the actions of T cell-derived cytokines. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

[00118] CD4+ T cells tend to differentiate into cells that principally secrete the cytokines IL-4, IL-5, IL-6, and IL-10 (TH2 cells) or into cells that mainly produce IL-2, IFN-□, and lymphotoxin (TH1 cells). The TH2 cells are very effective in helping B-cells develop into antibody-producing cells, whereas the TH1 cells are effective inducers of cellular immune responses, involving enhancement of microbicidal activity of monocytes and macrophages, and consequent increased efficiency in lysing microorganisms in intracellular vesicular compartments. Although the CD4+ T cells with the phenotype of TH2 cells (i.e., IL-4, IL-5, IL-6 and IL-10) are efficient helper cells, TH1 cells also have the capacity to be helpers. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

***T cells involved in Induction of Cellular Immunity***

[00119] T cells also may act to enhance the capacity of monocytes and macrophages to destroy intracellular microorganisms. In particular, interferon-gamma (IFN-γ) produced by helper T cells enhances several mechanisms through which mononuclear phagocytes destroy intracellular bacteria and parasitism including the generation of nitric oxide and induction of tumor necrosis factor (TNF) production. The TH1 cells are effective in enhancing the microbicidal action because they produce IFN-γ. By contrast, two of the major cytokines produced by TH2 cells, IL-4 and IL-10, block these activities. (Paul, W. E., "Chapter 1: The immune system: an introduction," Fundamental Immunology, 4th Edition, Ed. Paul, W. E., Lippicott-Raven Publishers, Philadelphia (1999)).

**Suppressor or Regulatory T (Treg) cells**

[00120] A controlled balance between initiation and downregulation of the immune response is important to maintain immune homeostasis. Both apoptosis and T cell anergy (a tolerance mechanism in which the T cells are intrinsically functionally inactivated following an antigen encounter (Scwartz, R. H., "T cell anergy," Annu. Rev. Immunol., 21: 305-334 (2003)) are important mechanisms that contribute to the downregulation of the immune response. A third mechanism is provided by active suppression of activated T cells by suppressor or regulatory CD4+ T (Treg) cells. (Reviewed in Kronenberg, M. et al., "Regulation of immunity by self-reactive T cells," Nature 435: 598-604 (2005)). CD4+ Tregs that constitutively express the IL-2 receptor alpha (IL-2R□) chain (CD4+ CD25+) are a naturally occurring T cell subset that are anergic and suppressive. (Taams, L. S. et l., "Human anergic/suppressive CD4+CD25+ T cells: a highly differentiated and apoptosis-prone population," Eur. J. Immunol., 31: 1122-1131 (2001)). Depletion of CD4+CD25+ Tregs results in systemic autoimmune disease in mice. Furthermore, transfer of these Tregs prevents development of autoimmune disease. Human CD4+CD25+ Tregs, similar to their murine counterpart, are generated in the thymus and are characterized by the ability to suppress proliferation of responder T cells through a cell-cell contact-dependent mechanism, the inability to produce IL-2, and the anergic phenotype in vitro. Human CD4+CD25+ T cells can be split into suppressive (CD25high) and nonsuppressive (CD25low) cells, according to the level of CD25 expression. A member of the forkhead family of transcription factors, FOXP3, has been shown to be expressed in murine and human CD4+CD25+ Tregs and appears to be a master gene controlling CD4+CD25+ Treg development. (Battaglia, M. et al., "Rapamycin promotes expansion of functional CD4+CD25+Foxp3+ regulator T cells of both healthy subjects and type 1 diabetic patients," J. Immunol., 177: 8338-8347 (200)).

**Cytotoxic T Lymphocytes (CTL)**

[00121] The CD8+ T cells that recognize peptides from proteins produced within the target cell have cytotoxic properties in that they lead to lysis of the target cells. The mechanism of CTL-induced lysis involves the production by the CTL of perforin, a molecule that can insert into the membrane of target cells and promote the lysis of that cell. Perforin-mediated lysis is enhanced by a series of enzymes produced by activated CTLs, referred to as granzymes. Many active CTLs also express large amounts of fas ligand on their surface. The interaction of fas ligand on the surface of CTL with fas on the surface of the target cell initiates apoptosis in the target cell, leading to the death of these cells. CTL-mediated lysis appears to be a major mechanism for the destruction of virally infected cells.

[00122] The term “matrix” as sued herein refers to a three dimensional network of fibers that contains voids (or “pores”) where the woven fibers intersect. The structural parameters of the pores, including the pore size, porosity, pore interconnectivity/tortuosity and surface area, affect how fluid, solutes and cells move in and out of the matrix.

[00123] The term “perfusion” as used herein refers to the process of nutritive delivery of arterial blood to a capillary bed in biological tissue. Perfusion (“F”) can be calculated with the formula F=((PA-Pv)/R) wherein PA is mean arterial pressure, Pv is mean venous pressure, and R is vascular resistance. Tissue perfusion can be measured in vivo, by, for example, but not limited to, magnetic resonance imaging (MRI) techniques. Such techniques include using an injected contrast agent and arterial spin labeling (ASL) (wherein arterial blood is magnetically tagged before it enters into the tissue of interest and the amount of labeling is measured and compared to a control recording). Tissue perfusion can be measured in vitro, by, for example, but not limited to, tissue oxygen saturation (StO2) using techniques including, but not limited to, hyperspectral imaging (HSI).

[00124] The terms “proliferation” and “propagation” are used interchangeably herein to refer to expansion of a population of cells by the continuous division of single cells into identical daughter cells.

[00125] The term “polymer” as used herein refers to a macromolecule formedby the chemical union of five or more identical combining units (monomers). Exemplary polymers by type include, without limitation, inorganic polymers (e.g., siloxane, sulfur chains, black phosphorus, boron-nitrogen, aluminosilicate, borosilicate, or boro-aluminosilicate, glass ceramics, ceramics, and semiconductor or crystalline materials (e.g. silicones); Organic polymers, including natural organic polymers e.g., polysaccharides, such

as starch, cellulose, pectin, seaweed gums (agar, etc), vegetable gums (Arabic, etc.); polypeptides (e.g., albumin, globulin); and hydrocarbons, e.g., polyisoprene; synthetic polymers, including thermoplastic polymers, such as polyvinyl chloride, polyethylene (linear), polystyrene, polypropylene, fluorocarbon resins, polyurethane, and acrylate resins, and thermosetting synthetic polymers, such as elastomers, polyethylene (cross-linked), penolics, and polyesters; and semisynthetic organic polymers, such as cellulosics (e.g., methylcellulose, cellulose acetate) and modified starches. Further examples of polymers include, without limitation, hydrophilic polyethylene, polystyrenes, polypropylenes, acrylates, methacrylates, polycarbonates, polysulfones, polyesterketones, poly- or cyclic olefins, polychlorotrifluoroethylene, and polyethylene therephthalate.

[00126] The term “reduce” or “reducing” as used herein refers to the limiting of an occurrence of a disease, disorder or condition in individuals at risk of developing the disorder.

[00127] The term “relapse” as used herein refers to the return of a disease or the signs and symptoms of a disease after a period of improvement.

[00128] The term “Rho” as used herein refers to a subfamily of proteins related to the RAS subgroup thought to be involved in cell transformation and the regulation of morphology and function of dendritic cells. Non-limiting examples of Rho proteins include RhoA, RhoB and RhoC, RhoG ,RhoH, RhoQ, RhoU RhoV, Rnd1, 2 and 3 (e.g., RhoE), and RAC1, 2, 3 and 4.

[00129] The term “ROCK”as used herein refers to Rho associated coil-coil kinase. ROCK proteins belong to the protein kinase A, G, and C family (AGC family) of classical serine/threonine protein kinases, a group that also includes other regulators of cell shape and motility, such as citron Rho-interacting kinase (CRIK), dystrophia myotonica protein kinase (DMPK), and the myotonic dystrophy kinase-related Cdc42-binding kinases (MRCKs). The main function of ROCK signaling is regulation of the cytoskeleton through the phosphorylation of downstream substrates, leading to increased actin filament stabilization and generation of actin-myosin contractility. (Morgan-Fisher et al., “Regulation of ROCK Activity in Cancer” (2013) 61:185-198, at 185).

[00130] Two homologous mammalian serine/threonine kinases, Rho-associated protein kinases I and II (ROCK I and II), are key regulators of the actin cytoskeleton acting downstream of the small GTPase Rho. ROCK I (alternatively called ROK $\beta$) and ROCK II (also known as Rho kinase or ROK $\alpha$) are 160-kDa proteins encoded by distinct genes. The

GeneBank accession number for human ROCK I is EF445027.1; the GeneBank accession number for human ROCK II is NP_004841. The mRNA of both kinases is ubiquitously expressed, but ROCK I protein is mainly found in organs such as liver, kidney, and lung, whereas ROCK II protein is mainly expressed in muscle and brain tissue. The two kinases have the same overall domain structure and have 64% overall identity in humans, with 89% identity in the catalytic kinase domain. Both kinases contain a coiled-coil region (55% identity) containing a Rho-binding domain (RBD) and a pleckstrin homology (PH) domain split by a C1 conserved region (80% identity). Despite a high degree of homology between the two ROCKs, as well as the fact that they share several common substrates, studies have shown that the two ROCK isoforms also have distinct and non-redundant functions. For example, ROCK I has been shown to be essential for the formation of stress fibers and focal adhesions, whereas ROCK II is required for myosin II-dependent phagocytosis.

[00131] ROCKs exist in a closed, inactive conformation under quiescent conditions, which is changed to an open, active conformation by the direct binding of guanosine triphosphate (GTP)-loaded Rho. (Morgan-Fisher et al., “Regulation of ROCK Activity in Cancer” (2013) 61:185-198). Rho is a small GTPase which functions as a molecular switch, cycling between guanosine diphosphate (GDP) and guanosine triphosphate (GTP) bound states under signaling through growth factors or cell adhesion receptors. (Morgan-Fisher et al., “Regulation of ROCK Activity in Cancer” (2013) 61:185-198, at 185) GTPases are hydrolase enzymes that bind and hydrolyze GTP. In a similar way to ATP, GTP can act as an energy carrier, but it also has an active role in signal transduction, particularly in the regulation of G protein activity. G proteins, including Rho GTPases, cycle between an inactive GDP-bound and an active GTP-bound conformation. The transition between the two conformational states occurs through two distinct mechanisms: activation by GTP loading and inactivation by GTP hydrolysis. GTP loading is a two-step process that requires the release of bound GDP and its replacement by a GTP molecule. Nucleotide release is a spontaneous but slow process that has to be catalyzed by RHO-specific guanine nucleotide exchange factors (RHOGEFs), which associate with RHO GTPases and trigger release of the nucleotide. The resulting nucleotide-free binary complex has no particular nucleotide specificity. However, the cellular concentration of GTP is markedly higher than that of GDP, which favors GTP loading, resulting in the activation of RHO GTPases.

[00132] Conversely, to turn off the switch, GTP has to be hydrolyzed. This is facilitated by RHO-specific GTPase-activating proteins (RHOGAPs), which stimulate the

intrinsically slow hydrolytic activity of RHO proteins. Although guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) are the canonical regulators of this cycle, several alternative mechanisms, such as post-translational modifications, may fine-tune the RHO switch. In addition, inactive RHO GTPases are extracted by RHO-specific guanine nucleotide dissociation inhibitors (RHOGDIs) from cell membranes to prevent their inappropriate activation and to protect them from misfolding and degradation. (R. Garcia-Mata et al. Nature Reviews Molecular Cell Biology (2011) 12:493-504; at 494).

[00133] Rho-ROCK signaling has been implicated in cell cycle regulation. Rho-ROCK signaling increases cyclin D1 and Cip1 protein levels, which stimulate G1/S cell cycle progression. (Morgan-Fisher et al., “Regulation of ROCK Activity in Cancer” (2013) 61:185-198, at 189). Polyploidization naturally occurs in megakaryocytes due to an incomplete mitosis, which is related to a partial defect in Rho-ROCK activation, and leads to an abnormal contractile ring lacking myosin IIA.

[00134] Rho-ROCK signaling also has been linked to apoptosis and cell survival. During apoptosis, ROCK I and ROCK II are altered to become constitutively-active kinases. Through proteolytic cleavage by caspases (ROCK I) or granzyme B (ROCK II), a carboxyl-terminal portion is removed that normally represses activity. Interaction with phosphatidyl inositol (3,4,5)-triphosphate (PIP3) provides an additional regulatory mechanism by localizing ROCK II to the plasma membrane where it can undertake spatially restricted activities, i.e. the regulation by localization of enzymatic activity. Phosphorylation at multiple specific sites by polo-like kinase 1 was found to promote ROCK II activation by RhoA. (Olson (2008) “Applications for ROCK kinase inhibition” Curr Opin Cell Biol 20(2): 242-248, at 242.) Additional Serine/Threonine and Tyrosine kinases may also regulate ROCK activity given that more phosphorylations have been identified. (Olson (2008) “Applications for ROCK kinase inhibition” Curr. Opin. Cell Biol. 20(2): 242-248, at 242.) Specifically, protein oligomerization induces N-terminal trans-phosphorylation. (K. Riento and A.J. Ridley, “ROCKs: multifunction kinases in cell behavior.” Nat. Rev. Mol. Cell Biol. (2003) 4:446-456) Other direct activators include intracellular second messengers such as arachodonic acid and sphingosylphosphorylcholine which can activate ROCK independently of Rho. Furthermore, ROCK1 activity can be induced during apoptosis. (Mueller, B.K. et al., “Rho Kinase, a promising drug target for neurological disorders.” (2005) Nat. Rev. Mol. Cell Biol. 4(6): 387-398.)

[00135] ROCK protein signaling reportedly acts in either a pro- or anti-apoptotic fashion depending on cell type, cell context and microenvironment. For instance, ROCK proteins are essential for multiple aspects of both the intrinsic and extrinsic apoptotic processes, including regulation of cytoskeletal-mediated cell contraction and membrane blebbing, nuclear membrane disintegration, modulation of Bcl2-family member and caspase expression/activation and phagocytosis of the fragmented apoptotic bodies (Figure 4) (B.K. Mueller et al. "Rho Kinase, a promising drug target for neurological disorders." (2005) Nature Rev.: Drug Discovery 4:387-398). In contrast, ROCK signaling also exhibits pro-survival roles (Figure 4). Though a wealth of data exists to suggest both pro- and anti-survival roles for ROCK proteins, the molecular mechanisms that modulate these pleitropic roles are largely unknown. (C.A. Street and B.A. Bryan, "Rho Kinase proteins – pleiotropic modulators of cell survival and apoptosis." (2011) Anticancer Res. 31(11):3645-3657).

[00136] The term "risk of intestinal graft-versus-host disease (GVHD)" as used herein refers to a potential for a population of T lymphocytes derived from a potential allogeneic donor to damage intestinal epithelial cells of a recipient subject.

[00137] The term "ROCK inhibitor" as used herein refers to any molecule that decreases the function of a ROCK protein.

[00138] The term "stimulate" in any of its grammatical forms as used herein refers to inducing activation or increasing activity.

[00139] As used herein, the terms "subject" or "individual" or "patient" are used interchangeably to refer to a member of an animal species of mammalian origin, including humans. The term "a subject in need thereof" is used to refer to a subject in need of allo-BMT or a subject at risk for the complication GVHD disease.

[00140] The term "suspension culture" as used herein refers to cells which do not require attachment to a substratum to grow, i.e. they are anchorage independent. Cell cultures derived from blood are typically grown in suspension. Cells can grow as single cells or clumps. To subculture the cultures which grow as single cells they can be diluted. However, the cultures containing clumps need to have the clumps disassociated prior to subculturing of the culture.

[00141] The term "symptom" as used herein refers to a phenomenon that arises from and accompanies a particular disease or disorder and serves as an indication of it.

[00142] The term "syndrome," as used herein, refers to a pattern of symptoms indicative of some disease or condition.

[00143] The term “therapeutic agent” as used herein refers to a drug, molecule, nucleic acid, protein, metabolite, composition or other substance that provides a therapeutic effect. The term “active” as used herein refers to the ingredient, component or constituent of the compositions of the described invention responsible for the intended therapeutic effect. The terms “therapeutic agent” and “active agent” are used interchangeably herein. The term "therapeutic component" as used herein refers to a therapeutically effective dosage (i.e., dose and frequency of administration) that eliminates, reduces, or prevents the progression of a particular disease manifestation in a percentage of a population. An example of a commonly used therapeutic component is the ED50 which describes the dose in a particular dosage that is therapeutically effective for a particular disease manifestation in 50% of a population.

[00144] The terms “therapeutic amount”, "therapeutically effective amount", an "amount effective", or “pharmaceutically effective amount” of an active agent is used interchangeably to refer to an amount that is sufficient to provide the intended benefit of treatment.

[00145] The term “therapeutic effect” as used herein refers to a consequence of treatment, the results of which are judged to be desirable and beneficial. A therapeutic effect can include, directly or indirectly, the arrest, reduction, or elimination of a disease manifestation. A therapeutic effect can also include, directly or indirectly, the arrest reduction or elimination of the progression of a disease manifestation.

[00146] General principles for determining therapeutic effectiveness, which may be found in Chapter 1 of Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Edition, McGraw-Hill (New York) (2001), incorporated herein by reference, are summarized below.

[00147] Pharmacokinetic principles provide a basis for modifying a dosage regimen to obtain a desired degree of therapeutic efficacy with a minimum of unacceptable adverse effects. In situations where the drug's plasma concentration can be measured and related to the therapeutic window, additional guidance for dosage modification can be obtained.

[00148] Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration. Two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions.

[00149] The term "therapeutic window" refers to a concentration range that provides therapeutic efficacy without unacceptable toxicity. Following administration of a dose of a drug, its effects usually show a characteristic temporal pattern. A lag period is present before the drug concentration exceeds the minimum effective concentration ("MEC") for the desired effect. Following onset of the response, the intensity of the effect increases as the drug continues to be absorbed and distributed. This reaches a peak, after which drug elimination results in a decline in the effect's intensity that disappears when the drug concentration falls back below the MEC. Accordingly, the duration of a drug's action is determined by the time period over which concentrations exceed the MEC. The therapeutic goal is to obtain and maintain concentrations within the therapeutic window for the desired response with a minimum of toxicity. Drug response below the MEC for the desired effect will be subtherapeutic, whereas for an adverse effect, the probability of toxicity will increase above the MEC. Increasing or decreasing drug dosage shifts the response curve up or down the intensity scale and is used to modulate the drug's effect. Increasing the dose also prolongs a drug's duration of action but at the risk of increasing the likelihood of adverse effects. Accordingly, unless the drug is nontoxic, increasing the dose is not a useful strategy for extending a drug's duration of action.

[00150] Instead, another dose of drug should be given to maintain concentrations within the therapeutic window. In general, the lower limit of the therapeutic range of a drug appears to be approximately equal to the drug concentration that produces about half of the greatest possible therapeutic effect, and the upper limit of the therapeutic range is such that no more than about 5% to about 10% of patients will experience a toxic effect. These figures can be highly variable, and some patients may benefit greatly from drug concentrations that exceed the therapeutic range, while others may suffer significant toxicity at much lower values. The therapeutic goal is to maintain steady-state drug levels within the therapeutic window. For most drugs, the actual concentrations associated with this desired range are not and need not be known, and it is sufficient to understand that efficacy and toxicity are generally concentration-dependent, and how drug dosage and frequency of administration affect the drug level. For a small number of drugs where there is a small (two- to three-fold) difference between concentrations resulting in efficacy and toxicity, a plasma-concentration range associated with effective therapy has been defined.

[00151] In this case, a target level strategy is reasonable, wherein a desired target steady-state concentration of the drug (usually in plasma) associated with efficacy and

minimal toxicity is chosen, and a dosage is computed that is expected to achieve this value. Drug concentrations subsequently are measured and dosage is adjusted if necessary to approximate the target more closely.

[00152] In most clinical situations, drugs are administered in a series of repetitive doses or as a continuous infusion to maintain a steady-state concentration of drug associated with the therapeutic window. To maintain the chosen steady-state or target concentration ("maintenance dose"), the rate of drug administration is adjusted such that the rate of input equals the rate of loss. If the clinician chooses the desired concentration of drug in plasma and knows the clearance and bioavailability for that drug in a particular patient, the appropriate dose and dosing interval can be calculated.

[00153] The term “two-dimensional tissue construct” as used herein refers to a collection of cells and the intercellular substances surrounding them in a geometric configuration having length and width.

[00154] The term “three-dimensional tissue construct” as used herein refers to a tissue like collection of cells and the intercellular substances surrounding them in a geometric configuration having length, width, and depth.

[00155] The term “transplantation” as used herein, refers to removal and transfer of cells, a tissue or an organ from one part or individual to another.

[00156] As used herein the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical symptoms of a condition, or substantially preventing the appearance of clinical symptoms of a condition. Treating further refers to accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting development of symptoms characteristic of the disorder(s) being treated; (c) limiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting recurrence of symptoms in patients that were previously asymptomatic for the disorder(s).

[00157] The term “tumor necrosis factor” (TNF) as used herein refers to a cytokine made by white blood cells in response to an antigen or infection, which induce necrosis (death) of tumor cells and possesses a wide range of pro-inflammatory actions. Tumor necrosis factor also is a multifunctional cytokine with effects on lipid metabolism, coagulation, insulin resistance, and the function of endothelial cells lining blood vessels.

[00158] The terms "VEGF-1" or "vascular endothelial growth factor-1" are used interchangeably herein to refer to a cytokine that mediates numerous functions of endothelial cells including proliferation, migration, invasion, survival, and permeability. VEGF is critical for angiogenesis.

## I. An Ex Vivo Model Of Persistent T Lymphocyte Stimulation Events Encountered By Circulating T Lymphocytes At Lymph Node And Tissue Levels In Vivo Comprising A Multiwell Plate-Based Perfusion Culture Device

[00159] According to one aspect, an ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo comprises

[00160] (a) A multiwell plate-based perfusion culture device, comprising, from top to bottom:

[00161] a bottomless multi-well plate comprising a plurality of bottomless wells;

[00162] a first micropatterned polymer layer attached to a bottom surface of the bottomless multi-well plate to form a plurality of adjacent wells, one or more of each pair of adjacent wells comprising a transparent polymer membrane placed within the one ore mover of each pair of adjacent wells;

[00163] a second micropatterned polymer layer comprising two or more holes that correspond to two or more adjacent wells, the second micropatterned polymer layer being attached to a bottom surface of the first micropatterned polymer layer, such that each hole of the second micropatterned polymer layer is aligned with the two or more adjacent wells in the first micropatterned polymer layer, one or more of each pair of adjacent wells comprising the transparent polymer membrane;

[00164] a microfluidic channel formed between the two adjacent wells that allows internal fluidic communication between the two adjacent wells;

[00165] one or more removable polymer plugs, each located at a top surface of each of the plurality of wells, and one or more tubes, each connected to the one or more polymer plugs;

[00166] a pump connected to a reservoir that removably connects to the tubes;

[00167] a transparent, optical grade glass layer attached to the bottom surface of the second micropatterned polymer layer that forms a bottom surface for the plurality of wells and that seals the multi-well plate perfusion culture device;

[00168] wherein one or more of the two adjacent wells is a culture chamber for culturing a population of cells;

[00169] (b) an expanded population of cells derived from a recipient subject comprising a cell-specific antigen in the first adjacent well of the device in (a);

[00170] (c) an expanded population of T lymphocytes derived from a potential donor of a BMT graft in the second adjacent well of the device in (a), wherein the potential donor is allogeneic to the recipient subject;

[00171] (d) A liquid culture medium that is flowable between the first adjacent well and the second adjacent well;

[00172] The model being characterized by:

[00173] (i) Circulation of the liquid medium from the first well into the second well and back to the first well through the microfluidic channel;

[00174] (ii) An interaction between the population of cells comprising the cell antigen derived from the recipient subject in the first well and the population of T lymphocytes is effective to generate alloreactive effector T lymphocytes; and

[00175] (iii)Alloreactive effector T lymphocyte-induced quantifiable damage to the population of cells comprising the cell antigen derived from the recipient by the population of alloreactive effector T lymphocytes from the donor allogeneic to the recipient.a multiwell plate-based perfusion culture device for culturing cells ex vivo with continuous perfusion of a liquid medium is used to replicate the interactions of human T cells and intestinal epithelial cells in vivo.

[00176] With respect to the multiwell plate-based perfusion culture device, according to one embodiment of the described invention, the device comprises a plurality of layers. According to some such embodiments, the multiwall plate-based perfusion culture device comprises a bottomless multi-well plate including a plurality of bottomless wells; a first micropatterned polymer layer comprising a plurality of transparent polymer membranes therein, a second micropatterned polymer layer comprising a plurality of holes therethrough, a third micropatterned polymer layer comprising a plurality of holes therethrough, one blank glass layer for use with plate readers; and a plurality of fluidic passages formed between the polymer membrane and the blank glass layer. The term “bottomless multi-well plate” as used herein refers to a multi-well plate without a bottom surface; and the term “bottomless wells” as used herein refers to wells of the multi-well plate without a bottom surface.

[00177] According to some embodiments, the device can comprise more than three micropatterned polymer layers.

[00178] According to some embodiments, the first micropatterned polymer layer is attached to a bottom surface of the bottomless multi-well plate such that each of the plurality of transparent polymer membranes corresponds to each of the plurality of wells when the number of the polymer membranes is equal to the number of the wells, wherein the second micropatterned polymer layer is attached to a bottom surface of the first micropatterned polymer layer such that each of the plurality of holes corresponds to each of the plurality of wells.

[00179] According to some embodiments, a polymer membrane is placed in every other well in the multiwell plate so that the number of the polymer membranes in the multiwall plate equals one-half of the number of the wells.

[00180] According to some embodiments, the third micropatterned polymer layer is attached to a bottom surface of the second micropatterned polymer layer such that each of the plurality of holes in the third micropatterned polymer layer corresponds to two adjacent wells, thereby creating a microfluidic channel between the two adjacent wells to allow internal fluidic communication between the two adjacent wells. According to some embodiments, the third micropatterned polymer layer is attached to a bottom surface of the second micropatterned polymer layer such that each of the plurality of holes in the third micropatterned polymer layer corresponds to more than two adjacent holes in the second micropatterned polymer layer.

[00181] According to some embodiments, the second micropatterned polymer layer is omitted, and the third micropatterned polymer layer is attached to the bottom surface of the first micropatterned polymer layer, such that each hole of the third micropatterned polymer layer corresponds to one or more adjacent polymer membranes in the first micropatterned polymer layer.

[00182] According to some embodiments, the microfluidic channel is 200 μm thick and 5 μm high.

[00183] According to some embodiments, one of the two adjacent wells is a culture chamber, which is used to culture cells or tissues; and the second adjacent well is an outlet chamber, which is used to direct the effluent streams to exit through the top of the device, wherein a first tubing attached to the culture chamber is an inlet and a second tubing attached to the outlet chamber is merely an outlet, thus providing re-circulation of liquid medium

together with non-adherent cells between two chambers. According to some such embodiments, the outlet chamber may or may not contain a polymer membrane.

[00184] According to some embodiments, both of the two adjacent wells are culture chambers, which are used to culture different cells or tissues. For example, according to an embodiment wherein both of the two adjacent wells are culture chambers, and these culture chambers are used to screen samples for determining a patient's risk of developing GVHD, the first chamber is used to culture epithelial cells, and the second is used to culture dendritic cells. According to some embodiments, the tubing connected to the first culture chamber is an inlet and another tubing connected to the second culture chamber is an outlet; thus providing re-circulation of liquid medium together with non-adherent cells between the two chambers.

[00185] According to some embodiments, the blank glass layer provides optical access through the bottom of the chambers for cell characterization with plate readers. According to some embodiments, the blank glass layer is attached to a bottom of the third micropatterned polymer layer to seal the multi-well plate culture device thereby forming a bottom surface thereof for the plurality of wells. According to some embodiments, the blank glass layer is about 1.2 mm-thick.

[00186] According to some embodiments, instead of comprising a plurality of layers, the well plate-based perfusion culture device comprises one polymer substrate which has multiple layers of holes therein, a first layer of holes comprises a plurality of holes, each corresponding to a shape and size and location of each of the plurality of wells, and a second layer of holes comprises a plurality of holes, each corresponding to a size and location of every two adjacent wells, thereby allowing internally fluidly connection between every two adjacent wells. According to some embodiments, each of the plurality of holes in the first layer of polymer substrate further has a transparent polymer membrane attached thereto.

[00187] According to some embodiments, the polymer substrate is made from polymer extrusion molding.

[00188] According to some embodiments, the micropatterned polymer layers are made of a polymer, e.g., polydimethyl siloxane (PMDS), polystyrene or the like.

[00189] According to some embodiments, the multi-well plate, the micropatterned polymer layers, and the glass layer are bonded (meaning joined securely to each other, for example, by an adhesive, a heat process, or pressure) using oxygen plasma treatments.

[00190] According to some embodiments, the multi-well plate-based perfusion culture device further comprises a plurality of removable polymer plugs (meaning a piece of material used to stopper an aperture), each located at a top surface of each of the plurality of wells; and a plurality of tubes (meaning a hollow, elongated body), each connected to each of the plurality of polymer plugs. According to some embodiments, the removable polymer plugs are made of a polymer, e.g., PDMS, polystyrene, or the like. According to some such embodiments, the removable polymer plugs made of PDMS are made by soft lithography. According to some such embodiments, the removable polymer plugs made of polystyrene (PS) are made by PS extrusion and bonding.

[00191] According to some embodiments, the device further comprises at least one pump connected to at least one reservoir, which removably connects to the tubes, e.g., the first tube and the second tube. According to some such embodiments; the pump controls flow rate of recirculation of the liquid medium, for example, via one or more valves, into and out of the wells.

[00192] According to some embodiments, the tube that connects the two adjacent chambers at the top of the device is a U-shaped tubing, and flow of a liquid medium is driven by the difference between an amount of liquid medium inside chamber 1 and chamber 2 until equilibrium is established.

[00193] According to some embodiments, a method for culturing cells in the multiwall plate device comprises (a) providing a liquid medium into a first well that is fluidly connected to a second well, such that the liquid medium flows from the first well into the second well, which is the well adjacent to the first well through the microfluidic channel, and (2) recycling the liquid medium back to the first well through a reservoir and pump or a U-tube externally connecting the two wells at the top of the device. According to some embodiments, the liquid medium flows at a rate of about 10-50 μL/min. According to some embodiments, the multi-well plate comprises at least 6, at least 12, at least 24, at least 48, at least 96, at least 384 or at least 1536 wells. The wells may have dimensions substantially same as the dimensions of the wells in plate currently commercially available for commercially available readers and dispensers. According to some embodiments, the multi-well plate has a substantially rectangular shape appropriate for commercially available readers and dispensers. According to some embodiments, the multi-well plate can have a shape different from rectangular.

[00194] According to some embodiments, the multi-well plate may be constructed of polymeric materials. Exemplary polymers include, without limitation, hydrophilic polyethylenes, polystyrenes, polypropylenes, acrylates, methacrylates, polycarbonates, polysulfones, polyesterketones, poly- or cyclic olefins, polychlorotrifluoroethylene, and polyethylene therephthalate. According to some embodiments, the multi-well plate can be constructed of polystyrene. According to some embodiments, the multi-well plate may be constructed of inorganic polymer materials.

[00195] According to some embodiments, the transparent polymer membrane provides optical access through the bottom surface of the culture chambers for cell characterization with plate readers. According to some embodiments, the transparent polymer membrane anchors tissue cells and biomaterials. According to some embodiments, the transparent polymer membrane is a transparent polycarbonate (PC) membrane. According to some embodiments, the transparent polymer membrane is a polyethylene terephthalate (PET) membrane. According to some such embodiments, the PET membrane has an average pore size of 8 μm.

[00196] According to some embodiments, the micropatterned polymer layers are used to anchor placement of the polymer membranes within the wells of the device that comprise one or more culture chambers.

[00197] According to some embodiments, the micropatterned polymer layers are constructed of a polymer. According to some such embodiments, the micropatterned polymer layers are made of polydimethyl siloxane (PMDS) or polystyrene. According to some such embodiments, the micropatterned polymer layers made of PMDS are made by soft lithography. According to some such embodiments, the micropatterned polymer layers made of polystyrene (PS) are made by PS extrusion and bonding.

[00198] According to some embodiments, the device further comprises biocompatible non-living material formed into a three-dimensional structure comprising interstitial spaces, for example, nanofibers or microbeads that are placed on a top surface of the polymer membrane. According to some embodiments, the microbeads comprise a polymer. According to some such embodiments, the microbeads comprise polystyrene. According to some such embodiments, the microbeads comprise biphasic calcium phosphate (BCP).

[00199] According to some embodiments, the polymer membrane is coated with a nanofiber mesh. According to some such embodiments, the nanofiber mesh comprises an

electrospun PCL/collagen mesh. According to some embodiments, the PCL/collagen mesh comprises a nanofiber matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass. According to some embodiments, the nanofiber matrix comprising the plurality of pores mimics the basement membrane of epithelial tissue and supports viability of the intestinal epithelial cells derived from the recipient subject.

[00200] According to some embodiments, the population of T lymphocytes in (c) is derived from peripheral blood lymphocytes of the potential donor. According to some embodiments, the population of T lymphocytes comprises a suspension of nonadherent cells. According to some embodiments, wherein the suspension of nonadherent cells contains T lymphocytes derived from the allogeneic donor. According to some embodiments, wherein the population of alloreactive effector T lymphocytes comprises alloreactive activated antigen presenting cells. According to some embodiments, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells. According to some embodiments, wherein the quantifiable damage to the population of cells comprising the cell-specific antigen derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death. According to some embodiments, wherein the population of cells comprising the cell-specific antigen in (b) are a population of intestinal epithelial cells derived from the recipient subject, and the alloreactive effector T lymphocyte-induced quantifiable damage to the population of intestinal epithelial cells of the recipient subject is an ex vivo measure of risk of graft vs. host disease.

## II. A Method For Optimizing Donor Selection For Allogeneic Transplantation And For Predicting Risk Of GVHD

[00201] A method for optimizing donor selection for allogeneic blood and marrow transplantation (BMT) therapy comprises, in order:

[00202] (a) acquiring a tissue sample from a recipient subject allogeneic to a potential donor of a BMT graft, the tissue sample comprising a population of primary intestinal epithelial cells comprising an intestinal epithelial cell-specific antigen;

[00203] (b) seeding the population of primary intestinal epithelial cells of (a) in a first adjacent well of a multiwall plate-based perfusion culture device, the first adjacent well comprising a transparent polymer membrane, expanding the population in a first liquid medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast

feeder layer and generating a population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising the intestinal cell-specific antigen derived from the recipient subject;

[00204] (c) acquiring a population of T lymphocytes from the potential donor allogeneic to the recipient;

[00205] (d) seeding and expanding in a second adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes derived from the potential donor of (c),

[00206] (e) co-culturing in a second liquid medium the CRIECs derived from the recipient subject in the first adjacent well and the T lymphocytes derived from the potential donor allogeneic to the recipient subject in the second adjacent well, the co-culturing being characterized by:

[00207] (i) the first adjacent well being fluidly connected to the second adjacent well so that the second liquid medium is flowable between the first adjacent well and the second adjacent well; and

[00208] (ii) an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes that is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor;

[00209] (f) measuring damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of a risk of intestinal graft versus host disease in the recipient subject;

[00210] (g) ranking a plurality of potential donors by the measure of the risk of intestinal graft versus host disease; and

[00211] (h) treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient subject whose T lymphocytes are characterized by a reduced risk of intestinal graft-versus-host disease.

[00212] According to some embodiments, the sample is a biopsy sample. According to some embodiments, the biopsy sample is a small biopsy sample of the order of 3 mm3. According to some embodiments, the biopsy sample is collected from intestinal tissue. According to some embodiments, the biopsy sample is collected from intestinal tissue by colonoscopy, endoscopy, or a combination thereof.

[00213] According to some embodiments the potential donor is a haploidentical donor (i.e., parent, child and other close relative),

[00214] According to some embodiments, the patient sample is acquired soon after diagnosis of a hematological malignancy for which allogeneic BMT is a potential therapeutic approach and stored for later use in the method. According to some embodiments, the patient sample is acquired in the relapse setting after chemotherapeutic interventions have been exhausted.

[00215] According to some embodiments, any cells of interest may be cultured. According to some embodiments, the cells to be cultured can include normal, diseased, stem, cancerous, and/or mutated cells.

[00216] According to some embodiments, the primary IECs are prepared from the small intestine, large intestine or colon of the recipient subject, and expanded using conditional reprogrammed cell technology, which comprises cultivating the primary IECs in a medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer. According to some embodiments the medium for cultivating the primary human IECs containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer is RPMI.

[00217] While expansion of conditionally reprogrammed cells is useful in expanding the IECs from biopsy samples, CRCs cannot be used for co-culture of CRIECs and T cells for 2-3 weeks due to adverse effects of CRC media additives (e.g., ROCK kinase inhibitor) on T cell motility and functionality. (Riento, et al., Molecular cell biology (2003) 4, 446-456; Iyengar, et al., Journal of the American Society for Blood and Marrow Transplantation, doi:10.1016/j.bbmt.2014.04.029 (2014)). According to some embodiments, the CRC medium is replaced with a complete RPI-1640 medium (defined as RPMI-1640 supplemented with 10% fetal bovine serum and 5% L-glutamine) to culture the T cells.

[00218] In native tissues, IECs reside on the thin fibrous basement membrane (BMa) consisting of intermingled networks of laminins and type IV collagen and provide cell anchoring and barrier functions. The membrane networks interact with cells through membranous integrin receptors and other plasma membrane molecules, influencing cell differentiation, migration, adhesion, phenotype and survival.

[00219] According to some embodiments, the first well comprises a nanofibrous coated transparent polymer membrane. According to some embodiments, the nanofibrous coating is prepared by electrospinning. According to some embodiments, the nanofibrous

coating comprises a fiber matrix of polycaprolactone in which extracellular matrix (ECM)-like molecules (e.g., collagen) is dispersed. According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to maintain the long term functionality of CRIECs and T cells using RPMI as a common culture medium. Wang's prior research (Fu, et al. Biomaterials (2014) 35, 1496-1506) has shown that, as a result of mimicking the morphological and dimensional characteristics of base membrane extracellular matrix (ECM) fibrils, nanofibrous meshes can support keratinocytes to form skin-like structures and maintain cobble stone-like morphology for 2 weeks (Huang, et al., Biomaterials (2012) 33, 1791-1800).

[00220] According to some embodiments, the method comprises providing polymer microbeads preconditioned with one or more adhesion-promoting agents to promote adhesion of cells to at least one surface of the microbeads. According to some such embodiments, the cells are dendritic cells (DCs). According to some embodiments, the adhesion promoting agent comprises an effective amount of lipopolysaccharides (LPS), wherein the LPS are effective to promote adhesion of the DCs to the microbeads surface.

[00221] According to some embodiments, the first well of the multiwall plate device contains a population of conditionally reprogrammed IECs prepared from a mammalian subject, and the second well fluidly connected to the first well contains T cells comprising dendritic cells from a mammal allogeneic to the mammalian subject. According to some such embodiments, the mammal is a mouse. According to some such embodiments, the mammal is a human.

[00222] According to some embodiments, a CRIEC culture chamber can be established by placing CRIECs into the first well on top of a polymer membrane coated with an electrospun PCL/collagen nanofibrous mesh.

[00223] According to some embodiment, an average open space (or pore size) in the nanofibrous mesh is within a range of about 1-10 μm. According to some such embodiments, the average open space (or pore size) in the nanofiber mesh is about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μm.

[00224] According to some embodiments, an antigen presenting cell (APC) culture chamber can be established by placing T cells comprising DCs in the liquid medium onto the pre-treated polymer microbeads in the second well. According to some embodiments, one or more cytokines can be added to the culture chamber to prolong T cell maintenance.

According to one embodiment, the population of T-cells suspended in the liquid medium comprises about 105 to 106 cells.

[00225] According to some embodiments, the method further comprises replenishing DCs with new DCs by opening a polymer plug on the top of the APC chamber and placing new DCs onto the top of the microbead assembly. According to some embodiments, the dendritic cell assembly can be replaced by a new microbead/dendritic cell assembly.

[00226] According to some embodiments, an average size of a polymer microbead is in a range of about 45-90 μm. According to some such embodiments, the average size of a polymer microbead is about 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90 μm.

[00227] According to some embodiments, the T cells comprising dendritic cells are derived from peripheral blood lymphocytes. According to some embodiments, mouse dendritic cells are enriched by injecting host mice with a B16-FLt3L tumor.

[00228] According to some embodiments, the polymer membrane has an average pore size that provides a sufficient opening for T cells to go through. According to some such embodiments, the average pore size of the polymer membrane is about 7-13 μm. According to some such embodiments, the average pore size of the polymer membrane is about 7, 8, 9, 10, 11, 12 or 13 μm. According to some such embodiments, an average diameter of a T cell is about 5 μm.

[00229] According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to anchor a population of cells. According to some embodiments, the polymer membrane comprises the population of human intestinal epithelial cells, the population of CRIECs, or a combination thereof.

[00230] According to some embodiments the cells to be cultured can be cultured in free suspensions, encapsulated in suitable hydrogels, encapsulated in matrices, and/or encapsulated in scaffolds. For example, according to some embodiments, the T cells comprising a suspension of about 106 T cells (e.g., eGFP m-T cells (harvested from an eGFP transgenic B6 mouse) or h-T cells) in a culture medium are flowable, i.e., they circulate with the liquid medium of the microfluidic well plate-based perfusion culture device. According to one embodiment, the culture medium contains retinoic acid, which facilitates the generation of T cells with superior IEC-killing avidity.

[00231] According to some embodiments, a ratio of CRIECs: T cells is in a range of from 2:1 to 20:1. According to some such embodiments, the ratio of CRIECS: T cells is

2:1, 34:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, or 20:1.

[00232] According to some embodiments, a ratio of T cells: DCs is in a range of from 1:1 to 20:1. According to some such embodiments, the ratio of T cells: DCs is 1:2, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12: 1, 13:1, 14:1, 15:1, 16:1, 17:1, 18: 1, 19:1, or 20:1. According to some such embodiments, the ratio of T cells: DCs is 5:1.

[00233] According to some embodiments, the co-culturing of the population of human IECs from the proposed recipient subject and the population of T lymphocytes from the donor allogeneic to the recipient subject is effective to generate alloreactive activated T-lymphocytes. According to some embodiments, the allogeneic activated/effector T cells recirculate through interconnected h-CRIEC and antigen presenting cell (APC) culture chambers. According to some embodiments, the alloreactive activated/effector T-lymphocytes comprise a population of antigen presenting cells. According to some such embodiments, the population of antigen presenting cells comprises a population of dendritic cells. According to some embodiments, the alloreactive T cells become activated by cognitive alloantigens on h-CRIECs. According to some embodiments, the alloreactive activated/effectorT cells comprise activated antigen presenting cells (APCs). According to some embodiments, the APCs comprise activated/effector dendritic cells. According to some embodiments, the alloreactive activated/effector T cells are effective to induce quantifiable damage to the population of h-CRIECs. According to some such embodiments, the device is clinically viable, i.e., it is effective to increase the critical number of functional T cells required to induce quantifiable alloreactivity in the CRIEC culture chamber within a diagnostic screening time frame of 2-3 weeks.

[00234] According to some embodiments, quantifiable damage to the population of CRIECs comprises measurable killing of the population of CRIECS. According to some embodiments, a pathological index (PIdx) is used to quantify T cell induced CRIEC damage. For example, the predictive capability of co-culture killing assays can be compared to known in vivo outcomes from well-established murine models of BMT.

[00235] According to some such embodiments, a panel of cell death analysis methods is used to quantify cell death. For example, annexin V/PI staining using flow cytometry and in situ detection of cleaved caspase-3 using immunofluorescence can be used to determine cell death. According to some embodiments, the percentage of dead cells is calculated as [% of Annexin V+/PI+ cells in co-cultures - % of Annexin V+/PI+ cells in IEC

alone cultures] (flow cytometric measurement). According to some embodiments, CD3 staining is performed to identify adherent T cells contributing to the response. According to some embodiments, the caspases-3 staining is conducted in the well plate to determine cell death as [fluorescence intensity in co-culture - fluorescence intensity in IEC alone cultures]/[fluorescence intensity of DAPI staining, as an indicator of the number of nucleated cells in the cultures].

[00236] According to some embodiments, cell death is evaluated at three or more T cell-IEC ratios (i.e., the effector: target, or E:T ratio). According to some embodiments, the E:T ratio is 30, 10, or 3. See Choksi, S. et al, "A cD8 DE loop peptide analog prevents graft versus host disease in a multiple minor histocompatibility antigen-mismatched bone marrow transplantation model," Biology of Blood and Marrow Transplantation: 10: 669-680, doi: 10.1016/j.bbmt.2004.06.005 (2004)).

[00237] According to some embodiments, the PIdx can be determined as the slope of the curve of percentage of dead cells vs. E:T ratios, where a steeper curve indicates a higher risk for developing GVHD. According to some embodiments, the PIdx can be determined at multiple time points post-co-culture.

[00238] According to some embodiments, the recipient subject and potential donor are mammals.

[00239] According to some embodiments, the recipient subject and potential allogeneic donor are mice.

[00240] According to some embodiments, the recipient subject and potential allogeneic donor are human.

[00241] According to some embodiments, the method further comprises identifying donor T cell clones responsible for the damage to the population of CRIECS, and selectively deleting the specific donor T cells clones responsible for the damage from the population of donor T lymphocytes.

[00242] According to some embodiments, the method comprises dissociating the donor T cell clones responsible for the damage to the population of CRIECS from donor T cell clones responsible for an anti-tumor specific T cell response, such that a therapeutically effective amount of the remaining donor T cell clones is effective to mediate GVT responses against tumor specific antigens.

[00243] According to some embodiments, the cells are cultured only in the first well, and the connected adjacent second well is an outlet well providing exit of the liquid medium from the top of the device.

[00244] According to some embodiments, cells of different types may be cultured at the same time in different fluidly connected wells of the plate-based perfusion device. For example, a first cell type can be seeded in and cultured in the first well while a second cell type can be seeded in and cultured in the second well at the same time.

## III. A method for minimizing risks and maximizing benefits of performing blood and marrow transplantation (BMT) in a recipient subject, wherein the recipient subject has a hematologic malignancy.

[00245] A method for minimizing risks and maximizing benefits of performing blood and marrow transplantation (BMT) in a recipient subject, wherein the recipient subject has a hematologic malignancy, comprising, in order:

(A) Evaluating a population of T lymphocytes derived from a potential donor, allogeneic to the recipient subject, for a potential to damage intestinal epithelial cells of the recipient subject according to steps (a) through (g) of the method above, i.e.,

[00246] (a) acquiring a tissue sample from a recipient subject allogeneic to a potential donor of a BMT graft, the tissue sample comprising a population of primary intestinal epithelial cells comprising an intestinal epithelial cell-specific antigen;

[00247] (b) seeding the population of primary intestinal epithelial cells of (a) in a first adjacent well of a multiwall plate-based perfusion culture device, the first adjacent well comprising a transparent polymer membrane, expanding the population in a first liquid medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer and generating a population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising the intestinal cell-specific antigen derived from the recipient subject;

[00248] (c) acquiring a population of T lymphocytes from the potential donor allogeneic to the recipient;

[00249] (d) seeding and expanding in a second adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes derived from the potential donor of (c),

[00250] (e) co-culturing in a second liquid medium the CRIECs derived from the recipient subject in the first adjacent well and the T lymphocytes derived from the potential donor allogeneic to the recipient subject in the second adjacent well, the co-culturing being characterized by:

[00251] (i) the first adjacent well being fluidly connected to the second adjacent well so that the second liquid medium is flowable between the first adjacent well and the second adjacent well; and

[00252] (ii) an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes that is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor;

[00253] (f) measuring damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of a risk of intestinal graft versus host disease in the recipient subject;

[00254] (g) ranking a plurality of potential donors by the measure of the risk of intestinal graft versus host disease; and**;**

[00255] (B) evaluating the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject for an effective graft versus tumor response against the tumor-specific antigens by:

[00256] (i) acquiring a specimen comprising a population of tumor cells derived from the recipient subject, the population of tumor cells comprising one or more tumor specific antigens;

[00257] (ii) seeding and expanding the population of tumor cells in the second liquid medium in a third adjacent well of the multiwell plate-based perfusion culture device; the third adjacent well comprising a transparent polymer membrane;

[00258] (iii) acquiring a population of T lymphocytes derived from the potential donor allogeneic to the recipient subject;

[00259] (iv) seeding and expanding in a fourth adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes of (iii),

[00260] (v) co-culturing in the second liquid medium the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject in the third adjacent well and the population of T lymphocytes derived from the potential donor

allogeneic to the recipient subject in the fourth adjacent well, the co-culturing being characterized by:

[00261] the third adjacent well being fluidly connected to the fourth adjacent well so that the second liquid medium is flowable between the third adjacent well and the fourth adjacent well;

[00262] an interaction between the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject that is effective to generate alloreactive effector T lymphocytes derived from the potential donor;

[00263] (vi) measuring damage to the population of tumor cells derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of an effective graft versus tumor (GVT) response against the tumor-specific antigens; and

[00264] (vii) ranking a plurality of potential donors by the measure of the effective GVT response against the tumor-specific antigens; and

[00265] C. Treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient, the selected donor being characterized by a reduced risk of intestinal graft versus host disease, and an effective GVT response against the tumor-specific antigens.

[00266] With respect to step (A), according to some embodiments, the tissue sample is a biopsy sample. According to some embodiments, the biopsy sample is a small biopsy sample of the order of 3 mm in diameter. According to some embodiments, the biopsy sample is collected from intestinal tissue. According to some embodiments, the biopsy sample is collected from intestinal tissue by colonoscopy, endoscopy, or a combination thereof.

[00267] According to some embodiments the potential donor is a haploidentical donor (i.e., parent, child and other close relative).

[00268] According to some embodiments, the patient sample is acquired soon after diagnosis of a hematological malignancy for which allogeneic BMT is a potential therapeutic approach and stored for later use in the method. According to some embodiments, the patient sample is acquired in the relapse setting after chemotherapeutic interventions have been exhausted.

[00269] According to some embodiments, the primary IECs are prepared from the small intestine, large intestine, or colon of a recipient subject, and expanded using conditional reprogrammed cell (CRC) technology, which comprises cultivating the primary IECs in a CRC medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer. According to some embodiments the medium for cultivating the primary human IECs containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer is RPMI.

[00270] According to some embodiments, the CRC medium is replaced with compete RPI-1640 medium (RPMI-1640 supplemented with 10% fetal bovine serum and 5% L-glutamine) to culture the T cells.

[00271] According to some embodiments, the first well comprises a nanofibrous coated transparent polymer membrane. According to some embodiments, the nanofibrous coating is prepared by electrospinning. According to some embodiments, the nanofibrous coating comprises a fiber matrix of polycaprolactone in which ECM-like molecules (e.g., collagen) is dispersed. According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to maintain the long-term functionality of CRIECs and T cells using RPMI as a common culture medium.

[00272] According to some embodiments, the method comprises providing polymer microbeads preconditioned with one or more adhesion-promoting agents to promote adhesion of cells to at least one surface of the microbeads. According to some such embodiments, the cells are dendritic cells (DCs). According to some embodiments, the adhesion promoting agent comprises an effective amount of lipopolysaccharides (LPS), wherein the LPS are effective to promote adhesion of the DCs to the microbeads surface.

[00273] According to some embodiments, the first well of the device contains a population of conditionally reprogrammed IECs prepared from a mammal, and the second well fluidly connected to the first well contains T cells comprising dendritic cells from an allogeneic mammal. According to some such embodiments, the mammal is a mouse. According to some such embodiments, the mammal is a human.

[00274] According to some embodiments, a CRIEC culture chamber can be established by placing CRIECs into the first well on top of a polymer membrane coated with an electrospun PCL/collagen nanofibrous mesh.

[00275] According to some embodiment, an average open space (or pore size) in the nanofibrous mesh is within a range of about 1-10 μm. According to some such

embodiments, the average open space (or pore size) in the nanofiber mesh is about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μm.

[00276] According to some embodiments, an antigen presenting cell (APC) culture chamber can be established by placing T cells comprising DCs in the liquid medium onto the pre-treated polymer microbeads in the second well. According to some embodiments, one or more cytokines can be added to the culture chamber to prolong T cell maintenance. According to one embodiment, the population of T-cells suspended in the liquid medium comprises about 105 to 106 cells.

[00277] According to some embodiments, the method further comprises replenishing DCs with new DCs by opening a polymer plug on the top of the APC chamber and placing new DCs onto the top of the microbead assembly. According to some embodiments, the dendritic cell assembly can be replaced by a new microbead/dendritic cell assembly.

[00278] According to some embodiments, an average size of a polymer microbead is in a range of about 45-90 μm. According to some such embodiments, the average size of a polymer microbead is about 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90 μm.

[00279] According to some embodiments, the T cells comprising dendritic cells are derived from peripheral blood lymphocytes. According to some embodiments, mouse dendritic cells are enriched by injecting host mice with a B16-FLt3L tumor.

[00280] According to some embodiments, the polymer membrane has an average pore size that provides a sufficient opening for T cells to go through. According to some such embodiments, the average pore size of the polymer membrane is about 7-13 μm. According to some such embodiments, the average pore size of the polymer membrane is about 7, 8, 9, 10, 11, 12 or 13 μm. According to some such embodiments, an average diameter of a T cell is about 5 μm.

[00281] According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to anchor a population of cells. According to some embodiments, the polymer membrane comprises the population of human intestinal epithelial cells, the population of CRIECs, or a combination thereof.

[00282] According to some embodiments the cells to be cultured can be cultured in free suspensions, encapsulated in suitable hydrogels, encapsulated in matrices, and/or encapsulated in scaffolds. For example, according to some embodiments, the T cells comprising a suspension of about 106 T cells (e.g., eGFP m-T cells (harvested from an eGFP

transgenic B6 mouse) or h-T cells) in a culture medium are flowable, i.e., they circulate with the liquid medium of the microfluidic well plate-based perfusion culture device. According to one embodiment, the culture medium contains retinoic acid, which facilitates the generation of T cells with superior IEC-killing avidity.

[00283] According to some embodiments, a ratio of CRIECs: T cells is in a range of from 2:1 to 20:1. According to some such embodiments, the ratio of CRIECS: T cells is 2:1, 34:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, or 20:1.

[00284] According to some embodiments, a ratio of T cells: DCs is in a range of from 1:1 to 20:1. According to some such embodiments, the ratio of T cells: DCs is 1:2, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12: 1, 13:1, 14:1, 15:1, 16:1, 17:1, 18: 1, 19:1, or 20:1. According to some such embodiments, the ratio of T cells: DCs is 5:1.

[00285] According to some embodiments, the co-culturing of the population of human IECs from the proposed recipient subject and the population of T lymphocytes from the donor allogeneic to the recipient subject is effective to generate alloreactive activated/effector T-lymphocytes. According to some embodiments, the allogeneic activated/effector T cells recirculate through interconnected h-CRIEC and antigen presenting cell (APC) culture chambers. According to some embodiments, the alloreactive activated/effector T-lymphocytes comprise a population of antigen presenting cells. According to some such embodiments, the population of antigen presenting cells comprises a population of dendritic cells. According to some embodiments, the alloreactive T cells become activated by cognitive alloantigens on h-CRIECs. According to some embodiments, the activated alloreactive T cells comprise activated antigen presenting cells (APCs). According to some embodiments, the APCs comprise activated/effector dendritic cells. According to some embodiments, the alloreactive activated/effector T cells are effective to induce quantifiable damage to the population of h-CRIECs. According to some such embodiments, the device is clinically viable, i.e., it is effective to increase the critical number of functional T cells required to induce quantifiable alloreactivity in the CRIEC culture chamber within a diagnostic screening time frame of 2-3 weeks.

[00286] According to some embodiments, quantifiable damage to the population of CRIECs comprises measurable killing of the population of CRIECS.

[00287] According to some embodiments, the recipient subject and potential allogeneic donor are mammals.

[00288] According to some embodiments, the recipient subject and potential allogeneic donor are mice. According to some embodiments, the recipient subject and potential allogeneic donor are human.

[00289] With respect to step B, according to some embodiments, the sample procured from the recipient patient with a hematologic malignancy is a blood sample, a bone marrow sample, or a leukapheresis sample. According to some embodiments, a sample is also procured from a normal, noncancerous subject to serve as a non-tumoral reference sample.

[00290] According to some embodiments, the patient sample is acquired soon after diagnosis of a hematological malignancy for which allogeneic BMT is a potential therapeutic approach and stored for later use in the method. According to some embodiments, the patient sample is acquired in the relapse setting after chemotherapeutic interventions have been exhausted.

[00291] According to some embodiments, the cell population of interest can be selected by any techniques known to the skilled artisan. For example, without limitation, according to some embodiments, cells expressing a particular cell antigen are selected by fluorescence activated cell sorting (FACS). According to some embodiments, cells expressing a particular cell antigen are selected by positive or negative immunoseparation techniques. According to some embodiments, isolation and/or purification of cells of interest from the bone marrow is based on cell fractionation methods based on size and cell density, efflux of metabolic dyes, or resistance to cytotoxic agents. According to some embodiments, RBCs are depleted by centrifugation. According to some embodiments, centrifugation at 1000×g for 20 minutes at ambient temperature is performed to separate a thin grayish white fraction of a blood sample that contains most of the white blood cells (leukocytes) (the buffy coat) from the RBCs.

[00292] According to some embodiments, the tumor samples are cultivated in a medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer (CRC medium) to generate expanded conditionally reprogrammed cells. According to some embodiments, the CRC medium is replaced with compete RPI-1640 medium (RPMI-1640 supplemented with 10% fetal bovine serum and 5% L-glutamine) to culture the T cells.

[00293] According to some embodiments, the third well comprises a nanofibrous coated transparent polymer membrane. According to some embodiments, the nanofibrous

coating is prepared by electrospinning. According to some embodiments, the nanofibrous coating comprises a fiber matrix of polycaprolactone in which ECM-like molecules (e.g., collagen) is dispersed. According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to maintain the functionality of the tumor cells and T cells using RPMI as a common culture medium.

[00294] According to some embodiments, the method comprises providing polymer microbeads preconditioned with one or more adhesion-promoting agents to promote adhesion of cells to at least one surface of the microbeads. According to some such embodiments, the cells are dendritic cells (DCs). According to some embodiments, the adhesion promoting agent comprises an effective amount of lipopolysaccharides (LPS), wherein the LPS are effective to promote adhesion of the DCs to the microbeads surface.

[00295] According to some embodiments, a tumor cell culture chamber can be established by placing tumor cells into the third well on top of a polymer membrane coated with an electrospun PCL/collagen nanofibrous mesh.

[00296] According to some embodiment, an average open space or pore size in the nanofibrous mesh is within a range of about 1-10 μm. According to some such embodiments, the average open space (or pore size) in the nanofiber mesh is about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μm.

[00297] According to some embodiments, the antigen presenting cell (APC) culture chamber can be established by placing T cells comprising DCs in the liquid medium onto the pre-treated polymer microbeads in the second well. According to some embodiments, one or more cytokines can be added to the culture chamber to prolong T cell maintenance. According to one embodiment, the population of T-cells suspended in the liquid medium comprises about 105 to 106 cells.

[00298] According to some embodiments, the method further comprises replenishing DCs with new DCs by opening a polymer plug on the top of the APC chamber and placing new DCs onto the top of the microbead assembly. According to some embodiments, the dendritic cell assembly can be replaced by a new microbead/dendritic cell assembly.

[00299] According to some embodiments, an average size of a polymer microbead is in a range of about 45-90 μm. According to some such embodiments, the average size of a polymer microbead is about 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90 μm.

[00300] According to some embodiments, the T cells comprising dendritic cells are derived from peripheral blood lymphocytes. According to some embodiments, mouse dendritic cells are enriched by injecting host mice with a B16-FLt3L tumor.

[00301] According to some embodiments, the polymer membrane has an average pore size that provides a sufficient opening for T cells to go through. According to some such embodiments, the average pore size of the polymer membrane is about 7-13 μm. According to some such embodiments, the average pore size of the polymer membrane is about 7, 8, 9, 10, 11, 12 or 13 μm. According to some such embodiments, an average diameter of a T cell is about 5 μm.

[00302] According to some embodiments, the nanofibrous coated transparent polymer membrane is effective to anchor a population of cells. According to some embodiments the cells to be cultured can be cultured in free suspensions, encapsulated in suitable hydrogels, encapsulated in matrices, and/or encapsulated in scaffolds. For example, according to some embodiments, the T cells comprising a suspension of about 106 T cells (e.g., eGFP m-T cells (harvested from an eGFP transgenic B6 mouse) or h-T cells) in a culture medium are flowable, i.e., they circulate with the liquid medium of the microfluidic well plate-based perfusion culture device.

[00303] According to some embodiments, a ratio of tumor cells: T cells is in a range of from 2:1 to 20:1. According to some such embodiments, the ratio of CRIECS: T cells is 2:1, 34:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, or 20:1.

[00304] According to some embodiments, a ratio of T cells: DCs is in a range of from 1:1 to 20:1. According to some such embodiments, the ratio of T cells: DCs is 1:2, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12: 1, 13:1, 14:1, 15:1, 16:1, 17:1, 18: 1, 19:1, or 20:1. According to some such embodiments, the ratio of T cells: DCs is 5:1.

[00305] According to some embodiments, the co-culturing of the population of tumor cells from the recipient subject and the population of T lymphocytes from the donor allogeneic to the recipient subject is effective to generate alloreactive activated T-lymphocytes. According to some embodiments, the allogeneic activated/effector T cells recirculate through the tumor cell culture chamber and the antigen presenting cell (APC) culture chamber, wherein the chambers are interconnected. According to some embodiments, the alloreactive activated T-lymphocytes comprise a population of antigen presenting cells. According to some such embodiments, the population of antigen presenting

cells comprises a population of dendritic cells. According to some embodiments, cognitive alloantigens on the tumor cells are effective to activate the alloreactive T cells. According to some embodiments, the activated alloreactive T cells comprise activated antigen presenting cells (APCs). According to some embodiments, the APCs comprise activated/effector dendritic cells. According to some embodiments, the alloreactive activated/effector T cells are effective to induce quantifiable damage to the population of tumor cells, compared to a normal control. According to some such embodiments, the device is clinically viable, i.e., it is effective to increase the critical number of functional T cells required to induce quantifiable alloreactivity in the tumor cell culture chamber within a diagnostic screening time frame of 2-3 weeks.

[00306] According to some embodiments, quantifiable damage to the expanded population of tumor cells comprises measurable killing of the expanded population of tumor cells.

[00307] According to some such embodiments, a panel of cell death analysis methods is used to quantify tumor cell death. For example, annexin V+/PI+ staining using flow cytometry and in situ detection of cleaved caspase-3 using immunofluorescence can be used to determine cell death. According to some embodiments, the percentage of dead cells is calculated as [% of Annexin V+/PI+ cells in co-cultures - % of Annexin V+/PI+ cells in tumor cells alone cultures] (flow cytometric measurement). According to some embodiments, CD3 staining is performed to identify adherent T cells contributing to the response. According to some embodiments, the caspases-3 staining is conducted in the well plate to determine cell death as [fluorescence intensity in co-culture - fluorescence intensity in tumor cells alone cultures]/[fluorescence intensity of DAPI staining, as an indicator of the number of nucleated cells in the cultures].

[00308] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges which may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[00309] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the described invention, exemplary methods and materials have been described. All publications mentioned herein are incorporated herein by reference to disclose and described the methods and/or materials in connection with which the publications are cited.

[00310] It must be noted that as used herein and in the appended claims, the singular forms “a”, “and”, and “the” include plural references unless the context clearly dictates otherwise.

[00311] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application and each is incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the described invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

## EXAMPLES

[00312] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

### *Example 1*

[00313] Generating conditionally reprogrammed IECs from murine samples. Successful isolation of primary murine m-IECs (small intestine) from adult 6-8 week old mice was performed as detailed by Evans et al. (“The development of a method for the

preparation of rat intestinal epithelial cell primary cultures," J. Cell Sci. 101 ( Pt 1), 219-231 (1992)), with modifications from techniques in Zilberberg's lab.

[00314] Expansion of m-IECs was performed using CR (Palechor-Ceron, N. et al., "Radiation induces diffusible feeder cell factor(s) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells," Am. J. Pathol. 183, 1862-1870, doi:10.1016/j.ajpath.2013.08.009 (2013); Liu, X. et al., "ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells," Am. J. Pathol 180, 599-607, doi: 10.1016/j.ajpath.2011.10.036 (2012); Suprynowicz, F. A. et al., "Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells," Proc. Nat. Acad. Sci. USA 109, 20035- 20040, doi: 10.1073/pnas. 1213241109 (2012)). Freshly isolated m-IECs and m-CRIEC were >98% positive pan cytokeratin and epithelial cell adhesion molecule (EpCAM) (ref. 14, 40) positive, confirming the purity of our cultures (see Fig. 2a and 2b). Gene expression analysis also corroborated that these cells significantly expressed cytokeratin 8 (KRT8; a specific marker for IECs) with low expression of cytokeratin 15 (KRT15; a marker for skin epithelium (Zhan, Q. et al., "Cytokeratin 15-positive basal epithelial cells targeted in graft-versus-host disease express a constitutive antiapoptotic phenotype, J. Invest. Dermatol. 127, 106-115, doi:10.1038/sj.jid.5700583 (2007); Whitaker-Menezes, D., et al, "An epithelial target site in experimental graft-versus-host disease and cytokine-mediated cytotoxicity is defined by cytokeratin 15 expression," Biology of Blood and Marrow Transplantation: 9, 559-570 (2003).) (data not shown).

[00315] Upon CR expansion with conditioned medium (Palechor-Ceron, N. et al., "Radiation induces diffusible feeder cell factor(s) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells," Am. J. Pathol. 183, 1862-1870, doi:10.1016/j.ajpath.2013.08.009 (2013).), mCRIECs acquired a stem-like phenotype (increased CD24 and Lgr5 in the case of IECs) as reported to be the case with other primary epithelial cells (Saenz, F. R. et al., "Conditionally reprogrammed normal and transformed mouse mammary epithelial cells display a progenitor-cell-like phenotype," PloS One 9, e97666, doi:10.1371/joumal.pone.0097666 (2014); (2014); Suprynowicz, F. A. et al., "Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells," Proc. Nat. Acad. Sci. USA 109, 20035- 20040, doi: 10.1073/pnas. 1213241109 (2012)) undergoing CR (Fig. 2b). These results with m-IECs suggest that CRIECs can be produced while preserving characteristic IEC functions.

*Example 2*

***Collecting human biospecimens***:

[00316] Donor and recipient peripheral blood lymphocytes (PBL) and allogeneic-BMT recipient GI biopsies (e.g., taken from colonoscopies) will be collected in accordance with the IRB approved protocol. GI specimens will be collected at the onset of GVHD if GI biopsies already are being performed. Transplant patients, undergoing a gut biopsy as part of their standard of care, will be asked to donate two extra cores of approximately 3 mm in size. Overly inflamed tissue samples will not be used in this study.
Blood collection will be performed prior to transplant to ensure the collection of viable cells (four 8.5 mL yellow top tubes per individual, containing $10^6$ cells/mL, of which half are T cells). Donor blood will be used to isolate T cells for killing assays, and patient blood will be utilized to develop DCs as specified below. PBL will be obtained by centrifugation of blood samples over Ficoll-Paque-Plus (Friedman, T. M. et al., "Overlap between in vitro donor antihost and in vivo posttransplantation TCR Vbeta use: a new paradigm for designer allogeneic blood and marrow transplantation," Blood 112, 3517- 3525, doi: 10.1182/blood-2008-03-145391 (2008)) and cryopreserved for later use in killing assays. Upon collection, tissue samples will be place in PBS at 4ºC.

*Example 3*

***Generating h-CRIECs from patients and murine models***:

[00317] h-CRIECs will be prepared following procedures developed for the generation of m-CRIECs (see, e.g., Saenz, F. R. et al. Conditionally reprogrammed normal and transformed mouse mammary epithelial cells display a progenitor-cell-like phenotype. PloS One 9, e97666, doi:10.1371/joumal.pone.0097666; (2014); Palechor-Ceron, N. et al. Radiation induces diffusible feeder cell factor(s) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells. Am. J. Pathol. 183, 1862-1870, doi:10.1016/j.ajpath.2013.08.009 (2013); Liu, X. et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells, Am. J. Pathol. 180, 599-607, doi: 10.1016/j.ajpath.2011.10.036 (2012)). All CRC will be cryopreserved until use.

*Example 4*

***Validating the utility of the in vitro GVHD (iGVHD) platform using clinically relevant murine models of allo-BMT***:

[00318] Table 1. Murine models and experiments for biological validation of the iGVHD concept and utility.

| Murine BMT model | Expected Outcome |
|---|---|
| miHA model<br>B6→BALB.B | Using the miHA model B6→BALB.B, with known GVHD potential (Zilberberg, J., McElhaugh, D., Gichuru, L. N., Korngold, R. & Friedman, T. M. Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-versus-host disease as determined by Vbeta spectratype analysis, J. Immunol.180, 5352-5359 (2008)), the percentage of killed IEC to aid the development of an empirical pathological index (PIdx). This PIdx will be put in practice to assess donor-patient pair reactivity in clinical samples. |
| Negative Control<br><br>B6→CXB-7 | B6→CXB-7 will be used as a negative control to identify the lower limit of the killing assay, i.e., to help determine what degree of IEC apoptosis can be expected in the absence of in vivo GVHD-induced lethality.. No substantial damage of CXB-7 IEC in this nonlethal miHA model, which has a subset of the miHA expressed by the BALB-B strain, is expected. Some apoptosis may occur, since some cachexia can be observed in recipient mice. (Korngold, R. & Wettstein, P. J. Immunodominance in the graft-vs-host disease T cell response to minor histocompatibility antigens. J. Immunol. 145, 4079-4088 (1990)). |
| Haploidentical transplant model, with three different potential donors and syngeneic negative control: B6→ B6D2F1; BALB.B →B6D2F1; C3H.SW→ B6D2F1; B6D2F1 – > B6D2F1. | To recapitulate the clinical scenario where haploidentical transplant recipients undergo cyclophosphamide treatment on day 3 post-BMT to eliminate highly alloreactive MHC_specific T cells and thereby lessen the severity of GVHD (Kanda, J., Chao, N. J. & Rizzieri, D. A. Haploidentical transplantation for leukemia, Cur. Oncol. Reports 12, 292-301, doi: 10.1007/sl 1912-010-0113-4 (2010); Luznik, L., O'Donnell, P. V. & Fuchs, E. J. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. Sem. Oncol. 39, 683-693, doi: 10.1053/j.seminoncol.2012.09.005 (2012)), cultures will also be treated with an analog of cyclophosphamide as described (Kanakry, C. G. et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. Sci. Translat. Med. 5, 21 Ira 157, doi: 10.1126/scitranslmed.3006960 (2013)). This would leave the T cell responses to be directed mostly to miHA differences.<br><br>The killing assays will thus be utilized here to predict the best donor for the B6D2F1 recipient, i.e., the donor that will incur the least degree of pathological damage (as determined by the PIdx). It is expected that the donor with the lowest PIdx score |

| Murine BMT model | Expected Outcome |
|---|---|
| | will likely induce less GVHD in vivo. This will be correlated with in vivo BMT GVHD experiments using methodology that has been described (Fanning, S. L. et al Unraveling graft-versus-host disease and graft-versus-leukemia responses using TCR Vbeta spectratype analysis in a murine bone marrow transplantation model. J. Immunol. 190,447-457, doi: 10.4049/jimmunol. 1201641 (2013); Zilberberg, J., et al., Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-versus-host disease as determined by Vbeta spectratype analysis. J. Immunol 180, 5352-5359 (2008)). The syngeneic negative control using B6D2F1 donor cells will provide the baseline for the PIdx. |

[00319] ***Preliminary results suitability of cRPMI***: Since CRC medium additives (e.g., ROCK kinase inhibitor) can ameliorate GVHD (Iyengar, S., Zhan, C., Lu, J., Korngold, R. & Schwartz, D. H. Treatment with a Rho Kinase Inhibitor Improves Survival from Graft-Versus-Host Disease in Mice after MHC-Haploidentical Hematopoietic Cell Transplantation. Biol. Blood Marrow Transplant.:, doi:10.1016/j.bbmt.2014.04.029 (2014)) and therefore should not be used for co-culture of m-CRIECs and T cells, the CRC medium was replaced with complete RPMI-1640 medium (cRPMI, RPMI medium supplemented with 10% FBS and 5% L-glutamine), which is conventionally used to culture T cells (Friedman, T. M. et al. Overlap between in vitro donor antihost and in vivo posttransplantation TCR Vbeta use: a new paradigm for designer allogeneic blood and marrow transplantation. Blood 112, 3517-3525, doi: 10.1182/blood-2008-03-145391 (2008)) **Fig. 2b**. m-CRIEC's upregulation of surface expression of major histocompatibility complexes I and II (MHCI and MHC II; the murine equivalent of human HLA) (Fig. 3). The increased expression of these molecules serves as a catalytic step without which T cells cannot recognize miHA or any antigen on the surface of host cells (Korngold, R. & Sprent, J. Graft-versus-host disease in experimental allogeneic bone marrow transplantation. Proc. Soc. Exp. Biol. Med. Soc. Exp. Biol. Med. 197, 12-18 (1991); Korngold, R. & Sprent, J. Surface markers of T cells causing lethal graft-vs-host disease to class I vs class II H-2 differences; J. Immunol. 135, 3004-3010 (1985)). The results indicate that, while other culture media partially hindered the upregulation of these molecules, cRPMI permitted maximum expression of MHC-I and MHC-II after 72 h of

cytokine exposure .

***Example 5***

***Feasibility of using nanofibrous mesh in maintaining the long-term functionality of CRIECS*:**

[00320] Basement membrane (BMa)-like fibrous meshes with random fiber organization were prepared by electrospinning (Yang, X., Ogbolu, K. R. & Wang, H. Multifunctional Nanofibrous Scaffold for Tissue Engineering. J. Exp. Nanoscience 3, 329 - 345 (2008)). To obtain stable and strong nanofibers, slow degradable, biocompatible polycaprolactone (PCL) was used as the fiber matrix phase in which Type IV collagen (representing ECM molecules) was dispersed.

[00321] As shown in Fig. 4, the combination of nanofibrous mesh and cRPMI culture medium enabled the maintenance of viable and morphologically sound CRIECs, even after 7 days in the absence of CR medium.

[00322] As shown in Table 2 flow cytometric analysis of annexin V+/propidium iodide (PI) + staining showed that mCRIEC viability decreased at an E:T ratio of 5:1 as determined by increased apoptotic cells (% Annexin V+). At a ratio of 10:1, 64.8% of m-CRIEC were dead (double+) by day 6. PIdx=3.83.

[00323] **Table 2**:

| **E:T ratio** | **% Annexin V+** | **% AnV+/PI+** |
|---|---|---|
| m-CRIEC | 14.5 | 11.8 |
| 5:1 | 71.3 | 19.3 |
| 10:1 | 67.4 | 64.8 |

[00324] **Also**, the above culture conditions were sufficient to enable anti-allogeneic T cell responses capable of inducing quantifiable reaction to m-CRIECs in an MHC-mismatched setting (Fig. 5a, and Table 1). The pathological index (PIdx) was calculated to be 3.83.

***Example 6***

***Experiments to establish the pathological index (PIdx) to quantify T cell induced IEC damage and killing:***

[00325] The predictive capability of co-culture killing assays and later iGVHD will be compared to known in vivo outcomes from well-established murine models of BMT(Table 1). These models represent different degrees of alloantigenic barriers and hence distinct clinical scenarios:

[00326] the miHA-disparate C57BL6/J (B6) → C.B10-H2b/LiMedJ (BALB.B) and B6 → CXB-7/By (CXB-7) models (see Zilberberg, J. et al, "Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-versus-host disease as determined by Vbeta spectratype analysis," J. Immunol. 180: 5352-59 (2008); Korngold, R. & Wettstein, P.J. "Immunodominance in the graft vs host disease T cell response to minor histocompatibility antigens," J. Immunol. 145: 4079-4088 (1990); Jones, S.C. et al, "Specific donor Vbeta-associated CD4 T-cell responses correlate with severe acute graft versus host disease directed to multiple minor histocompatibility antigens. Biol.Blood Marrow Transplant. 10: 91-105, doi: 10.1016/j.bbmt.2003.10.002 (2004); Jones et al, "Importance of minor histocompatibility antigen expression by nonhematopoietic tissues in a CD4+ T cell-mediated graft-versus-host disease model," J. Clin. Invest. 112: 1880-86, doi: 10.1172/JC119427 (2003); Friedman, T.M., et al, "Vbeta spectratype analysis reveals heterogeneity of CD4+ T cell responses to minor histocompatibility antigens involved in graft-versus-host disease: correlations with epithelial tissue infiltrate," Biol. Blood Marrow Transplant. 7: 2-13, doi: 10.1053/bbmt.2001.v7.pm11215694 (2001); Friedman, T.M. et al, "Repertoire analysis of CD8+ T cell responses to minor histocompatibility antigens involved in graft-versus-host disease, J. Immunol 161: 41-48 (1998)), where both donor and recipients are MHC ($H2^b$ – matched); and

[00327] the haploidentical-MHC model (see Zilberberg, J. et al, "Inter-strain tissue-infiltrating T cell responses to minor histocompatibility antigens involved in graft-verus-host disease as determined by Vbeta spectratype analysis," J. Immunol. 180: 5352-59 (2008); Korngold, R. & Wettsstein, P.J. "immunodominance in the graft vs host disease T cell response to mino histocompatibility antigens," J. Immunol. 145: 4079-4088 (1990); Jones, S.C. et al, "Specific donor Vbeta-associated CD4 T-cell responses correlate with

severe acute graft versus host disease directed to multiple minor histocompatibility antigens. Biol. Blood Marrow Transplant.10: 91-105, doi: 10.1016/j.bbmt.2003.10.002 (2004); Jones et al, "Importance of minor histocompatibility antigen expression by nonhematopoietic tissues in a CD4+ T cell-mediated graft-versus-host disease model," J. Clin. Invest. 112: 1880-86, doi: 10.1172/JC119427 (2003); Friedman, T.M., et al, "Vbeta spectratype analysis reveals heterogeneity of CD4+ T cell responses to minor histocompatibility antigens involved in graft-versus-host disease: correlations with epithelial tissue infiltrate," Biol.Blood Marrow Transplant.7: 2-13, doi: 10.1053/bbmt.2001.v7.pm11215694 (2001); Friedman, T.M. et al, "Repertoire analysis of CD8+ T cell responses to minor histocompatibility antigens involved in graft-versus-host disease, J. Immunol 161: 41-48 (1998)) B6 → (B6xDBA/2)F1[B6D2F1(H2b/d)] (Patterson, A.E. and Korngold, R., "Infusion of select leukemia-reactive TCR Vbeta+ T cells provides graft-versus-leukemia responses with minimization of graft-versus-host disease following murine hematopoietic stem cell transplantation," Biol.Blood Marrow Transplant.7: 187-196 (2001)). In brief, m-IECs from small and large intestine can be isolated from recipient strains and expanded using CR technology. The m-CRIECs can be cryopreserved for later use in co-culture experiments. The m-CRIECs can be cultured on nanofibrous matrices in the presence of complete RPMI supplemented with TNF-α and IFN-γ to induce upregulation of MHC-1 and MHC-II molecules.

[00328] In brief, for each of the experimental murine models proposed in Table 1, m-IECs (from small and large intestine) will be isolated from recipient strains and expanded using CR technology. The m-CRIECs will be cryopreserved for later use in co-culture experiments. The m-CRIECs will be cultured on nanofibrous matrices in the presence of cRPMI supplemented with TNFα and IFNγ to induce upregulation of MHC-1 and MHC-II molecules; an indispensable state to generate tissue-directed alloresponses (Fig. 3).

[00329] Although TNFα is best known for its inflammatory effects, it also can induce upregulation of programmed death ligand 1 (PDL-1) on the surface of cells, which acts as an immunological checkpoint and can shut down effector T cells. Preliminary data (not shown) indicates that epithelial cells upregulate PDL-1 under inflammatory conditions (Wu, Y. Y. et al Increased programmed death-ligand-1 expression in human gastric epithelial cells in Helicobacter pylori infection. Clin. Exp. Immunol. 161, 551-559, doi: 10.1111/j. 1365- 2249.2010.04217.x (2010)), and thus TNFα can play an important regulatory role in allogeneic transplantation (Alderson, K. L. et al Regulatory and conventional CD4+ T cells

show differential effects correlating with PD-1 and B7-H1 expression after immunotherapy. J. Immunol. 180, 2981-2988 (2008); Tanaka, K. et al PDL1 is required for peripheral transplantation tolerance and protection from chronic allograft rejection. J. Immunol. 179, 5204-5210 (2007); Saha, A. et al Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. Blood 122, 3062-3073, doi: 10.1182/blood-2013-05- 500801 (2013)). A PDL-1 blocker (e.g., MPDL3280A, Genentech) , will be introduced in order to ensure that T cell reactivity is not negatively modulated through this pathway.

[00330] Likewise, to better recapitulate tissue-induced damage by preconditioning regimens (Ferrara, J. L., Levine, J. E., Reddy, P. & Holler, E. Graft-versus-host disease. Lancet 373, 1550-1561, doi: 10.1016/SO 140-6736(09)60237-3 (2009)), IEC can be treated with the same chemotherapeutic agents that patients typically receive prior to transplant. This may induce the expression of MHC-I and MHC-II on the IEC, priming the T cells for a more robust response.

***Mixed Lymphocyte Culture***

[00331] To mimic the early activation/proliferation stage of T cells in the described in vitro system, a mixed lymphocyte culture (MLC) will be used. (Fanning, S. L. et al Unraveling graft-versus-host disease and graft-versus-leukemia responses using TCR Vbeta spectratype analysis in a murine bone marrow transplantation model. J. Immunol. 190,447-457, doi: 10.4049/jimmunol. 1201641 (2013); Friedman, T. M. et al. Overlap between in vitro donor antihost and in vivo posttransplantation TCR Vbeta use: a new paradigm for designer allogeneic blood and marrow transplantation. Blood 112, 3517- 3525, doi: 10.1182/blood-2008-03-145391 (2008)). In brief, donor T cells (i.e., responders; R) will be cultured with irradiated (30 Gy) recipient lymphocytes (i.e., stimulators; S) at a 1:2 R:S ratio. For human MLC, enriched PBL from the patients will be used to stimulate responding T cells from their donors. Natural killer cells will be depleted from donor T cells to diminish non-specific target cell killing by this subpopulation of lymphocytes. After 9 days, human MLC responders will be harvested and re-stimulated for another 8 days as before, with the addition of 20 U/ml of rIL-2.

MLC will be carried out in the antigen presenting cells (APC) culture chamber, as part of the iGVHD platform, to facilitate activation, expansion and concentration of alloreactive T cells. Dendritic cells, as opposed to bulk lymphocytes, will be used in iGVHD, with a T cell-DC (R:S) ratio of 5:1. Activated T cells from MLC will then be placed in CRIEC on nanofibers

to monitor for epithelial cell death. Killing assays with specimens from murine models of allo-BMT (Table 1), where the GVHD response has been characterized in vivo, will be used in order to designate an empirical PIdx to quantitate the response.

***Objective Cell Death Analysis Methods***

[00332] A panel of objective cell death analysis methods (e.g., Annexin V/PI staining) using flow cytometry and in situ detection of cleaved caspase-3 using immunofluorescence will be utilized to determine cell death. The percentage of dead cells is calculated as [% of Annexin V+/PI+ cells in co-cultures - % of Annexin V+/PI+ cells in IEC alone cultures] (flow cytometric measurement). CD3 staining also will be performed to identify adherent T cells contributing to the response.

[00333] Caspase-3 staining also will be conducted in the well plate (and later in the microfluidic chambers to determine cell death as [fluorescence intensity in co-culture - fluorescence intensity in IEC alone cultures]/[fluorescence intensity of DAPI staining, as an indicator of the number of nucleated cells in the cultures].

[00334] Cell death will be evaluated at three or more (if determined to be necessary) T cell-IEC ratios (i.e., the effector: target, or E:T ratio). According to some embodiments, the E:T ratio is 30, 10, or 3. (See Choksi, S. et al, “A cD8 DE loop peptide analog prevents graft versus host disease in a multiple minor histocompatibility antigen-mismatched bone marrow transplantation model,” Biol.Blood Marrow Transplant.10: 669-680, doi: 10.1016/j.bbmt.2004.06.005 (2004)).

[00335] The PIdx will be determined as the slope of the curve of percentage of dead cells vs. E:T ratios, where a steeper slope indicates a higher risk for developing GVHD. The PIdx will be determined at 4 different time points (day 3, day 7, day 14 and day 21 post co-culture) in order to maximize the opportunity to observe a response while ensuring that faster reactions do not reach plateau before obtaining a quantifiable PIdx, and that slow-to-develop GVHD responses also can be captured.

[00336] Statistical considerations. Continuous random variables (i.e., flow cytometric data, in situ staining/caspase-3 readout, PIdx) will be summarized as mean (standard deviation) or median (interquartile range) depending on whether or not they are normally distributed. Categorical random variables (i.e., GVHD grading) will be presented as count (percentage). Comparison of continuous random variables between groups (i.e., comparing different murine allo-BMT models) will be performed using two-sided Student’s

t-test or 2-sided Wilcoxon rank sum test, analysis of variance (ANOVA), Kruskal-Wallis, as appropriate. Categorical variables will be compared using Fisher's exact test or Pearson's Chi-square test, as appropriate. Median survival of transplanted mice will be estimated by the Kaplan-Meier method. Any $p<0.05$ will be considered statistically significant. For reproducibility of PIdx and staining methods, repeated (test-retest) measurements of PIdx will be compared using two-sided paired t-tests or Wilcoxon signed rank test. Correlation of the replicate PIdx measurements will be examined using Pearson correlation coefficient or Spearman correlation coefficients. Reliability of the PIdx will be evaluated using intra-class correlation coefficient, coefficient of variation. To examine the effect of culture time on PIdx, a mixed model repeated measures analysis will be conducted with PIdx at different time points. The Bland-Altman plot will be used to assess agreement between flow cytometry and in situ staining.

***Example 7***

***Use of the Multiwell Plate-Based Microfluidic Perfusion Culture Device to mimic interactions of circulating murine T cells (m-T cells) with m-CRIECs and murine dendritic cells (m-DCs):***

[00337] Our current prototype device (Fig. 6) was used to mimic interactions of circulating murine T cells (m-T cells) with m-CRIECs and murine dendritic cells (m-DCs) as illustrated in Fig. 1. One practical design feature of the device is the use of removable polydimethylsiloxane (PDMS) plugs at the top of the culture chambers to: (1) allow the convenient placement of cells and biomaterials into the culture chambers at various time points during culture; (2) externally interconnect culture the m-CRIEC and m-DC culture chambers using polyethylene tubing and a peristaltic pump (Model 78023-02, ISMATEC); and (3) recirculate m-T cells suspended in the RPMI common culture medium. The device uses transparent polycarbonate (PC) membranes (TCTP02500, Millipore) to: (1) anchor tissue cells and biomaterials, and (2) provide optical access through the bottom of the chambers for cell characterization with plate readers.

[00338] As shown in Fig. 6c, the prototype device was assembled with: (1) a commercial polystyrene (PS) bottomless 96-well plate (Model 655-000, Greiner Bio-One); 2) three micropatterned PDMS layers made by soft lithography, and (3) one blank glass layer. The PDMS layers were used to: (1) provide a microfluidic channel of 200 μm thick and 5 mm wide for use as an internal fluidic passage between the chambers, and (2) anchor the

placement of the PC membranes within the culture chambers. The bottom of the device was sealed with the 1.2 mm-thick glass layer for use with plate readers. These parts were bonded using oxygen plasma treatments.

[00339] For our preliminary study, the average pore of the PC membranes was selected to be 10 μM in order to provide sufficient opening for m-T cells to go through, since the average diameter of m-T cells is about 5 μm. For the m-CRIEC culture chamber, the membrane was coated with electrospun PCT/collagen nanofibrous meshes (Fig. 6e).

[00340] The device was used to culture m-CRIECs prepared from the small intestine of a B6 mouse. The cells develop a confluent layer while maintaining their viability up to 7 days (Fig. 7a).

[00341] After the m-CRIEC culture was established, eGFP m-T cells harvested from an eGFP transgenic B6 mouse were suspended in the culture medium (106 cells total) and introduced and circulated through the device. The SEM and fluorescence images in Figs. 7b and 7c show that T cells were able to travel through the PC membrane and the nanofibrous meshes and interact with m-CRIECs through physical contact. Accordingly, these results show that the device can be used to promote physical interactions between m-CRIECs and circulating m-T cells. The a biomaterial that has been commonly used for 3D lEC/organoid cultures (Sato, T. et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterol. 141, 1762-1772, doi:10.1053/j.gastro.2011.07.050 (2011)).

[00342] For the m-APC culture chamber, PS microbeads of 90 pm were assembled with m-DCs (from BALB.B mice) to form a 250 pm-thick assembly on the PC membrane surface. Microbeads were pre-conditioned with 100 ng/mL lipopolysaccharides (LPS) to promote the adhesion of m-DCs to the microbead surface (Abdi, K., Singh, N. J. & Matzinger, P. Lipopolysaccharide-activated dendritic cells: "exhausted" or alert and waiting? J.Immunol. 188, 5981-5989, doi: 10.4049/jimmunol.l 102868 (2012)). m-T cells (from eGFP transgenic B6 mice) were labeled with eFIuro® 670 and introduced 24 h later from the top of the microbeads assembly at a R:S of 5:1. Since the packed 90 pm microbeads provide interstitial openings of -14 pm, T cells were able to infiltrate through the microbeads assembly and interact with m-DCs, which were attached to the microbead surface. The m-T cells were circulated for 4 days. As shown in **Fig. 8**, analyses of live cells (as determined by light forward and side scatters) the P.:S ratio of m-T cells to m-DCs: (1) increased from 5:1 to -10:1 (i.e., m-T cells=90%, m-DCs=9%) in the 3D culture chamber and (2) decreased to -2:1

(i.e., m-T cells=60%, m-DCs=30%) in 2D plate culture. Likewise, while T cells underwent one round of proliferation in both culture conditions (for explanation of analyses, see Zhang et al. Patient-Specific 3D Microfluidic Tissue Model for Multiple Myeloma. Tissue engineering. Part C, Methods 20: 663-670, doi: 10.1089/ten.TEC.2013.0490 (2014)), the percentage of proliferating cells (labeled as "P" in Fig. 8) was greater in 3D than in 2D (40 vs. 33%).

[00343] As hypothesized, these results suggest that the circulatory 3D perfusion culture is an effective approach in enhancing the viability, proliferation, and activation of T cells in comparison to conventional 2D co-culture. These enhancements are attributed to the synergistic use of both microbeads and circulatory perfusion in providing m-T cells with significantly higher chances of interacting with m-DCs.

[00344] Taken together, these preliminary results strongly support that the device of the described invention can be used for: (1) biomaterials-guided cultures of CRIECs and DCs and (2) T cell circulation through these chambers to facilitate and enhance the viability, proliferation, and activation of reactive T cell population.

***Example 8***

***Experiments to further optimize the use of the device in replicating the stimulation, circulation and proliferation events that donor T cells encounter in the patient body and predicting the pathologic potential of donor T cells against host epithelium:***

[00345] ***Experiments to establish that 80% of unstimulated T cells can be circulated through the CRIEC and DC culture chambers for up to 1 week***. The effects of biomaterials, flow conditions, and tissue cell presence on the re-circulation of unstimulated T cells from transgenic eGFP B6 mice, in the range of $10^5$ to $10^6$ cells will be quantified. These baseline experiments will be primarily conducted with murine cells, but main results from the experiments will be confirmed using human cells. When tissue cells are not present in the device, it is anticipated that culture medium flow rate, pore size of nanofibrous mesh, and microbead size will have major influences as to how T cells can travel through the culture chambers. (1) The flow rate will be varied in the range of 10 to 50 uL/min; (2) the mesh pore size will be varied from 5 to 10 inn by controlling electrospinning process parameters, and (3) the PS microbead size will be varied, i.e., 45, 75, and 90 urn as these sizes are commercially available (Polyscience). In addition to qualitative visual and microscopic observations at various locations of the device, the percentage changes of circulating T cells (vs. cells that get

entrapped in the device) will be quantified by sampling 50-100 uL of the effluent each day for a 1 week period and counting the cells in the collected medium using an automated cell counter. Also, flow cytometry will be performed to follow changes in cell viability on a daily basis for the 1-week period. For the sampling purpose and medium replenishment, a sampling port will be added in the external circulation loop.

[00346] After the empty device characterization, how the presence of CRIECs and APCs (i.e., DCs) in these chambers will interfere with the movement of T cells will be studied. For the epithelial culture chamber, experiments after CRIECs reach confluence will be performed, which initial observations indicate takes about 1-4 days.

[00347] In preliminary experiments, no evidence of m-CRIECs blocking T cell movements was seen, although such observations to date are limited. The flow rate and biomaterial parameters will be optimized to ensure that >80% can freely be recirculated through the chambers for up to 1 week.

[00348] ***Experiments to establish that T cells become activated and persist for 3 weeks due to biomimetic recirculation***. Due to the recirculatory attribute of iGVHD, GI miHA-specific T cells continuously stimulated in the APC and tissue chambers are expected to persist and expand over the 3-week benchmark period to cause measurable CRIEC damage. The operation of the APC chamber that can be initially seeded with $10^5$ DCs (sufficient for the stimulation of $10^6$ T cells) will be optimized. m-DCs will be prepared by injecting host mice with B16-FLt3L tumor, which promotes the enrichment of DCs in tumor bearing mice. (Anandasabapathy, N. et al. Classical Flt3L-dependent dendritic cells control immunity to protein vaccine. J. Exptl Med. 211, 1875-1891, doi:10.1084/jem.20131397 (2014); Anandasabapathy, N. et al. Flt3L controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. J. Exptl Med. 208, 1695-1705, doi: 10.1084/jem.20102657 (2011)).

[00349] Human DCs (h-DCs) will be derived from patient PBL monocytes (Santodonato, L. et al. Monocyte-derived dendritic cells generated after a short-term culture with IFN- alpha and granulocyte-macrophage colony-stimulating factor stimulate a potent Epstein-Barr virus- specific CD8+ T cell response. J. Immunol. 170, 5195-5202 (2003)).

[00350] The following experiments will be performed with murine cells first and later confirmed with human cells. As preliminary results suggest, m-DCs can infiltrate into the microbeads assembly and become adhered to the microbead surface. Upon the introduction of T cells and their physical contact, T cells will be activated. Since DCs are

programmed to die after maturation (typically within 5 days) and therefore in order to provide constant stimulations (Abdi, K., Singh, N. J. & Matzinger, P. Lipopolysaccharide-activated dendritic cells: "exhausted" or alert and waiting? J. Immunol. 188, 5981-5989, doi: 10.4049/jimmunol.1 102868 (2012)) the capability to replenish dead DCs with new DCs will need to be developed. Simply adding new DCs at 5-day intervals by opening the PDMS plug and placing them onto the top of the microbeads assembly is planned. It is expected that dead cell debris will be washed away and the microbead surface will become available again for the arrival and adhesion of new DCs, since T cells do not adhere to the microbead surface (as observed in preliminary experiments). The effectiveness of the replenishment approach at providing constant T cell stimulation will be evaluated by measuring cell viability, activation, and proliferation at various replenish time intervals (3, 7, 14, 21 days) over 3 weeks. After the APC chamber is optimized, the synergistic effects of CRIECs on T cell viability, activation, and proliferation will be investigated and compared with DCs only. Annexin V/PI staining will be used to determine the viability of T cells. T cell activation will be determined by percent changes in CD25 and CD69 expressions. For the proliferation assay (Zhang, W., Lee, W. Y., Siegel, D. S., Tolias, P. & Zilberberg, J. Patient-Specific 3D Microfluidic Tissue Model for Multiple Myeloma. Tissue Engineering. Part C, Methods 20, 663-670, doi: 10.1089/ten.TEC.2013.0490 (2014)), T cells will be labeled with cell trace carboxyfluorescein succinimidyl ester (CFSE) proliferation dye and analyzed using flow cytometry.

[00351] If DCs cannot be replenished by the infiltration approach, replacing the whole assembly and place a new microbead/dendtric cell assembly with T cells separated from the old assembly and re-introduced will be considered. Also, cytokines (e.g., IL-2) can be added to the culture chamber in order to prolonged T cell maintenance (Hedfors, I. A. & Brinchmann, J. E. Long-term proliferation and survival of in vitro-activated T cells is dependent on Interleukin-2 receptor signalling but not on the high-affinity IL-2R. Scandinavian journal of immunology 58, 522-532 (2003)). Moreover, retinoic acid could be added to generate gut-tropic DCs, which should facilitate the generation of T cells with superior lEC-killing avidity (Gorfu, G., Rivera-Nieves, J. & Ley, K. Role of beta7 integrins in intestinal lymphocyte homing and retention. Current Molec. Med. 9, 836-850 (2009); Agace, W. W. T-cell recruitment to the intestinal mucosa. Trends in Immunol. 29, 514-522, doi: 10.1016/j.it.2008.08.003 (2008)).

[00352] The relatively large hole-to-hole distance in the current membrane material may limit the T cell movement through the membranes. Although this was not seen in preliminary experiments with 106 circulating T cells, this may be an issue when the number of circulating T cells is significantly increased to achieve high E:T ratios. If this becomes a problem, using polyethylene terephthalate (PET) membrane (Greiner Bio-One) with the average pore size of 8 μm and the surface pore density of 1.5x106 cm-2 (vs. 105 cm-2 for the current PC membrane) will be considered.

[00353] ***Experiments to Establish that iGVHD can facilitate CRIECs killing within 2 or 3 weeks***: After operative procedures are optimized from the above tasks, iGVHD will be used to determine PIdx values using cells from murine GVHD models (**Table 1**). T cell recirculation is expected to: (1) lower the E:T cell ratio (i.e., the seeding ratio of CRIECs and T cells in iGVHD) to achieve measurable m-CRlEC killing and (2) speed up the killing for the reasons articulated earlier. For these experiments, the E:T ratios will be titrated in the range of 2:1 to 20:1. Because of the plate reader assay capability of the platform, measurement of % cell death for calculation of PIdx is expected to be streamlined using in situ determination of cell death by caspase-3 staining (Luft, T. et al. Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. Blood 110,4535-4542, doi: 10.1182/blood-2006-10-049817 (2007); Disbrow, G. L. et al. Dihydroartemisinin is cytotoxic to papillomavirus-expressing epithelial cells in vitro and in vivo. Cancer Res. 65, 10854-10861, doi:10.1158/0008-5472.CAN-05-1216 (2005)). After iGVHD's facilitated killing capability is established with murine cells, the results will be confirmed using patient- derived cells. Based on these results, overall iGVHD design features and operational protocols will be reviewed and revised as necessary.

[00354] ***Experiments to Correlate statistically GVHD risk predictions from iGVHD from 24 patient-donor samples with patient outcomes***: For each patient-donor pair, PIdx will be determined using the iGVHD device and the protocols developed in the previous tasks. The recirculation and high-throughput capabilities of the device will be utilized to evaluate 3 or more E:T ratios. PIdx values determined from 24 patient-donor samples will be compared to patient outcomes as follows. The main outcome of interest, severity of GVHD (grades 0-IV), will be dichotomized in low severity (LS:0-l) and high severity (HS: 11, III, IV). Discriminant validity of PIdx will be examined by comparing PIdx from LS and HS groups using a two-sided Student's t-test or Wilcoxon rank sum test, as appropriate. Logistic regression analysis will be performed to assess the capability of PIdx as a risk predictor of

GVHD. The results of this analysis will be presented as odds ratios (OR), 95% confidence interval, P-value. Area under receiver operating characteristics (ROC) curve will be used to quantify probability of accurate classification of LS vs. HS outcomes. ROC analysis and optimal cut point function based on Youden Index will be used to determine the cutoff value for PIdx. Hochberg procedure will be utilized to adjust for multiple testing. Using the determined cutoff value, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), overall accuracy will be calculated and reported using standard 2 by 2 tables for categorical analysis.

[00355] ***Follow-On Clinical Study***. A follow-on clinical study with prospective sample collection is anticipated after proof of principle of predicting GVHD using human samples is established. Samples of 3 to 4 donors for haploidentical cases can be screened, although corroboration of the predictive outcome will be only for the selected haploidentical donor. Nonetheless, these cases will be use as a proof-of-principle that responses between multiple donors using this approach can be discerned.

***Example 9***

***Circulation of Primary Murine T Cells Through Primary Murine Intestine Epithelial Cells Maintained on Nanofibrous Mesh:***

[00356] Adoptive T cell therapy in the form of allogeneic blood and marrow transplantation (allo-BMT) has proven to be one of the few curative treatments for patients suffering from a number of drug-resistant hematological malignancies. However, the full exploitation of this clinical intervention is greatly limited by graft versus host disease (GVHD), as one of the major BMT complications. This disease is characterized by severe and potentially lethal tissue damage to skin, liver, and gut tissues of transplanted patients, mediated by donor T cells responding to host alloantigens.35–37 In particular, GVHD of the gastrointestinal tissues is closely associated with non-relapse mortality following allo-BMT (A. C. Harris, J. E. Levine and J. L. Ferrara, Clin. Haematol., 2012, 25, 473–478). Currently, there is no way to predict which patient–donor pairs will develop GVHD after BMT. Our long-term interest is to explore the possibility of emulating the potential killing of patient-derived intestinal epithelial cells (IECs) by donor T cells, where IECs are the primary population targeted in GI GVHD (A. M. Hanash, J. A. Dudakov, G. Hua, M. H. O'Connor, L. F. Young, N. V. Singer, M. L. West, R. R. Jenq, A. M. Holland, L. W. Kappel, A. Ghosh, J. J. Tsai, U. K. Rao, N. L. Yim, O. M. Smith, E. Velardi, E. B. Hawryluk, G. F. Murphy, C.

Liu, L. A. Fouser, R. Kolesnick, B. R. Blazar and M. R. M. van den Brink, Immunity, 2013, 37, 339–350; R. El-Asady, R. Yuan, K. Liu, D. Wang, R. E. Gress, P. J. Lucas, C. B. Drachenberg and G. A. Hadley, J. Exp. Med., 2005, 201, 1647–1657). In native tissues, IECs reside on a thin fibrous basement membrane (BMa) consisting of the intermingled networks of laminins and collagens and provides cell anchoring and barrier functions. The membrane networks interact with cells through membranous integrin receptors and other plasma membrane molecules, influencing cell differentiation, migration, adhesion, phenotype, and survival.

[00357] As an initial step towards this application, we used our prototype device to: (1) culture and maintain primary conditionally reprogrammed murine IECs isolated from the small intestine of a C57Bl/6-TgIJCAG-OVA)916 Jen/J mouse (B6-SIINFEKL) and (2) assess the device's capability in supporting the circulation of primary murine T cells through the IECs (FIG. 9a for conceptual illustration of the experimental approach).

[00358] As shown by the scanning electron microscopic (SEM) image in FIG. 9b, nanofiber mesh was used to mimic the BMa of the epithelial tissue as well as to support the long-term viability of IECs. The latter role is particularly important, since primary IECs cannot be kept viable during conventional culture. The nanofiber mesh was produced by electronspinning polycaprolactone (PCL)/type I collagen onto the PC membrane surface prior to the device assembly (X. Yang, K. R. Ogbolu and H. Wang, J. Exp. Nanosci., 2008, 3, 329–345). The average open space in the nanofiber mesh was controlled to about 6 μm (FIG. 9b) since the average diameter of T cells is about 5 μm. For the same reason, we also selected the average pore of the PC membrane to be 10 μm (FIG. 9b). With the use of nanofiber mesh, IECs were able to develop into a confluent layer and exhibit cobblestone morphology while remaining viable in the perfusion device for up to 7 days (FIG. 9c).

[00359] After IECs became confluent (approximately 4 days post seeding), enriched T cells obtained from an eGFP transgenic C57Bl6/J mouse were introduced and circulated through the culture chambers (2.5 × 105 cells per chamber). As illustrated in FIG. 9a, a peristaltic pump was used to circulate T cells in RPMI complete medium. Visually, we did not see the entrapment of T cells in any part of the culture device and external circulatory pathways. T cell viability was quantified by sampling the culture medium at various time points and counting live and dead T cell numbers suspended in the medium. As shown in FIG. 9d, the overall viability of T cells decreased during the 72 h culture period. This was expected since it is well known that the viability of T cells cannot be maintained in vitro

unless they are stimulated by antigen-presenting cells or maintained via the addition of cytokines like IL-2 (P. Marrack and J. Kappler, Annu. Rev. Immunol., 2004, 22, 765–787). However, interestingly, there were more viable T cells when they were circulated through the IEC layer. The results suggest that T cells were activated by IECs, resulting in the increased viability of T cells. Although both B6-SIINFEKL IECs and T cells were of B6 background, it is likely that minor antigen differences between the B6-SIINFEKL and the eGFP-B6 strains could have elicited activation of T cells and potentially other cells like natural killer (NK) cells. FIG. 9e shows that IECs were spreading on the nanofiber mesh surface, and were in physical contact with T cells. Since the membrane pores (10 μm) and opening spaces (>6 μm) between nanofibers were larger than the T cells (5 μm), they were able to go through the IEC layer without getting trapped in the culture chamber.

[00360] While the described invention has been described with reference to the specific embodiments thereof it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adopt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the described invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. An ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo comprising

(a) a multiwell plate-based perfusion culture device, comprising, from top to bottom:

a bottomless multi-well plate comprising a plurality of bottomless wells;

a first micropatterned polymer layer attached to a bottom surface of the bottomless multi-well plate to form a plurality of adjacent wells, one or more of each pair of adjacent wells comprising a transparent polymer membrane placed within the one ore mover of each pair of adjacent wells;

a second micropatterned polymer layer comprising two or more holes that correspond to two or more adjacent wells, the second micropatterned polymer layer being attached to a bottom surface of the first micropatterned polymer layer, such that each hole of the second micropatterned polymer layer is aligned with the two or more adjacent wells in the first micropatterned polymer layer, one or more of each pair of adjacent wells comprising the transparent polymer membrane;

a microfluidic channel formed between the two adjacent wells that allows internal fluidic communication between the two adjacent wells;

one or more removable polymer plugs, each located at a top surface of each of the plurality of wells, and one or more tubes, each connected to the one or more polymer plugs;

a pump connected to a reservoir that removably connects to the tubes;

a transparent, optical grade glass layer attached to the bottom surface of the second micropatterned polymer layer that forms a bottom surface for the plurality of wells and that seals the multi-well plate perfusion culture device;

wherein one or more of the two adjacent wells is a culture chamber for culturing a population of cells;

(b) an expanded population of cells derived from a recipient subject comprising a cell-specific antigen in the first adjacent well of the device in (a);

(c) an expanded population of T lymphocytes derived from a potential donor of a BMT graft in the second adjacent well of the device in (a), wherein the potential donor is allogeneic to the recipient subject;

(d) a liquid culture medium that is flowable between the first adjacent well and the second adjacent well;

the model being characterized by:

circulation of the liquid medium from the first well into the second well and back to the first well through the microfluidic channel;

an interaction between the population of cells comprising the cell antigen derived from the recipient subject in the first well and the population of T lymphocytes is effective to generate alloreactive effector T lymphocytes;

alloreactive effector T lymphocyte-induced quantifiable damage to the population of cells comprising the cell antigen derived from the recipient by the population of alloreactive effector T lymphocytes from the donor allogeneic to the recipient.

2. The ex vivo model according to claim 1, wherein the population of T lymphocytes in (c) is derived from peripheral blood lymphocytes of the potential donor.

3. The ex vivo model according to claim 1, wherein the population of T lymphocytes comprises a suspension of nonadherent cells.

4. The ex vivo model according to claim 1, wherein the first micropatterned polymer layer and the second micropatterned polymer layer comprises an organic polymer.

5. The ex vivo model according to claim 4, wherein the organic polymer is polydimethyl siloxane (PMDS) or polystyrene.

6. The ex vivo model according to claim 1, wherein the transparent polymer membrane comprises a nanofibrous mesh.

7. The ex vivo model according to claim 6, wherein the nanofibrous mesh is placed on a top surface of the transparent polymer membrane to coat the top surface of the transparent polymer membrane.

8. The ex vivo model according to claim 6, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

9. The ex vivo model according to claim 1, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an adhesion-promoting agent in an amount effective to promote adhesion of a population of cells to a surface of the microbeads.

10. The ex vivo model according to claim 9, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of a subpopulation of the population of T lymphocytes to the microbead surface.

11. The ex vivo model according to claim 10, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

12. The ex vivo model according to claim 3, wherein the suspension of nonadherent cells contains T lymphocytes derived from the allogeneic donor.

13. The ex vivo model according to claim 1, wherein the population of alloreactive effector T lymphocytes comprises alloreactive activated antigen presenting cells.

14. The ex vivo model according to claim 13, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

15. The ex vivo model according to claim 1, wherein the quantifiable damage to the population of cells comprising the cell-specific antigen derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

16. The ex vivo model according to claim 1, wherein the population of cells comprising the cell-specific antigen in (b) are a population of intestinal epithelial cells derived from the recipient subject, and the alloreactive effector T lymphocyte-induced quantifiable damage to the population of intestinal epithelial cells of the recipient subject is an ex vivo measure of risk of graft vs. host disease.

17. A method for optimizing donor selection for allogeneic blood and marrow transplantation (BMT) therapy comprising, in order:

(a) acquiring a tissue sample from a recipient subject allogeneic to a potential donor of a BMT graft, the tissue sample comprising a population of primary intestinal epithelial cells comprising an intestinal epithelial cell-specific antigen;

(b) seeding the population of primary intestinal epithelial cells of (a) in a first adjacent well of a multiwall plate-based perfusion culture device, the first adjacent well comprising a transparent polymer membrane, expanding the population in a first liquid medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer and generating a population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising the intestinal cell-specific antigen derived from the recipient subject;

(c) acquiring a population of T lymphocytes from the potential donor allogeneic to the recipient;

(d) seeding and expanding in a second adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes derived from the potential donor of (c),

(e) co-culturing in a second liquid medium the CRIECs derived from the recipient subject in the first adjacent well and the T lymphocytes derived from the potential donor allogeneic to the recipient subject in the second adjacent well, the co-culturing being characterized by:

the first adjacent well being fluidly connected to the second adjacent well so that the second liquid medium is flowable between the first adjacent well and the second adjacent well;

an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes that is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor;

(f) measuring damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of a risk of intestinal graft versus host disease in the recipient subject;

(g) ranking a plurality of potential donors by the measure of the risk of intestinal graft versus host disease; and

(h) treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient subject whose T lymphocytes are characterized by a reduced risk of intestinal graft-versus-host disease.

18. The method according to claim 17, wherein the potential donor of the BMT graft is haploidentical to the recipient subject.

19. The method according to claim 17, wherein the tissue sample comprising a population of primary intestinal epithelial cells is derived from small intestine, large intestine or colon of the recipient subject.

20. The method according to claim 17, wherein the transparent polymer membrane comprises a nanofibrous mesh to which the population of CRIECs is adherent.

21. The method according to claim 20, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

22. The method according to claim 17, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads.

23. The method according to claim 22, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

24. The method according to claim 23, wherein the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject.

25. The method according to claim 24, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

26. The method according to claim 17, wherein the expanded population of T lymphocytes derived from the donor allogeneic to the recipient subject in (d) comprise a suspension of nonadherent cells.

27. The method according to claim 17, wherein the population of alloreactive effector T lymphocytes comprises a population of alloreactive activated antigen presenting cells.

28. The method according to claim 27, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

29. The method according to claim 17, wherein the quantifiable damage to the population of CRIECs derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

30. The method according to claim 17, further comprising (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of CRIECs derived from the recipient subject; and (ii) selectively deleting the T lymphocyte clones from the population of T lymphocytes derived from the donor.

31. A method for minimizing risks and maximizing benefits of performing allogeneic blood and marrow transplantation (BMT) therapy in a recipient subject, wherein the recipient subject has a hematologic malignancy comprising, in order:

(A) evaluating a population of T lymphocytes derived from a potential donor allogeneic to the recipient subject for a potential to damage intestinal epithelial cells of the recipient subject according to the method of claim 17 steps (a) through (g);

(B) evaluating the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject for an effective graft versus tumor response against the tumor-specific antigens by:

(i) acquiring a specimen comprising a population of tumor cells derived from the recipient subject, the population of tumor cells comprising one or more tumor specific antigens;

(ii) seeding and expanding the population of tumor cells in the second liquid medium in a third adjacent well of the multiwell plate-based perfusion culture device; the third adjacent well comprising a transparent polymer membrane;

(iii) acquiring a population of T lymphocytes derived from the potential donor allogeneic to the recipient subject;

(iv) seeding and expanding in a fourth adjacent well of the multiwall plate-based perfusion culture device the population of T lymphocytes of (iii),

(v) co-culturing in the second liquid medium the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject in the third adjacent well and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject in the fourth adjacent well, the co-culturing being characterized by:

the third adjacent well being fluidly connected to the fourth adjacent well so that the second liquid medium is flowable between the third adjacent well and the fourth adjacent well;

an interaction between the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject that is effective to generate alloreactive effector T lymphocytes derived from the potential donor;

(vi) measuring damage to the population of tumor cells derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of an effective graft versus tumor (GVT) response against the tumor-specific antigens;

(vii) ranking a plurality of potential donors by the measure of the effective GVT response against the tumor-specific antigens; and

(C) treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient, the selected donor being characterized by a reduced risk of intestinal graft versus host disease, and an effective GVT response against the tumor-specific antigens.

32. The method according to claim 31, wherein the potential donor of the BMT graft is haploidentical to the recipient subject.

33. The method according to claim 31 part B, wherein the specimen comprising the population of tumor cells derived from the recipient subject in (i) is a blood sample, a bone marrow sample, or a leukapheresis sample.

34. The method according to claim 31 part B, wherein the transparent polymer membrane comprises a nanofibrous mesh to which the population of tumor cells is adherent.

35. The method according to claim 31 part B, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

36. The method according to claim 31 part B, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads.

37. The method according to claim 36, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

38. The method according to claim 36, wherein the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject.

39. The method according to claim 38, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

40. The method according to claim 31 part B, wherein the expanded population of T lymphocytes derived from the allogeneic donor in (iv) comprise a suspension of nonadherent cells.

41. The method according to claim 31 part B, wherein the population of alloreactive effector T lymphocytes in (v) comprises a population of alloreactive activated antigen presenting cells.

42. The method according to claim 41, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

43. The method according to claim 31, part B, wherein the quantifiable damage to the population of tumor cells derived from the recipient subject induced by the population of nonadherent alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient comprises cell death.

44. The method according to claim 31, further comprising enriching the population of T lymphocytes derived from the potential donor for an effective GVT therapeutic effect by (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of tumor cells derived from the recipient subject; and (ii) selecting the T lymphocyte clones from the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject; and (iii) expanding the T lymphocyte clones to obtain a therapeutic amount of the T cell clones effective to mediate a GVT response against the tumor-specific antigens.

AMENDED CLAIMS
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What is claimed is:

1. An ex vivo model of persistent T lymphocyte stimulation events encountered by circulating T lymphocytes at lymph node and tissue levels in vivo comprising

(a) a multiwell plate-based perfusion culture device, comprising, from top to bottom:

a bottomless multi-well plate comprising a plurality of bottomless wells;

a first micropatterned polymer layer attached to a bottom surface of the bottomless multi-well plate to form a plurality of adjacent wells, one or more of each pair of adjacent wells comprising a transparent polymer membrane placed within the one or more of each pair of adjacent wells;

a second micropatterned polymer layer comprising two or more holes that correspond to two or more adjacent wells, the second micropatterned polymer layer being attached to a bottom surface of the first micropatterned polymer layer, such that each hole of the second micropatterned polymer layer is aligned with the two or more adjacent wells in the first micropatterned polymer layer, one or more of each pair of adjacent wells comprising the transparent polymer membrane;

a microfluidic channel formed between the two adjacent wells that allows internal fluidic communication between the two adjacent wells;

one or more removable polymer plugs, each located at a top surface of each of the plurality of wells, and one or more tubes, each connected to the one or more polymer plugs;

a pump connected to a reservoir that removably connects to the tubes;

a transparent, optical grade glass layer attached to the bottom surface of the second micropatterned polymer layer that forms a bottom surface for the plurality of wells and that seals the multi-well plate perfusion culture device;

wherein one or more of the two adjacent wells is a culture chamber for culturing a population of cells;

(b) an expanded population of cells derived from a recipient subject comprising a cell-specific antigen in the first adjacent well of the device in (a);

(c) an expanded population of T lymphocytes derived from a potential donor of a BMT graft in the second adjacent well of the device in (a), wherein the potential donor is allogeneic to the recipient subject;

(d) a liquid culture medium that is flowable between the first adjacent well and the second adjacent well;

the model being characterized by:

circulation of the liquid medium from the first well into the second well and back to the first well through the microfluidic channel;

an interaction between the population of cells comprising the cell antigen derived from the recipient subject in the first well and the population of T lymphocytes is effective to generate alloreactive effector T lymphocytes;

alloreactive effector T lymphocyte-induced quantifiable damage to the population of cells comprising the cell antigen derived from the recipient by the population of alloreactive effector T lymphocytes from the donor allogeneic to the recipient.

2. The ex vivo model according to claim 1, wherein the population of T lymphocytes in (c) is derived from peripheral blood lymphocytes of the potential donor.

3. The ex vivo model according to claim 1, wherein the population of T lymphocytes comprises a suspension of nonadherent cells.

4. The ex vivo model according to claim 1, wherein the first micropatterned polymer layer and the second micropatterned polymer layer comprises an organic polymer.

5. The ex vivo model according to claim 4, wherein the organic polymer is polydimethyl siloxane (PMDS) or polystyrene.

6. The ex vivo model according to claim 1, wherein the transparent polymer membrane comprises a nanofibrous mesh.

7. The ex vivo model according to claim 6, wherein the nanofibrous mesh is placed on a top surface of the transparent polymer membrane to coat the top surface of the transparent polymer membrane.

8. The ex vivo model according to claim 6, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

9. The ex vivo model according to claim 1, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an adhesion-promoting agent in an amount effective to promote adhesion of a population of cells to a surface of the microbeads.

10. The ex vivo model according to claim 9, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of a subpopulation of the population of T lymphocytes to the microbead surface.

11. The ex vivo model according to claim 10, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

12. The ex vivo model according to claim 3, wherein the suspension of nonadherent cells contains T lymphocytes derived from the allogeneic donor.

13. The ex vivo model according to claim 1, wherein the population of alloreactive effector T lymphocytes comprises alloreactive activated antigen presenting cells.

14. The ex vivo model according to claim 13, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

15. The ex vivo model according to claim 1, wherein the quantifiable damage to the population of cells comprising the cell-specific antigen derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

16. The ex vivo model according to claim 1, wherein the population of cells comprising the cell-specific antigen in (b) are a population of intestinal epithelial cells derived from the recipient subject, and the alloreactive effector T lymphocyte-induced quantifiable damage to the population of intestinal epithelial cells of the recipient subject is an ex vivo measure of risk of graft vs. host disease.

17. A method for optimizing donor selection for allogeneic blood and marrow transplantation (BMT) therapy comprising, in order:

(a) acquiring a tissue sample from a recipient subject allogeneic to a potential donor of a BMT graft, the tissue sample comprising a population of primary intestinal epithelial cells comprising an intestinal epithelial cell-specific antigen;

(b) seeding the population of primary intestinal epithelial cells of (a) in a first adjacent well of a multiwell plate-based perfusion culture device, the first adjacent well comprising a transparent polymer membrane, expanding the population in a first liquid medium containing ROCK inhibitor Y-27632 and an irradiated Swiss 3T3-J2 fibroblast feeder layer and generating a population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising the intestinal cell-specific antigen derived from the recipient subject;

(c) acquiring a population of T lymphocytes from the potential donor allogeneic to the recipient;

(d) seeding and expanding in a second adjacent well of the multiwell plate-based perfusion culture device the population of T lymphocytes derived from the potential donor of (c),

(e) co-culturing in a second liquid medium the CRIECs derived from the recipient subject in the first adjacent well and the T lymphocytes derived from the potential donor allogeneic to the recipient subject in the second adjacent well, the co-culturing being characterized by:

the first adjacent well being fluidly connected to the second adjacent well so that the second liquid medium is flowable between the first adjacent well and the second adjacent well;

an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes that is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor;

(f) measuring damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of a risk of intestinal graft versus host disease in the recipient subject;

(g) ranking a plurality of potential donors by the measure of the risk of intestinal graft versus host disease; and

(h) treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient subject whose T lymphocytes are characterized by a reduced risk of intestinal graft-versus-host disease.

18. The method according to claim 17, wherein the potential donor of the BMT graft is haploidentical to the recipient subject.

19. The method according to claim 17, wherein the tissue sample comprising a population of primary intestinal epithelial cells is derived from small intestine, large intestine or colon of the recipient subject.

20. The method according to claim 17, wherein the transparent polymer membrane comprises a nanofibrous mesh to which the population of CRIECs is adherent.

21. The method according to claim 20, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

22. The method according to claim 17, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads.

23. The method according to claim 22, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

24. The method according to claim 23, wherein the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject.

25. The method according to claim 24, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

26. The method according to claim 17, wherein the expanded population of T lymphocytes derived from the donor allogeneic to the recipient subject in (d) comprise a suspension of nonadherent cells.

27. The method according to claim 17, wherein the population of alloreactive effector T lymphocytes comprises a population of alloreactive activated antigen presenting cells.

28. The method according to claim 27, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

29. The method according to claim 17, wherein the quantifiable damage to the population of CRIECs derived from the recipient induced by the population of nonadherent alloreactive effector T lymphocytes from the donor allogeneic to the recipient comprises cell death.

30. The method according to claim 17, further comprising (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of CRIECs derived from the recipient subject; and (ii) selectively deleting the T lymphocyte clones from the population of T lymphocytes derived from the donor.

31. A method for minimizing risks and maximizing benefits of performing allogeneic blood and marrow transplantation (BMT) therapy in a recipient subject, wherein the recipient subject has a hematologic malignancy comprising, in order:

(A) evaluating a population of T lymphocytes derived from a potential donor allogeneic to the recipient subject for a potential to damage intestinal epithelial cells of the recipient subject according to the method of claim 17 steps (a) through (g);

(B) evaluating the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject for an effective graft versus tumor response against the tumor-specific antigens by:

(i) acquiring a specimen comprising a population of tumor cells derived from the recipient subject, the population of tumor cells comprising one or more tumor specific antigens;

(ii) seeding and expanding the population of tumor cells in the second liquid medium in a third adjacent well of the multiwell plate-based perfusion culture device; the third adjacent well comprising a transparent polymer membrane;

(iii) acquiring a population of T lymphocytes derived from the potential donor allogeneic to the recipient subject;

(iv) seeding and expanding in a fourth adjacent well of the multiwell plate-based perfusion culture device the population of T lymphocytes of (iii),

(v) co-culturing in the second liquid medium the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject in the third adjacent well and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject in the fourth adjacent well, the co-culturing being characterized by:

the third adjacent well being fluidly connected to the fourth adjacent well so that the second liquid medium is flowable between the third adjacent well and the fourth adjacent well;

an interaction between the population of tumor cells comprising one or more tumor-specific antigens that is derived from the recipient subject and the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject that is effective to generate alloreactive effector T lymphocytes derived from the potential donor;

(vi) measuring damage to the population of tumor cells derived from the recipient subject induced by the alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient subject, wherein the damage is a measure of an effective graft versus tumor (GVT) response against the tumor-specific antigens;

(vii) ranking a plurality of potential donors by the measure of the effective GVT response against the tumor-specific antigens; and

(C) treating the recipient subject with a BMT graft derived from a selected donor allogeneic to the recipient, the selected donor being characterized by a reduced risk of intestinal graft versus host disease, and an effective GVT response against the tumor-specific antigens.

32. The method according to claim 31, wherein the potential donor of the BMT graft is haploidentical to the recipient subject.

33. The method according to claim 31 part B, wherein the specimen comprising the population of tumor cells derived from the recipient subject in (i) is a blood sample, a bone marrow sample, or a leukapheresis sample.

34. The method according to claim 31 part B, wherein the transparent polymer membrane comprises a nanofibrous mesh to which the population of tumor cells is adherent.

35. The method according to claim 31 part B, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject can pass.

36. The method according to claim 31 part B, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an amount of an adhesion-promoting agent effective to promote adhesion of a population of cells to at least one surface of the microbeads.

37. The method according to claim 36, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells.

38. The method according to claim 36, wherein the population of cells is a subpopulation of the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject.

39. The method according to claim 38, wherein the subpopulation of the population of T lymphocytes comprises a population of dendritic cells.

40. The method according to claim 31 part B, wherein the expanded population of T lymphocytes derived from the allogeneic donor in (iv) comprise a suspension of nonadherent cells.

41. The method according to claim 31 part B, wherein the population of alloreactive effector T lymphocytes in (v) comprises a population of alloreactive activated antigen presenting cells.

42. The method according to claim 41, wherein the alloreactive activated antigen presenting cells comprise a population of alloreactive activated dendritic cells.

43. The method according to claim 31, part B, wherein the quantifiable damage to the population of tumor cells derived from the recipient subject induced by the population of nonadherent alloreactive effector T lymphocytes derived from the potential donor allogeneic to the recipient comprises cell death.

44. The method according to claim 31, further comprising enriching the population of T lymphocytes derived from the potential donor for an effective GVT therapeutic effect by (i) identifying T lymphocyte clones responsible for the quantifiable damage to the population of tumor cells derived from the recipient subject; and (ii) selecting the T lymphocyte clones from the population of T lymphocytes derived from the potential donor allogeneic to the recipient subject; and (iii) expanding the T lymphocyte clones to obtain a therapeutic amount of the T cell clones effective to mediate a GVT response against the tumor-specific antigens.

45. A multiwell plate-based perfusion culture device, comprising, from top to bottom:

a bottomless multi-well plate comprising a plurality of bottomless wells;

a first micropatterned polymer layer attached to a bottom surface of the bottomless multi-well plate to form a plurality of adjacent wells, one or more of each pair of adjacent wells comprising a transparent polymer membrane placed within the one or more of each pair of adjacent wells;

a second micropatterned polymer layer comprising two or more holes that correspond to two or more adjacent wells, the second micropatterned polymer layer being attached to a bottom surface of the first micropatterned polymer layer, such that each hole of the second micropatterned polymer layer is aligned with the two or more adjacent wells in the first micropatterned polymer layer, one or more of each pair of adjacent wells comprising the transparent polymer membrane;

a microfluidic channel formed between the two adjacent wells that allows internal fluidic communication between the two adjacent wells;

one or more removable polymer plugs, each located at a top surface of each of the plurality of wells, and one or more tubes, each connected to the one or more polymer plugs;

a pump connected to a reservoir that removably connects to the tubes; and

a transparent, optical grade glass layer attached to the bottom surface of the second micropatterned polymer layer that forms a bottom surface for the plurality of wells and that seals the multi-well plate perfusion culture device;

wherein one or more of the two adjacent wells is a culture chamber for culturing a population of cells; and

(d) a liquid culture medium that is flowable between the first adjacent well and the second adjacent well.

46. The multiwell plate-based perfusion culture device according to claim 45, wherein circulation of the liquid medium occurs from the first well into the second well and back to the first well through the microfluidic channel.

47. The multiwell plate-based perfusion culture device according to claim 45, wherein the first micropatterned polymer layer and the second micropatterned polymer layer comprises an organic polymer.

48. The multiwell plate-based perfusion culture device according to claim 47, wherein the organic polymer is polydimethyl siloxane (PMDS) or polystyrene.

49. The multiwell plate-based perfusion culture device according to claim 45, wherein the transparent polymer membrane comprises a nanofibrous mesh.

50. The multiwell plate-based perfusion culture device according to claim 49, wherein the nanofibrous mesh is placed on a top surface of the transparent polymer membrane to coat the top surface of the transparent polymer membrane.

51. The multiwell plate-based perfusion culture device according to claim 49, wherein the nanofibrous mesh comprises a nanofibrous matrix comprising a plurality of pores through which a population of cells can pass.

52. The multiwell plate-based perfusion culture device according to claim 45, wherein the transparent polymer membrane comprises a plurality of microbeads preconditioned with an adhesion-promoting agent in an amount effective to promote adhesion of a population of cells to a surface of the microbeads.

53. The multiwell plate-based perfusion culture device according to claim 52, wherein the adhesion promoting agent comprises a lipopolysaccharide in an amount effective to promote adhesion of the population of cells to the surface of the microbeads.

54. The multiwell plate-based perfusion culture device according to claim 45, wherein

the first adjacent well of the multiwell plate-based perfusion culture device in (a) contains an expanded population of cells derived from a recipient subject comprising a cell-specific antigen;

the second adjacent well of the multiwell plate-based perfusion culture device in (a) contains an expanded population of T lymphocytes derived from a potential donor of a blood and marrow transplantation (BMT) graft, wherein the potential donor is allogeneic to the recipient subject; and

an interaction in vitro between the population of cells comprising the cell antigen derived from the recipient subject in the first well and the population of T lymphocytes

derived from the donor in the second well is effective to generate alloreactive effector T lymphocytes.

55. The multiwell plate-based perfusion culture device according to claim 45, wherein

the first adjacent well of the multiwell plate-based perfusion culture device in (a) contains an expanded population of conditional reprogrammed intestinal epithelial cells (CRIECs) comprising an intestinal cell-specific antigen from a potential donor allogeneic to a recipient subject;

the second adjacent well of the multiwell plate-based perfusion culture device in (a) contains an expanded population of T lymphocytes derived from a potential donor of a blood and marrow transplantation (BMT) graft, wherein the potential donor is allogeneic to the recipient subject;

an interaction between the population of CRIECs derived from the recipient subject and the population of T lymphocytes is effective to generate alloreactive effector T lymphocytes derived from the potential allogeneic donor; and

damage to the population of CRIECs derived from the recipient subject induced by the alloreactive effector T cells derived from the potential donor allogeneic to the recipient subject is a measure of a risk of intestinal graft versus host disease in the recipient subject.

## Statement under Article 19(1)

Pursuant to Rule 46.5, Section 205 and PCT Applicant's Guide 9.006, the amendment of claim 1 and the addition of claims 45-55 has no impact on the description and drawings as originally filed, since the amendments do not go beyond the disclosure in the international application as filed.

* * *

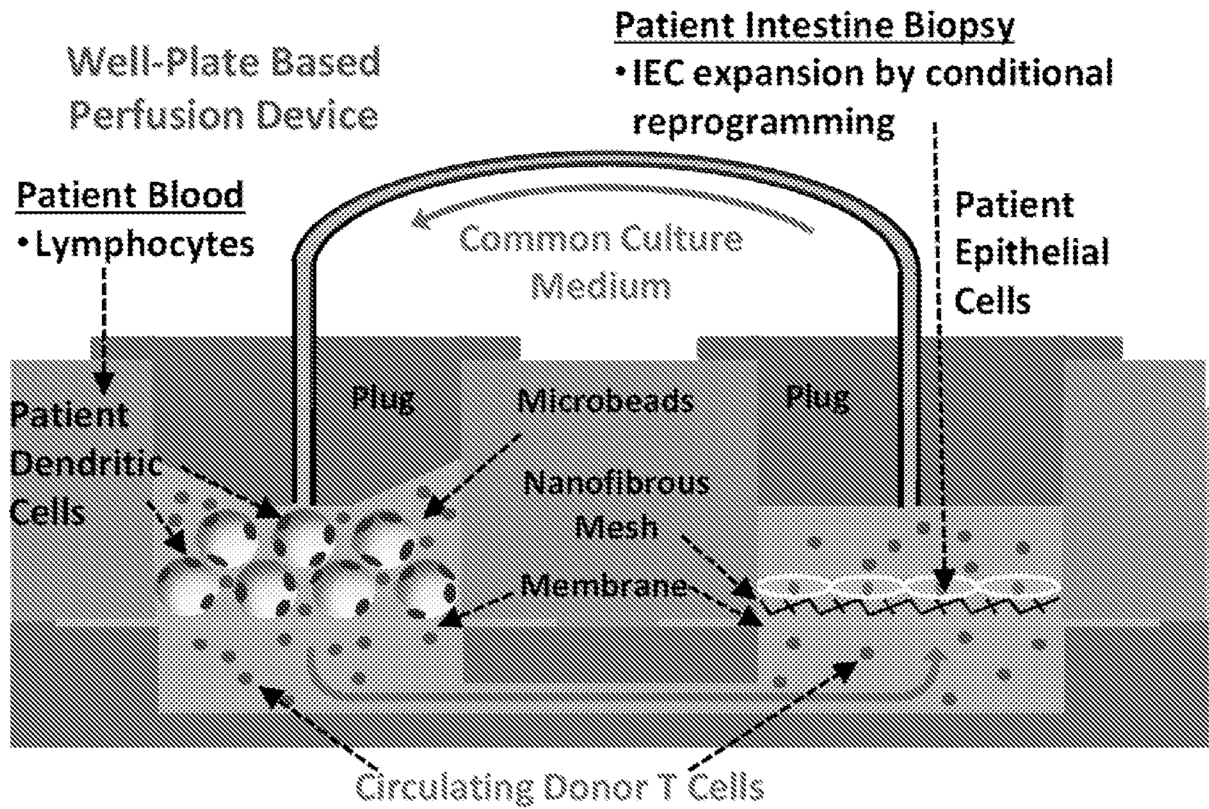
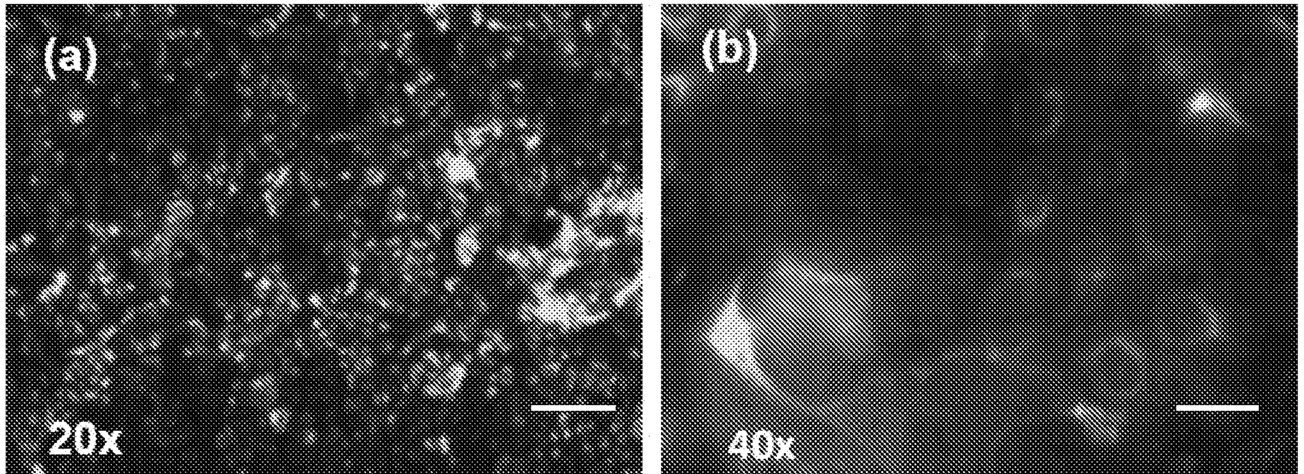



Figure 1



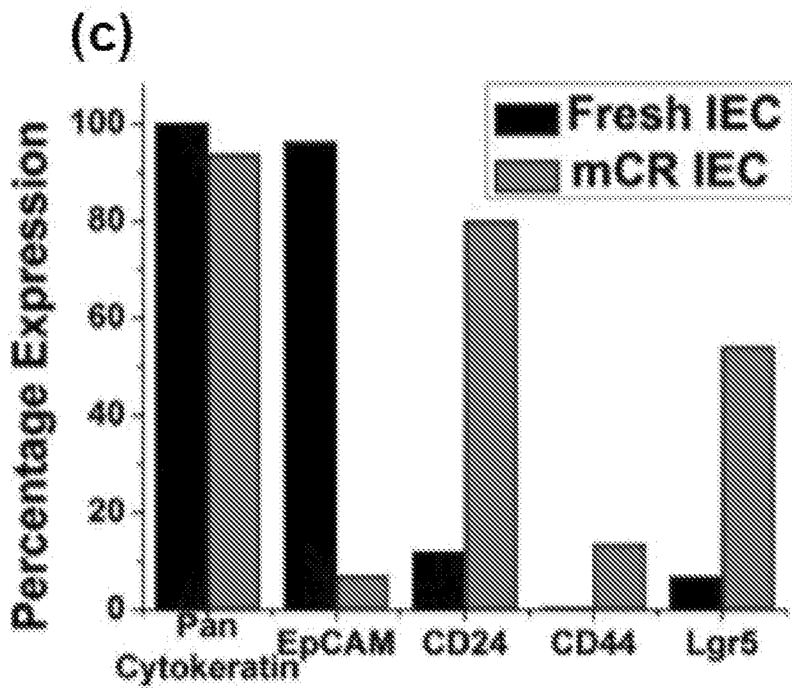




Figure 2

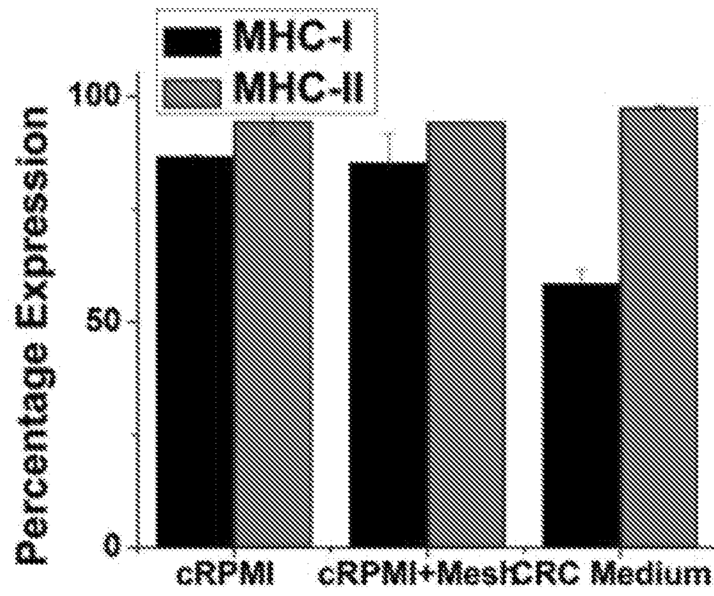



Figure 3

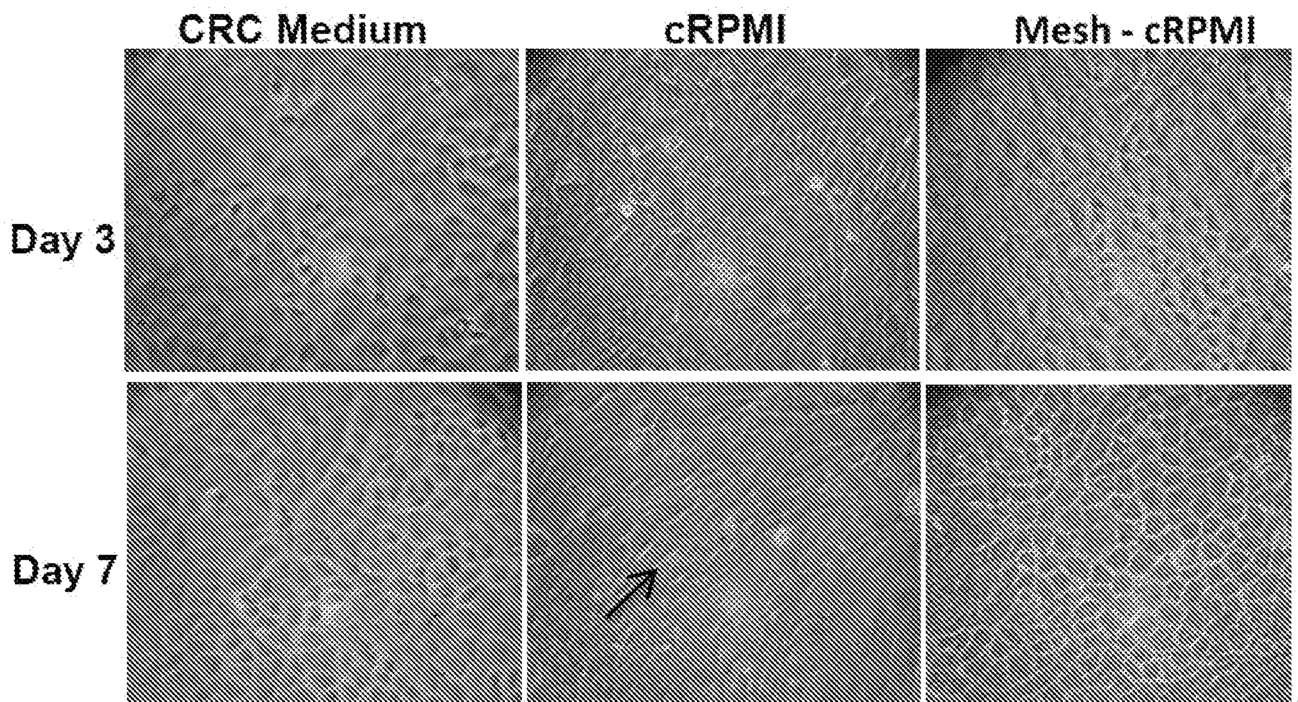



Figure 4

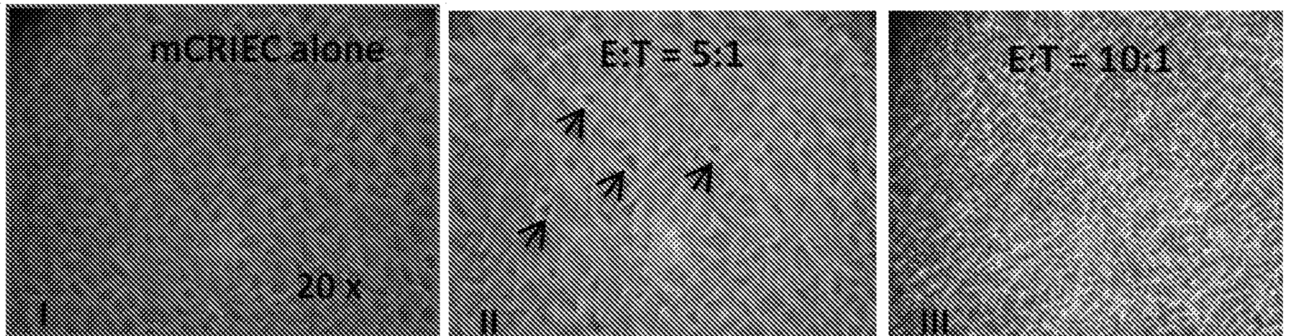



Figure 5

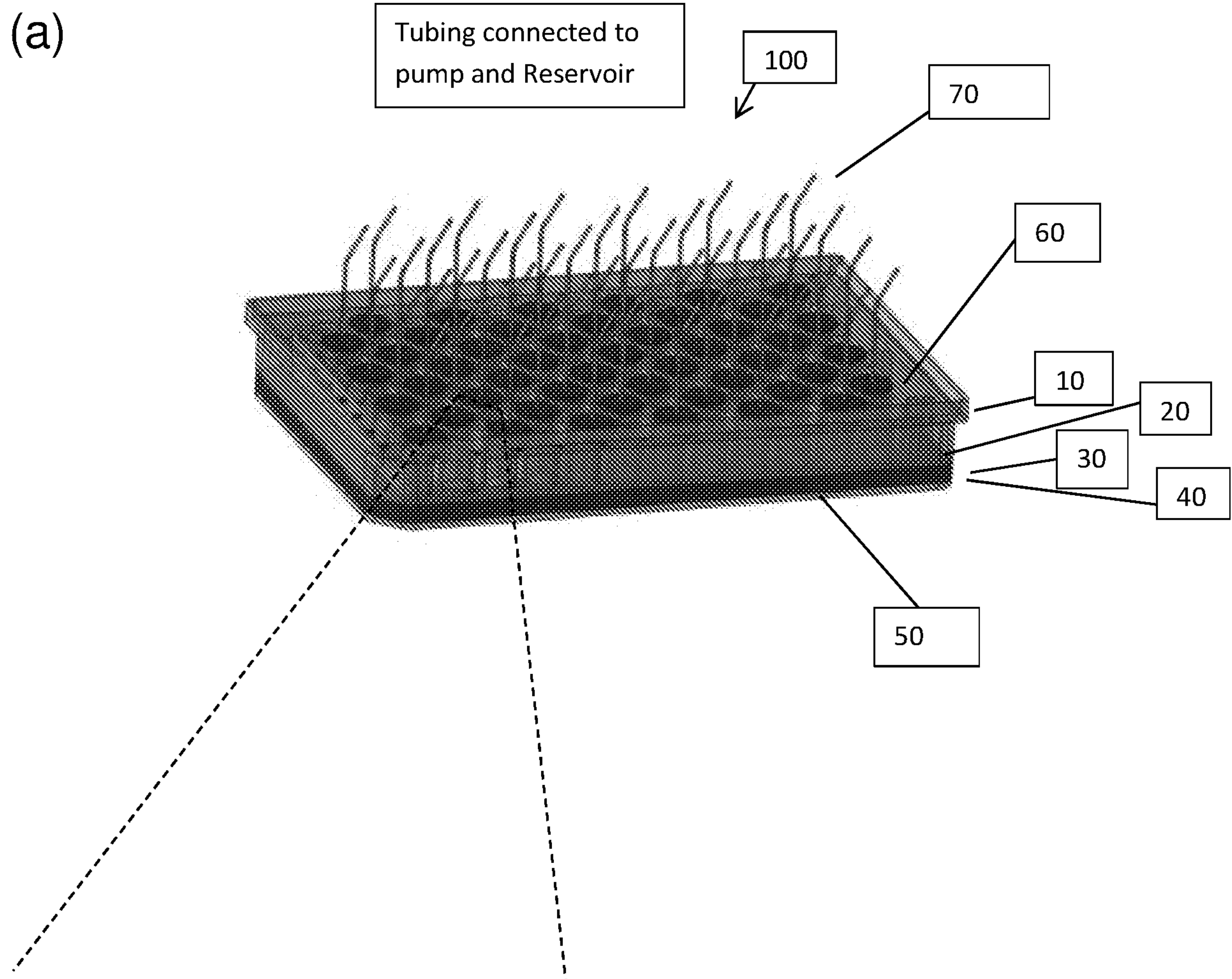



Figure 6

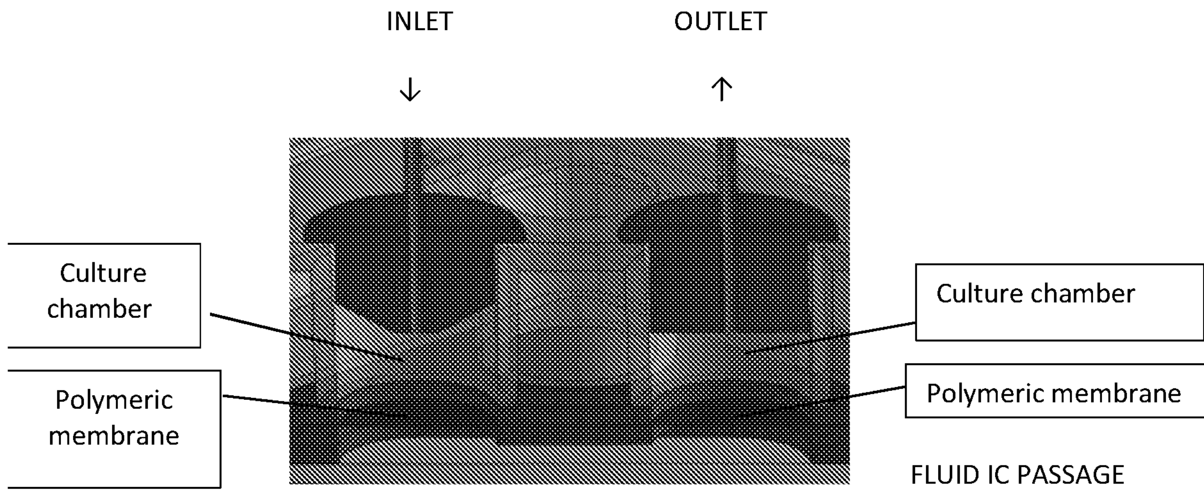



Figure 6

(c)

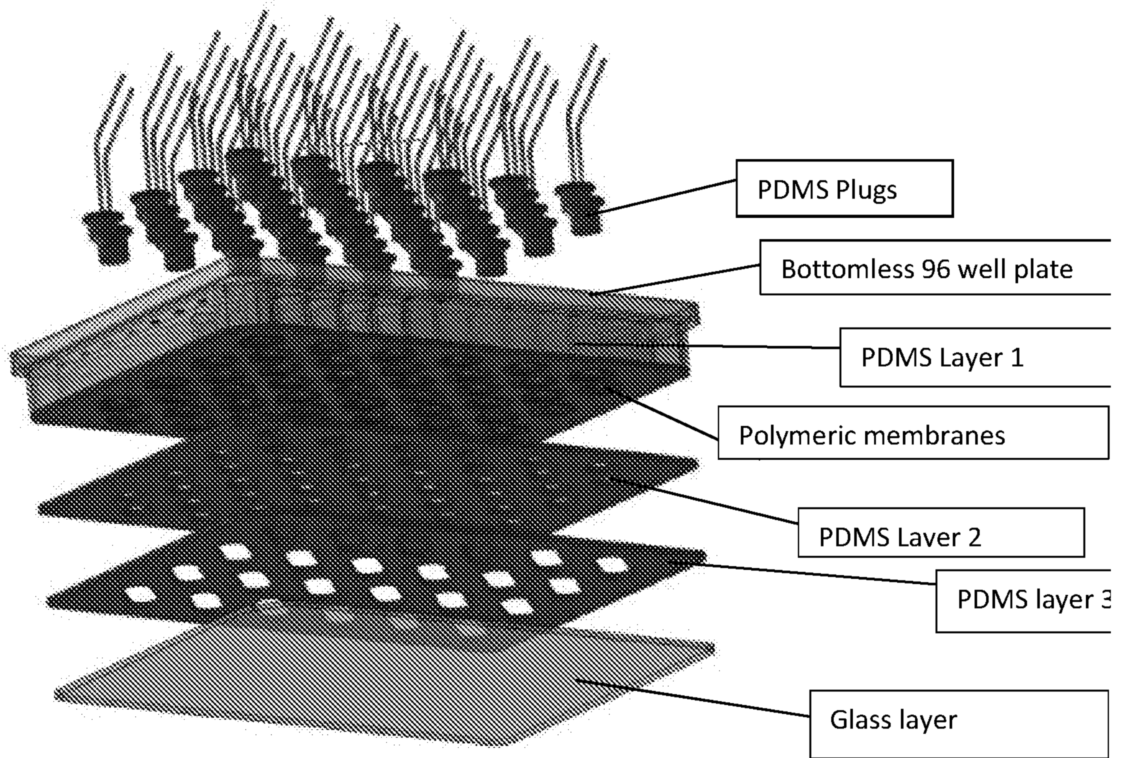



Figure 6

(d)

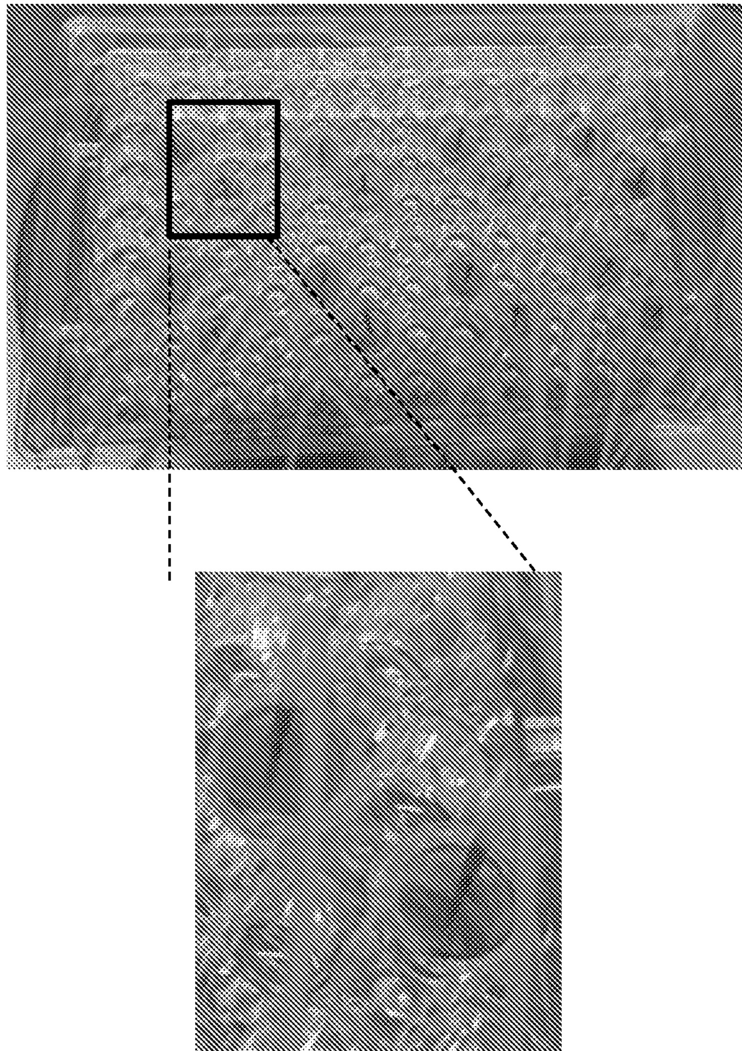


Figure 6

(e)

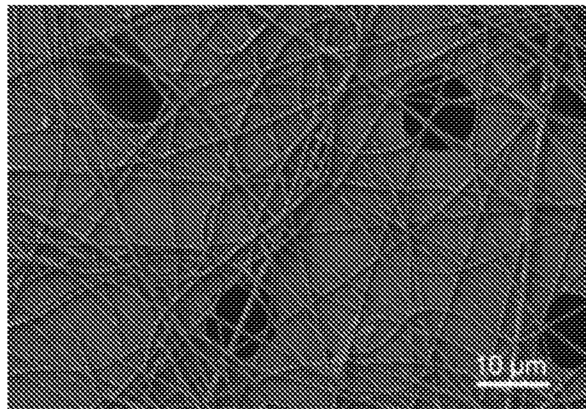



Figure 6

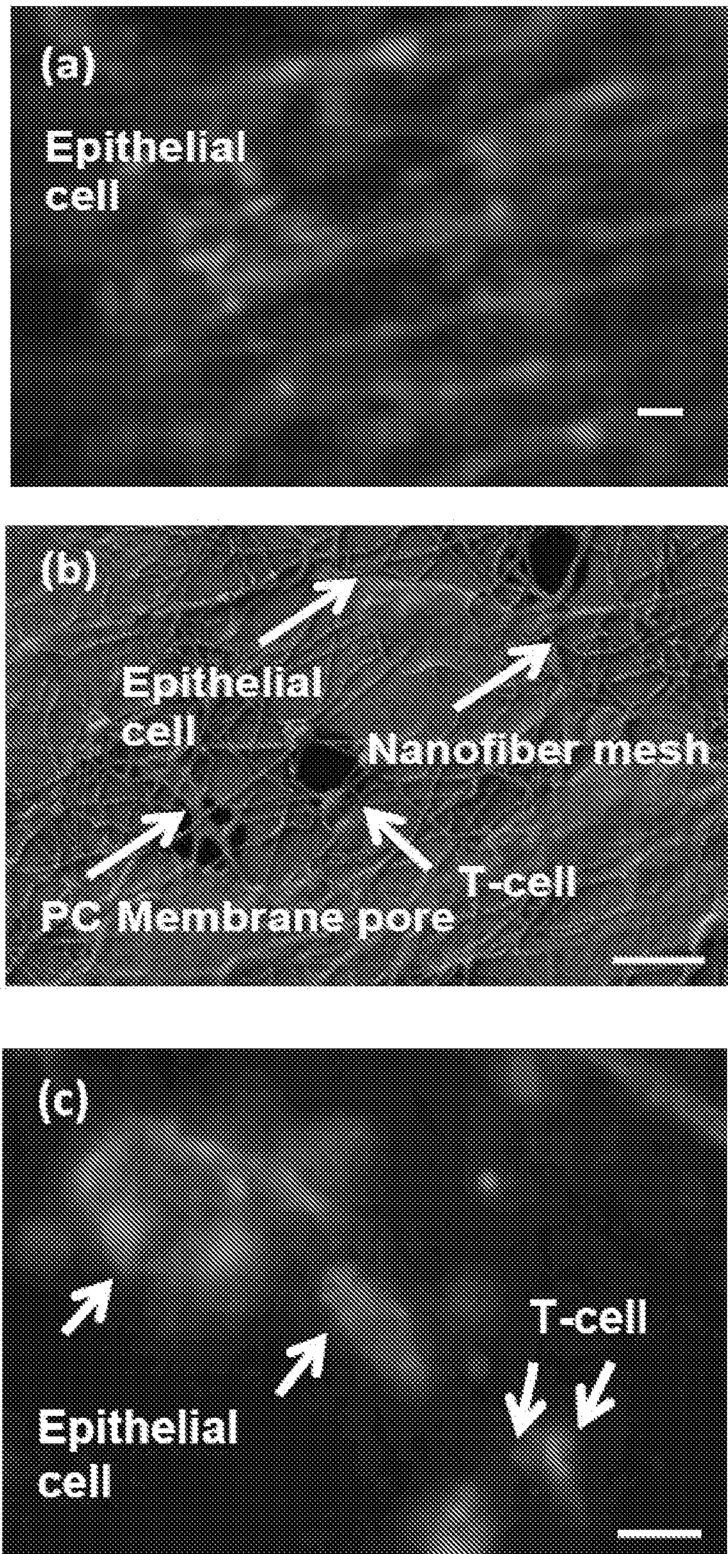



Figure 7

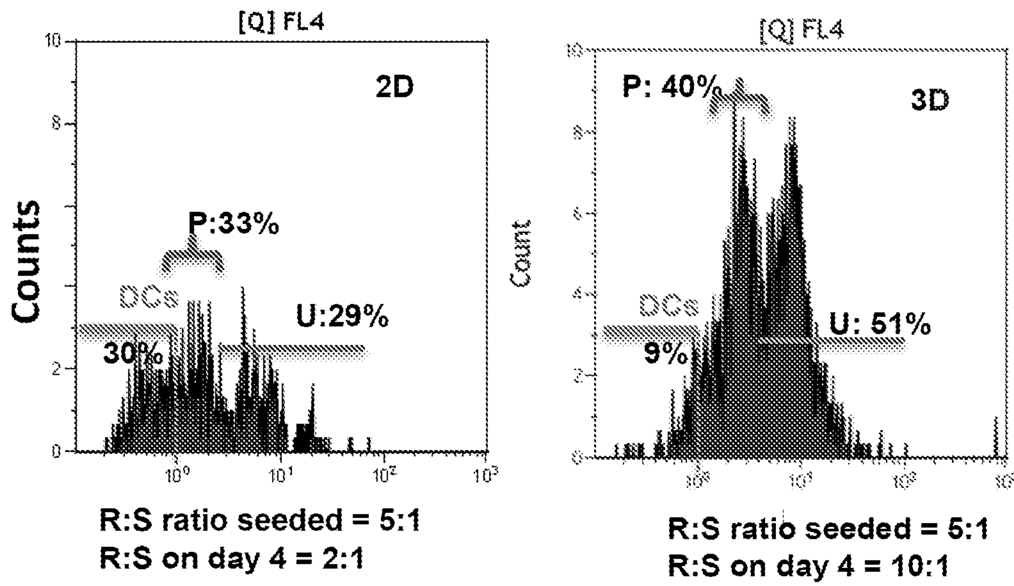



Figure 8

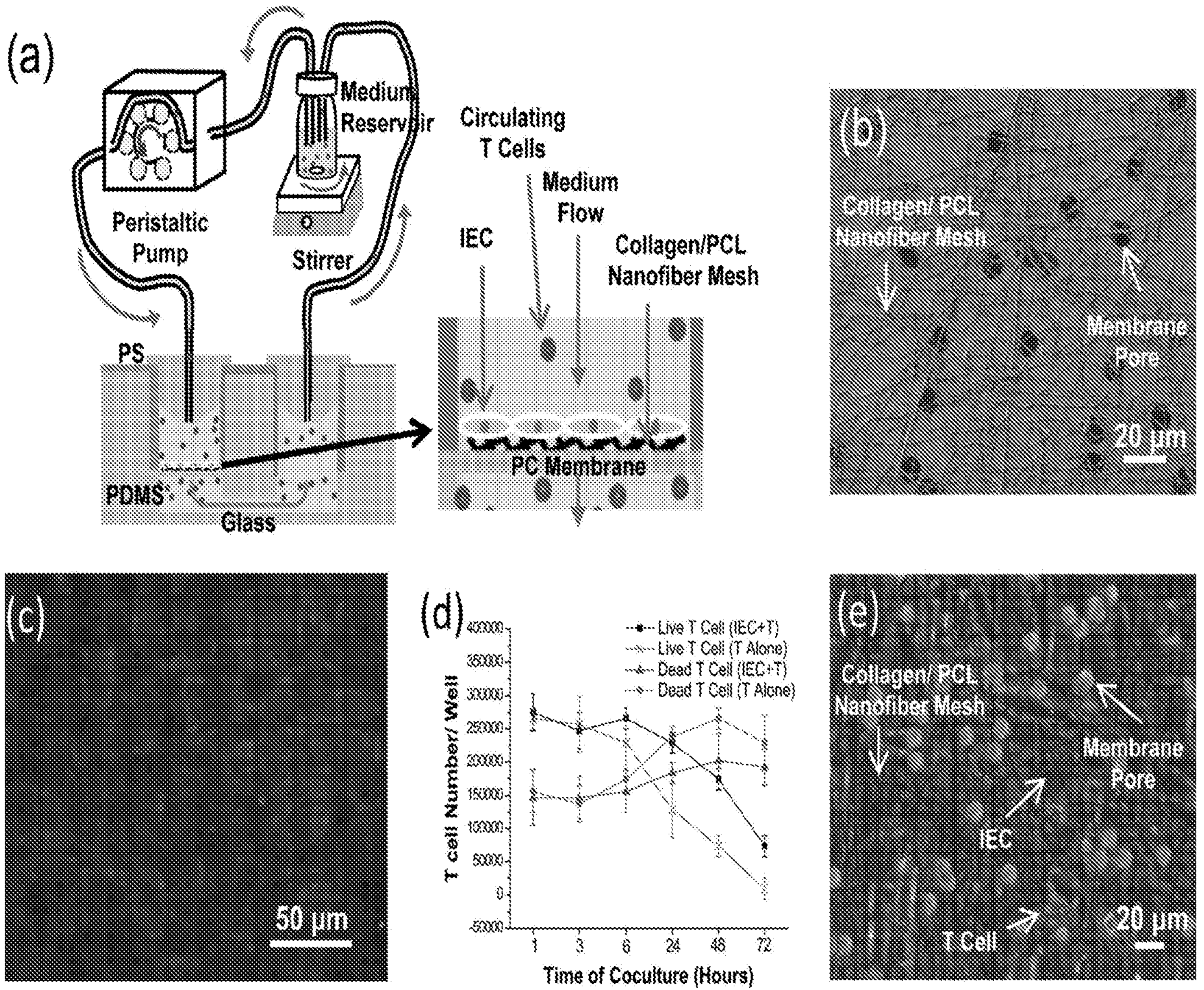



Figure 9

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/012573

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC(8) - A61K 35/17 (2016.01)
CPC - A61K 35/17 (2016.02)
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)
IPC(8) - A61K 35/17, 35/36; G01N 33/48 (2016.01)
CPC - A61K 35/17, 35/36; C12M 23/16; G01N 33/48, 33/4833, 33/5044, 33/505 (2016.02)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/154.1; 435/289.1, 297.2, 371 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar.
Search terms used: lymphocyte, T cells, epithelial, intestinal, allogeneic, transplantation, model, coculture, microfluidic, first, second, adjacent, immune

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 2011/0027804 A1 (YARMUSH et al) 03 February 2011 (03.02.2011) entire document | 1-44 |
| A | SAMSONOV et al. "Differential activation of human T cells to allogeneic endothelial cells, epithelial cells and fibroblasts in vitro," Transplant Res. 24 April 2012 (24.04.2012), Vol. 1, No. 1, Pgs. 1-10. entire document | 1-44 |
| A | STYBAYEVA et al. "Micropatterned co-cultures of T-lymphocytes and epithelial cells as a model of mucosal immune system,"Biochem Biophys Res Commun. 31 January 2009 (31.01.2009), Vol. 380, No. 3, Pg. 575-80. entire document | 1-44 |
| A | US 2014/0038279 A1 (INGBER et al) 06 February 2014 (06.02.2014) entire document | 1-44 |
| A | WO 2008/060449 A2 (BUTTE et al) 22 May 2008 (22.05.2008) entire document | 1-44 |
| A | US 2004/0259177 A1 (LOWERY et al) 23 December 2004 (23.12.2004) entire document | 1-44 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 11 April 2016 | 03 MAY 2016 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, VA 22313-1450<br>Facsimile No. 571-273-8300 | Blaine R. Copenheaver<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |