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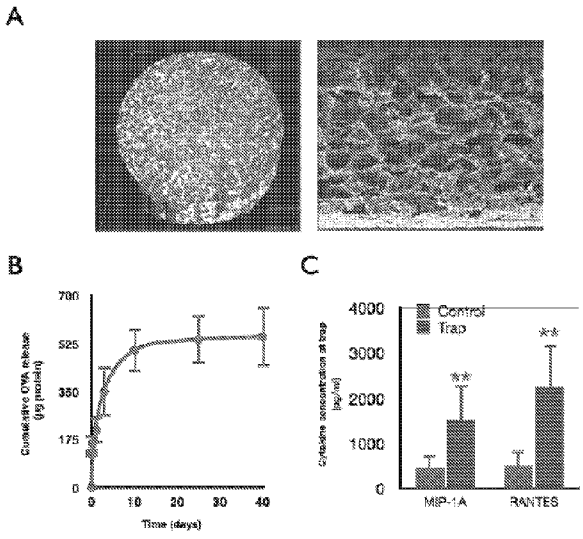

FIG. 1

(57) Abstract: Embodiments herein described provide devices for identifying and collecting rare cells or cells which occur at low frequency in the body of a subject, such as, antigen-specific cells or disease-specific cells. More specifically, the devices are useful for trapping immune cells and the devices contain a physiologically-compatible porous polymer scaffold, a plurality of antigens, and an immune cell -recruiting agent, wherein the plurality of antigens and the immune cell recruiting agent attract and trap the immune cell in the device. Also provided are pharmaceutical compositions, kits, and packages containing such devices. Additional embodiments relate to methods for making the devices, compositions, and kits/packages. Further embodiments relate to methods for using the devices, compositions, and/or kits in the diagnosis or therapy of diseases such as autoimmune diseases or cancers.

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IMMUNE CELL TRAPPING DEVICES AND METHODS FOR MAKING AND USING THE SAME

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 62/146,205, filed on April 10, 2015. The entire contents of this application are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Cells of the immune system play a central role in distinguishing between foreign and self-antigens in the context of autoimmunity and neoplastic diseases. The process of thymic (central) tolerance provides a basis for the immune cells to confer homeostatic balance between the two disease states. At one end, high-affinity self-antigen-specific T cells are eliminated from the system, leading to the prevention of autoimmunity. At the other end, cells that fail to recognize self-antigens entirely are also eliminated and this ensures that tumor cells are held in check. The result of this complex process is that only the T cells that recognize self-antigens with intermediate affinity are spared and allowed to propagate. This development of peripheral tolerance is central to keeping potentially autoreactive lymphocytes in check because recirculating lymphocytes exposed to tissue antigens under non-inflammatory conditions are normally in a tolerant, anergic state. However, in the presence of danger stimuli, such as those elicited by infection and tissue damage, the balance between tolerance and autoimmunity can be broken. Conversely, if self-reactive cells are depleted from the immune repertoire, then the immune system may fail to recognize tumors expressing altered levels or patterns of self antigens.

It is now recognized that many autoimmune diseases are mediated by auto-reactive T cell responses. For example, in the context of type-1 diabetes, auto-reactive T cells have been implicated in the destruction of pancreatic beta cells. Likewise, pathophysiology of multiple sclerosis is often characterized by auto-reactive T-cell mediated targeting and apoptosis of neuronal cells. However, proper diagnosis and identification of autoimmune disease is fraught with difficulty because they often require performing many laboratory tests (usually, involving complete blood count, comprehensive metabolic panel, acute phase reactants, immunologic studies, serologies, flow cytometry, cytokine analysis, and HLA typing). These tests are cumbersome and expensive to administer.

At the cellular level, diagnosing auto-immune disorders based on immune-cell karyotyping is similarly problematic due to the lack of accessibility of functional T cells from the site of tissue destruction. In many diseases, auto-reactive cells occur at such low frequencies (1:100,000 cells) that detection of specific immune cells is technically challenging. Additionally, in many instances, the population of auto-reactive cells are not necessarily disease specific. Even when the disease-specific

T-cells are isolated, they have limited use in diagnostic or therapeutic applications because they cannot be frozen or cultured without affecting their functional capacities.

Similarly, in the context of cancer, existing therapeutic strategies generally involve adoptive transfer of antigen-specific T cells that are extracted from the host. In such instances, tumor infiltrating leukocytes (TILs) are extracted from tumor biopsies or peripheral blood, manipulated to add antigen specificity or homing capacity, expanded ex vivo, and injected into patients to target tumor cells. However, the quantities of these cells in the blood or in diseased sites are often of low frequency and lack ideal functionality.

There is, therefore, an unmet need for compositions and methods that allow isolation of immune cells, which can be analyzed for diagnosing autoimmune disorders and cancers, and for the therapy of human diseases, such as autoimmune disorders, cancers and infectious diseases. Embodiments of the instant invention, which are described in detail below, address these needs.

SUMMARY OF THE INVENTION

The present invention provides a solution to the problem of identifying and collecting rare cells or cells with occur at low frequency in the body. Particular embodiments described herein relate to collection devices, cell trapping devices, which are useful in the collection of such cells. The devices include a scaffold composition which incorporates or is coated with a plurality of antigens and, optionally, recruiting agents, allowing the device to attract, adhere to, and capture or sequester targeted cells, such as immune cells. The device executes these functions by a variety of methods that include direct or indirect interaction with the antigens, recruiting agents, or other molecules present therein. Depending on the application for which the device is used, the device regulates capture and survival of the targeted cells through the physical or chemical characteristics of the scaffold itself. For example, the scaffold composition is differentially permeable, allowing cell passage only in certain physical areas of the scaffold. The permeability of the scaffold composition is regulated, for example, by selecting or engineering a material for greater or smaller pore size, density, polymer cross-linking, stiffness, toughness, ductility, or visco-elascticity. The scaffold composition may contain physical channels or paths through which targeted cells interact with the device and/or move into a specific compartment or region of the device. To facilitate the compartmentalization, the scaffold composition is optionally organized into compartments or layers, each with a different permeability, so that cells are sorted or filtered to allow access to only a certain sub-population of cells. Sequestration of target cell populations in the device may also be regulated by the degradation, de- or re-hydration, oxygenation, chemical or pH alteration, or ongoing self-assembly of the scaffold composition. Following their capture, the targeted cells, *e.g.,* immune cells, may be allowed to grow or expand within the device with the help of stimulatory molecules, cytokines, and other co-factors present in the device. In some embodiments, non-targeted cells which have otherwise infiltrated the device may be rejected or removed using negative selection agents.

The cells that are trapped within the devices of the invention are primarily immune cells. In certain embodiments, the invention relates to T-cell traps. In other embodiments, the invention relates to B-cell traps. Yet in other embodiments, the invention relates to a combination of traps, *e.g.*, a combination of T-cell traps and B-cell traps. The traps described herein may also be configured to trap antigen presenting cells (APCs), such as, for example, autoreactive APCs. Examples of auto-reactive APCs that may be trapped include, for example, dendritic cells (DCs), macrophages, or a combination of thereof. In other embodiments, a plurality of traps may be employed, *e.g.*, traps which are configured for trapping both lymphocytes and APCs. For example, antigen-specific cells or disease specific-cells such as T cells, dendritic cells (DCs), or macrophages may be trapped, either separately or together, for analyses, reprogramming or depletion. The trapped immune cells, *e.g.*, lymphocytes and APCs, are optionally harvested and analyzed to identify and characterize targets for disease diagnosis or immunotherapy. The trapped cells may also be reprogrammed or expanded for developing compositions or formulations that are to be used in therapy.

Accordingly, in one aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device. The antigens may be absorbed onto the polymer scaffold or encapsulated by the polymer scaffold.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device by binding to the plurality of immune cells.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device does not contain an agent that kills or eliminates immune cells.

In yet another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device further comprises an immune cell recruiting agent. The immune cell recruiting agent may be, for example, a T-cell recruiting agent, a dendritic cell recruiting agent, or a macrophage recruiting agent, or a combination thereof. Particularly, the immune cell recruiting agent may be a natural killer (NK) cell recruiting agent, a CD3+ T-cell recruiting agent, a CD4+ T-cell recruiting agent, a CD8+ T cell recruiting agent, a CD8+ T cell recruiting agent, a regulatory T-cell (Treg) recruiting agent, or a combination thereof. In related aspects, the immune cell recruiting agent may be a growth factor, a cytokine, an interleukin, an adhesion signaling molecule, an integrin signaling molecule, an interferon, a lymphokine, or a chemokine, or a fragment

thereof, or a combination thereof. Particularly, the immune cell recruiting agent may be an interleukin which is selected from the group consisting of IL-1, IL-2, IL-4, IL-5, IL-10 IL-12 and IL-17.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device comprises an agent which enhances infiltration of the immune cells into the device.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified cancer antigens, wherein the plurality of cancer antigens attract and trap a plurality of immune cells specific to the plurality of cancer antigens in the device. According to this aspect, the cancer antigen may be selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, CEA, Tyrosinase, midkin, BAGE, CASP-8, β-catenin, β- catenin, γ-catenin, CA-125, CDK-1, CDK4, ESO-1, gp75, gp100, MART-1, MUC-1, MUM-1, p53, PAP, PSA, PSMA, ras, trp-1, HER-2, TRP-1, TRP-2, IL13Ralpha, IL13Ralpha2, AIM-2, AIM-3, NY-ESO-1, C9orf 112, SART1, SART2, SART3, BRAP, RTN4, GLEA2, TNKS2, KIAA0376, ING4, HSPH1, C13orf24, RBPSUH, C6orf153, NKTR, NSEP1, U2AF1L, CYNL2, TPR, SOX2, GOLGA, BMI1, COX-2, EGFRvIII, EZH2, LICAM, Livin, Livinβ, MRP-3, Nestin, OLIG2, ART1, ART4, B-cyclin, Gli1, Cav-1, cathepsin B, CD74, E-cadherin, EphA2/Eck, Fra-1/Fosl 1, GAGE-1, Ganglioside/GD2, GnT-V, β1,6-N, Ki67, Ku70/80, PROX1, PSCA, SOX10, SOX11, Survivin, UPAR, WT-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (AD Abp), cyclophilin b, Colorectal associated antigen (CRC)- C017-1A/GA733, T-cell receptor/CD3-zeta chain, GAGE-family of tumor antigens, RAGE, LAGE-I, NAG, GnT-V, , RCASl, α-fetoprotein, pl20ctn, Pmel117, PRAME, brain glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-I, SSX-4, SSX-5, SCP-I, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, PlA, Connexin 37, Ig-idiotype, pl5, GM2, GD2 gangliosides, Smad family of tumor antigens, lmp-1, EBV-encoded nuclear antigen (EBNA)-I, UL16-binding protein-like transcript 1 (Mult1), RAE-1 proteins, H60, MICA, MICB, and c-erbB-2, or an immunogenic peptide thereof, and combinations thereof.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified non-self antigens, wherein the plurality of non-self antigens attract and trap a plurality of immune cells specific to the plurality of non-self antigens in the device. According to this aspect, the non-self antigens may be pathogenic antigens derived from a pathogen selected from the group consisting of a virus, a bacteria, a protozoan, a parasite, and a fungus. Further according to this aspect, the pathogen may be selected from the group consisting of *Mycobacterium bovis*, Human Papillomavirus (HPV), Human immunodeficiency virus, a pox virus, smallpox virus, ebola virus, marburg virus, dengue fever virus,

influenza virus, parainfluenza virus, respiratory syncytial virus, rubeola virus, varicella-zoster virus, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, JC virus, rhabdovirus, rotavirus, rhinovirus, adenovirus, papillomavirus, parvovirus, picornavirus, poliovirus, virus that causes mumps, virus that causes rabies, reovirus, rubella virus, togavirus, orthomyxovirus, retrovirus, hepadnavirus, coxsackievirus, equine encephalitis virus, Japanese encephalitis virus, yellow fever virus, Rift Valley fever virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, SARS CoV, MERS CoV, Enterovirus, Borrelia species, Bacillus anthracis, Borrelia burgdorferi, Bordetella pertussis, Camphylobacter jejuni, Chlamydia species, Chlamydial psittaci, Chlamydial trachomatis, Clostridium species, Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Corynebacterium diphtheriae, Coxiella species, an Enterococcus species, Erlichia species, Escherichia coli, Francisella tularensis, Haemophilus species, Haemophilus influenzae, Haemophilus parainfluenzae, Lactobacillus species, a Legionella species, Legionella pneumophila, Leptospirosis interrogans, Listeria species, Listeria monocytogenes, Mycobacterium species, Mycobacterium tuberculosis, Mycobacterium leprae, Mycoplasma species, Mycoplasma pneumoniae, Neisseria species, Neisseria meningitidis, Neisseria gonorrhoeae, Pneumococcus species, Pseudomonas species, Pseudomonas aeruginosa, Salmonella species, Salmonella typhi, Salmonella enterica, Rickettsia species, Rickettsia ricketsii, Rickettsia typhi, Shigella species, Staphylococcus species, Staphylococcus aureus, Streptococcus species, Streptococccus pnuemoniae, Streptococcus pyrogenes, Streptococcus mutans, Treponema species, Treponema pallidum, a Vibrio species, Vibrio cholerae, Yersinia pestis, Methicillin-resistant *Staphylococcus aureus*, Aspergillus species, Aspergillus, funigatus, Aspergillus flavus, Aspergillus calvatus, Candida species, Candida albicans, Candida tropicalis, Cryptococcus species, Cryptococcus neoformans, Entamoeba histolytica, Histoplasma capsulatum, Leishmania speceis, Nocardia asteroides, Plasmodium falciparum, *Stachybotrys chartarum,* Toxoplasma gondii, Trichomonas vaginalis, Toxoplasma species, Trypanosoma brucei, Schistosoma mansoni, Fusarium species, Trichophyton species, Plasmodium species, Toxoplasma species, Entamoeba species, Babesia species, Trypanosoma species, Leshmania species, Pneumocystis species, Pneumocystis jirovecii, Trichomonas species, Giardia species, Schisostoma species, Cryptosporidium species, Plasmodium species, Entamoeba species, Naegleria species, Acanthamoeba species, Balamuthia species, Toxoplasma species, Giardia species, Trichomonas species, Leishmania species, and Trypanosoma species.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified self antigens, wherein the plurality of self antigens attract and trap a plurality of immune cells specific to the plurality of self antigens in the device. According to this aspect, the self antigens may be derived from a lysate of a cell to which an autoimmune response is directed. Further according to this aspect, the antigen may be a pancreatic beta cell antigen, a neuronal cell antigen, a bone or joint associated autoimmune disease associated antigen, or a gastrointestinal-disease associated antigen. Still further

according to this aspect, the antigen may be an antigen associated with type I diabetes; an antigen associated with multiple sclerosis; an antigen associated with rheumatoid arthritis; an antigen associated with inflammatory bowel disease; or an antigen associated with Crohn's disease.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, a plurality of purified antigens and another agent which is an RGD peptide, a CpG oligonucleotide or a granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokine, or a fragment thereof or a combination thereof, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device. In an alternate aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold and a plurality of purified antigens, but the device does not contain an RGD peptide, a CpG oligonucleotide or a granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokine or a fragment thereof or a combination thereof.

In another aspect, the present invention provides an immune-cell trapping device which preferentially attracts T-cells compared to other immune cells. According to this aspect, the device comprises a physiologically-compatible porous polymer scaffold and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device further comprises a chemokine selected from the group consisting of CCL1, CCL2 (MCP-1), CCL-3, CCL-4, CCL-5 (RANTES), CCL-17, CCL-22, CXCL12 and XCL1, or a fragment thereof, or a combination thereof. Still further according to this aspect, the chemokine may be selected from the group consisting of:

(a) CCL1 (accession numbers for human homolog: NM_002981.2; GI:523498696 and accession numbers for mouse homolog: NM 011329; GI:257153404),

(b) CCL2 (MCP-1)(accession numbers for human homolog: NM_002982.3; GI:56119169 and accession numbers for mouse homolog: NM_011333.3; GI:141803162),

(c) CCL3 (accession numbers for human homolog: NM_002983.2; GI: 121582465 and accession numbers for mouse homolog: NM 011337.2; GI: 126432552),

(d) CCL4 (accession numbers for human homolog: NM 002984.3; GI:748585189 NM 013652.2 and accession numbers for mouse homolog: NM_013652.2; GI:126366031),

(e) CCL5 (RANTES)(accession numbers for human homolog: NM_002985.2; GI: 22538813 and accession numbers for mouse homolog: NM_013653.3; GI: 164698427),

(f) CCL17 (accession numbers for human homolog: NM_002987.2; GI:22538801 and NM_011332.3; accession numbers for mouse homolog: NM_011332.3; GI:225735578),

(g) CCL19 (accession numbers for human homolog: NM_006274.2; GI:22165424 and accession numbers for mouse homolog: NM 011888.2; GI: 10518345),

(h) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695),

(i) CXCL12 (accession numbers for human homolog: NM_199168.3; GI: 291045298 and accession numbers for mouse homolog: NM_001277990.1; GI: 489406389), and
(j) XCL1 (accession numbers for human homolog: NM_002995.2; GI:312434026 and accession numbers for mouse homolog: NM 008510.1; GI: 6678711).

In another aspect, the present invention provides an immune-cell trapping device which preferentially attracts dendritic cells (DC) compared to other immune cells. According to this aspect, the device comprises a physiologically-compatible porous polymer scaffold and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device further comprises a chemokine selected from the group consisting of CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 or a fragment thereof, or a combination thereof. Still further according to this aspect, the chemokine may be selected from the group consisting of:
(a) CCL2 (accession numbers for human homolog: NM 002982.3; GI:56119169 and accession numbers for mouse homolog: NM 011333.3; GI:141803162),
(b) CCL3 (accession numbers for human homolog: NM_002983.2; GI: 121582465 and accession numbers for mouse homolog: NM_011337.2; GI: 126432552),
(c) CCL5 (Variant 1) (accession numbers for human homolog: NM 002985.2; GI: 22538813 and accession numbers for mouse homolog: NM_013653.3; GI: 164698427),
(d) CCL7 (accession numbers for human homolog: NM_006273.3; GI:428673540 and accession numbers for mouse homolog: NM_013654.3; GI:226958664),
(e) CCL8 (accession numbers for human homolog: NM_005623.2; GI:22538815 and accession numbers for mouse homolog: NM 021443.3; GI:255708468),
(f) CCL13 (accession numbers for human homolog: NM_005408.2; GI:22538799 and accession numbers for mouse homolog: NM_011333.3; GI:141803162),
(g) CCL17 (accession numbers for human homolog: NM_002987.2 GI:22538801 and accession numbers for mouse homolog: NM 011332.3; GI:225735578), and
(h) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695).

In another aspect, the present invention provides an immune-cell trapping device which preferentially attracts both T-cells and dendritic cells (DC) compared to other immune cells. According to this aspect, the device comprises a physiologically-compatible porous polymer scaffold and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device further comprises a chemokine selected from the group consisting of CCL17 and CCL22, or a fragment thereof, or a combination thereof. Still further according to this aspect, the chemokine may be selected from the group consisting of:

(a) CCL17 (accession numbers for human homolog: NM_002987.2 GI:22538801 and accession numbers for mouse homolog: NM 011332.3; GI:225735578), and
(b) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695).

In yet another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, a plurality of purified antigens, and a plurality of adhesion receptors, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device. According to this aspect, the adhesion receptors may be adhesion receptors for T-cells selected from the group consisting of LFA-1, MAdCAM-1, VCAM-1, CD28 and CTLA-4 or a fragment thereof or a combination thereof. Still further according to this aspect, the adhesion receptor may be selected from the group consisting of:
(a) LFA (accession numbers for human homolog: NM 000211.4; GI:735367774 and accession numbers for mouse homolog: M60778.1; GI:198785),
(b) MAdCAM-1 (accession numbers for human homolog: NM 130760.2 GI:109633021 and accession numbers for mouse homolog: D50434.2; GI: 60391311),
(c) VCAM-1 (accession numbers for human homolog: NM 001078.3; GI:315434269 and accession numbers for mouse homolog: X67783.1; GI: 298116),
(d) CD28 (accession numbers for human homolog: NM 006139.3; GI: 340545506 and accession numbers for mouse homolog: BC064058.1; GI: 39850201), and
(e) CTLA-4 (accession numbers for human homolog: NM 005214.4; GI: 339276048 and accession numbers for mouse homolog: U90270.1; GI:4099836).

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device comprises about 10 μg to about 2.0 mg of the antigens.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device comprises about 0.1 μg to about 400 μg of the antigens per gram of dry weight of the scaffold.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device and the device has a porosity of between about 40% to about 90%.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold having pores having a diameter of about 10 μM

to about 500 μM, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device.

In another aspect, the present invention provides an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold having a compound selected from the group consisting of polylactic acid (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA), alginate or a derivative thereof, gelatin, collagen, fibrin, hyaluronic acid (HA), agarose, a polysaccharide, a polyamino acid, a polypeptide, a polyester, a polyanhydride, a polyphosphazine, a polyvinyl alcohol (PVA), a polyalkylene oxides (PAO), a polyallyl amines)(PAM), a polyacrylate, a modified styrene polymer, a pluronic polyol, a polyoxamer, a polyuronic acid, and a polyvinylpyrrolidone or a co-polymer thereof, or a graft polymer thereof, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device.

In yet another aspect, the present invention provides a pharmaceutical composition comprising an immune-cell trapping device and a pharmaceutically acceptable carrier, wherein the device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device. According this aspect, the pharmaceutical composition may be formulated for intravenous administration, subcutaneous administration, intraperitoneal administration, or intramuscular administration. Still further according to this aspect, the pharmaceutical composition may be formulated for subcutaneous administration as a microneedle patch.

In another aspect, the present invention provides a method of treating a disease in a subject in need thereof, comprising administering an immune-cell trapping device to the subject, wherein the device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens that are specific for the disease, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device; collecting a plurality of immune cells trapped in the device; and administering the plurality of immune cells into the subject, thereby treating the disease in the subject. According to this aspect, the immune cells may be collected by explanting the device from the subject. Still further according to this aspect, the therapeutic method may include expanding the plurality of immune cells, thereby generating an expanded population of immune cells that are specific for the disease; and administering the expanded population of immune cells into the subject. The subject to be treated according to this aspect is preferably a human subject. Still further, the method according to this aspect may involve administering the device subcutaneously or intravenously into the subject. Under one embodiment of the therapeutic aspect, the plurality of immune cells are collected about 1 day to about 60 days after the device is administered to the subject.

In a related aspect, the present invention provides a method of treating cancer in a subject in need thereof, comprising administering an immune-cell trapping device to the subject, wherein the

device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens that are specific for the cancer, wherein the plurality of cancer antigens attract and trap a plurality of immune cells specific to the cancer antigens; collecting a plurality of immune cells trapped in the device; and administering the plurality of immune cells into the subject, thereby treating the cancer in the subject. According to this aspect, the method may be used to treat a cancer selected from the group consisting of head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, esophageal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, glioblastoma, leukemia, lymphoma, mantle cell lymphoma, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. The subject to be treated according to this aspect is preferably a human subject.

In yet another related aspect, the present invention provides a method of treating an autoimmune disease in a subject in need thereof, comprising administering an immune-cell trapping device to the subject, wherein the device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens that are specific for the autoimmune disease, wherein the plurality of antigens specific for the autoimmune disease attract and trap a plurality of immune cells specific to the antigens; collecting a plurality of immune cells trapped in the device; and administering the plurality of immune cells into the subject, thereby treating the autoimmune disease in the subject. According to this aspect, the method may be used to treat an autoimmune disease selected from the group consisting of type I diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and Crohn's disease. Still according to this aspect, the immune cells that are trapped may include regulatory or suppressor T-cells. The subject to be treated according to this aspect is preferably a human subject.

In yet another related aspect, the present invention provides a method of treating a pathogenic disease in a subject in need thereof, comprising administering an immune-cell trapping device to the subject, wherein the device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens that are specific for the pathogenic disease, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the antigens specific for the pathogenic disease; collecting a plurality of immune cells trapped in the device; and administering the plurality of immune cells into the subject, thereby treating the pathogenic disease in the subject. According to this aspect, the pathogen is selected from the group consisting of a virus, a bacteria, a protozoan, a parasite, and a fungus. The subject to be treated according to this aspect is preferably a human subject.

In another aspect, the instant invention provides for a method for obtaining immune cells that are specific for an antigen. The method comprises administering an immune-cell trapping device, which comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device to a subject; and harvesting immune cells trapped in the device, thereby obtaining immune cells specific for the antigen. The device may be explanted from the

subject to collect the immune cells. The subject from which the immune cells are to be obtained according to this aspect is preferably a human subject. According to this aspect, the device may be subcutaneously or intravenously administered into the subject. Still according to this aspect, the plurality of immune cells may be collected about 1 day to about 60 days after the device is administered to the subject.

In another aspect, the instant invention provides method for determining whether a subject has an autoimmune disease. The method comprises administering an immune-cell trapping device into a subject, wherein the device comprises a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens that are specific for the autoimmune disease, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device; collecting immune cells trapped in the device, wherein the immune cells are specific to the autoimmune disease; and determining the number of immune cells specific to the autoimmune disease trapped in the device, thereby determining whether a subject has an autoimmune disease. The device may be explanted from the subject to collect the immune cells. The subject from which the immune cells are to be obtained according to this aspect is preferably a human subject. According to this aspect, the device may be subcutaneously or intravenously administered to the subject. Still according to this aspect, the plurality of immune cells may be collected about 1 day to about 60 days after the device is administered to the subject.

In yet another aspect, the instant invention provides for a method for making an immune-cell trapping device, comprising a physiologically-compatible porous polymer scaffold, and a plurality of purified antigens, wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device. The method comprises incubating plurality of purified antigens with a physiologically compatible polymer to generate a polymer-antigen mixture; freezing, lyophilizing and mixing the polymer-antigen mixture with a porogen; compression molding the mixture to produce a disc; subjecting the disk to a high-pressure CO_2 environment and rapidly reducing the pressure to expand and fuse the polymer into an interconnected scaffold structure; and leaching the porogen from the scaffold structure by immersing the structure in water to generate a porous article, thereby making the device. According to this aspect, the polymer may include a copolymer of lactide and glycolide (PLG) or alginate. Still further according to this aspect, the porogen is NaCl or sucrose.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. References cited are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Various features and advantages of the embodiments herein described can be fully appreciated as the same becomes better understood when considered in light of the accompanying drawings:

FIG. 1A is a photograph showing surface microcomputed tomograph and cross-sectional SEM images of PLG based T cell Trap.

FIG. 1B is a line graph showing cumulative release of ovalbumin (OVA) protein as a model antigen from OVA-T cell traps.

FIG. 1C is a bar graph showing the local cytokine concentration of MIP-la and RANTES at site of traps implanted subcutaneously in the backs of mice for 14 days. The values in B and C represent the average and standard deviation (n=5). Figs. lA-C show characterization of a model T cell Trap.

FIG. 2A is a series of fluorescence activated cell sorting (FACS) plots of cells isolated from control (Control) and ovalbumin (OVA; Antigen) loaded traps. Cells were stained with anti-CD8 and OVA class I tetramers. The gates represent the OVA-specific, CD8(+) T cells and numbers provide the percentage of positive cells. Traps were implanted into OT-1 mice for 14 days.

FIG. 2B is a line graph showing the kinetics of CD8(+)OVA tetramer(+) T cells accumulating in traps (OVA Trap) and controls (Control). Scaffolds were implanted in OT-1 mice for 7, 14 and 28 weeks.

FIG. 2C is a series of FACS plots. Mice vaccinated with CFA-OVA at day 0 (DO) were implanted with traps containing OVA (OVA trap) and melanoma cell lysate (melanoma trap) at day 7 (D7) and the traps were then assayed at day 21 (D21). CD3(+) T cells were isolated and sorted from OVA and melanoma traps and stained with anti-CD8, Trp2 tetramer and OVA tetramer. Gates in FACS plots represent the CD8(+) T cells positive for OVA or Trp2 tetramer staining and numbers represent the percentage of CD8 T cells as indicated.

FIG. 2D is a bar graph showing the number of OVA specific and Trp2 specific T cells isolated from OVA Traps and Melanoma Traps. The values in B and D represent the average and standard deviation (n=5). **P<0:01. Figs. 2A-D demonstrate that the traps are useful in discriminating T cells via antigen directed homing.

FIG. 3A is a line graph showing cumulative release of protein from Traps loaded with pancreatic beta cell lysate antigen.

FIG. 3B is a schematic indicating the degree of T cell Infiltration in islets of NOD mice during implantation of T cell Traps. Traps were implanted when NOD mice were 9, 12, and 15 weeks old and assayed 2 weeks later.

FIG. 3C is a series of FACS histograms displaying the levels of CD3(+) T cells harvested from controls (solid blue) and (3-cell traps (open red line) isolated from 11, 14 and 17 week old NOD mice.

FIG. 3D is a line graph showing the total number of CD3(+) cells isolated from controls (Controls) and -cell loaded traps (Trap) explanted from NOD mice at 11, 14 and 17 weeks of age.

FIG. 3E is a bar graph showing the percentage of total cells isolated from traps and controls that are CD3(+)CD4(+) and CD3(+)CD8(+) T cells. The values in A, D and E represent the average and standard deviation (n=5). **P<0:01. These figures demonstrate T cell trapping during diabetes progression in non-obese diabetic mice.

FIG. 4A is a photograph of an IL-2 ELISPOT assay results of beta cell targets cultured with T cells harvested from antigen loaded Traps and Controls. T cells were harvested 14 days after implantation in NOD mice at ages 11, 14 and 17 weeks. Each well represents reactions from T cells harvested from a single mouse.

FIG. 4B is a line graph showing a comparison of the number of IL-2 positive spots (normalized to 1600 T cells) generated by T cells extracted from traps versus spleens. Spots were developed and quantified in wells containing beta cells cultured with T cells from antigen loaded traps (Trap), control scaffolds (Control), spleens of mice implanted with Traps (Spleen-Trap), or spleens of mice implanted with control scaffolds (Spleen-Control). Beta cell targets were modified to overexpress either MHC class I H2(Kd) ((3 -cell I(D) or MHC class II I-A(g7) ((3 -cell G7). T cells (T cells only) and Beta cells ((3-cells) were assayed alone as controls. Values in B represent the mean and standard deviation. These figures demonstrate that the traps are effective for enrichment of Beta Cell specific T cells.

FIG. 5A is a histogram depicting CD3(+) T cell infiltration into blank (Control) and ovalbumin (Antigen) loaded traps. Dot plots depicting CD8(+) OVA tetramer(+) T cells infiltrating blank (Control) and ovalbumin (Antigen) loaded traps. Gate and number represents percent of OVA(+) cytotoxic T cells isolated from traps.

FIG. 5B is a bar graph showing the number of CD3(+) T cells trapped in blank (control) and ovalbumin-loaded (Trap) alginate cryogels.

FIG. 5C is a bar graph showing the number of CD8(+)OVA(+) T cells trapped in blank (control) and antigen-loaded (Trap) alginate cryogels. Values in B and C represent the mean and standard deviation (n=4). *P<0.05. The data shown in FIGS. 5A-C demonstrate successful T cell capture and retention into alginate cryogel traps that contain an antigen lure. Traps with antigen alone are sufficient, and the addition of GM-CSF and CpG as adjuvants to the antigen enhances the T cell infiltrate.

FIG. 6A-D show recruitment of cancer-specific T-cells *in vivo*.

FIG. 6A shows FACS plots of cells isolated from control control) and ovalbumin (OVA; antigen) loaded traps (which were implanted into OT-1 mice for 14 days). Cells were stained with anti-CD8 and OVA class I tetramers. The gates represent the OVA-specific, CD8(+) T cells and numbers provide the percentage of positive cells.

FIG. 6B shows the kinetics of CD8(+)OVA tetramer(+) T cells accumulating in traps (OVA trap) and controls (Control). Scaffolds were implanted in OT-1 mice for 7, 14 and 28 weeks.

FIG. 6C shows FACS analysis of CD3(+) T-cells. Mice were vaccinated with CFA-OVA at day 0 (D0) and implanted with traps containing OVA (OVA trap) and melanoma cell lysate (melanoma trap) at day 7 (D7). The traps were then assayed at day 21 (D21). CD3(+) T cells were isolated and sorted from OVA and melanoma traps and stained with anti-CD8, Trp2 tetramer and OVA tetramer. Gates in FACS plots represent the CD8(+) T cells positive for OVA or Trp2 tetramer staining and numbers represent the percentage of CD8 T cells as indicated.

FIG. 6D shows the number of OVA specific and Trp2 specific T cells isolated from OVA Traps and Melanoma Traps. The values in B and D represent the average and standard deviation (n=5). **P<0:01.

FIG. 7 shows the kinetics of diabetes incidence after transfer of cells from traps. Fifteen week old NOD mice were implanted with either blank PLG scaffolds (Control) or scaffolds containing beta-cell lysate (Traps). At 17 weeks 2 x 10^6 cells extracted from control and trap scaffolds along with splenocytes from untreated NOD mice (spleen) were intravenously injected into NOD-SCID mice. The plasma glucose levels in mice were monitored weekly and mice were considered diabetic after registering glucose levels higher than 250 mg/dL for two consecutive weeks (n=8 or 9).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a solution to the problem of identifying and collecting rare cells or cells with occur at low frequency in the body. Particular embodiments described herein relate to collection devices, for example cell traps, which are useful in the collection of such cells. The device includes a scaffold composition which incorporates or is coated with a plurality of antigens and, optionally, recruiting agents, allowing the device to attract, adhere to, and capture or sequester targeted cells. The device executes these functions by a variety of methods that include direct or indirect interaction with the antigens, recruiting agents, or other molecules present therein. Depending on the application for which the device is used, the device regulates capture and survival of the targeted cells through the physical or chemical characteristics of the scaffold itself. For example, the scaffold composition is differentially permeable, allowing cell passage only in certain physical areas of the scaffold. The permeability of the scaffold composition is regulated, for example, by selecting or engineering a material for greater or smaller pore size, density, polymer cross-linking, stiffness, toughness, ductility, or visco-elascticity. The scaffold composition may contain physical channels or paths through which targeted cells interact with the device and/or move into a specific compartment or region of the device. To facilitate the compartmentalization, the scaffold composition is optionally organized into compartments or layers, each with a different permeability, so that cells are sorted or

filtered to allow access to only a certain sub-population of cells. Sequestration of target cell populations in the device may also be regulated by the degradation, de- or re-hydration, oxygenation, chemical or pH alteration, or ongoing self-assembly of the scaffold composition. Following their capture, the targeted cells may be allowed to grow or expand within the device with the help of stimulatory molecules, cytokines, and other co-factors present in the device. In other instances, non-targeted cells which have otherwise infiltrated the device may be rejected or removed using negative selection agents.

The cells that are trapped within the devices of the invention are primarily immune cells. In certain embodiments, the invention relates to T-cell traps. In other embodiments, the invention relates to B-cell traps. Yet in other embodiments, the invention relates to a combination of traps, *e.g.*, a combination of T-cell traps and B-cell traps. The traps described herein may also be configured to trap antigen presenting cells (APCs), such as, for example, autoreactive APCs. Examples of auto-reactive APCs that may be trapped include, for example, dendritic cells (DCs), macrophages, or a combination of thereof. In other embodiments, a plurality of traps may be employed, *e.g.*, traps which are configured for trapping both lymphocytes and APCs. For example, antigen-specific cells or disease specific-cells such as T cells, dendritic cells (DCs), or macrophages may be trapped, either separately or together, for analyses, reprogramming or depletion. The trapped immune cells, *e.g.*, lymphocytes and APCs, are optionally harvested and analyzed to identify and characterize targets for disease diagnosis or immunotherapy. The trapped cells may also be reprogrammed or expanded for developing compositions or formulations that are to be used in therapy.

The invention is further described in more detail in the subsections below.

I. Devices for recruiting and trapping immune cells

In one aspect, the present invention provides cell-trapping devices. The devices contain a physiologically compatible porous polymer scaffold, a plurality of antigens and, optionally, one or more recruiting agents which attract an immune cell.

In one embodiment, the devices contain scaffolds which are made up of physiologically-compatible and optionally biodegradable polymers. Examples of polymers that are employable in the devices are known in the art. See, for example, US pub. No. 2011/0020216, the entire contents of which are incorporated herein by reference. Representative examples of such polymers include, but are not limited to, poly(lactide)s, poly(glycolide)s, poly(lactic acid)s, poly(glycolic acid)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactones, polyesteramides, polycarbonates, polycyanoacrylates, polyurethanes, polyacrylates, and blends or copolymers thereof. Biodegradable scaffolds may comprise biodegradable materials, *e.g.*, collagen, alginates, polysaccharides, polyethylene glycol (PEG), poly(glycolide) (PGA), poly(L-lactide) (PLA), or poly(lactide-co-glycolide) (PLGA) or silk. In one embodiment, the scaffold or scaffold device may comprises a biocompatible polymer matrix that is wholly or partly

biodegradable. A hydrogel is one example of a suitable polymer matrix material. Examples of materials which can form hydrogels include polylactic acid, polyglycolic acid, PLGA polymers, alginates and alginate derivatives, gelatin, collagen, agarose, natural and synthetic polysaccharides, polyamino acids such as polypeptides particularly poly(lysine), polyesters such as polyhydroxybutyrate and poly-ε-caprolactone, polyanhydrides; polyphosphazines, poly(vinyl alcohols), poly(alkylene oxides) particularly poly(ethylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers such as poly(4-aminomethylstyrene), pluronic polyols, polyoxamers, poly(uronic acids), poly(vinylpyrrolidone) and copolymers of the above, including graft copolymers. In another embodiment, the scaffolds may be fabricated from a variety of synthetic polymers and naturally-occurring polymers such as, but not limited to, collagen, fibrin, hyaluronic acid, agarose, and laminin-rich gels. In the case of biodegradable structures, the composition is degraded by physical or chemical action, *e.g.*, level of hydration, heat or ion exchange or by cellular action, *e.g.*, elaboration of enzyme, peptides, or other compounds by nearby or resident cells. The consistency varies from a soft/pliable (*e.g.*, a gel) to glassy, rubbery, brittle, tough, elastic, stiff. The structures contain pores, which are nanoporous, microporous, or macroporous, and the pattern of the pores is optionally homogeneous, heterogeneous, aligned, repeating, or random.

In one embodiment, the polymers are hydrogel-forming agents, *e.g.*, glycolides and/or alginates. Alginates are versatile polysaccharide based polymers that may be formulated for specific applications by controlling the molecular weight, rate of degradation and method of scaffold formation. Coupling reactions can be used to covalently attach bioactive epitopes, such as the cell adhesion sequence RGD to the polymer backbone. Glycolide/alginate polymers can be formed into a variety of scaffold types. Injectable hydrogels can be formed from low MW alginate solutions upon addition of a cross-linking agents, such as calcium ions, while macroporous scaffolds are formed by lyophilization of high MW alginate discs. Differences in scaffold formulation control the kinetics of scaffold degradation. Release rates of morphogens or other bioactive substances from alginate scaffolds is controlled by scaffold formulation to present morphogens in a spatially and temporally controlled manner. This controlled release not only eliminates systemic side effects and the need for multiple injections, but can be used to create a microenvironment that activates host cells at the implant site and transplanted cells seeded onto a scaffold.

Methods for fabricating the scaffold compositions are known in the art. See, for example, Martinsen et al. (Biotech. & Bioeng., 33 (1989) 79-89), (Matthew et al. (Biomaterials, 16 (1995) 265-274), Atala et al. (J Urology, 152 (1994) 641-643), and Smidsrod (TIBTECH 8 (1990) 71-78), the disclosures in which are incorporated by reference herein in their entirety.

Exemplary devices utilize glycolides or alginates of a relatively low molecular weight, preferably of size which, after dissolution, is at the renal threshold for clearance by humans, *e.g.*,

the alginate or polysaccharide is reduced to a molecular weight of 1000 to 80,000 daltons. Preferably, the molecular mass is 1000 to 60,000 daltons, particularly preferably 1000 to 50,000 daltons. It is also useful to use an alginate material of high guluronate content since the guluronate units, as opposed to the mannuronate units, provide sites for ionic cross-linking through divalent cations to gel the polymer. For example, U.S. Pat. No. 6,642,363, which incorporated herein by reference, discloses methods for making and using polymers containing polysaccharides such as alginates.

The scaffolds of the invention may be porous such that the scaffolds can sustain antigen presentation and attract and trap immune cells. In one embodiment, the devices contain porous scaffolds, wherein the pores have a diameter between 10 nm to 500 μm. In these embodiments, the invention utilizes devices comprising nanoporous scaffolds, wherein the pores have a diameter of less than about 10 nm (*e.g.*, about 0.5 nm, about 1 nm, about 3 nm, about 5 nm, about 7 nm, about 9 nm, and greater). In another embodiment, the scaffolds are microporous wherein the pores have a diameter in the range of about 100 nm-20 μm (*e.g.*, about 200 nm, about 500 nm, about 700 nm, about 1 μm, about 2 μm, about 5 μm, about 7 μm, about 10 μm, about 12 μm, about 15 μm, about 17 μm, about 20 μm or more). In another embodiment, the scaffolds are macroporous wherein the diameter of the pores about 20 μm-500 μm (*e.g.*, 30 μm, 50 μm, 70 μm, 100 μm, 200 μm, 300 μm, 400 μm, or more). Particularly, the diameter of the pores is greater than about 100 μm and preferably, greater than about 400 μm. In the exemplified embodiments, the scaffold is macroporous with aligned pores of about 200-500 μm in diameter.

Methods of making polymer matrices having the desired pore sizes and pore alignments are described in the art, *e.g.*, US pub. No. 2011/0020216 and US patent No. 6,511,650, the pertinent disclosures in which are incorporated herein by reference.

II. Antigens

The devices of the invention include one or more antigens, which may be naturally-occurring, synthetically produced, or recombinant compounds, *e.g.*, peptides, polypeptides, proteins, nucleic acids, small molecules, haptens, carbohydrates, or other agents, including fragments thereof or combinations thereof. More specifically, the antigens are peptides or proteins or immunologically active fragments thereof. In one embodiment, the antigens described herein are purified. Purified compounds contain at least 60% by weight (dry weight) of the compound of interest. Particularly, the antigens are at least 75% pure, preferably at least 90% pure, and more preferably at least 99% pure. Purity is measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The antigens may be self-antigens or non-self antigens.

Representative examples of non-self antigens include, for example, antigens derived from a pathogen selected from the group consisting of a virus, a bacteria, a protozoan, a parasite, and a fungus. In these embodiments, the non-self antigens may be derived from *Mycobacterium bovis*, Human Papillomavirus (HPV), Human immunodeficiency virus, a pox virus, smallpox virus, Ebola virus, Marburg virus, dengue fever virus, influenza virus, parainfluenza virus, respiratory syncytial virus, rubella virus, varicella-zoster virus, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, JC virus, rhabdovirus, rotavirus, rhinovirus, adenovirus, papillomavirus, parvovirus, picornavirus, poliovirus, virus that causes mumps, virus that causes rabies, reovirus, rubella virus, togavirus, orthomyxovirus, retrovirus, hepadnavirus, coxsackievirus, equine encephalitis virus, Japanese encephalitis virus, yellow fever virus, Rift Valley fever virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, SARS CoV, MERS CoV, Enterovirus, Borrelia species, Bacillus anthracis, Borrelia burgdorferi, Bordetella pertussis, Camphylobacter jejuni, Chlamydia species, Chlamydial psittaci, Chlamydial trachomatis, Clostridium species, Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Corynebacterium diphtheriae, Coxiella species, an Enterococcus species, Erlichia species, Escherichia coli, Francisella tularensis, Haemophilus species, Haemophilus influenzae, Haemophilus parainfluenzae, Lactobacillus species, a Legionella species, Legionella pneumophila, Leptospirosis interrogans, Listeria species, Listeria monocytogenes, Mycobacterium species, Mycobacterium tuberculosis, Mycobacterium leprae, Mycoplasma species, Mycoplasma pneumoniae, Neisseria species, Neisseria meningitidis, Neisseria gonorrhoeae, Pneumococcus species, Pseudomonas species, Pseudomonas aeruginosa, Salmonella species, Salmonella typhi, Salmonella enterica, Rickettsia species, Rickettsia ricketsii, Rickettsia typhi, Shigella species, Staphylococcus species, Staphylococcus aureus, Streptococcus species, Streptococccus pnuemoniae, Streptococcus pyrogenes, Streptococcus mutans, Treponema species, Treponema pallidum, a Vibrio species, Vibrio cholerae, Yersinia pestis, Methicillin-resistant *Staphylococcus aureus*, Aspergillus species, Aspergillus, funigatus, Aspergillus flavus, Aspergillus calvatus, Candida species, Candida albicans, Candida tropicalis, Cryptococcus species, Cryptococcus neoformans, Entamoeba histolytica, Histoplasma capsulatum, Leishmania speceis, Nocardia asteroides, Plasmodium falciparum, *Stachybotrys chartarum,* Toxoplasma gondii, Trichomonas vaginalis, Toxoplasma species, Trypanosoma brucei, Schistosoma mansoni, Fusarium species, Trichophyton species, Plasmodium species, Toxoplasma species, Entamoeba species, Babesia species, Trypanosoma species, Leshmania species, Pneumocystis species, Pneumocystis jirovecii, Trichomonas species, Giardia species, Schisostoma species, Cryptosporidium species, Plasmodium species, Entamoeba species, Naegleria species, Acanthamoeba species, Balamuthia species, Toxoplasma species, Giardia species, Trichomonas species, Leishmania species, and Trypanosoma species.

Alternately, the devices contain a plurality of self-antigens, which are optionally linked to or associated with a disease or disorder. Preferably, the self-antigens are specifically associated with a

human disease or a disorder. In one embodiment, the self-antigen is associated with an autoimmune disorder selected from the group consisting of rheumatoid arthritis , lupus, celiac disease, inflammatory bowel disease or Crohn's disease, sjögren's syndrome polymyalgia rheumatic, multiple sclerosis, ankylosing spondylitis, Type 1 diabetes, alopecia areata, vasculitis, temporal arteritis, etc. Specific types of antigens, including fragments thereof, which are associated with type 1 diabetes, multiple sclerosis, Crohn's disease, and rheumatoid arthritis and the like have been characterized in literature. For example, rheumatoid arthritis-related antigen is a 47kDa protein (RA-A47). See Hattori et al, J Bone Miner Metab., 18(6):328-34 (2000). In Crohn's disease, the antigen may be bacterial flagellin. See, Lodes et al., J Clin Invest. 113(9):1296-306 (2004). Likewise, major myelin proteins such as myelin basic protein (MBP) and proteolipid protein (PLP), are likely to be of importance in the course of multiple sclerosis (MS). See, deRosbo et al., J Clin Invest. 92(6): 2602–260 (1993). In the context of type 1 diabetes, a plurality of autoantigens may be involved, such as, preproinsulin (PPI), islet-specific glucose-6-phosphatase (IGRP), glutamate decarboxylase (GAD65), insulinoma antigen-2 (IA-2), chromogranin A and heat shock protein 60. See Roep et al., Cold Spring Harb Perspect Med.2(4), 2012 (PMID: 22474615).

In another embodiment, the self-antigens are associated with a cancer. Representative types of cancer antigens include, for example, MAGE-1, MAGE-2, MAGE-3, CEA, Tyrosinase, midkin, BAGE, CASP-8, β-catenin, β- catenin, γ-catenin, CA-125, CDK-1, CDK4, ESO-1, gp75, gp100, MART-1, MUC-1, MUM-1, p53, PAP, PSA, PSMA, ras, trp-1, HER-2, TRP-1, TRP-2, IL13Ralpha, IL13Ralpha2, AIM-2, AIM-3, NY-ESO-1, C9orf 112, SART1, SART2, SART3, BRAP, RTN4, GLEA2, TNKS2, KIAA0376, ING4, HSPH1, C13orf24, RBPSUH, C6orf153, NKTR, NSEP1, U2AF1L, CYNL2, TPR, SOX2, GOLGA, BMI1, COX-2, EGFRvIII, EZH2, LICAM, Livin, Livinβ, MRP-3, Nestin, OLIG2, ART1, ART4, B-cyclin, Gli1, Cav-1, cathepsin B, CD74, E-cadherin, EphA2/Eck, Fra-1/Fosl 1, GAGE-1, Ganglioside/GD2, GnT-V, β1,6-N, Ki67, Ku70/80, PROX1, PSCA, SOX10, SOX11, Survivin, UPAR, WT-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (AD Abp), cyclophilin b, Colorectal associated antigen (CRC)- C017-1A/GA733, T-cell receptor/CD3-zeta chain, GAGE-family of tumor antigens, RAGE, LAGE-I, NAG, GnT-V, , RCASl, α-fetoprotein, pl20ctn, Pmel117, PRAME, brain glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-I, SSX-4, SSX-5, SCP-I, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, PlA, Connexin 37, Ig-idiotype, pl5, GM2, GD2 gangliosides, Smad family of tumor antigens, lmp-1, EBV-encoded nuclear antigen (EBNA)-I, UL16-binding protein-like transcript 1 (Mult1), RAE-1 proteins, H60, MICA, MICB, and c-erbB-2, or an immunogenic peptide thereof, and combinations thereof.

III. Combination of scaffold compositions and antigens

The antigens may be combined with the scaffold compositions using any known methods, including covalent and non-covalent interactions. Types of non-covalent interactions include, for

example, electrostatic interactions, van der Waals' interactions, π-effects, hydrophobic interactions, etc. The antigens may also be attached or tethered to scaffold compositions via covalent interactions. Methods for attaching antigens to scaffolds/surfaces are known in the art, *e.g.*, surface absorption, physical immobilization, *e.g.*, using a phase change to entrap the substance in the scaffold material. In one specific embodiment, an antigenic composition containing a protein is mixed with the scaffold composition while it is in an aqueous or liquid phase, and after a change in environmental conditions (*e.g.*, pH, temperature, ion concentration), the liquid gels or solidifies thereby entrapping the antigen. Alternatively, covalent coupling via alkylating or acylating agents may be used to provide a stable, long-term presentation of an antigen on the scaffold in a defined conformation. Exemplary reagents and methods for covalently coupling peptides/proteins to polymers are known in the art. See, for example, US patent No. 6,001,395, the contents of which as they relate to the present invention are incorporated herein by reference. In other embodiments, the antigens are encapsulated into the scaffolds. Methods for encapsulating antigens into suitable scaffolds, *e.g.*, PLGA microspheres, are known in the art. See, for example, US patent No. 6,913,767 and WO/1995/011010, the contents of which as they relate to the present invention are incorporated herein by reference.

The antigens may be formulated to interact with the immune cell via direct binding or indirect binding. Types of direct binding include, for example, engagement or coupling of the antigen with the cognate receptor, *e.g.*, B-cell receptor or T-cell receptor. Indirect binding may occur through the intermediacy of one or more secondary agents or cell-types. For example, the antigen may first bind to a B-cell or an antigen-presenting cell (APC), get processed (*e.g.*, degraded) and presented on cell-surface major-histocompatibility complexes (MHC), to which the target cell population, *e.g.*, T-cell, binds. Alternately, the antigen may recruit other intermediary cells that secrete various cytokines, growth factors, chemokines, etc., which in turn attract the target immune cell population to the devices. Whatever the mechanism may be, the recited components act in concert to recruit and sequester immune cells to the devices of the instant invention.

The antigen may be derived from a cell lysate, a fractionated cell lysate, freshly harvested cells, biological fluids (including blood, serum, ascites), tissue extracts, etc. In one embodiment, the antigens are derived from lysates of target cells to which the desired immune cells, *e.g.*, B-cells or T cells bind. In these embodiments, the antigens are first fractionated in the cell lysate prior to loading the cell traps. The lysates may be derived from an autoimmune disease-specific cells, *e.g.*, pancreatic beta cells associated with type I diabetes, neuronal cells associated with multiple sclerosis, a bone/joint antigen associated with rheumatoid arthritis, an antigen associated with inflammatory bowel disease or Crohn's disease. Alternately, the lysates may be derived from cancer cells, *e.g.*, individual cells obtained from tumor samples or tissue cultures or bulk tumor cells obtained from biopsies or histological preparations.

IV. Recruitment agents

The devices of the invention may also contain one or more recruiting agents. The recruiting agent may be an agent selected from the group consisting of a T-cell recruiting agent, a B-cell recruiting agent, a dendritic cell recruiting agent, and a macrophage recruiting agent, or a combination thereof.

In one embodiment, the devices contain T-cell recruiting agents. Non-limiting examples of T-cell recruiting agents include, but are not limited to, CCL1, CCL2 (MCP-1), CCL-3, CCL-4, CCL-5 (RANTES), CCL-17, CCL-22, CXCL12 and XCL1, or a fragment thereof, a variant thereof, or a combination thereof. Various homologs of the aforementioned T-cell recruiting agents, including functional fragments thereof, or variants thereof, are known in the art. Representative examples of homologs include related proteins from fly, mouse, rat, pig, cow, monkey, humans or the like. The homologs preferably include human or mouse homologs of the aforementioned recruiting agents having the NCBI accession numbers shown in Table 1.

Table 1: Chemokines for trapping T-cells, including related NCBI accession numbers:

Chemokine	NCBI Accession No.
CCL1	NM_002981.2 (human) GI:523498696 (human) NM 011329.3 (mouse) GI:257153404 (mouse)
CCL2 **(MCP-1)**	NM_002982.3 (human) GI:56119169 (human) NM_011333.3 (mouse) GI:141803162 (mouse)
CCL3	NM_002983.2 (human) GI: 121582465 (human) NM 011337.2 (mouse) GI: 126432552 (mouse)
CCL4	NM 002984.3 (human) GI:748585189 (human) NM 013652.2 (mouse) GI:126366031 (mouse)

Chemokine	NCBI Accession No.
CCL5 (RANTES)	NM_002985.2 (human) GI: 22538813 (human) NM_013653.3 (mouse) GI: 164698427 (mouse)
CCL17	NM_002987.2 (human) GI:22538801 (human) NM_011332.3 (mouse) GI:225735578 (mouse)
CCL19	NM_006274.2 (human) GI:22165424 (human) NM 011888.2 (mouse) GI: 10518345 (mouse)
CCL22	NM_002990.4 (human) GI:300360575 (human) NM_009137.2 (mouse) GI: 154240695 (mouse)
CXCL12	NM_199168.3 (human) GI: 291045298 (human) NM_001277990.1 (mouse) GI: 489406389 (mouse)
XCL1	NM_002995.2 (human) GI:312434026 (human) NM 008510.1 (mouse) GI: 6678711 (mouse)

The devices may also contain any combination of the aforementioned recruiting agents, for example, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or greater number of the recruiting agents listed in Table 1. In related embodiments, the devices contain all the recruiting agents listed in Table 1.

In other embodiments, the invention provides devices for recruiting dendritic cells or macrophages or a combination thereof. Non-limiting examples of dendritic cell/macrophage recruiting agents include, but are not limited to, CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 or a functional fragment thereof, a variant thereof, or a combination thereof. Representative examples of homologs of the dendritic cell/macrophage recruiting agents include related proteins from fly, mouse, rat, pig, cow, monkey, humans or the like. The homologs preferably include human or mouse homologs of the aforementioned recruiting agents having the NCBI accession numbers shown in Table 2.

Table 2. Chemokines for trapping dendritic cells and/or macrophages, including related NCBI accession numbers:

Chemokine	NCBI Accession No.
CCL2	NM 002982.3 (human) GI:56119169 (human) NM 011333.3 (mouse) GI:141803162 (mouse)
CCL3	NM_002983.2 (human) GI: 121582465 (human) NM_011337.2 (mouse) GI: 126432552 (mouse)
CCL5	NM 002985.2 (human variant 1) GI: 22538813 (human) NM_013653.3 (mouse) GI: 164698427 (mouse)
CCL7	NM_006273.3 (human) GI:428673540 (human) NM_013654.3 (mouse) GI:226958664 (mouse)
CCL8	NM_005623.2 (human) GI:22538815 (human) NM 021443.3 (mouse)

Chemokine	NCBI Accession No.
	GI:255708468 (mouse)
CCL13	NM_005408.2 (human)
	GI:22538799 (human)
CCL17	NM_002987.2 (human)
	GI:22538801 (human)
	NM 011332.3 (mouse)
	GI:225735578 (mouse)
CCL22	NM_002990.4 (human)
	GI:300360575 (human)
	NM_009137.2 (mouse)
	GI: 154240695 (mouse)

The aforementioned devices for recruiting DC/macrophage-recruiting may contain any combination of the aforementioned recruiting agents, for example, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, or greater number of the recruiting agents listed in Table 2. In related embodiments, the devices contain all the recruiting agents listed in Table 2.

In related embodiments, the invention provides devices for recruiting B-cells. Non-limiting examples of B-cell recruiting agents include, but are not limited to, SDF-1, BLC, BCL-2 and BCA-1 or a functional fragment thereof, a variant thereof, or a combination thereof. Representative examples of homologs of the B-cell recruiting agents include homologs of the aforementioned proteins in fly, mouse, rat, pig, cow, monkey, humans, or the like. The homologs preferably include human or mouse homologs of the aforementioned recruiting agents having the NCBI accession numbers shown in Table 3.

Table 3. Chemokines for trapping B-cells, including related NCBI accession numbers:

Chemokine	NCBI Accession No.
SDF-1	NM_000609.6 (human)
	GI:489406302 (human)
	NM 001012477.2 (mouse)
	GI:270309154 (mouse)
BLC	NM_006419.2 (human)
	GI:194733765 (human)
	NM_018866.2 (mouse)

Chemokine	NCBI Accession No.
	GI:118130712 (mouse)
BCL-2	NM_000657.2 (human) GI:72198345 (human) NM 009741.4 (mouse) GI:545477919 (mouse)
BCA-1	NM_006419.2 (human) GI:194733765 (human) NM_018866.2 (mouse) GI:118130712 (mouse)

The aforementioned devices for recruiting B-cells may contain any combination of the aforementioned recruiting agents, for example, at least 2, at least 3, or all 4 of the recruiting agents listed in Table 3, or variants thereof or fragments thereof. Preferably, the devices contain all recruiting agents listed in Table 3 or fragments thereof.

Further embodiments relate to devices containing agents that are capable of recruiting a plurality of immune cells, *e.g.*, a combination of T-cells and DC/macrophages, a combination of T-cells and B-cells, a combination of B-cells and DC/macrophages, etc. Accordingly, in one embodiment, the invention provides for devices that recruit both T-cells and dendritic cells/macrophages, comprising, recruitment agents selected from at least one agent from Table 1 and at least one agent from Table 2. In a related embodiment, the invention provides for devices that recruit both B-cells and dendritic cells/macrophages, comprising, recruitment agents selected from at least one agent from Table 2 and at least one agent from Table 3. In yet another embodiment, the invention provides for devices that recruit both T-cells and B-cells, comprising, recruitment agents selected from at least one agent from Table 1 and at least one agent from Table 3.

In devices that are capable of recruiting a combination of immune cells, various combinations and sub-combinations of recruitment agents from the aforementioned tables may be employed. For example, devices designed for recruiting both T-cells and DC/macrophages may contain at one agent from Table 1 and two agents from Table 2, two agents from Table 1 and one agent from Table 2, two agents from Tables 1 and 2, etc. Likewise, devices designed for recruiting both B-cells and DC/macrophages may contain at one agent from Table 2 and two agents from Table 3, two agents from Table 2 and one agent from Table 3, two agents from Tables 2 and 3, etc.; devices designed for recruiting both T-cells and B-cells may contain at one agent from Table 1 and two agents from Table 3, two agents from Table 1 and one agent from Table 3, two agents from Tables 1 and 3, etc. Thus, in one representative embodiment, the device

is adapted for the recruitment of a combination of T-cells and dendritic cells/macrophages, comprising, CCL17, CCL22, a variant thereof or a fragment thereof, or a combination thereof. Representative examples of homologs of the recruiting agents, including fragments or variants thereof include related proteins from fly, mouse, rat, pig, cow, monkey, humans or the like. The homologs preferably include human or mouse homologs the aforementioned recruiting agents having the NCBI accession numbers shown in Table 2.

The devices/traps may also be adapted to preferentially recruit certain types of immune cell combinations, *e.g.*, a combination of lymphocytes and APCs, compared to other hematopoietic cells, *e.g.*, platelets, red-blood cells, etc. Such devices may contain any combination of recruitment agents listed in Tables 1, 2, and 3.

The devices of the instant invention are adapted for the preferential recruitment of a single type or single sub-type of cell, for example, preferential recruitment of T-cells and particularly a subset of Treg cells or NK cells. Preferential recruitment is characterized by an accumulation of at least 10%, at least 20%, at least 30%, at least 50%, at least 75%, at least 100%, at least 2-fold, at least 5-fold, at least 8-fold, at least 10-fold, or greater increase in one or more of a particular type of immune cells (*e.g.*, T cells, B-cells, DC/macrophages) in the device compared to other types of immune cells in the device (or in control traps that are devoid of recruitment agents). In devices that are adapted to recruit a combination of immune cells, *e.g.*, a combination of T-cells and DC/macrophages, preferential recruitment is characterized where the total percentage of recruited cells is at least 10%, at least 20%, at least 30%, at least 50%, at least 75%, at least 100%, at least 2-fold (i.e., 200%), at least 5-fold, at least 8-fold, at least 10-fold, or greater than other types of immune cells in the device (or in control traps). Particularly, preferential recruitment is characterized by 1-10 fold increase in the number of the cells of interest compared to other immune cells.

V. Additional agents

Embodiments of the invention further provide for devices for trapping immune cells which comprise a plurality of additional agents. In such embodiments, the additional agent may comprise a growth factor, a cytokine, a chemokine, an interleukin, an adhesion signaling molecule, an integrin signaling molecule or a fragment thereof or a combination thereof.

Representative examples of growth factors/cytokines include, but are not limited to, adrenomedullin (AM), angiopoietin (Ang), autocrine motility factor, bone morphogenetic proteins (BMPs), brain-derived neurotrophic factor (BDNF), epidermal growth factor (EGF), erythropoietin (EPO), fibroblast growth factor (FGF), foetal Bovine Somatotrophin (FBS) glial cell line-derived neurotrophic factor (GDNF), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like growth

factor (IGF), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), myostatin (GDF-8), nerve growth factor (NGF), neurotrophins, platelet-derived growth factor (PDGF), thrombopoietin (TPO), T-cell growth factor (TCGF), transforming growth factor (TGF-α or TGF-β), tumor necrosis factor-alpha(TNF-α), vascular endothelial growth factor (VEGF), Wnt, placental growth factor (PGF), or functional fragment thereof, or a combination thereof.

Representative types of interleukins include, but are not limited to, IL-1 (activates T cells, B-cells, NK cells, and macrophages), IL-2 (activates B-cells and NK cells), IL-3 (stimulates non-lymphoid cells), IL-4 (growth factor for activated B cells, resting T cells, and mast cells), IL-5 (for differentiation of activated B cells), IL-6 (growth factor for plasma cells and T-cells), IL-7 (growth factor for pre B-cells/pre T-cells and NK cells), IL-10 (activates macrophages, B-cells, mast cells, Th1/Th2 cells), IL-12 (activates T cells and NK cells), IL-17 (activates Th cells). Functional fragments of interleukins, which are characterized by their ability to modulate the activity of target cells, may also be employed. Representative types of interleukin molecules, including, NCBI accession numbers of human and/or mouse homologs thereof, are provided in Table 4.

Table 4. Types of interleukins that may be employed in the devices.

Interleukins	**NCBI Accession Nos.**
IL-1	BC008678.1; GI:14250476 (human) BC011437.1; GI:15030320 (mouse)
IL-2	NM_000586.3 GI:125661059 (human) K02292.1; GI:198330 (mouse)
IL-4	NM_172348.2; GI: 391224449 (human) NM 021283.2; GI:226874825 (mouse)
IL-5	NM 000879.2; GI:28559032 (human) NM 010558.1; GI: 6754335 (mouse)
IL 10	NM 000572.2; GI:24430216 (human) NM 010548.2; GI:291575143 (mouse)
IL-12 (Beta2)	U64198.1; GI:1685027 (human) U64199.1; GI:1685029 (mouse)
IL-17 (A)	NM 002190.2; GI:27477085 (human) NM 010552.3; GI:142367609 (mouse)

Optionally, the devices may contain adhesion molecules, which may also serve as signaling agents. Representative examples of adhesion signaling molecules include, but are not limited to, fibronectin, laminin, collagen, thrombospondin 1, vitronectin, elastin, tenascin, aggrecan, agrin, bone sialoprotein, cartilage matrix protein, fibronogen, fibrin, fibulin, mucins, entactin, osteopontin, plasminogen, restrictin, serglycin, SPARC/osteonectin, versican, von Willebrand Factor, polysaccharide heparin sulfate, connexins, collagen, RGD (Arg-Gly-Asp) and YIGSR (Tyr-Ile-Gly-Ser-Arg) peptides and cyclic peptides, glycosaminoglycans (GAGs), hyaluronic acid (HA), condroitin-6-sulfate, integrin ligands, selectins, cadherins and members of the immunoglobulin superfamily. Other examples include neural cell adhesion molecules (NCAMs), intercellular adhesion molecules (ICAMs), vascular cell adhesion molecule (VCAM-1), platelet-endothelial cell adhesion molecule (PECAM-1), L1, and CHL1. Functional fragments of the adhesion molecules, which are characterized by their ability to modulate the binding of target cells to the devices of the invention, may also be employed. Particularly, adhesion molecules comprise peptides or cyclic peptides containing the amino acid sequence arginine-glycine-aspartic acid (RGD), which is known as a cell attachment ligand and found in various natural extracellular matrix molecules. A polymer matrix with such a modification provides cell adhesion properties to the scaffold of the invention, and sustains long-term survival of mammalian cell systems, as well as supporting cell growth and differentiation. The adhesion molecules may be coupled to the polymer matrix is accomplished using synthetic methods which are in general known to one of ordinary skill in the art and are described in the examples. See, *e.g.*, Hirano et al., Advanced Materials, p. 17-25 (2004); Hermanson et al., Bioconjugate Techniques, p. 152-185 (1996); Massia and Hubbell, J. Cell Biol. 114:1089-1100 (1991; Mooney et al., J. Cell Phys. 151:497-505 (1992; and Hansen et al., Mol. Biol. Cell 5:967-975, (1994), the disclosures in which are incorporated by reference.

Depending on the target cell type, it may be preferable to employ adhesion signaling molecules that are specific for the target cells. Thus, in one embodiment, the devices contain adhesion receptors that are useful in the recruitment of T-cells. In other embodiments, the devices contain adhesion receptors that are useful in the recruitment of dendritic cells/macrophages. Yet in another embodiment, the devices contain adhesion receptors that are useful in the recruitment of B-cells.

In these embodiments, the devices may contain T-cell specific adhesion molecules, for example, a receptor selected from the group consisting of LFA-1, MAdCAM-1, VCAM-1, CD28 and CTLA-4 or a variant thereof, a fragment thereof or a combination thereof. Representative examples of human and mouse homologs of such T-cell specific adhesion molecules are provided in Table 5.

Table 5. Various types of T-cell adhesion receptors and the NCBI and GI accession numbers for known human and mouse homologs thereof:

Receptor	NCBI Accession No.	GI Accession No.
LFA-1	NM 000211.4 (human) M60778.1 (mouse)	GI:735367774 (human) GI:198785 (mouse)
MAdCAM-1	NM 130760.2 (human) D50434.2 (mouse)	GI:109633021 (human) GI: 60391311 (mouse)
VCAM-1	NM 001078.3 (human) X67783.1 (mouse)	GI:315434269 (human) GI: 298116 (mouse)
CD28	NM 006139.3 (human) BC064058.1 (mouse)	GI: 340545506 (human) GI: 39850201 (mouse)
CTLA-4	NM 005214.4 (human) U90270.1 (mouse)	GI: 339276048 (human) GI:4099836 (mouse)

Devices for recruiting DC/macrophages or B-cells may be similarly formulated to contain adhesion molecules specific for DC/macrophages or B-cells. Representative examples of B-cell-specific adhesion molecules include, for example, N-CAM (type 2), laminin or fibronectin, etc. Representative examples of adhesion molecules that are specific for macrophages or dendritic cells include, for example, ICAM-1 or VCAM-1. Depending on need, the devices may be specifically formulated to contain a subset of recruitment agents and adhesion molecules from the aforementioned Tables 1-5. For example, devices for recruiting both T-cells and dendritic cells may be formulated to contain a combination of CCL17 and CCL22 (from the list of chemokines) and VCAM-1 (from the list of adhesion molecules). Devices for recruiting both T and B lymphocytes may be similarly formulated by selective incorporation of various recruitment agents and adhesion molecules, which are optionally incorporated with one or more interleukins and/or cytokines.

VI. Devices for recruiting specific subtypes of immune cells

The devices of the invention may also be formulated for preferentially recruiting one or more sub-populations of cells by using agents that specifically bind to cell-surface markers that are expressed in the cells. For example, in the context of T-cells, the devices may be adapted for the preferential recruitment of helper T-cells (T_H cells; which differentially express CD4+),

cytotoxic T-cells (T_c cells; which differentially express CD8+), memory T-cells (T_m cells; which differentially express CD45RO), suppressor T-cells (T_s which cells), regulatory T-cells (Tregs; further characterized as FOXP3+ Treg cells and FOXP3– Treg), natural killer T-cells (NK cells; differentially express CD1d+), mucosal associated invariant (MAITs; differentially express MR1), gamma delta T cells, (γδ T cells; comprise TCRs containing one γ-chain and one δ-chain). Such agents which bind to cell-surface markers may include, for example, haptens, peptides, ligands, antibodies, or the like. Other routine techniques for enriching the isolates with one or more cell subtype may be optionally used *in situ* or *ex situ*.

Likewise, in the context of B-cells, the devices may be formulated for the preferential recruitment of specific B-cell subtype(s) using agents that selectively bind to the cell-surface markers expressed therein. Representative examples of B-cell subtypes that may be preferentially trapped include plasmablasts, plasma cells (differentially express CD27+/CD138+/CD319+), memory B cell (differentially express a combination of markers disclosed in Airoldi et al., Clin Cancer Research, 2004 10; 144), follicular (FO) B cell (express high levels of CD23 but are negative for CD1 or CD5), marginal zone (MZ) B cell (positive for CD21, CD1, CD9 but negative for CD23, CD5, and CD11b), B-1 cell (having a marker profile, *e.g.*, CD20+CD27+CD43+CD70-), B-2 cell (similar to FO B cells and MZ B cells), regulatory B (Breg) cell (*e.g.*, having a marker profile CD24+/CD38+), etc.

The devices may also be adapted to preferentially recruit various sub-types of antigen presenting cells. For example, in the context of dendritic cells, the devices may be adapted for the recruitment of myeloid dendritic cells (mDC, which includes the more common mDC-1 and the less common mDC-2) compared to plasmacytoid dendritic cells (pDC) or vice versa. In the context of macrophages, the devices may be adapted for the preferential recruitment of M1 (i.e., "killer" macrophages) compared to M2 (i.e., activated macrophages) or vice versa. The differences between the two is recognized in the art. For example, the M1 subtype secretes high levels of IL-12 and low levels of IL-10; the reverse is true for the M2 subtype. Tumor-associated macrophages are mainly of the M2 phenotype, and seem to actively promote tumor growth.

The device may also be adapted for recruiting immune cells that are specific for a disease. For example, a plurality of T cells that are specific for a particular type of autoimmune disease may be recruited. Thus in one embodiment, devices that are useful in the diagnosis of autoimmune disorders may be formulated to contain recruitment agents that are specific to the immune cells implicated in the disorder. Such recruitment agents may, for example, be specific to regulatory T cells (Tregs), suppressor T cells (Ts) or a combination thereof. In a related embodiment, devices that are useful in the diagnosis of cancers may be formulated to contain recruitment agents for preferentially recruiting cancer-specific T-cell types, *e.g.*, cytotoxic T cells (Tc), natural killer cells (NK) or a combination thereof.

In certain embodiments, the device is useful to pan for disease-specific cells. Such may include, for example, cells that directly promote disease progression. In the context of many autoimmune diseases, the disease may mediated and promoted via targeted killing of specific population of cells, *e.g.*, beta cells of pancreas in T1D and neuronal cells in multiple sclerosis. In other automimmune diseases, the disease may be precipitated by targeted attack of specific epitopes such as, for example, rheumatoid factors (RF) and citrullinated peptides (ACPA) in the context of rheumatoid arthritis and antigens present in the gut flora in the context of Crohn's disease. The targeted destruction of the cells generally involves specific type or subset of immune cells. Thus, based on the nature and properties of the cellular targets, immune cells that are specific thereto may be preferentially trapped using the devices of the instant invention.

In the aforementioned embodiments, the devices are provided with antigens to which disease-specific immune cells, *e.g.*, T cells, B cells, dendritic cells, macrophages, etc., bind. These autoimmune cells can be trapped and optionally re-programmed to a non-autoimmune phenotype. Methods of reprogramming T-cells to pluripotency are known in the art. See, Nishimura et al., Stem Cell 12, 114–126 (2013); Themeli et al., Nature Biotechnology 31, 928–933 (2013). In certain instances, particularly in the context of cancer-specific T-cells, the reprogrammed cells may be rejuvenated to target the cancer. Alternately, in the context of T-cells that are specific to autoimmune diseases, the trapped cells may be eliminated (removed from the body) or killed.

VII. Methods of making/using the devices

The devices of the invention are porous devices engineered to sustain antigen presentation. Representative examples of devices are shown in FIG. 1A and 1B. As shown in the Drawings, the pores allow space for T cell infiltration and expansion. Methods for fabricating porous scaffolds have been described in the art. See, for example, US pub. Nos. 2011/0020216, 2013/0202707, 2011/0020216 and US patent No. 8,067,237, the disclosures in which are incorporated by reference herein. For example, traps may be fabricated with copolymers of lactide and glycolide (PLG) to generate PLG cell traps using a gas forming process (as described in detail in the Examples). Antigens are physically adsorbed onto PLG polymer, by incubating the composition of polymers and the antigen. Alternately, PLG microspheres encapsulating the antigens may be made using a standard double emulsion. PLG-antigen spheres are then frozen and lyophilized and mixed with a porogen or a pore-forming agent, and compression molded. The resulting disc is allowed to equilibrate within a high-pressure CO_2 environment, and a rapid reduction in pressure causes the polymer particles to expand and fuse into an interconnected scaffold structure. The porogen is leached from the scaffolds by immersion in water yielding antigen-laden cell traps that are about 90% porous. The porosity may be altered by routine methods, *e.g.*, by varying the concentration of the porogen and/or using other biopolymers as

scaffolding agents. The resulting porosity of the device is between 40% and 99%, preferably between 50% and 90%, particularly preferably between 60% and 80% or more.

Device implantation/removal

The device can be implanted by any means known in the art, such as intravenous administration, implantation, *e.g.*, subcutaneous implantation or implantation as a microneedle patch, intraperitoneal administration or intramuscular injection. The period of implantation which is sufficient for the cells of interest to migrate into the porous matrix can be between two hours and a few months, *e.g.*, 0.5, 1, 2, 5, 7, 14, 25, 30 35, 38, 45, 50, 60 or more days. The period of implantation may be at least one day, during which time, 1 x 10^9 to 1 x 10^{10} cells accumulate and/or are extracted from the device after residence in a bodily tissue.

A plurality of devices may be implanted into a subject. In one embodiment, at least 1, at least 3, at least 10, at least 30, at least 50, at least 100, at least 300, at least 500, at least 1000, or more devices are implanted into a subject. Application of the devices may be uniform (*e.g.*, each device containing the same antigen) or varied (e.g., multiple devices containing different antigens).

The device need not be removed from the body for isolation, characterization, analysis and/or expansion/reprogramming of cells. In one embodiment, the cells are removed without physically removing the device from the site of the implant. In one embodiment, cells that are in the periphery of the device, *e.g.*, cells that are located within a radius of about 1 μm, about 2 μM, about 5 μM, about 10 μm, about 20 μm, about 60 μm, about 100 μm, about 300 μm, about 0.5 mm, about 1.0 mm, about 3.0 mm, 5.0 mm, about 10 mm, 50 mm, etc. of the device may be removed and optionally expanded or reprogramed. Cells may also be purified through cell sorting techniques (FACS or MACS) or resolution techniques, *e.g.*, using FICOLL gradients, to achieve a more homogenous population of cells. In another embodiment, the cells that are attached onto or located within the device may be removed and optionally expanded or reprogramed without removing the device. Methods for removing cells from implanted devices are known in the art. Such may include, for example, chemical or enzymatic treatments. For instance, combinations of different classes of detergents in low dosages, for example, a nonionic detergent, Triton X-100, and an anionic detergent, sodium dodecyl sulfate, may disrupt membranes and aid in the removal of cells from implants. Alternately, connective tissue enzymes such as collagenase, Trypsin, dispase may be used to dislodge cells. The Traps may also be subjected to sensitive mechanical disruptions (such as mortor and pistal, or tissue homogenization) to enhance removal of resident cells. Additional steps could be taken to eliminate any residual detergents or enzymes in the tissue matrix, so as to avoid interference with the later repopulating of the implant with viable cells. A similar approach is described in US pub. No. 2008/0131966, which is incorporated by reference.

In another embodiment, the device is removed from the site of the implant. The devices can be removed by any method known in the art. In order to aid in removal, the device may have a small length of wire or small lip that would allow the mechanical removal of the device without damaging the device. The device may be partially or completely removed for retrieving the cells.

After removal of the device, the trapped cells can be retrieved by any method known in the art, such as by digesting the cell attachments to the porous matrix. This can be achieved by, for example, flowing a trypsin containing buffer through the device. The trapped cells can then be expelled from the device by any method known in the art. In a preferred embodiment, the device contains an outlet covered by a membrane which can be burst, for example, by the application of positive pressure through the device, allowing the expulsion of the trapped cells from the device. Alternatively, the porous matrix with the trapped cells can be expelled from the device and the trapped cells can then be separated from the porous membrane by any method known in the art. In a preferred embodiment, the device contains an outlet covered by a membrane which can be burst, for example, by the application of positive pressure through the device, allowing the expulsion of the porous matrix with the trapped cells from the device.

Once the trapped cells have been retrieved from the device, trapped cells of interest can be separated from the other trapped cells. This can be done by any means known in the art such as, for example, combining the trapped cells with microbeads with a binding partner to a surface marker present on the other trapped cells but not the trapped cells of interest. Removing the microbeads with the bound other trapped cells leaves only the trapped cells of interest. In another embodiment, the trapped cells of interest can be separated from the other trapped cells by combining the trapped cells with microbeads with a binding partner to a surface marker present on the trapped cells of interest but not the other trapped cells. The trapped cells of interest bind to the microbeads and can be removed. For example, T-cells may be removed by using binding partners that specifically bind to T-cell surface receptors or markers but not to B-cell, dendritic cells, or macrophages.

Alternately, a binding partner capable of binding to the other infiltrating cells but not the cells of interest, which binding partners are capable of being bound (covalently or noncovalently) to a microbead may be used. For example, such binding partners can include aptamers, or preferably antibodies or antibody fragments, where the binding site is preferably specific for a cell surface marker present on the surface of such infiltrating cells but not the cells of interest. Alternately, a reverse approach of using binding partners that are specific for cells of interest may be used. For example, when the trapped cells of interest are macrophages, a preferred microbead has antibodies specific for macrophage-specific cell surface receptors. The skilled artisan could formulate a binding partner for any particular cell of interest or a combination thereof.

Aptamers are single stranded oligonucleotides or oligonucleotide analogs that bind to a particular target molecule, such as a protein or small molecule. Thus, aptamers are the

oligonucleotide analogs of antibodies. Both RNA and single-stranded DNA, or DNA analog, aptamers are known. Aptamers that bind to virtually any particular target can be selected by using an iterative process called SELEX (Systematic Evolution of Ligands by EXponential enrichment).

An antibody or antibody fragments can be polyclonal, monoclonal, or recombinant and can be of any animal, such as a rodent or a human, or a mixture of animals, such as humanized mouse.

Any type of microbead can be used to bind the other trapped cells. For example, the microbeads can be heavy particles that are pelleted under centrifugal conditions, but which do not pellet the trapped cells of interest. Alternatively, the microbeads can be buoyant particles that are not pelleted under centrifugal conditions that pellet the trapped cells of interest. In a preferred embodiment, the microbeads are magnetic beads.

The isolated cells may be subjected to further analysis. Such further analysis may include quantification of the cells, or analysis of mRNA or protein expression. For example, the trapped cells of interest can be quantified, in order to approximate the number of trapped cells of interest in a given amount of tissue, in order to compare the number of trapped cells of interest to the amount of the other trapped cells. The trapped cells of interest can be quantified by any method known in the art, such as by microscopic observation.

Preferably, the trapped cells are separated and/or sorted for analysis using cytometric techniques, *e.g.*, fluorescence-activated cell sorting (FACS). This may be accomplished using a plurality of antibodies or other binding molecules described above and other secondary agents. The trapped cells may be phenotyped using art known methods. See, Autissier et al., J Immunol Methods. 360(1-2): 119–128 (2010). For example, T cells are characterized by or identified by cell surface markers such as CD4 or CD8. Dendritic cells are characterized by or identified by expression of CD11c. Macrophages are characterized by or identified by expression of CD11c and/or CD68. Exemplary markers (both to positively identify and also to exclude other cells) include CD11c (to select for DCs), Gr-1 (to select for macrophages), CD204 (to select for macrophages), CD16 (to select for monocytes and to negatively select for (*e.g.*, exclude) non-monocyte cells, i.e., cells that do not have a monocyte phenotype or cell surface marker phenotype), CD45 (to select for hematopoetic cells, *e.g.*, immune cells), CD49b [to select for natural killer (NK cells) as well as a subset of T cells that express this marker], CD3 (to select for T cells), CD4 (T-cells), CD8 (T-cells), CD19 and B220.

In some preferred embodiments, mRNA or protein expression is determined in the trapped cells of interest. For example, T cell and B cell receptors of trapped cells may be sequenced to identify the antigen-specificity of disease and drug targets for autoimmunity. In related embodiments, the mRNA or protein expression of the trapped cells of interest may then be compared to the expression of the same gene or genes in other cell types or identical cells from other subjects. When analysis of mRNA or protein expression of more than one gene is desired,

microarray technology can be employed. This well-established technology can analyze mRNA or protein expression patterns of many genes at once, allowing comparison between, for example, an entire genome of trapped cells of interest and other cells. In the event that the amount of biological material recovered from the devices is insufficient for analysis, mRNA from the trapped cells of interest can be amplified prior to determination of gene expression. Such amplification can be done by any method known in the art, such as by reverse transcription and cDNA amplification.

Antigen-specific cells

In some embodiments, the antigen-specific trapped cells or a sub-population thereof (*e.g.*, antigen-specific T cells or B-cells) are manipulated for formulating compositions that are suitable for diagnostic or therapeutic use. Examples of manipulation include, for example, activation, division, differentiation, growth, expansion, reprogramming, anergy, quiescence, senescence, apoptosis or death. The cells need not be physically removed from the device to be manipulated. Thus, in one embodiment, the cells are manipulated *in situ* (*e.g.*, at or near the implant site). In other embodiments, the cells are manipulated *ex situ* (*e.g.*, at a site that is different from the implant site).

In some embodiments, the antigen-specific cells, *e.g.*, T-cells, are manipulated (*e.g.*, activated and/or expanded) *in situ* by providing devices containing a plurality of antigens optionally together with antigen-presenting cells (or antigens which attract such APCs). In other embodiments, the antigen-specific cells are manipulated in cell culture by incubation with the antigen (*e.g.*, autoimmune antigen) optionally together with an antigen-presenting cell. Once activated, cells undergo a complex cascade of cell signaling which leads to the transcription and expression of many gene products. The gene products specific for activated cells may be analyzed further for identifying and isolating cells with desired antigen specificity.

(a) T-cell

Embodiments described herein are directed to a method for manipulating antigen-specific T cells *ex situ*. It is noted that the technique described herein can be varied for carrying out the process *in situ*. A sample comprising T cells is incubated with a particular antigen, which causes the activation of a T cell specific for the antigen of interest. The T-cell containing sample may be incubated with the antigen for about 1 day to 7 days. Antigen-specific T-cells are then be isolated by selecting for T cells that express gene products of T cells activated as described above. The antigen of interest could be a self-antigen or a non-self antigen or a fragment thereof. The antigen of interest may also be a combination of two or more individual antigens of interest.

The gene product for identifying or negatively selecting for activated T cells may be a cell surface marker or cytokine, or a combination thereof. Cell surface markers for identifying activated T cells include, but are not limited to, CD69, CD4, CD8, CD25, HLA-DR, CD28, and CD134. CD69 is an early activation marker found on B and T lymphocytes, NK cells and granulocytes. CD25 is an IL-2 receptor and is a marker for activated T cells and B cells. CD4 is a

TCR coreceptor and is marker for thymoctes, TH1- and TH2-type T cells, monocytes, and macrophages. CD8 is also a TCR coreceptor and is marker for cytotoxic T cells. CD134 is expressed only in activated CD4+ T cells.

Cell surface markers for negatively selecting for activated T cells include, but are not limited to, CD36, CD40, and CD44. CD28 acts as a stimulatory T-cell activation pathway independent of the T-cell receptor pathway and is expressed on CD4+ and CD8+ cells. CD36 is a membrane glycoprotein and is a marker for platelets, monocytes and endothelial cells. CD40 is a marker for B cells, macrophages and dendritic cells. CD44 is a marker for macrophages and other phagocytic cells. Subsets of T cells may be isolated by using positive selection, negative selection, or a combination thereof for expression of cell surface gene products of helper T cells or cytotoxic T cells (*e.g.*, CD4 vs. CD8). Cytokines for identifying activated T cells of the present invention include, but are not limited to cytokines produced by TH1-type T cells (cell-mediated response) and TH2-type T cells (antibody response). Cytokines for identifying activated TH1-type T cells include, but are not limited to, IL-2, gamma interferon (γIFN) and tissue necrosis factor alpha (TNFα). Cytokines for identifying activated TH2-type T cells include, but not limited to, IL-4, IL-5, IL-10 and IL-13. Subsets of T cells may also be isolated by using positive selection, negative selection, or a combination thereof for expression of cytokine gene products of helper T cells or cytotoxic T cells (*e.g.*, γIFN vs. IL4).

An activated TH1-type T cell specific for an antigen of interest may be isolated by identifying cells that express CD69, CD4, CD25, IL-2, IFNγ, TNFα, or a combination thereof. An activated TH1-type T cell specific for an antigen of interest may also be isolated by identifying cells that express CD69 and CD4 together with IFNγ or TNFα. An activated TH2-type T cell specific for an antigen of interest may be isolated by identifying cells that express CD69, CD4, IL-4, IL-5, IL-10, IL-13, or a combination thereof. A combination of an activated TH1-type T cell and a TH2-type T cell specific for an antigen of interest may be isolated by identifying cells that express CD69, CD4, CD25, IL-2, IFNγ, TNFα, or a combination thereof and cells that express CD69, CD4, IL-4, IL-5, IL-10, IL-13, or a combination thereof.

The gene products used for positive or negative selection of the activated T cells of the present invention may be identified by immunoselection techniques known to those in the art which utilize antibodies including, but not limited to, fluorescence activated cell sorting (FACS), magnetic cell sorting, panning, and chromatography. Immunoselection of two or more markers on activated T cells may be performed in one or more steps, wherein each step positively or negatively selects for one or more markers. When immunoselection of two or more markers is performed in one step using FACS, the two or more different antibodies may be labeled with different fluorophores. Alternately, as described above, cells may be sorted using microbeads.

For cell-surface expressed gene products, the antibody may directly bind to the gene product and may be used for cell selection. For cell-surface gene products expressed at low

concentrations, magnetofluorescent liposomes may be used for cell selection. At low levels of expression, conventional fluorescently labeled antibodies may not be sensitive enough to detect the presence of the cell surface expressed gene product. Fluorophore-containing liposomes may be conjugated to antibodies with the specificity of interest, thereby allowing detection of the cell surface markers.

For intracellular gene products, such as cytokines, the antibody may be used after permeabilizing the cells. Alternatively, to avoid killing the cells by permeabilization, the intracellular gene product if it is ultimately secreted from the cell may be detected as it is secreted through the cell membrane using a "catch" antibody on the cell surface. The catch antibody may be a double antibody that is specific for two different antigens: (i) the secreted gene product of interest and (ii) a cell surface protein. The cell surface protein may be any surface marker present on T cells, in particular, or lymphocytes, in general, (*e.g.*, CD45). The catch antibody may first bind to the cell surface protein and then bind to the intracellular gene product of interest as it is secreted through the membrane, thereby retaining the gene product on the cell surface. A labeled antibody specific for the captured gene product may then be used to bind to the captured gene product, which allows the selection of the activated T cell. Certain forms of cytokines are also found expressed at low concentration on the cell surface. For example, γIFN is displayed at a low concentration on the cell surface with kinetics similar to those of intracellular γIFN expression (Assenmacher, et al. Eur J. Immunol, 1996, 26:263-267). For forms of cytokines expressed on the cell surface, conventional fluorescently labeled antibodies or fluorophore containing liposomes may be used for detecting the cytokine of interest. One of ordinary skill in the art will recognize other techniques for detecting and selecting extracellular and intracellular gene products specific for activated T cells.

The T cells isolated by the present invention may be enriched by at least 40%-90% from whole blood. The T cells may also be enriched by at least 95% from whole blood. The T cells may also be enriched by at least 98% from whole blood. The T cells may also be enriched at least 99.5% from whole blood. Similar methods may be used in the *in situ* or *ex situ* manipulation of B-cells.

(b) B-cells

Antigen specific B-cells may be obtained using slight variations to the aforementioned methods. For example, DYNABEAD-M-450 can be used to couple disease specific antigens, which are then incubated with a composition containing the cells trapped with the devices of the invention. Optionally, the trapped cells may be subjected to a screening process to enrich the sample, *e.g.*, using agents that bind to cell surface markers that are specific for B-cells. The B-cells are then incubated with the antigen-coupled DYNABEADS and cultured for a period of about 7-10 days to generate rosette cells. The rosette cells are optionally typed by analyzing the

secreted antibodies, *e.g.*, based on their ability to bind to the disease specific antigens. Clones that are identified as positives may be transformed to produce hybridomas using routine techniques.

Embodiments described herein further relate to isolated cells that are specific for an antigen of interest, which cells are isolated by the method describeds above. The isolated cells may be monoclonal cells or polyclonal antigen-specific immune cells, *e.g.* antigen-specific T-cells.

Embodiments of the invention further relate to methods of determining the relative frequency of antigen-specific immune cells by determining the number of immune cells trapped in the devices of the present invention. Preferably, the antigen-specific immune cells are T-cells or B-cells. Particularly preferably, the antigen-specific immune cells are T-cells.

Diagnosis of disease/disorders

Embodiments described herein further relate to methods for detecting or diagnosing a disease or a disorder in a subject. In one embodiment, the disease is cancer. In another embodiment, the disease is an autoimmune disorder. In a third embodiment, the disease is a pathogenic disease.

In these embodiments, a subject with a disease may be diagnosed by first administering a device of the invention to the subject, wherein the antigens in the device are specific to the disease. The devices are then allowed to remain in the subject for a period, *e.g.*, 0.5 day, 1 day, 2 days, 5 days, 7 days, 14 days, 25 days, 30 days, 35 days, 38 days, 45 days, 50 days, 60 days or more and the cells contained therein are analyzed using one or more of the aforementioned techniques. For example, in the context of autoimmune diseases, the cells that are analyzed may include T-cells, B-cells, antigen presenting cells, or a combination thereof. In the context of cancer diagnosis, the cells that are analyzed my include T-cells or B-cells. In the context of pathogenic diseases, the cells that are analyzed may include antigen presenting cells.

The analytical step may be carried out using any routine methods. Accordingly, in one embodiment, the analytical step may involve determining the number of immune cells that are specific to the autoimmune disease. Any routine technique may be used to determine antigen-binding specificity of immune cells, *e.g.*, loading cell samples onto antigen-coated surfaces, washing away non-specifically bound cells, and quantitating the number of antigen-specific cells (either in free form by releasing the bound cells or in bound form) using a detection agent (*e.g.*, an antibody that binds to a cell-surface epitope located on the antigen-specific cells). In another embodiment, the analytical step may involve determining the physical or biological characteristics of the antigen-specific immune cells. Examples of physical characteristics include, for example, size, shape, reflectivity, morphology, density. Examples of biological characteristics include, for example, expression of particular cell surface markers, secretion of cytokines, reactivity to particular antigens or agents, patterns of gene expression.

The analytical step may be tied to a correlation step, wherein, the results of the analytical step are correlated to the parameter of interest. Representative types of parameters include, presence (or absence of disease), type of disease (*e.g.*, aggressive vs. non-aggressive autoimmune disorder; druggable vs. non-druggable disease, *e.g.*, antibiotic susceptible vs. antibiotic resistant bacterial infection, immunotherapy-resistant vs. immunotherapy-sensitive cancer), stage of disease, progression/regression of disease (over time), etc. In one embodiment, the parameter relates to presence or absence of disease (which can be expressed in binary terms). In another embodiment, the parameter relates to staging of disease (which can be expressed in a nominal scale, *e.g.*, stage I-IV, with stage IV being the highest). Yet in another embodiment, the parameter relates to odds or likelihood of occurrence of the disease, *e.g.*, least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 2-fold, 3-fold, 5-fold, 10-fold, 20-fold or more.

In the aforementioned diagnostic methods the parameters may be compared to a baseline value. The baseline value may be a value that is pre-determined, *e.g.*, in a population of healthy subjects. For example, where the antigen of interest is rheumatoid arthritis (RA) antigen, a baseline level of RA-specific antibodies (or T-cells) in healthy subjects may be used in the correlation step. Alternately, the baseline value may be experimentally identified using suitable controls, *e.g.*, devices that only contain scaffolds (i.e., negative for antigen, recruitment agent, or both) or devices that contain random or non-disease specific antigens. The skilled worker can use routine techniques to correlate and/or draw inferences between various subject groups.

For example, in one embodiment, devices containing disease-specific antigens (*e.g.*, MBP70 derived from *Mycobacterium bovis*) are implanted into a group of subjects that are suspected to have been exposed to the pathogen and a group of healthy (or random) subjects for a given time period (*e.g.*, two weeks). Separately, a small number of subjects in each group are provided with control devices (containing random or non-MBP antigens). The devices are explanted and the number of antigen-specific T-cells in the experimental group (*e.g.*, suspect group implanted with the MBP70+ device) and the control groups are measured and compared. Positive diagnosis is made if more (*e.g.*, at statistical significance) antigen-specific T-cells are detected in devices recovered from the suspect group than those recovered from the control group(s). Additionally, depending on the diagnosis, the suspect group may be treated with a therapeutic agent. The number of antigen-specific T-cells in treated subjects may be compared over time and also compared to those present in healthy subjects. Based on the results, the researcher could make inferences on the effectiveness of the therapy and also monitor therapeutic progress/regression of the disease over time. A similar protocol may be used in the diagnosis, monitoring and management of cancer patients and subjects suffering from autoimmune diseases.

The subject is an animal, preferably a mammal or a bird. Particularly preferably, the subject is selected from the group consisting of humans, dogs, cats, pigs, cows, buffalo and horses. Most preferably, the subject is a human.

Any immune cell may be used in the diagnosis of the disease or disorder. Preferably, diagnosis is performed with a lymphocyte, *e.g.*, T-cells, B-cells, dendritic cells or APCs, *e.g.*, macrophages, or a combination thereof. It is especially preferable to use T-cells in the diagnosis.

Any disease or disorder may be detected or diagnosed using the aforementioned methods. Particularly preferably, the disease is an autoimmune disease selected from the group consisting of rheumatoid arthritis , lupus, celiac disease, inflammatory bowel disease or Crohn's disease, sjögren's syndrome polymyalgia rheumatic, multiple sclerosis, ankylosing spondylitis, Type 1 diabetes, alopecia areata, vasculitis, temporal arteritis, etc. In other embodiments, the disease is a cancer which is selected from the group consisting of head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, esophageal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, glioblastoma, leukemia, lymphoma, mantle cell lymphoma, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer.

In yet another embodiment, the disease is a pathogenic disease selected from the group consisting of a bacterial disease, a viral disease and a fungal disease or a combination thereof.

Accordingly, embodiments of the invention relate to detecting or diagnosing autoimmune disease, cancer, or a pathogenic disease in a subject by administering the device of the invention containing antigens which are specific to the autoimmune disease, cancer disease, or pathogenic disease, and analyzing the immune cells contained therein. The devices may be optionally explanted to recover the immune cells. It may be possible to carry out the detection/analytical step *in situ*.

Related embodiments are directed to methods of monitoring the progression of a disease in a subject. The method comprises administering, to a subject, the devices of the invention containing antigens that are specific to the disease and analyzing the immune cells contained therein. The number/types of immune cells trapped in the device may offer valuable cues as to the progression of the disease. Alternately, wherein the subject has undergone therapeutic intervention, analogous methods may be used to monitor the therapy of disease and/or disease management.

The aforementioned methods may be used to monitor the progression/therapy of autoimmune disorders, cancers, pathogenic diseases, and the like. Preferably, the immune cells that are used in the diagnostic methods are T-cells or B-cells.

In the context of autoimmune disorders, the progression of the disease may be monitored by analyzing the number and/or type of autoreactive T cells. Depending on the result of the analysis, methods of intervention and/therapy may be designed to minimize the severity of the symptoms. In other instances, preventive methods may be undertaken, including providing recommendations to subjects on dietary, nutritional and/or other lifestyle changes.

Production of novel compositions and vaccines for the therapy of diseases

Embodiments described herein further relate to methods for devising and producing novel compositions for treating a disease. The method comprises administering the devices of the invention containing disease specific antigens to a subject, which then trap immune cells that are specific to the disease, optionally isolating, enriching, and expanding the immune cells trapped in the device, and then administering the immune cells back to the subject. Alternately, products derived from such immune cells may be administered to the subjects. Examples of products derived from the immune cells include, nucleic acids (including vectors and cells containing such nucleic acids), peptides, proteins, antibodies, cytokines, etc.

Preferably, the disease is an autoimmune disease. Under this embodiment, autoreactive T cells which have been isolated (and optionally expanded in culture as described herein) by the aforementioned methods may be inactivated *in situ* or *ex situ*. Methods of inactivating T cells are known in the art. Examples include, but not limited to, chemical inactivation or irradiation. The autoreactive T cells may be preserved either before or after inactivation using a number of techniques known to those skilled in the art including, but not limited to, cryopreservation. As described below, the composition may be used as a vaccine to deplete autoreactive T cells in autoimmune patients.

In one embodiment, the vaccine may comprise autoreactive T cells comprising homogeneous ("monoclonal") or heterogeneous ("polyclonal") patterns of Vβ-Dβ-Jβ gene usage. Clinical studies indicate that autoimmune patients receiving autologous monoclonal T cell vaccination may show a gradual decline in the immunity against autoreactive T cells. In some cases, the reappearing autoreactive T cells may originate from different clonal populations, suggesting that the T cells may undergo clonal shift or epitope spreading potentially associated with the ongoing disease process. Clonal shift or epitope spreading may be a problem in autoimmune diseases mediated by autoreactive T cells. A vaccine comprising polyclonal autoreactive T cells capable of depleting multiple populations of autoreactive T cells may avoid problems with clonal shift or epitope spreading.

Embodiments described herein further relate to compositions and vaccines produced by the aforementioned methods. The composition may be a pharmaceutical composition, which may be produced using methods well known in the art. Pharmaceutical compositions used as preclinical and clinical therapeutics in the treatment of disease or disorders may be produced by those of skill, employing accepted principles of diagnosis and treatment.

Therapy of disease

Embodiments described herein further relate to methods for treating a disease or a disorder in a subject. In one embodiment, the disease is cancer. In another embodiment, the disease is an autoimmune disorder. In a third embodiment, the disease is a pathogenic disease.

In these embodiments, a subject with a disease may be treated by first administering a device of the invention to the subject, wherein the antigens in the device are specific to the disease. The devices are then allowed to remain in the subject for a period, *e.g.*, 0.5 day, 1 day, 2 days, 5 days, 7 days, 14 days, 25 days, 30 days, 35 days, 38 days, 45 days, 50 days, 60 days or more, and the cells contained therein are manipulated using one or more of the aforementioned techniques. For example, in the context of autoimmune diseases, the cells that are manipulated may include T-cells, B-cells, antigen presenting cells, or a combination thereof. In the context of cancer treatment, the cells that are manipulated may include T-cells or B-cells. In the context of pathogenic diseases, the cells that are manipulated may include antigen presenting cells. Examples of manipulation include, for example, activation, division, differentiation, growth, expansion, reprogramming, anergy, quiescence, senescence, apoptosis, death, etc. The cells need not be physically removed from the device to be manipulated. Thus in one embodiment, the cells are manipulated *in situ* (*e.g.*, at or near the implant site). In other embodiments, the cells are manipulated *ex situ* (*e.g.*, at a site that is different from the implant site).

Accordingly, embodiments of the instant invention provide for methods for treating cancer in a subject. The method comprises administering a device of the instant invention to a subject, wherein the plurality of antigens are specific for the cancer, collecting a plurality of immune cells trapped in the device, wherein the plurality of immune cells are specific to the plurality of antigens, and administering the plurality of immune cells or products derived therefrom into the subject, thereby treating the cancer.

Representative examples of cancer antigens include, but are not limited to, MAGE-1, MAGE-2, MAGE-3, CEA, Tyrosinase, midkin, BAGE, CASP-8, β-catenin, β- catenin, γ-catenin, CA-125, CDK-1, CDK4, ESO-1, gp75, gp100, MART-1, MUC-1, MUM-1, p53, PAP, PSA, PSMA, ras, trp-1, HER-2, TRP-1, TRP-2, IL13Ralpha, IL13Ralpha2, AIM-2, AIM-3, NY-ESO-1, C9orf 112, SART1, SART2, SART3, BRAP, RTN4, GLEA2, TNKS2, KIAA0376, ING4, HSPH1, C13orf24, RBPSUH, C6orf153, NKTR, NSEP1, U2AF1L, CYNL2, TPR, SOX2, GOLGA, BMI1, COX-2, EGFRvIII, EZH2, LICAM, Livin, Livinβ, MRP-3, Nestin, OLIG2, ART1, ART4, B-cyclin, Gli1, Cav-1, cathepsin B, CD74, E-cadherin, EphA2/Eck, Fra-1/Fosl 1, GAGE-1, Ganglioside/GD2, GnT-V, β1,6-N, Ki67, Ku70/80, PROX1, PSCA, SOX10, SOX11, Survivin, UPAR, WT-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (AD Abp), cyclophilin b, Colorectal associated antigen (CRC)- C017-1A/GA733, T-cell receptor/CD3-zeta chain, GAGE-family of tumor antigens, RAGE, LAGE-I, NAG, GnT-V, , RCASl, α-fetoprotein, pl20ctn, Pmel117, PRAME, brain glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-I, SSX-4, SSX-5, SCP-I, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, PlA, Connexin 37, Ig-idiotype, pl5, GM2, GD2 gangliosides, Smad family of tumor antigens, lmp-1, EBV-encoded nuclear antigen (EBNA)-I, UL16-binding protein-like transcript 1 (Mult1), RAE-1 proteins, H60, MICA, MICB, and c-erbB-2, or an immunogenic peptide thereof, and combinations thereof.

Preferably, the immune cells are T-cells or B-cells which bind with specificity to one or more of the aforementioned antigens. Cell products that are useful in practicing the cancer therapy methods of the instant invention include, for example, hybridomas of B-cells, stable lineages of T-cells, antibodies derived from B-cells or hybridomas thereof, receptors which bind to the cancer antigens (receptors which bind to MHC molecules presenting the antigens), including fragments thereof, nucleic acids encoding the receptors or antigen-binding domains thereof, nucleic acids encoding antibodies, including whole cells.

Embodiments of the instant invention provide for methods for treating a pathogenic disease in a subject. The method comprises administering a device of the instant invention to a subject, wherein the plurality of antigens are specific for the pathogenic disease, collecting a plurality of immune cells trapped in the device, wherein the plurality of immune cells are specific to the plurality of antigens, and administering the plurality of immune cells or products derived therefrom into the subject, thereby treating the pathogenic disease. In some instances, the immune cells or compositions derived therefrom may be administered prophylactically, *e.g.*, before the onset of the disease symptoms in the subject.

Pathogenic diseases that may be treated in accordance with the aforementioned embodiment include, bacterial diseases, viral diseases, fungal diseases, or a combination thereof.

Cell products that are useful in treating pathogenic diseases of the instant invention include, for example, hybridomas of B-cells, stable lineages of T-cells, antibodies derived from B-cells or hybridomas thereof, receptors which bind to the pathogenic antigens (and/or receptors which bind to MHC molecules presenting the pathogenic antigens), fragments of such antibodies or receptors, nucleic acids encoding the receptors, antibodies, or antigen-binding domains thereof, including whole cells.

Embodiments of the instant invention provide for methods for treating an autoimmune disease in a subject. The method comprises administering a device of the instant invention to a subject, wherein the plurality of antigens are specific for the autoimmune disease, collecting a plurality of immune cells trapped in the device, wherein the plurality of immune cells are specific to the plurality of antigens, and administering the plurality of immune cells or regulators thereof into the subject, thereby treating the autoimmune disease.

In the context of autoimmune diseases, it may be preferable not to administer active immune cells (as these are autoreactive) but rather quiescent, senescent or inactivated immune cells. Preferably, the immune cells are T-cells. Alternately, regulators of immune cells may be administered. Such may include, for example, suppressor T cells or regulatory T cells. Accordingly, in some embodiments, the invention provides for a method for treating autoimmune disease by administering to subject in need thereof, the device of the invention, wherein the plurality of antigens in the device are specific for the autoimmune disease, collecting a plurality of regulatory or suppressor T-cells trapped in the device, wherein the plurality of regulatory or

suppressor T-cells are specific to the antigens, and administering the plurality of regulatory T-cells or suppressor T-cells or products derived therefrom into the subject, thereby treating the autoimmune disease.

Cell products that are useful in practicing the therapy of autoimmune diseases include, for example, antibodies and receptors which bind to autoreactive cells, regulatory proteins located in suppressor or regulatory T-cells, including nucleic acid sequences which encode such molecules.

In the therapeutic embodiments described above, cells may be formulated at total cell concentrations including from about 5×10^2 cells/ml to about 1×10^9 cells/ml. Preferred doses of T cells range from about 2×10^6 cells to about 9×10^7 cells.

Embodiments of the instant invention further relate to therapy of diseases by administering one or more of the aforementioned compositions. The composition may be a pharmaceutical composition, which is administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intraarterial, intradermal, intramuscular, intraperitoneal, transdermal, transmucosal, intracerebral, intrathecal, or intraventricular routes. Alternatively, or concurrently, administration may be by the oral route. The pharmaceutical compositions may be administered parenterally by bolus injection or by gradual perfusion over time.

The dosage administered may be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The dose ranges for the administration of the pharmaceutical compositions may be large enough to produce the desired effect, whereby, for example, autoreactive T cells are depleted and/or the autoimmune disease is significantly prevented, suppressed, or treated. The doses may not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Determination of repertoire of immune cells associated with a disease

Embodiments disclosed herein further relate to methods for determining the repertoire of nucleic acids encoding one or more immune cell receptors, or a portion thereof, in a subject suffering from a disease. The methods comprise amplifying nucleic acids encoding one or more immune cell receptors obtained from cells trapped by the devices of the instant invention. Preferably, the immune cells are T-cells or B-cells and the immune cell receptors are T-cell receptors (TCR) and B-cell receptors (BCR), respectively. Methods for targeting and amplifying targeted nucleic acid constructs from whole cell lysates are known in the art. See, Maryanski et al., Immunity, Vol. 4, Pages 47–55, 1996, which is incorporated by reference.

In these embodiments, the subject is suffering from an autoimmune disease such as type I diabetes, Multiple Sclerosis, rheumatoid arthritis (RA), inflammatory bowel disease (IBD) or Crohn's Disease (CD), or and other gastrointestinal autoimmune conditions. The population of

cells collected from the devices may be further selected with various antigens associated with the aforementioned diseases and repertoire of immune cells selected via antigen priming may be characterized based on the sequences of cell-surface receptors expressed therein. In one representative method, the immune cells are T-cells and are characterized based on the sequences of T-cell receptors involved in binding to the disease antigen, *e.g.*, an antigen associated with T1D.

Trapping of cells for analysis of pathogens and/or various strains thereof

The immune system uses a variety of players for detecting and removing pathogens from the body. For example, pathogens may be taken up by macrophages, which digest and process them to present them on cell-surface major histocompatibility complex (MHC) molecules, allowing for lymphocytes to recognize them. Accordingly, embodiments of the instant invention provide for detecting and analyzing pathogens via analysis of the immune cells that are specific to the pathogens. The pathogen may be a bacterium, a virus, a fungus, or a combination thereof.

Thus, in accordance with the aforementioned embodiment, the instant invention provides for traps which recruit immune cells, *e.g.*, T-cells, B-cells, macrophages or dendritic cells, which immune cells are specific for the pathogen of interest. In particular, the immune cells are T-cells or B-cells which bind to pathogenic antigens. Alternately, the immune cells are antigen presenting cells such as dendritic cells or macrophages. In these embodiments, the devices containing a plurality of antigens that are specific to a pathogen are administered into a subject for a period (*e.g.*, 1 week to 3 weeks) and immune cells contained therein are analyzed for specificity and/or binding to the pathogenic antigen. The cells may be further divided into distinct sub-populations based on their reactivity to particular epitopes contained in the antigen. Optionally, the cells may be formulated into immune compositions for the therapy of pathogenic diseases. Preferably, the immune composition is a dendritic cell composition.

A representative list of pathogens and pathogenic antigens, including NCBI/GI accession numbers thereof, are provided in Table 6.

In a related embodiment, the instant invention provides for devices for trapping immune cells that are specific to new bacterial antigens. For example, MPB70 and MPB83 have been well-studied and generally well-understood in the context of mycobacteria. However, because they are less abundantly expressed in *M. tuberculosis* (more highly expressed in *Mycobacterium bovis*), their involvement in pathogenicity of *M. tuberculosis* is not well-understood. The devices of the instant invention may be implemented to identify new/key pathogenic antigens in related strains which can be specifically targeted by immune cells and/or their products. For example, devices containing MPB70 and MPB83 proteins derived from *Mycobacterium bovis* can be implanted into subjects to isolate a population of immune cells which can be tested for cross-reactivity to *M. tuberculosis*. The

immune cells that cross-react can be typed for determining receptors and molecules involved in cross-reactivity. Thus, novel targets and therapeutic agents binding thereto can be identified.

Table 6. Various types of pathogens, exemplary forms of pathogenic antigens and their corresponding NCBI and GI accession numbers:

Pathogen	**Antigens**	**Accession Nos. of antigens**
Mycobacterium bovis	MPB70	D38230.1 GI:1008918
	MPB83	EU683972.1 GI:188523816
	MPB64 **MPB80**	
Human Papillomavirus (HPV)	E2	AF115832.1 GI:5257208
	E6	KC190291.1 GI:425892430
	E7	JX896422.1 GI:414090986
Human immunodeficiency virus	p17	AJ294941.1 GI:10800847
	Gag p24	ACI05538.1 GI:206129896
Methicillin-resistant *Staphylococcus aureus*	PBP2a	KF895401.1 GI:586616552

VIII. Kits

In certain embodiments, the present invention provides kits comprising, in one or separate compartments, the devices of the instant invention. The kits may further comprise additional ingredients, *e.g.*, gelling agents, emollients, surfactants, humectants, viscosity enhancers, emulsifiers in one or more compartments. The kits may optionally comprise instructions for formulating the devices for diagnostic or therapeutic applications. The kits may also comprise instructions for using the components, either individually or together, in the therapy or diagnosis of various disorders and/or diseases.

In a related embodiment, the present invention provides kits comprising the devices of the invention along with reagents for selecting, culturing, expanding, sustaining, and/or transplanting the trapped cells of interest. Representative examples of cell selection kits, culture kits, expansion kits, transplantation kits for T-cells, B-cells and antigen presenting cells are known in the art. For example, where the target cells of interest are B-cells, such may be initially sorted using DYNABEADS, MACS-beads (Miltenyi Biosciences), maintained in ROSETTESEP culture media (Stem Cell

Technologies) and expanded with CELLEXVIVO Human B Cell Expansion Kit (R&D Systems). The cells may be enriched in the sample by using centrifugation techniques known to those in the art including, but not limited to, FICOLL® gradients. Cells may also be enriched in the sample by using positive selection, negative selection, or a combination thereof for expression of gene products thereof.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

EXAMPLE 1: Device Fabrication and Characterization

In accordance with the aforementioned methods, polymeric scaffolds for the devices were engineered to sustain antigen presentation. In short, device fabrication was carried out as follows: Copolymers of lactide and glycolide (PLG) (Alkermes, Cambridge, MA) were utilized in a gas-foaming process to form PLG scaffolds. To physically adsorb antigen onto PLG polymer, a gas foaming particulate leaching was used to fabricate scaffolds to be used as traps. FIG. 1A shows a photomicrograph of the cross-sectional scanning electron microscope (SEM) images of a model device made with PLG (the picture on the right shows displays an enlarged view of the pores). Alternatively, other polymers such as alginate may be used as the structural element of the devices. The pores allow space for T cell infiltration and expansion. FIG. 1B shows that OVA-T cell traps are capable of releasing ovalbumin (OVA) protein (model antigen).

The devices were further implanted subcutaneously in the backs of mice for a period of 14 days. FIG. 1C is a bar graph showing the local cytokine concentration of MIP-la and RANTES at site of implantation. The values in B and C represent the average and standard deviation (n=5). It can be seen that compared to controls, local cytokine concentration for each cytokine was significantly higher at the site of implantation of the traps. Normally, antigen-specific T cells have the ability to enter into inflamed tissues presenting the corresponding antigen and proliferating upon T cell receptor (TCR)-antigen recognition. When T cell traps displaying antigens are administered subcutaneously, they promote local MIP-la and RANTES production, which are similar to sites of inflammation and autoimmune lesions. Traps contain an antigen (or mixture of antigens) that engages a specific TCR on the T cell to become sticky and remain in device. This antigen interaction also causes T cells to proliferate within the device, thereby enriching the local area with these cells.

EXAMPLE 2: Implantation of devices

Traps loaded with the model antigen, ovalbumin (OVA), and control traps were implanted into OT-1 mice that have a pre-existing pool of OVA-specific T cells. After 14 days, the scaffolds were explanted and cells harvested and stained for CD8 and OVA tetramer. Traps containing OVA were enriched for OVA-specific T cells (FIG. 2A) and had approximately 5-8 fold more T cells relative to controls (FIG. 2B). When two different traps containing a unique antigen mix, either for OVA or melanoma antigens, were implanted into mice, T cells accumulated preferentially in traps loaded with the cognate antigen (FIG. 2C). OVA specific T cells accumulated significantly in OVA traps, whereas Trp2 (melanoma antigen) specific T cells accumulated significantly in melanoma traps (FIG. 2C and FIG. 2D). The OVA and melanoma specific cells were essentially absent in traps not containing the corresponding antigen (FIG. 2C and FIG. 2D).

EXAMPLE 3: Trapping of T Cells during Diabetes Progression

Antigen-specific trapping was demonstrated in an art-recognized mouse model for type 1 diabetes. Diabetic T cell traps were fabricated by loading PLG scaffolds with beta-cell lysate (FIG. 3A). These traps were then implanted into non-obese diabetic (NOD) mice at ages 9, 12, and 15 weeks for 2 weeks when they were assayed for T cell infiltration (FIG. 3B). In NOD mice, insulitis or T cell infiltration of islets increases predictably with age as illustrated in Fig. 3B. In particular, traps were found to significantly accumulate T cells relative to controls, and the infiltration correlated with insulitis in the pancreas (FIG. 3B and FIG. 3D). The total number of T cells in traps not only increased with diabetes progression, but the T cell populations were also enriched. There was a three to five fold difference in the number of T-cells in traps compared to controls.

EXAMPLE 4: Characterization of trapped T-cells

T cells were harvested 14 days after implantation in NOD mice at ages 11, 14 and 17 weeks. Panceratic beta cell targets were cultured with the harvested T cells. IL-2 secretion was measured using an ELISPOT assay. Results are shown in FIG. 4A. It was found that a significant subset of T cells isolated from traps (12% of T cells) was responsive to beta-cell targets *in vitro* as demonstrated by ELISPOT (FIG. 4A). In contrast, control traps were completely devoid of T cells responsive to pancreatic beta cells. The traps contained enriched levels of beta-cell specific T cells relative to control traps and spleens of mice, indicating that higher T cell numbers and enriched specificity could be obtained from traps relative to T cell levels in the blood. T cells in traps were analyzed and compared to T cells in spleens as a marker of blood levels. Diabetes-specific cells were identified in the traps were enriched by approximately 4000% relative to blood concentration (FIG. 4B).

EXAMPLE 5: Trapping and harvesting cancer specific T cells

Porous scaffolds were loaded with antigens and other molecular patterns of the disease. One set of traps contained a unique mixture of OVA antigens, the other set contained a unique mixture of melanoma antigens. It was found that T cells that are specific to an antigen accumulated preferentially in traps loaded with the cognate antigen (FIG. 5C). More specifically, OVA specific T cells accumulated significantly in OVA traps whereas Trp2 (melanoma antigen) specific T cells accumulated significantly in melanoma traps (FIG. 5D). Conversely, the OVA and melanoma specific cells were absent from the traps which did not contain the corresponding antigen (FIG. 5C and FIG. 5D). These results show that antigen-specific T cells can be trapped and harvested based on the antigens that are provided in the scaffold.

EXAMPLE 6: Transferring functional T-cells to host

T cell traps and blank scaffolds were implanted subcutaneously into 15 week-old NOD mice for 14 days. Cells were then isolated from these scaffolds and 2 x 10^6 cells were transferred to NOD SCID mice via retro-orbital injection. Splenocytes from 17 week-old NOD mice were also injected into NOD-SCID recipients as a positive control. After cell transfer, the mice were monitored for the onset of diabetes by measuring blood glucose.

NOD-SCID mice receiving NOD derived splenocytes began to develop diabetes at 15 weeks after cell injection and 88% became diabetic by week 20 (FIG. 6). Trapped cells induced diabetes with faster kinetics as 20% of mice became diabetic by week 9 and 100% of mice were diabetic at 20 weeks. Cells extracted from control scaffolds completely failed to induce diabetes in SCID mice. These data indicate that Traps are able to specifically recruit functional, T-cells that can be transferred into the host.

Taken together, Examples 5 and 6 demonstrate that T cells can be generated and/or harvested *in situ* using porous scaffolds of the invention that are loaded with antigen(s) and molecular patterns of the disease. The results show that antigen-specific T cells are attracted and generated at these traps in vivo that can be harvested, similar to how they home and expand at primary sites of disease. These cells are generated *in vivo* and may also be expanded further via delivery of growth factors/cytokines from the trap. Surprisingly and unexpectedly, it was found that the functionality of trapped, disease-specific T cells is maintained after adoptive transfer into a host.

EXAMPLE 7: Trapping of cells for clinical or analytical use

T cell traps, B cell traps, and/or dendritic cell traps are constructed using the aforementioned manufacturing processes. For example, T cell traps can utilize different chemokines/cytokines as shown in Table 1. Likewise, exemplary traps for dendritic cells/macrophages may utilize the cytokines/ chemokines shown in Table 2. Similarly, B-cell traps may include the various cytokines/chemokines shown in Table 3. For each type of trap, additional agents, such as, for

example, interleukins, growth factors, sequestering agents, adhesion receptors, or other compounds/molecules may be optionally employed. Table 4 provides a list of interleukins that may be optionally employed in the T-cell, B-cell, or DC/macrophage traps. Table 5 provides a list of T-cell adhesion receptors.

Loading with antigens is an effective homing agent to differentiate T cells from DCs. These immune cells are then analyzed to understand disease pathogenesis and to identify therapeutic targets such as antigens. Disease-causing T cells are currently inaccessible as they concentrate only in the lesions of autoimmunity. The trap technology described herein provide a means to collect these cells.

The devices are implanted in subjects for at least a day and up to 14 days or more as described above. Cells may proliferate within the device, and the device loaded with immune cells can be explanted anywhere from days 1-28, or longer, *e.g.*, 30 days, 60 days, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 36 months, 48 months or more. The devices may be monitored periodically.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. An immune-cell trapping device, comprising

a physiologically-compatible porous polymer scaffold, and

a plurality of purified antigens,

wherein the plurality of antigens attract and trap a plurality of immune cells specific to the plurality of antigens in the device.

2. The device of claim 1, wherein the antigens are absorbed onto the polymer scaffold.

3. The device of claim 1, wherein the antigens are encapsulated by the polymer scaffold.

4. The device of claim 1, wherein the plurality of antigens bind to a receptor on the plurality of immune cells.

5. The device of claim 1, wherein the device does not contain an agent that kills or eliminates immune cells.

6. The device of claim 1, wherein the device further comprises an immune cell recruiting agent.

7. The device of claim 6, wherein the immune cell recruiting agent comprises an agent selected from the group consisting of a T-cell recruiting agent, a dendritic cell recruiting agent, and a macrophage recruiting agent, or a combination thereof.

8. The device of claim 6, wherein the immune cell recruiting agent comprises an agent selected from the group consisting of natural killer (NK) cell recruiting agent, a CD3+ T-cell recruiting agent, a CD4+ T-cell recruiting agent, a CD8+ T cell recruiting agent, a CD8+ T cell recruiting agent, a regulatory T-cell (Treg) recruiting agent, or a combination thereof.

9. The device of claim 6, wherein the immune cell recruiting agent is a growth factor, a cytokine, an interleukin, an adhesion signaling molecule, an integrin signaling molecule, an interferon, a lymphokine, or a chemokine, or a fragment thereof, or a combination thereof.

10. The device of claim 9, wherein the interleukin is selected from the group consisting of IL-1, IL-2, IL-4, IL-5, IL-10 IL-12 and IL-17.

11. The device of claim 1, wherein the device further comprises an agent which enhances infiltration of the immune cells into the device.

12. The device of claim 1, wherein the plurality of antigens are cancer antigens.

13. The device of claim 12, wherein the cancer antigens are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, CEA, Tyrosinase, midkin, BAGE, CASP-8, β-catenin, β- catenin, γ-catenin, CA-125, CDK-1, CDK4, ESO-1, gp75, gp100, MART-1, MUC-1, MUM-1, p53, PAP, PSA, PSMA, ras, trp-1, HER-2, TRP-1, TRP-2, IL13Ralpha, IL13Ralpha2, AIM-2, AIM-3, NY-ESO-1, C9orf 112, SART1, SART2, SART3, BRAP, RTN4, GLEA2, TNKS2, KIAA0376, ING4, HSPH1, C13orf24, RBPSUH, C6orf153, NKTR, NSEP1, U2AF1L, CYNL2, TPR, SOX2, GOLGA, BMI1, COX-2, EGFRvIII, EZH2, LICAM, Livin, Livinβ, MRP-3, Nestin, OLIG2, ART1, ART4, B-cyclin, Gli1, Cav-1, cathepsin B, CD74, E-cadherin, EphA2/Eck, Fra-1/Fosl 1, GAGE-1, Ganglioside/GD2, GnT-V, β1,6-N, Ki67, Ku70/80, PROX1, PSCA, SOX10, SOX11, Survivin, UPAR, WT-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (AD Abp), cyclophilin b, Colorectal associated antigen (CRC)- C017-1A/GA733, T-cell receptor/CD3-zeta chain, GAGE-family of tumor antigens, RAGE, LAGE-I, NAG, GnT-V, , RCASl, α-fetoprotein, pl20ctn, Pmel117, PRAME, brain glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-I, SSX-4, SSX-5, SCP-I, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, PlA, Connexin 37, Ig-idiotype, pl5, GM2, GD2 gangliosides, Smad family of tumor antigens, lmp-1, EBV-encoded nuclear antigen (EBNA)-I, UL16-binding protein-like transcript 1 (Mult1), RAE-1 proteins, H60, MICA, MICB, and c-erbB-2, or an immunogenic peptide thereof, and combinations thereof.

14. The device of claim 1, wherein the plurality of antigens are non-self antigens.

15. The device of claim 14, wherein the non-self antigens are pathogenic antigens derived from a pathogen selected from the group consisting of a virus, a bacteria, a protozoan, a parasite, and a fungus.

16. The device of claim 15, wherein the pathogen is selected from the group consisting of *Mycobacterium bovis*, Human Papillomavirus (HPV), Human immunodeficiency virus, a pox virus, smallpox virus, ebola virus, marburg virus, dengue fever virus, influenza virus, parainfluenza virus, respiratory syncytial virus, rubeola virus, varicella-zoster virus, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, JC virus, rhabdovirus, rotavirus, rhinovirus, adenovirus, papillomavirus, parvovirus, picornavirus, poliovirus, virus that causes mumps, virus that causes rabies, reovirus, rubella virus, togavirus, orthomyxovirus, retrovirus, hepadnavirus, coxsackievirus, equine encephalitis virus, Japanese encephalitis virus, yellow fever virus, Rift Valley fever virus,

hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, SARS CoV, MERS CoV, Enterovirus, Borrelia species, Bacillus anthracis, Borrelia burgdorferi, Bordetella pertussis, Camphylobacter jejuni, Chlamydia species, Chlamydial psittaci, Chlamydial trachomatis, Clostridium species, Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Corynebacterium diphtheriae, Coxiella species, an Enterococcus species, Erlichia species, Escherichia coli, Francisella tularensis, Haemophilus species, Haemophilus influenzae, Haemophilus parainfluenzae, Lactobacillus species, a Legionella species, Legionella pneumophila, Leptospirosis interrogans, Listeria species, Listeria monocytogenes, Mycobacterium species, Mycobacterium tuberculosis, Mycobacterium leprae, Mycoplasma species, Mycoplasma pneumoniae, Neisseria species, Neisseria meningitidis, Neisseria gonorrhoeae, Pneumococcus species, Pseudomonas species, Pseudomonas aeruginosa, Salmonella species, Salmonella typhi, Salmonella enterica, Rickettsia species, Rickettsia ricketsii, Rickettsia typhi, Shigella species, Staphylococcus species, Staphylococcus aureus, Streptococcus species, Streptococccus pnuemoniae, Streptococcus pyrogenes, Streptococcus mutans, Treponema species, Treponema pallidum, a Vibrio species, Vibrio cholerae, Yersinia pestis, Methicillin-resistant *Staphylococcus aureus*, Aspergillus species, Aspergillus, funigatus, Aspergillus flavus, Aspergillus calvatus, Candida species, Candida albicans, Candida tropicalis, Cryptococcus species, Cryptococcus neoformans, Entamoeba histolytica, Histoplasma capsulatum, Leishmania speceis, Nocardia asteroides, Plasmodium falciparum, *Stachybotrys chartarum,* Toxoplasma gondii, Trichomonas vaginalis, Toxoplasma species, Trypanosoma brucei, Schistosoma mansoni, Fusarium species, Trichophyton species, Plasmodium species, Toxoplasma species, Entamoeba species, Babesia species, Trypanosoma species, Leshmania species, Pneumocystis species, Pneumocystis jirovecii, Trichomonas species, Giardia species, Schisostoma species, Cryptosporidium species, Plasmodium species, Entamoeba species, Naegleria species, Acanthamoeba species, Balamuthia species, Toxoplasma species, Giardia species, Trichomonas species, Leishmania species, and Trypanosoma species.

17. The device of claim 1, wherein the antigens are self-antigens.

18. The device of claim 17, wherein the antigens are derived from a lysate of a cell to which an autoimmune response is directed.

19. The device of claim 17, wherein the antigen is a pancreatic beta cell antigen, a neuronal cell antigen, a bone or joint associated autoimmune disease associated antigen, or a gastrointestinal-disease associated antigen.

20. The device of claim 19, wherein the pancreatic beta cell antigen is an antigen associated with type I diabetes; the neuronal cell antigen is an antigen associated with multiple sclerosis; the bone or joint

associated antigen is an antigen associated with rheumatoid arthritis; the gastrointestinal disease associated antigen is an antigen associated with inflammatory bowel disease or Crohn's disease.

21. The device of claim 1, wherein the device further comprises an RGD peptide, a CpG oligonucleotide or a granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokine, or a fragment thereof or a combination thereof.

22. The device of claim 1, wherein the device does not contain an RGD peptide, a CpG oligonucleotide or a granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokine or a fragment thereof or a combination thereof.

23. The device of claim 1, wherein the device preferentially attracts T-cells compared to other immune cells and wherein the device further comprises a chemokine selected from the group consisting of CCL1, CCL2 (MCP-1), CCL-3, CCL-4, CCL-5 (RANTES), CCL-17, CCL-22, CXCL12 and XCL1, or a fragment thereof, or a combination thereof.

24. The device of claim 23, wherein the chemokine is a chemokine selected from the group consisting of:
(a) CCL1 (accession numbers for human homolog: NM_002981.2; GI:523498696 and accession numbers for mouse homolog: NM 011329; GI:257153404),
(b) CCL2 (MCP-1)(accession numbers for human homolog: NM_002982.3; GI:56119169 and accession numbers for mouse homolog: NM_011333.3; GI:141803162),
(c) CCL3 (accession numbers for human homolog: NM_002983.2; GI: 121582465 and accession numbers for mouse homolog: NM 011337.2; GI: 126432552),
(d) CCL4 (accession numbers for human homolog: NM 002984.3; GI:748585189 NM 013652.2 and accession numbers for mouse homolog: NM_013652.2; GI:126366031),
(e) CCL5 (RANTES)(accession numbers for human homolog: NM_002985.2; GI: 22538813 and accession numbers for mouse homolog: NM_013653.3; GI: 164698427),
(f) CCL17 (accession numbers for human homolog: NM_002987.2; GI:22538801 and NM_011332.3; accession numbers for mouse homolog: NM_011332.3; GI:225735578),
(g) CCL19 (accession numbers for human homolog: NM_006274.2; GI:22165424 and accession numbers for mouse homolog: NM 011888.2; GI: 10518345),
(h) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695),
(i) CXCL12 (accession numbers for human homolog: NM_199168.3; GI: 291045298 and accession numbers for mouse homolog: NM_001277990.1; GI: 489406389), and

(j) XCL1 (accession numbers for human homolog: NM_002995.2; GI:312434026 and accession numbers for mouse homolog: NM 008510.1; GI: 6678711).

25. The device of claim 1, wherein the device preferentially attracts dendritic cells (DC) or macrophages compared to other immune cells and wherein the device further comprises a chemokine selected from the group consisting of CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 or a fragment thereof, or a combination thereof.

26. The device of claim 25, wherein the chemokine is a chemokine selected from the group consisting of:
(a) CCL2 (accession numbers for human homolog: NM 002982.3; GI:56119169 and accession numbers for mouse homolog: NM 011333.3; GI:141803162),
(b) CCL3 (accession numbers for human homolog: NM_002983.2; GI: 121582465 and accession numbers for mouse homolog: NM_011337.2; GI: 126432552),
(c) CCL5 (Variant 1) (accession numbers for human homolog: NM 002985.2; GI: 22538813 and accession numbers for mouse homolog: NM_013653.3; GI: 164698427),
(d) CCL7 (accession numbers for human homolog: NM_006273.3; GI:428673540 and accession numbers for mouse homolog: NM_013654.3; GI:226958664),
(e) CCL8 (accession numbers for human homolog: NM_005623.2; GI:22538815 and accession numbers for mouse homolog: NM 021443.3; GI:255708468),
(f) CCL13 (accession numbers for human homolog: NM_005408.2; GI:22538799 and accession numbers for mouse homolog: NM_011333.3; GI:141803162),
(g) CCL17 (accession numbers for human homolog: NM_002987.2 GI:22538801 and accession numbers for mouse homolog: NM 011332.3; GI:225735578), and
(h) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695).

27. The device of claim 1, wherein the device preferentially attracts both T-cells and dendritic cells (DC) compared to other immune cells and wherein the device further comprises a chemokine selected from the group consisting of CCL17 and CCL22, or a fragment thereof, or a combination thereof.

28. The device of claim 27, wherein the chemokine is a chemokine selected from the group consisting of:
(a) CCL17 (accession numbers for human homolog: NM_002987.2 GI:22538801 and accession numbers for mouse homolog: NM 011332.3; GI:225735578), and

(b) CCL22 (accession numbers for human homolog: NM_002990.4; GI:300360575 and accession numbers for mouse homolog: NM_009137.2; GI: 154240695).

29. The device of claim 1, further comprising a plurality of adhesion receptors for T-cells selected from the group consisting of LFA-1, MAdCAM-1, VCAM-1, CD28 and CTLA-4 or a fragment thereof or a combination thereof.

30. The device of claim 29, wherein the adhesion receptors are selected from the group consisting of
(a) LFA (accession numbers for human homolog: NM 000211.4; GI:735367774 and accession numbers for mouse homolog: M60778.1; GI:198785),
(b) MAdCAM-1 (accession numbers for human homolog: NM 130760.2 GI:109633021 and accession numbers for mouse homolog: D50434.2; GI: 60391311),
(c) VCAM-1 (accession numbers for human homolog: NM 001078.3; GI:315434269 and accession numbers for mouse homolog: X67783.1; GI: 298116),
(d) CD28 (accession numbers for human homolog: NM 006139.3; GI: 340545506 and accession numbers for mouse homolog: BC064058.1; GI: 39850201), and
(e) CTLA-4 (accession numbers for human homolog: NM 005214.4; GI: 339276048 and accession numbers for mouse homolog: U90270.1; GI:4099836).

31. The device of claim 1, wherein the device comprises about 10 μg to about 2.0 mg of the antigens.

32. The device of claim 1, wherein the device comprises about 0.1 μg to about 400 μg of the antigens per gram of dry weight of the scaffold.

33. The device of claim 1, wherein the device has a porosity of between about 40% to about 90%.

34. The device of claim 1, wherein the porous polymer scaffold comprises pores having a diameter of about 10 μM to about 500 μM.

35. The device of claim 1, wherein the porous polymer scaffold comprises a compound selected from the group consisting of polylactic acid (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA), alginate or a derivative thereof, gelatin, collagen, fibrin, hyaluronic acid (HA), agarose, a polysaccharide, a polyamino acid, a polypeptide, a polyester, a polyanhydride, a polyphosphazine, a polyvinyl alcohol (PVA), a polyalkylene oxides (PAO), a polyallyl amines)(PAM), a polyacrylate, a modified styrene polymer, a pluronic polyol, a polyoxamer, a polyuronic acid, and a polyvinylpyrrolidone or a co-polymer thereof, or a graft polymer thereof.

36. A pharmaceutical composition comprising the device of claim 1 and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36, wherein the composition is formulated for intravenous administration, subcutaneous administration, intraperitoneal administration, or intramuscular administration.

38. The pharmaceutical composition of claim 37, wherein the composition is formulated for subcutaneous administration as a microneedle patch.

39. A method of treating a disease in a subject in need thereof, comprising

administering the device of claim 1 to a subject, wherein the plurality of antigens are specific for the disease;

collecting a plurality of immune cells trapped in the device, wherein the plurality of immune cells are specific to the plurality of antigens; and

administering the plurality of immune cells into the subject, thereby treating the disease in the subject.

40. The method of claim 39, further comprising explanting the device from the subject.

41. The method of claim 39, further comprising

expanding the plurality of immune cells, thereby generating an expanded population of immune cells that are specific for the disease; and

administering the expanded population of immune cells into the subject.

42. The method of claim 39, wherein the disease is a cancer and the device comprises an antigen that is specific to the cancer.

43. The method of claim 39, wherein the cancer is selected from the group consisting of head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, esophageal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, glioblastoma, leukemia, lymphoma, mantle cell lymphoma, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer.

44. The method of claim 39, wherein the disease is an autoimmune disease and the device comprises an antigen that is specific to the autoimmune disease.

45. The method of claim 44, wherein the autoimmune disease is selected from the group consisting of type I diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and Crohn's disease.

46. The method of claim 39, wherein the disease is a caused by a pathogen and the device comprises an antigen that is specific to the pathogen.

47. The method of claim 46, wherein the pathogen is selected from the group consisting of a virus, a bacteria, a protozoan, a parasite, and a fungus.

48. The method of claim 39, wherein the subject is human.

49. The method of claim 39, wherein the device is subcutaneously administered into the subject.

50. The method of claim 39, wherein the device is intravenously administered to the subject.

51. The method of claim 39, wherein the plurality of immune cells are collected about 1 day to about 60 days after the device is administered to the subject.

52. A method for obtaining immune cells specific for an antigen, comprising

administering the device of claim 1 to a subject; and

harvesting immune cells trapped in the device, thereby obtaining immune cells specific for the antigen.

53. The method of claim 52, further comprising explanting the device from the subject.

54. The method of claim 52, wherein the subject is human.

55. The method of claim 52, wherein the device is subcutaneously administered into the subject.

56. The method of claim 52, wherein the device is intravenously administered to the subject.

57. The method of claim 52, wherein the plurality of immune cells are collected about 1 day to about 60 days after the device is administered to the subject.

58. A method for determining whether a subject has an autoimmune disease, comprising

administering the device of claim 1 into a subject, wherein the plurality of antigens are specific for the autoimmune disease;

collecting immune cells trapped in the device, wherein the immune cells are specific to the autoimmune disease; and

determining the number of immune cells specific to the autoimmune disease trapped in the device, thereby determining whether a subject has an autoimmune disease.

59. The method of claim 58, further comprising explanting the device from the subject.

60. The method of claim 58, wherein the subject is human.

61. The method of claim 58, wherein the device is subcutaneously administered into the subject.

62. The method of claim 58, wherein the device is intravenously administered to the subject.

63. The method of claim 58, wherein the plurality of immune cells are collected about 1 day to about 60 days after the device is administered to the subject.

64. A method for making the device of claim 1, comprising

incubating plurality of purified antigens with a physiologically compatible polymer to generate a polymer-antigen mixture;

freezing, lyophilizing and mixing the polymer-antigen mixture with a porogen;

compression molding the mixture to produce a disc;

subjecting the disk to a high-pressure CO_2 environment and rapidly reducing the pressure to expand and fuse the polymer into an interconnected scaffold structure; and

leaching the porogen from the scaffold structure by immersing the structure in water to generate a porous article, thereby making the device.

65. The method of claim 64, wherein the polymer is a copolymer of lactide and glycolide (PLG) or alginate.

66. The method of claim 64, wherein the porogen is NaCl or sucrose.

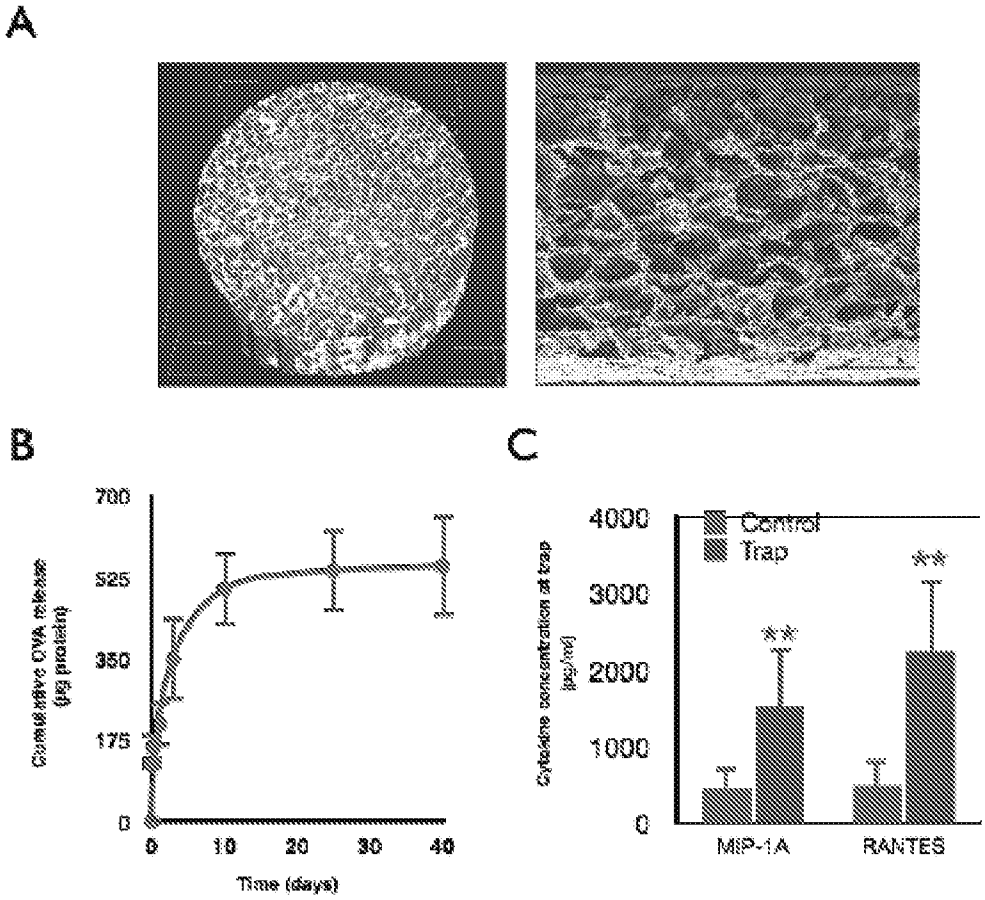
A
B
C
700
525
350
175
0
0
10
20
30
40
Time (days)
4000
3000
2000
1000
0
Control
Trap
**
**
MIP-1A
RANTES

FIG. 1

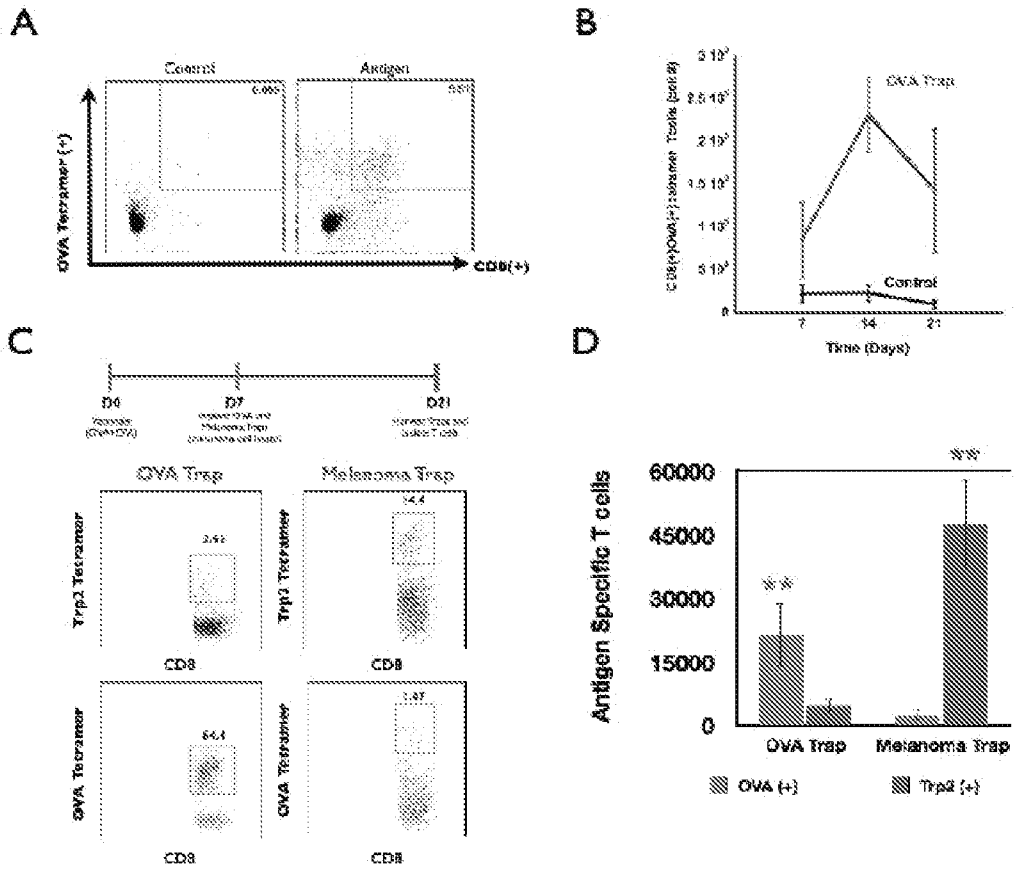
A
Control
Antigen
OVA Tetramer (+)
CD8(+)
B
OVA Trap
Control
Time (Days)
C
D0
D7
D21
OVA Trap
Melanoma Trap
Trp1 Tetramer
Trp2 Tetramer
OVA Tetramer
CD8
D
Antigen Specific T cells
60000
45000
30000
15000
0
OVA Trap
Melanoma Trap
OVA (+)
Trp2 (+)

FIG. 2

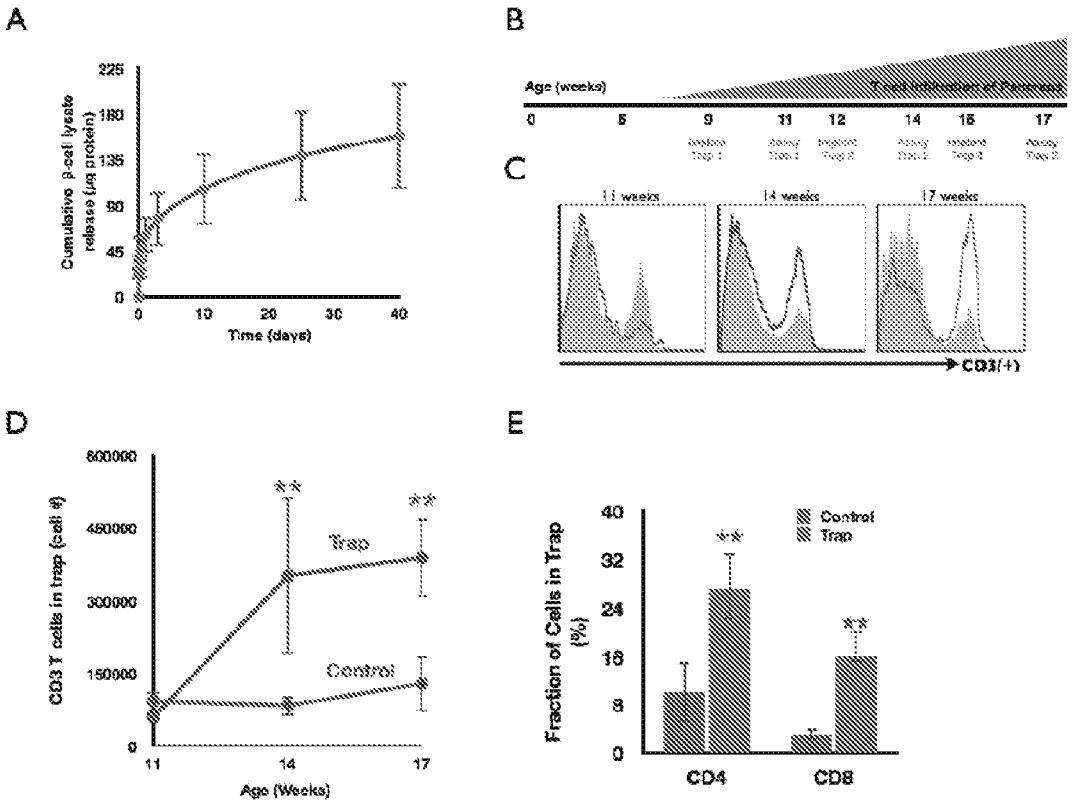
A
Cumulative β-cat lysate release (μg protein)
225
180
135
90
45
0
0
10
20
30
40
Time (days)
B
Age (weeks)
0
5
9
11
12
14
15
17
C
11 weeks
14 weeks
17 weeks
CD3(+)
D
CD3 T cells in trap (cell #)
600000
450000
300000
150000
0
Trap
Control
11
14
17
Age (Weeks)
E
Fraction of Cells in Trap (%)
40
32
24
16
8
0
Control
Trap
CD4
CD8

FIG. 3

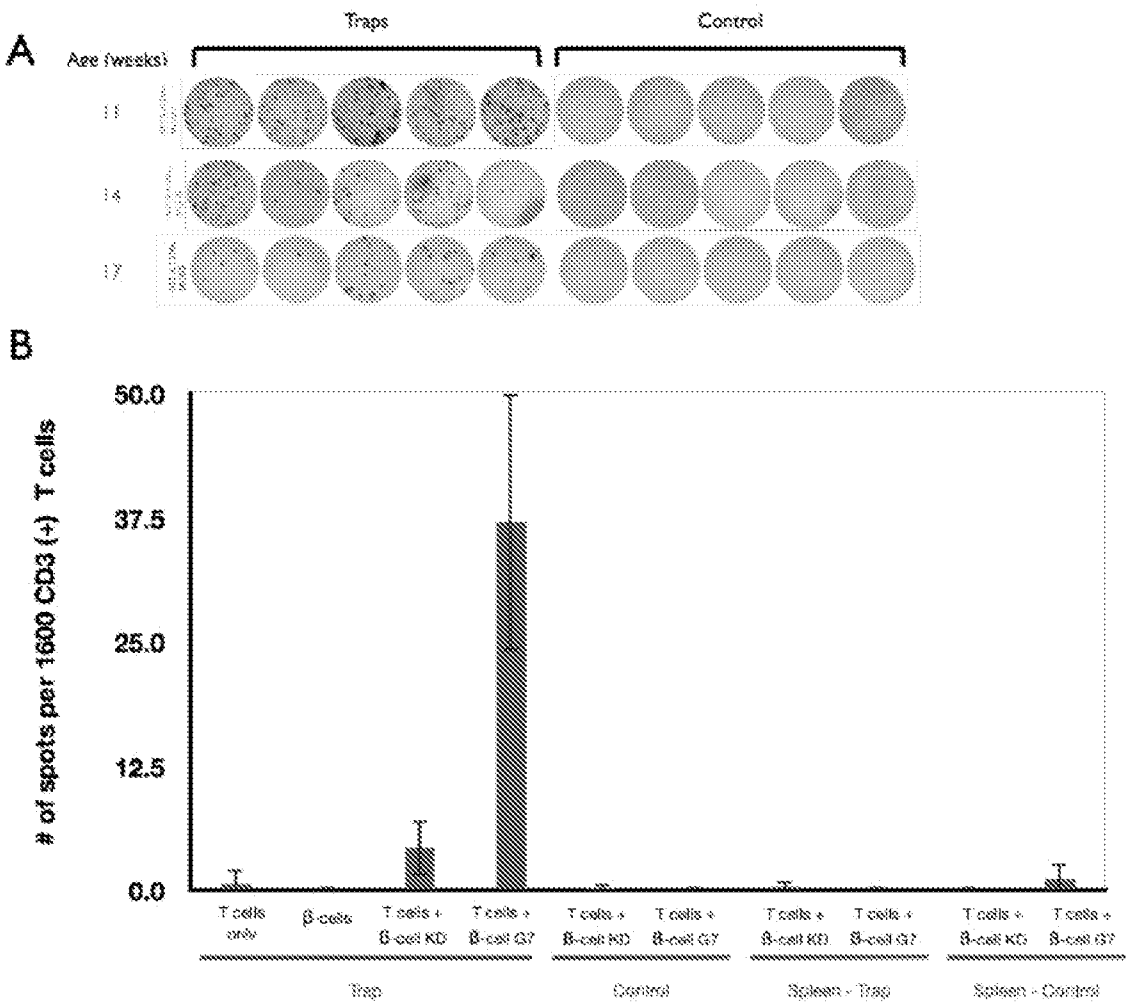
A
Traps
Control
Age (weeks)
11
14
17
B
# of spots per 1600 CD3 (+) T cells
50.0
37.5
25.0
12.5
0.0
T cells only
β-cells
T cells + β-cell KD
T cells + β-cell G7
T cells + β-cell KD
T cells + β-cell G7
T cells + β-cell KD
T cells + β-cell G7
T cells + β-cell KD
T cells + β-cell G7
Trap
Control
Spleen - Trap
Spleen - Control

FIG. 4

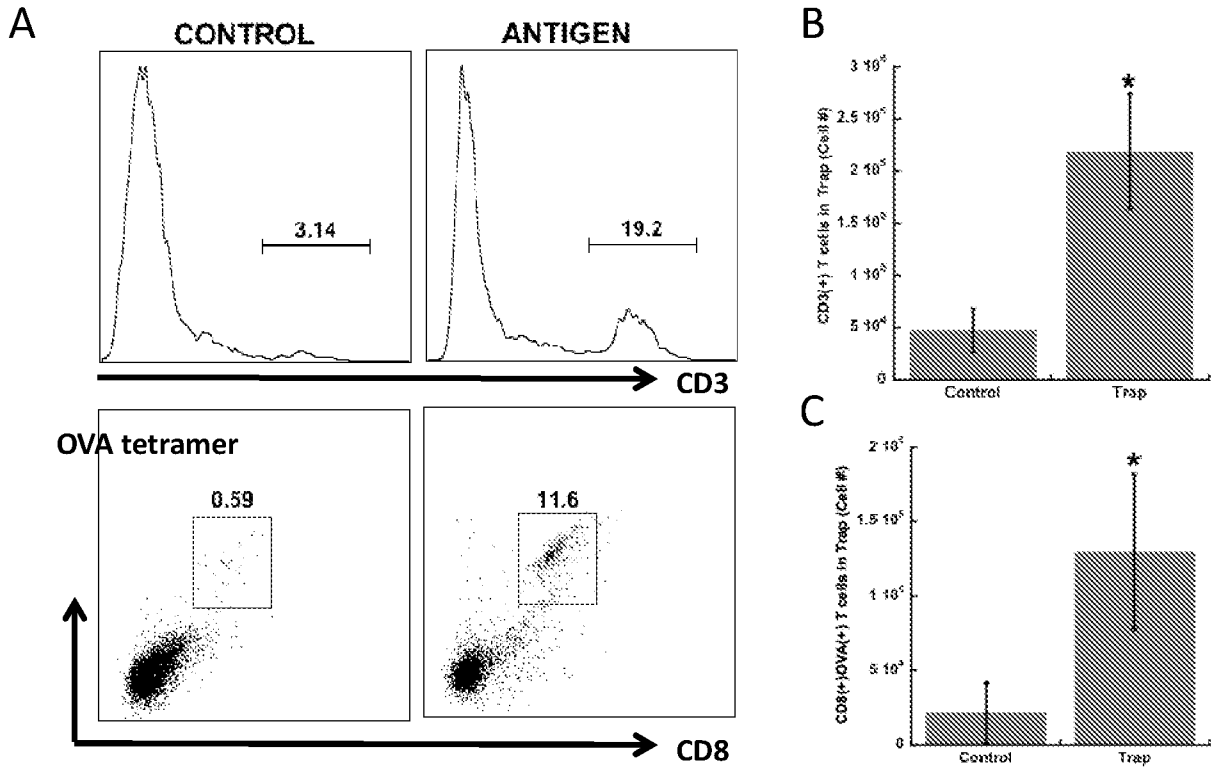
A
CONTROL
ANTIGEN
3.14
19.2
CD3
OVA tetramer
0.59
11.6
CD8
B
Control
Trap
C
Control
Trap

FIG. 5

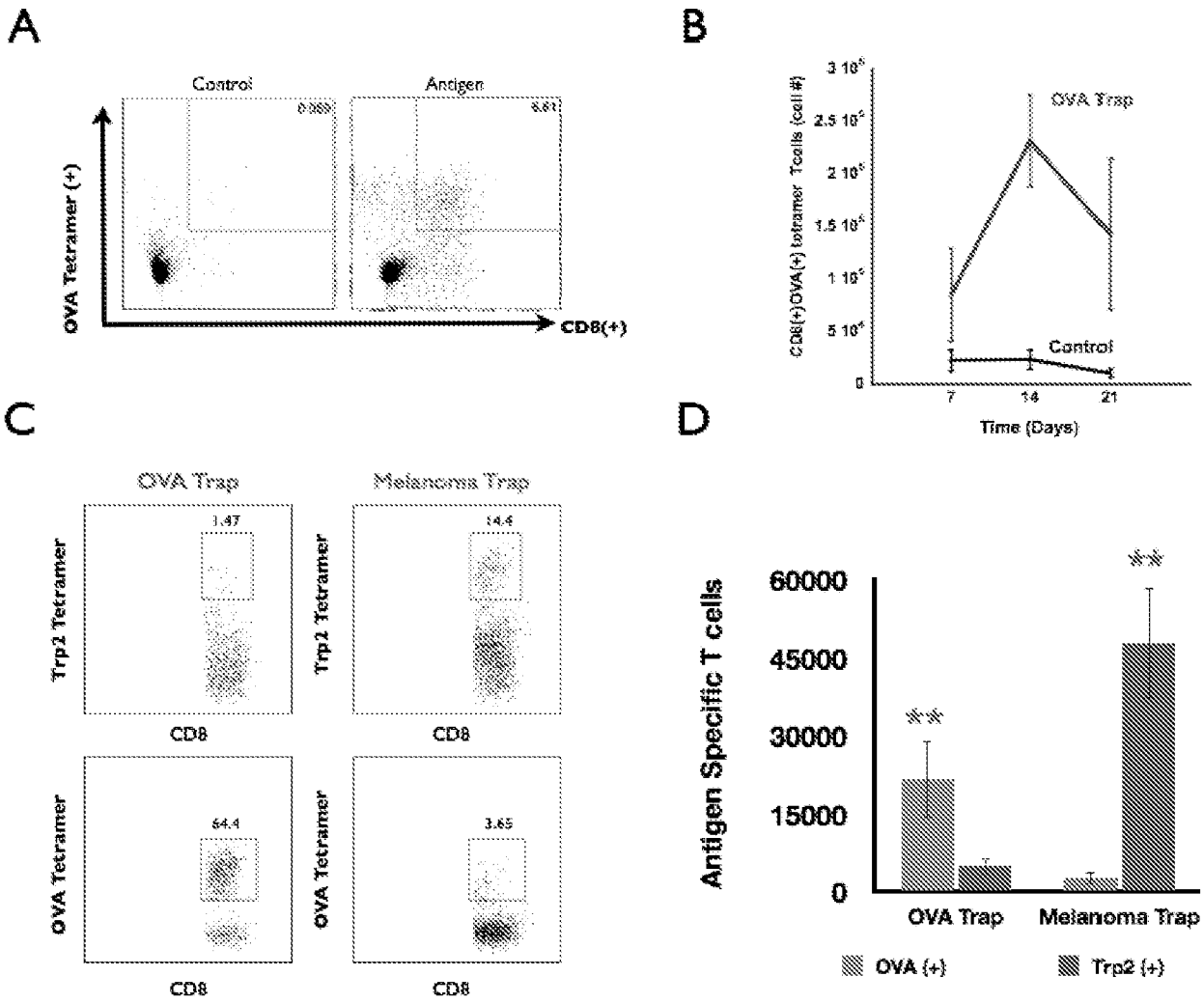
A
Control
Antigen
0.069
8.61
OVA Tetramer (+)
CD8(+)
B
OVA Trap
Control
CD8(+)OVA(+) tetramer Tcells (cell #)
Time (Days)
7
14
21
C
OVA Trap
Melanoma Trap
Trp2 Tetramer
OVA Tetramer
CD8
1.47
14.4
64.4
3.65
D
Antigen Specific T cells
60000
45000
30000
15000
0
OVA Trap
Melanoma Trap
OVA (+)
Trp2 (+)

FIG. 6

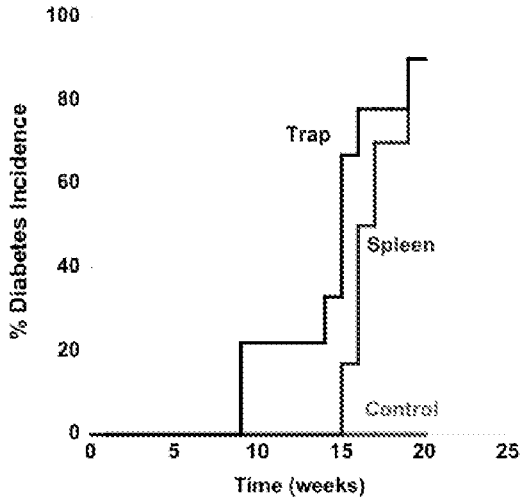
100
80
60
40
20
0
% Diabetes Incidence
Trap
Spleen
Control
0
5
10
15
20
25
Time (weeks)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/26617

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61L 27/54, 27/56, 27/36; C08 J9/26; A61K 35/12, 35/13, 35/14, 35/15, 35/17 (2016.01)
CPC - A61L 27/54, 27/56, 27/3683, 27/3691; C08J 9/26; A61K 35/12, 35/13, 35/15, 35/17
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): A61L 27/54, 27/56, 27/36, 27/38, 37/58, 27/54, 27/22; C08J 9/26; A61K 35/12, 35/13, 35/14, 35/15, 35/17, 38/16 (2016.01)
CPC: A61L 27/54, 27/56, 27/3683, 27/3691, 27/38, 27/58; C08J 9/26; A61K 35/12, 35/13, 35/15, 35/17, 39/0011, 38/193

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; Pubmed; EBSCO
Keywords: polymer, scaffold, antigen, immune cell, attract, trap, collect, eliminat*, dendritic cells, T-cells, chemokines, intravenous, subcutaneous, micorneedle, patch, autoimmune disease, cancer, microb*, infection, treat*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0020216 A1 (MOONEY, DJ et al.) 27 January 2011; abstract; paragraphs [0006], [0012]-[0018], [0020]-[0022], [0024], [0031], [0035], [0037], [0039]-[0040], [0042], [0046], [0068], [0070], [0076]-[0077], [0083]; claims 8, 26-27	1-8, 14-20, 22, 33-37, 39, 42-50, 58, 60-62 --- 9-13, 21, 23-32, 38, 40-41, 51-57, 59, 63-66
Y	US 2012/0100182 A1 (MOONEY, DJ et al.) 26 April 2012; paragraphs [0079], [0098]-[0099], [0190], [0194]	9-13, 21
Y	(DESHMANE, SL et al.) Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. J Interferon Cytokine Res. June 2009; Vol. 29, No. 6; pages 313-326; page 315, column 1, paragraph 2; page 318, column 2, paragraph 3; table 1; DOI: 10.1089/jir.2008.0027	23-24
Y	(PATTERSON, AM et al.) Differential Binding of Chemokines to Macrophages and Neutrophils in the Human Inflamed Synovium. Arthritis Res. 31 January 2002; Vol. 4, No. 3; pages 1-9; page 5, column 2, paragraph 3; page 7, column 1, paragraph 1	25-26
Y	(IELLEM, A et al.) Unique Chemotactic Response Profile and Specific Expression of Chemokine Receptors CCR4 and CCR8 by CD4+CD25+ Regulatory T Cells. J Exp Med. 17 September 2001; Vol. 194, No. 6; pages 847-853; page 847, column 1, paragraph 1; page 848, column 1, paragraph 2; page 851, column 1, paragraph 3	27-28
Y	(AMBROSINI, E et al.) Astrocytes Produce Dendritic Cell-Attracting Chemokines In Vitro and in Multiple Sclerosis Lesions. J Neuropathol Exp Neurol. August 2005; Vol. 64, No. 8; pages 706-715; page 709, column 1, paragraph 1	27-28

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2016 (14.06.2016)	11 JUL 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/26617

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(WEEKS, S et al.) The Effects of Chemokine, Adhesion and Extracellular Matrix Molecules on Binding of Mesenchymal Stromal Cells to Poly(L -lactic acid). Cytotherapy. 19 July 2012; Vol. 14, No. 9; pages 1080-1088; abstract; page 1083, column 2, paragraph 2; DOI: 10.3109/14653249.2012.700704	29-30
Y	(KIM, J et al.) Injectable, Spontaneously Assembling Inorganic Scaffolds Modulate Immune Cells in vivo and Increase Vaccine Efficacy. Nat Biotechnol. 8 December 2014; Vol. 33, No. 1; pages 1-25; page 12, paragraph 3; DOI: 10.1038/nbt.3071	31-32
Y	WO 2013/172967 A1 (EXTEND BIOSCIENCES, INC) 21 November 2013; page 47, paragraph 3	38
Y	US 8067237 B2 (MOONEY, DJ et al.) 29 November 2011; column 9, lines 18-19; column 25, lines 15-17; column 27, lines 16-19, 25-56; figures 15A-15B, 16C	40-41, 51-57, 59, 63
Y	US 2002/0045672 A1 (HARRIS, LJ et al.) 18 April 2002; abstract; paragraphs [0004], [0007], [0019], [0021], [0065], [0071]; claims 1, 8-9, 14	64-66