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(54) **Title:** IMMUNOMODULATION WITH IMPLANTATION OF CELLULAR ALLOGRAFT BONE INTO A NON-BONY SITE

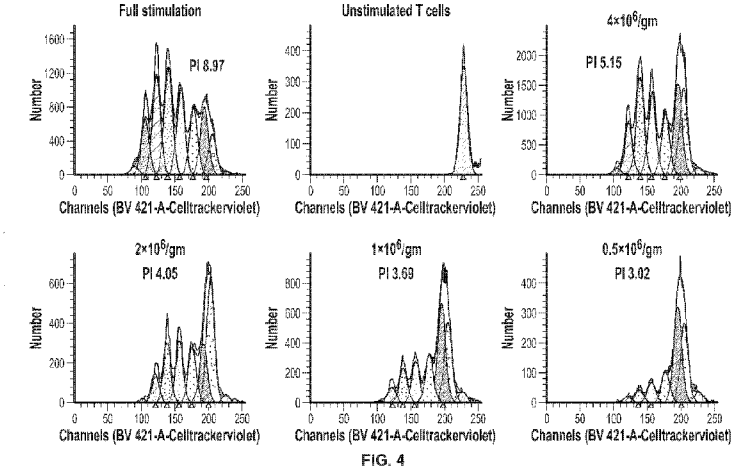

FIG. 4

(57) **Abstract:** An implant for the treatment of immune disorders is provided. Methods for use of the implant in the suppression of an immune response and treatment of autoimmune disorders are provided.

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IMMUNOMODULATION WITH IMPLANTATION OF CELLULAR ALLOGRAFT

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Patent Application Serial No. 62/263,945, filed on December 7, 2015, the entire contents of which are hereby expressly incorporated by reference into this disclosure as if set forth in their entireties herein.

FIELD

The present disclosure relates generally to a method of treating autoimmune diseases, specifically implantation of immunodepleted cellular allograft bone in a confined non-resorbable container in a soft tissue (non-bony) site. Such methods and devices are described.

BACKGROUND

Multipotential stromal cells including mesenchymal stem cells (hereafter "MSCs") have been used in the treatment of autoimmune diseases. MSCs were first used as graft-enhancing agents to prevent graft vs. host disease (GVHD) in patients receiving hematopoietic stem cell transplants. MSCs have low acute toxicity, and their apparent immune privilege, relative availability (e.g., bone marrow, adipose tissue, placental products), and capacity for ex vivo expansion has expanded the clinical application to include ischemic injury such as myocardial infarction (MI). There are numerous case studies reporting the effect of MSC treatment on the immunomodulation of autoimmune disorders including scleroderma, multiple sclerosis (MS), Crohn's, lupus nephritis, systemic lupus erythematosus (SLE).

MSCs have been shown to inhibit the proliferation and function of T cells, natural killer T (NKT) cells, regulatory T cells (Treg), B cells, and dendritic cells (DC). The immunomodulatory effect of MSC is mediated by cell-to-cell contact and by secretion of immune regulatory molecules. The MSC-derived soluble factors with immunosuppressive effects include prostaglandin E2 (PGE2), transforming growth factor-βî (TGF-β), indoleamine

2,3-dioxygenase (IDO), nitric oxide, hepatocyte growth factor (HGF), interleukin 6 (IL-6) and IL-10. MSCs are able to inhibit T cell proliferation induced by mitogens, alloantigens, and activation by cross-linking with CD3 and CD28 antibodies. MSCs can also modulate immune responses by induction of Tregs.

Autoimmune Disorders

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting many joints, including those in the hands and feet. Annual excess health care costs of RA patients are $8.4 billion, and costs of other RA consequences are $10.9 billion. These costs translate to a total annual cost of $19.3 billion.

Type 1 diabetes (T1D) is a chronic condition in which the pancreas produces little or no insulin. Each year type 1 diabetes costs in the United States are $14.4 billion (11.5-17.3) in medical costs and lost income. In terms of lost income, type 1 patients incur a disproportionate share of type 1 and type 2 costs. Further, if the disease were eliminated by therapeutic intervention, an estimated $10.6 billion (7.2-14.0) incurred by a new cohort and $422.9 billion (327.2-519.4) incurred by the existing number of type 1 diabetic patients over their lifetime would be avoided.

Multiple sclerosis (MS) is a disease in which the immune system eats away at the protective covering of nerves. Patients with MS have healthcare expenses of typical patients with health insurance, with some studies suggesting average annual direct treatment costs exceeding $20,000 per patient. Nationwide treatment costs in the United States exceeded $7 billion per year and MS treatment costs have risen during the past several years with the introduction of disease-modifying agents, such as interferon beta and glatiramer acetate.

Vasculitis is an inflammation of the blood vessels that causes changes in the blood vessel walls.

Alopecia areata is a sudden hair loss that starts with one or more circular bald patches that may overlap.

Lupus (SLE) is an inflammatory disease caused when the immune system attacks its own tissues. The mean annual total costs of lupus in the United States (combining direct costs and productivity costs for subjects of employment age) was $20,924. The Lupus Foundation of America estimates that 1.5 million Americans have a form of lupus equating to approximately $31 billion.

Polymyalgia rheumatic is an inflammatory disorder causing muscle pain and stiffness around the shoulders and hips.

Ankylosing spondylitis (AS) is an inflammatory arthritis affecting the spine and large joints. Although ankylosing spondylitis affects 0.1-0.2% of the population, the economic consequences of AS are largely unknown. Annual total costs averaged $6,720 (in 1999 US dollars; median $1,495). Indirect costs comprised 73.6% and direct costs comprised *26.4%* of total costs.

Temporal arteritis is an inflammation of blood vessels, called arteries, in and around the scalp.

Sjogren's syndrome is an immune system disorder characterized by dry eyes and dry mouth. With upwards of 4,000,000 Americans suffering from Sjogren's, it is one of the most prevalent autoimmune disorders. Nine out of 10 patients are women. About half of the time Sjogren's occurs alone, and the other half it occurs in the presence of another autoimmune connective tissue disease such as rheumatoid arthritis, lupus, or scleroderma.

Celiac disease is an immune reaction to eating gluten, a protein found in wheat, barley, and rye. Up to 66% of patients with celiac disease also have osteopenia or osteoporosis. Up to 51.4% of those with celiac disease also have neurologic disorders. Healthcare costs per untreated celiac in the US is between $5,000 - $12,000 annually. Total US healthcare cost for all untreated celiacs is $14.5 - $34.8 billion annually.

Each of these disorders is characterized by the inappropriate activation of the patient's immune system, resulting in an autoreactive immune response that leads to the destruction of

self-tissues and the manifestation of the symptoms of the disorder. Those symptoms vary depending upon the target of the immune response. As detailed above, the costs of these autoimmune disorders, including treatment and loss of productivity, exceeds $100 billion annually. The disorders also result in a loss of quality of life for the patients, and in many cases a reduced life expectancy. Despite decades of research, currently there is no method for preventing or curing these disorders, merely treatment of the symptoms. Thus, it is clear that there is a need for a better mechanism to suppress the immune systems of the individuals suffering from these disorders in order to improve quality of life measures, and decrease the costs of the disorders and their treatment.

MSCs Suppress Immune Activation

MSCs have been shown to inhibit immune cell proliferation, maturation, and function both *in vitro* and *in vivo,* in a non-MHC restricted manner. They express low levels of major histocompatibility complex (MHC) class I, no MHC class II, and have low expression of costimulatory molecules. *Ex vivo* expanded MCSs have been shown to affect the activation of T cells, NKT cells, DCs, B cells, neutrophils, monocytes, and macrophages.

In vitro studies have demonstrated that suppression of T cell function appears to be mediated by soluble factors including PGE2, IDO, hepatocyte growth factor (HGF), and TGF-βï . It has also been established that IFN-γ plays a role in the T cell-suppressive activity of MSCs. Some effects of MSC on NKT cells appear to require cell-to-cell contact. Other effects are mediated by soluble factors including PGE2 and TGF-βI. MSC can suppress several functions of B cells including proliferation, differentiation, chemotaxis, and migration. MSCs block the differentiation and maturation of DCs. DCs matured in the presence of MSCs showed reduced up-regulation of costimulatory molecules CD80 and CD86, as well as preventing upregulation of MHC class II expression. Co-culture of monocytes with MSCs has been shown to inhibit their differentiation to DCs.

In vivo studies in mice have also demonstrated the immunomodulatory effects of MSCs.

Administration of MSCs in mouse models have been shown to prolong the survival of skin grafts, ameliorate experimental autoimmune encephalomyelitis (a murine model of multiple sclerosis), ameliorate autoimmune type 1 diabetes pathenogenesis, and protect from severe sepsis.

There have also been promising clinical data from treatment of immune diseases in human patients. Injection of MSCs has been shown to improve GVHD, including a complete recovery in some patients. The effects were observed for MSCs isolated from both HLA-matched and HLA-mismatched bone marrow aspirate donors.

Numerous clinical trials are underway to test the efficacy of MSC treatment in various autoimmune disorders including MS, RA, Crohn's disease, and SLE. These trials have demonstrated the relative safety of MSC treatment, with no severe adverse effects being observed.

Immune disorders including GVHD, HVGD, and autoimmune reactions against self-tissues affect a large population of individuals worldwide, and the incidence of autoimmune disorders is on the rise. While antigen-specific treatments for autoimmune disorders are being contemplated, due to the complexity of the disorders and the variability in the immune responses between individuals suffering from the same disorder, such treatments may be of limited efficacy. Further, the compounds used for systemic immunosuppression to prevent GVHD and HVGD result in an increased risk of certain types of cancers. Intravenous infusion of MSCs has been used for systemic immunomodulation as a treatment for GHVD, and more recently in clinical trials for autoimmune disorders. MSC treatment is generally well-tolerated, without the adverse effects of other immunosuppressive treatments. However, infused MSCs have a relatively short life span. Thus, there is a need in the art for an alternative mechanism for delivery of MSCs to treat GVHD and other autoimmune conditions wherein the MSCs are retained and not rapidly destroyed, to provide a sustained immunomodulatory effect. This application addresses this need.

SUMMARY

The needs above, as well as others, are addressed by embodiments of an implant for treatment of immune disorders which comprises cellular allograft bone material contained inside a biocompatible container.

A method for suppressing an immune response in a patient is provided. The method comprises implanting the one of the implants described herein in a non-bony tissue of the patient.

A method for treating an autoimmune disorder in a patient is provided. The method comprises implanting the one of the implants described herein in a non-bony tissue of the patient.

The above presents a simplified summary in order to provide a basic understanding of some aspects of the claimed subject matter. This summary is not an extensive overview. It is not intended to identify key or critical elements or to delineate the scope of the claimed subject matter. Its sole purpose is to present some concepts in a simplified form as a prelude to the more detailed description that is presented later.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Flow cytometry gating strategy of MSCs and enumeration and using MSC specific markers, HSC specific markers and FACS counting beads

FIG 2. Egression outcome corrected to mg of weight of the cancellous bone samples

FIG. 3. Paired comparison of the egression outcome corrected to mg of weight of the cancellous bone samples

FIG. 4. Immunomodulation tracker dye dilution plots, Modfit software showing unstimulated T-cells and full stimulated T-cells at day5, the second row of figures represents dye dilution plots of the bone mixed with increasing concentrations of T-cells (from 0.5 to $4xl0^{6}$/gram of bone).

FIG. 5. T-cell suppression assay showing immunomodulatory effects of cancellous bone as

described by T-cell proliferation index.

FIG. 6. PGE2 levels in the supernatants harvested after 5 days of immunomodulation incubation.

FIG. 7. TGF-βI levels in the supernatants harvested after 5 days of immunomodulation incubation.

DETAILED DESCRIPTION

Illustrative embodiments of methods and devices for immunomodulation through implantation of cellular allograft bone are described below. In the interest of clarity, not all features of an actual implementation of the methods and devices are described in this specification. It will of course be appreciated that in the development of any such actual methods and devices for implantation, numerous implementation-specific decisions must be made to achieve the developers' specific goals, such as a compliance with system-related and business-related constraints, which will vary from one implementation to another. Moreover, it will be appreciated that such a development effort might be complex and time-consuming, but would nevertheless be a routine undertaking for those of ordinary skill in the art having the benefit of this disclosure. The methods and devices for immunomodulation through implantation of cellular allograft bone disclosed herein boast a variety of inventive features and components that warrant patent protection, both individually and in combination.

Definitions

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art of this disclosure. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well known functions or constructions may not be described in detail for brevity or clarity.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

The terms "first", "second", and the like are used herein to describe various features or elements, but these features or elements should not be limited by these terms. These terms are only used to distinguish one feature or element from another feature or element. Thus, a first feature or element discussed below could be termed a second feature or element, and similarly, a second feature or element discussed below could be termed a first feature or element without departing from the teachings of the present disclosure.

The term "consisting essentially of means that, in addition to the recited elements, what is claimed may also contain other elements (steps, structures, ingredients, components, etc.) that do not adversely affect the operability of what is claimed for its intended purpose as stated in this disclosure. Importantly, this term excludes such other elements that adversely affect the operability of what is claimed for its intended purpose as stated in this disclosure, even if such other elements might enhance the operability of what is claimed for some other purpose.

The terms "about" and "approximately" shall generally mean an acceptable degree of error or variation for the quantity measured given the nature or precision of the measurements. Typical, exemplary degrees of error or variation are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. For biological systems, the term "about" refers to an acceptable standard deviation of error, preferably not more than 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

The terms "prevention", "prevent", and "preventing", as used herein refer to a course of action initiated prior to the onset of a clinical manifestation of a disease state or condition so as to prevent or reduce such clinical manifestation of the disease state or condition. Such preventing need not be absolute to be useful.

The terms "suppression", "suppress" and "suppressing" as used herein refer to a detectable reduction in the amount or severity of a clinical manifestation of a disease state or condition. Such reduction need not be absolute to be useful.

The terms "treatment", "treat" and "treating" as used herein refers a course of action initiated after the onset of a clinical manifestation of a disease state or condition so as to eliminate or reduce such clinical manifestation of the disease state or condition. Such treating need not be absolute to be useful.

The term "in need of treatment" as used herein refers to a judgment made by a caregiver that a patient requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the patient is ill, or will be ill, as the result of a condition that is treatable by a method or device of the present disclosure.

The term "individual", "subject" or "patient" as used herein refers to any animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and humans. The term may specify male or female or both, or exclude male or female.

The term "therapeutically effective amount" as used herein refers to an amount that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease state or condition. Such effect need not be absolute to be beneficial.

The terms "multipotential stromal cells", "mesenchymal stem cells", or "MSCs" as used herein refers to $CD45^{-}CD271^{+}CD90^{+}CD73^{+}$ multipotent cells capable of proliferation in culture, with a fibroblast-like morphology.

The term "cellular allograft" as used herein refers to bone and cells donated from a human and processed such that cells maintain their viability. The cells may be native to the harvested bone and retained in the processed bone fragments, or the cells may be harvested from other tissue types and added to the bone. Generally, the allograft bone is derived from

cadaveric donors. Processing makes the allograft bone suitable for implantation into a human patient.

The term "matrix" as used herein refers to fragments of cancellous bone, whether finely ground or in large chips, which contains the viable cells native to the harvested bone.

The term "implant" as used herein refers to the cellular allograft bone matrix packed into a containment device and suitable for implantation into a subject.

The term "autoimmune condition" as used herein refers to any immune condition in a patient which results in an undesirable and pathogenic immune activation against the patient's own tissue, or against tissues transplanted into the patient. Such conditions include, without limitation, graft versus host disease (GVHD), host versus graft disease (HVGD), rheumatoid arthritis, type 1 diabetes, multiple sclerosis, vasculitis, alopecia areata, lupus, polymyalgia rheumatic, ankylosing spondylitis, temporal arteritis, autoimmune thyroiditis, myasthenia gravis, Sjogren's syndrome, Addison's disease, and Crohn's disease, and celiac disease.

The term "immunomodulatory molecule" as used herein refers to any molecule released by MSCs which is capable of effecting the proliferation or activation of the cells of a patient's immune system. Such molecules include, without limitation, prostaglandin E2 (PGE2), transforming growth factor-βï (TGF-β), indoleamine 2,3-dioxygenase (IDO), nitric oxide, hepatocyte growth factor (HGF), interleukin 6 (IL-6) and interleukin 10 (IL-10).

Enhanced donor screening and the use of highly sensitive viral nucleic acid testing has increased the safety of allografts by reducing the risk of disease transmission from donor to recipients. Implementation of enhanced screening has increased the usage of allografts in bone repair treatments. While prior treatments resulted in the complete removal of donor cells, in recent years there has been a shift toward "viable" cellular bone grafts. These grafts incorporate the selective removal of donor immune cells, while retaining the osteogenic and non-immune cells. The allograft material consists of "viable" cancellous bone fragments and

acellular/nonviable demineralized bone matrix (DBM) particles. The cells remaining in a viable cellular bone graft include MSCs. It has been found that the proportion of native MSCs in cellular allograft material exceeds the number in freshly obtained iliac crest (IC) and bone marrow (BM) aspirate.

Flow cytometric analysis of cell populations from cultures of freshly-thawed cellular bone allograft material has shown the cells to be positive for expression of MSC markers CD73, CD90, and CD 105, and negative for expression of hematopoietic-lineage markers such as CD45, CD19, and CD14. Further comparison of the cell populations, compared with freshly prepared IC bone samples showed a greatly increased proportion of $CD45^{-}CD271^{+}$ MSCs in the cellular bone allograft (avg. 30.4%, n=9) compared to age-matched IC bone (avg. 0.3%, n=6). The percentage of $CD45^{+}CD271^{-}$ hematopoietic-lineage cells (HLC) was much lower in cellular bone allograft (5.6%, range 3.1-9.1%) compared to IC bone (93%, range 89-95%). Thus, there was an average 101-fold increase in MSC in the cellular bone allograft than in age-matched IC bone. The average ratio of MSCs to HLCs was 6:1 in the cellular allograft, and 1:370 in the IC bone. Thus, during processing, there is efficient removal of HLC from the cellular bone allograft. A comparison of the total number of $CD45^{+}CD271^{-}$ cells indicated an average of 1.3 million HSCs/g of IC bone and 0.53 million HSCs/g of cellular bone allograft.

Example 1: Identification of MSCs in cancellous bone

The cancellous bone chips were frozen at -80°C to mimic the standard preparation of the cellular allograft preparations. Cancellous bone chips were washed thoroughly with PBS, weighed using digital electronic scale and placed in 0.5 mL 0.25% collagenase (Stem Cell Technologies, Grenoble, France) per 0.2 g of tissue and digested (1 gram of cancellous bone fragments used per each sample) for 4 hours with gentle rotation to obtain single-cell suspensions in slow rotator 37 °C incubator. The liquid fraction containing cells was filtered through a 70 μm filter (Miltenyi biotec), centrifuged (400 x g for 5 min), washed in PBS and diluted in MACS buffer (PBS, 0.5% bovine serum albumin and 2 mM EDTA). Nonspecific

binding was blocked using FcR blocking reagent (Miltenyi Biotec) at 1:10 ratio in MACs buffer and incubated for 10 min. The cells were stained with CD90-FITC, CD105-PE (both from BioRad, UK), CD73-PerCP-Cy5.5, CD45-PE-Cy7 (both from BD Biosciences), CD271-APC (Miltenyi Biotec), aqua fluorescent dye and Calcein AM from the live/ dead violet viability/vitality kit (Thermofisher). The cells were incubated for 30 min in the Fridge 2-8 °C in the dark before adding 50 μL of CountBright beads (Molecular Probes/Life Technologies, Paisley, UK) to permit absolute cell quantification. Total volume was 450 μL. The total number of CD271+ (MSCs) and CD45+ (HCs) per gram of bone was calculated.

Fig. 1 shows representative flow cytometry profiles and gating strategies to identify the MSC and HSC populations. Gating was performed to identify live cells (Fig. IB). Staining for CD271 and CD45 was used to discriminate between the MSC and HSC populations (Fig. 1C). The CountBright beads allowed enumeration of cells (Fig. ID).

Example 2: Egression velocity and MSC growth rates in explant cultures.

Cancellous bone chips were washed thoroughly with PBS and transferred into 100 mm culture dishes containing 10 mL of PBS. At least 5 replicates per donor sample were placed into 6-well culture plates. Explant cultures were initiated by replacing PBS with 15 mL of non-hematopoietic expansion medium (Miltenyi Biotec, Bisley, UK). Six-well culture dishes were fed twice-weekly with half-media changes each time. The cultures were monitored weekly for 6th weeks and then evaluated. The outgrowth cells were trypsinized and manually counted and frozen.

Egression off of the chips was comparable between cancellous bone paired samples. The Sample 2 egression average 680 MSC cell/mg (Range 240-1320) compared to the Sample 1 average 768 MSC cell/mg (Range 211-1778). (Fig. 2) Pairwise there was no significant difference between the egression between samples. (Fig. 3)

Example 3: Immunosuppression by allograft bone in co-culture with T-cells

Single donor T-cells were purified from peripheral blood mononuclear cells by

depletion of non-CD4+ cells using a CD4+ T-cell isolation kit (Miltenyi Biotec) and depleted from Tregs (CD25+) cells by positive selection. The T-cells were labeled with the carbocyfluorescein succinimidyl ester dye analogue, CellTrace™ Violet (Invitrogen). T-cell proliferation was stimulated using MSC Suppression Inspector containing Anti-Biotin MACSiBead™ particles preloaded with biotinylated CD2, CD3, and CD28 antibodies (Miltenyi Biotec) according to the weight of the cancellous bone fragments. Cancellous bone chips (replicates of the same weight) were placed in 6-well culture plates and co-cultured with different increasing ratio of T-cells. The cancellous bone chips (MSCs): T-cell ratios were calculated based on weight of the fragments and worked out to be 0.5, 1.0, 2.0, 4.0, and 8.0 x 10^6 T-cells / gm of the cancellous bone. Control groups included: Cancellous bone chips only Labeled but not stimulated T-cells (no stimulation control) Labeled but not stimulated T-cells with bone chips (bone stimulation control) Labeled and stimulated T-cells without MSCs (No MSC control) Co-cultures were maintained in Glutamax™ RPMI 1640 (Invitrogen) in 24 well plate, 1 mL per well), supplemented with 10% human AB serum (Sigma, Dorset, UK) for 5 days. At 5 days, the cells were pelleted by centrifugation and the liquid supernatant fraction from each well was collected and frozen (-80 °C) for later detection of cytokine production. T-cell proliferation suppression was detected by divisions/generations detection and calculated using ModFit software version 3.2 (Verity Software House, Topsham, ME. USA). MSC suppression was calculated using relative division index; and calculated as an 'average' T-cell generation (calculated as a geometric mean) in MSC-containing wells and normalized to the 'average' T-cell generation in no MSC control (100%).

Immunomodulation power of the cancellous bone samples was assessed by T-cell proliferation assay, all pairs tested showed significant suppression of T-cell proliferation. The T-cell proliferation was evaluated using the Modfit software and Proliferation index was used as the measuring output for that (Fig. 4). Modfit software showing unstimulated T-cells and full stimulated T-cells at day5, the second row of figures represents dye dilution plots of the

cancellous bone samples and mixed with increasing concentrations of T-cells (from 0.5 to 4xl0 ^{6}/gram of bone).

The proliferation index for each condition was corrected for each pair of bone samples towards the full stimulation and plotted (Fig. 5).

The supernatants collected from immunomodulation set of experiments were analyzed for PGE2 (Fig. 6) and TGF-βI (Fig. 7) using ELISA. The analysis revealed that viable bone chips product both PGE2 and TGF-βI. This indicates that immunomodulation effect was due not only to direct contact between cancellous bone and T-cells but also due to remote effect via soluble mediators. Spontaneous secretion of both mediators was noted as indicated by their detection in supernatants of bone cultured in the absence of T-cells (control groups).

Conclusions

Cancellous bone pairs were tested for their content of MSCs and HSCs. Immunomodulation capacity of cancellous bone samples showed immunomodulatory suppression of the activated CD4+ (Tregs depleted) T-cell proliferation, as indicated by the reduction in the proliferation index. Secretion of TGF-βI and PGE2 (immunomodulation secretory molecules) was also similar between the pairs.

Allograft Implants

According to an exemplary embodiment, cancellous bone is harvested from a donor, washed to remove blood and other tissue and processed to selectively remove hematopoietic lineage cells (i.e. immunodepleted). The immunodepleted cancellous bone comprises viable bone lining cells, which are mesenchymal stem cells, osteoprogenitor cells and osteoblasts as well as the mineral-embedded osteocytes. According to some embodiments, the cancellous bone is milled into a particulate. According to an exemplary embodiment, the immunodepleted cancellous bone is combined with a cryopreservative and stored in a sealed container and may be frozen until it is used for treatment.

In an alternative embodiment, MSCs are extracted from adipose tissue, bone marrow or

the cancellous bone, cultured and expanded before the expanded culture is re-introduced and attached to the contained cancellous bone matrix to enhance the number of MSCs in the cancellous bone. In such embodiments, the culture and expansion process may increase the MSC population in the cellular allograft implant beyond the MSCs that are native to the donor bone. In alternative embodiments, autologous MSCs from adipose tissue or bone marrow are added to the cellular allograft bone without expansion in culture.

In an exemplary embodiment, an implant is prepared by separating the cellular allograft from the cryopreservative and packing or placing the cellular allograft into a biocompatible containment device. The containment device may be formed in any shape, size or configuration to fit in a desired location and may include at least one attachment member in any location thereon. The containment device should be sufficiently porous to allow the free diffusion of immunosuppressive molecules, yet also retain the bone matrix within the device. Any suitable technique may be used to form the containment device. For example, the device may be formed as a sheet, a mesh, or as a textile-type material. Where it is formed as a sheet, any of the techniques Icnown to those of ordinary skill in the art may be used to make the outer surface of the containment device porous to allow the diffusion of immunomodulatory molecules. In some embodiments, the enclosure may be rigid. In other embodiments, the enclosure may flexible and capable of deformation.

In some embodiments, the amount of cellular allograft in the implant is between approximately 5cc and approximately 40cc. In some embodiments the amount of cellular allograft is approximately 5, 10, 15, 20, 25, 30, 35, or 40cc or even dosed based upon the patients weight (XXcc/Kg).

In one embodiment, the biocompatible containment device may be a non-resorbable pre-formed enclosure with pores of a size to allow the free diffusion of cytokines and chemokines, while retaining the bone matrix within the enclosure. In another embodiment, the biocompatible containment device is a non-resorbable woven mesh bag or a radiologically

traced bag that is porous enough to allow immunomodulatory molecules to pass, but still contain the cellular bone matrix.

In some embodiments, the biocompatible container is non-resorbable. Implantation of the cellular allograft in a non-resorbable container allows the cancellous bone to be contained within a confined space and prevents dispersal of the cancellous bone. Examples of a suitable non-degradable biocompatible materials include, but are not limited to, polytetrafluoroethylene, perfluorinated polymers such as fluorinated ethylene propylene, silicone elastomer, polyurethane, polyethylene, polyethylene teraphthalate, polysulfone, non-degradable polycarboxylate, non-degradable polycarbonate, non-degradable polyester, polypropylene, polymethylmethacrylate, poly(hydroxymethacrylate), and polyamide such as polyesteramide, and copolymers, block copolymers, and blends thereof.

In some embodiments, the biocompatible containment device is biodegradable. Examples of a suitable degradable biocompatible material include, but are not limited to, non-highly cross-linked collagen, non-highly cross-linked hyaluronic acid, hydrolyzable polyester such as polylactic acid and polyglycolic acid, polyorthoester, degradable polycarbonate, degradable polycarboxylate, polycaprolactone, polyanhydride, and copolymers, block copolymers, and blends thereof. The implant, comprising a containment device and the cellular allograft bone matrix, may be implanted into a non-bony soft tissue site of a patient in need of treatment. In one embodiment, the implant is implanted into the intraperitoneal space. In an alternative embodiment, the implant is placed within the lymph system for systemic effect. In yet another embodiment, the implant is placed near a diseased organ for direct focused effect. Placement of the implant near a target organ may be suited to the therapy of organ transplantation rejection reactions (HVGD).

A Method of Suppressing an Immune Response

According to one embodiment of a method for suppressing an immune response, an implant containing a therapeutic dose of the cellular allograft bone is implanted into a subject.

In some embodiments, the subject is a patient in need of treatment for an immune condition. In some embodiments, the immune condition is an autoimmune disorder. The implant described herein may be used to treat a variety of autoimmune diseases including but not limited to: rheumatoid arthritis, type 1 diabetes, multiple sclerosis, vasculitis, alopecia areata, lupus, polymyalgia rheumatic, ankylosing spondylitis, temporal arteritis, Sjogren's syndrome, celiac disease, among others.

According to one embodiment of a method for suppressing an immune response in a patient in need thereof, the therapeutic dose of is between 5g and 75g of cellular allograft material comprising cancellous bone fragments. In some embodiments, the therapeutic dose is between 20g and 60g, or between 25g and 50 g of cellular allograft material.

According to one embodiment, the therapeutic dose of MSC for suppressing an immune response in a patient in need thereof, is between 1 million and 100 million MSCs per cc of allograft. In some embodiments, the therapeutic dose is between 2.5 million and 37.5 million MSCs per cc of allograft. In some embodiments, the therapeutic dose is between 5 million and 30 million MSCs per cc of allograft. In some embodiments, the therapeutic dose is between 10 million and 25 million MSCs per cc of allograft.

According to one embodiment of a method for suppressing an immune response in a patient in need thereof, the implant is implanted in the intraperitoneal space. In some embodiments, the implant is implanted near a lymph node. In other embodiments, the implant is implanted into a tissue or organ affected by an immune disorder.

According to one embodiment of a method for suppressing an immune response in a patient in need thereof, the implant is replenished to maintain the immunomodulatory effects of the treatment. In some embodiments, the replenishment consists of removing the implant from the patient, replacing the cellular allograft material within the containment device, and implanting the replenished implant in the patient. In some embodiments, the replenishment consists of removing the implant from the patient, supplementing the existing cellular allograft

material within the containment device, and implanting the replenished implant in the patient. In some embodiments, the replenishment consists of removing the implant from the patient and implanting a new implant in the patient. In some embodiments the replenished implant is implanted in the same location as the previous implant. In other embodiments, the replenished implant is moved to a different location.

According to one embodiment of a method for suppressing an immune response in a patient in need thereof, the implant is replenished at a predetermined interval to maintain the delivery of immunomodulatory factors. In other embodiments, the implant is replenished at regular intervals to maintain the delivery of immunomodulatory factors. In still further embodiments, the implant is replaced when a clinical indication of decreased immunomodulation is observed.

A Method of Treating Autoimmune Disease

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, an implant containing a therapeutic dose of the cellular allograft bone is implanted into a subject. In some embodiments, the autoimmune disease is one of: rheumatoid arthritis, type 1 diabetes, multiple sclerosis, vasculitis, alopecia areata, lupus, polymyalgia rheumatic, ankylosing spondylitis, temporal arteritis, Sjogren's syndrome, celiac disease, among others.

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, the therapeutic dose of is between 5g and 75g of cellular allograft material comprising cancellous bone fragments. In some embodiments, the therapeutic dose is between 20g and 60g, or between 25g and 50 g of cellular allograft material.

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, the therapeutic dose of MSCs is between 1 million and 100 million MSCs per cc of allograft. In some embodiments, the therapeutic dose of MSCs is between 2.5 million and 37.5 million MSCs per cc of allograft. In some embodiments, the therapeutic dose

is between 5 million and 30 million MSCs per cc of allograft. In some embodiments, the therapeutic dose is between 10 million and 25 million MSCs per cc of allograft.

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, the implant is implanted in the intraperitoneal space. In some embodiments, the implant is implanted near a lymph node. In other embodiments, the implant is implanted into a tissue or organ affected by the autoimmune disorder.

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, the implant is replenished to maintain the immunomodulatory effects of the treatment. In some embodiments, the replenishment consists of removing the implant from the patient, replacing the cellular allograft material within the containment device, and implanting the replenished implant in the patient. In some embodiments, the replenishment consists of removing the implant from the patient, supplementing the existing cellular allograft material within the containment device, and implanting the replenished implant in the patient. In some embodiments, the replenishment consists of removing the implant from the patient and implanting a new implant in the patient. In some embodiments the replenished implant is implanted in the same location as the previous implant. In other embodiments, the replenished implant is moved to a different location.

According to one embodiment of a method for treating an autoimmune disease in a patient in need thereof, the implant is replenished at a predetermined interval to maintain the delivery of immunomodulatory factors. In other embodiments, the implant is replenished at regular intervals to maintain the delivery of immunomodulatory factors. In still further embodiments, the implant is replaced when a clinical indication of decreased immunomodulation is observed.

CLAIMS

1. An implant for the treatment of an immune disorder in a patient in need thereof, said implant comprising a cellular allograft bone material contained within a biocompatible, container.

2. The implant of claim 1 wherein the cellular allograft material comprises therapeutic dose of immunodepleted cancellous bone chips with a population of viable cells.

3. The implant of claim 2 wherein the population of viable cells comprises MSCs.

4. The implant of any one of claims 1-3 wherein the cellular allograft bone material comprises at least 5g of immunodepleted cancellous bone chips.

5. The implant of any one of claims 2-4 wherein the therapeutic dose comprises at least 1 million MSCs per cc of cancellous bone chips.

6. The implant of any one of claims 2-5 wherein the population of viable cells in the cellular allograft material comprises between 25-100% MSCs.

7. The implant of any one of claims 2-6 wherein the population of viable cells in the cellular allograft material comprises between 50-95% MSCs.

8. The implant of any one of claims 2-7 wherein the population of resident cells in the cellular allograft material comprises between 75-90% MSCs.

9. The implant of any one of claims 1-8 wherein the cellular allograft material further comprises cultured MSCs.

10. The implant of claim 9 wherein the cultured MSCs are extracted from one of adipose tissue, bone marrow or cancellous bone.

11. The implant of any one of claims 1-10 further comprising an attachment mechanism on the container.

12. The implant of any claim 10 wherein the attachment mechanism is a tether.

13. The implant of any one of claims 1-12 wherein the biocompatible container is deformable.

14. The implant of any one of claims 1-13 wherein the biocompatible container is cylindrical in shape.

15. The implant of any one of claims 1-14 wherein the biocompatible container is formed from one or more non-resorbable materials.

16. The implant of any one of claims 15 wherein the non-resorbable material is selected from the group consisting of: polytetrafluoroethylene, fluorinated ethylene propylene, silicone elastomer, polyurethane, polyethylene, polyethylene teraphthalate, polysulfone, non-degradable polycarboxylate, non-degradable polycarbonate, non-degradable polyester, polypropylene, polymethylmethacrylate, poly(hydroxymethacrylate), and polyamide.

17. The implant of any one of claims 1-16 wherein the biocompatible container is a non-resorbable woven mesh bag.

18. The implant of any one of claims 1-17 wherein the biocompatible container is a non-resorbable radiologically traced woven bag.

19. The implant of any one of claims 1-14 wherein the biocompatible container is formed from one or more resorbable materials.

20. The implant of any one of claims 19 wherein the resorbable material is selected from the group consisting of: non-highly cross-linked collagen, non-highly cross-linked hyaluronic acid, polylactic acid, polyglycolic acid, polyorthoester, degradable polycarbonate, degradable polycarboxylate, polycaprolactone, and polyanhydride.

21. A method of suppressing an immune response in a patient in need thereof, said method comprising implanting the implant of any one of claims 1-20 in a non-bony tissue of the patient.

22. The method of claim 21 wherein the non-bony tissue is a site within an intraperitoneal cavity.

23. The method of any one of claims 21-22, wherein the non-bony tissues is a site proximal to a lymph node.

24. The method of any one of claims 21-23, wherein the non-bony tissue is a site proximal

to an organ affected by the immune disorder.

25. The method of any one of claims 21-24, further comprising replacing the implant at a specified time.

26. The method of claim 25, wherein the specified time is a predetermined interval.

27. The method of claim 25, wherein the specified time is a time point where a clinical indication is observed.

28. The method of claim 27, wherein the clinical indication is calcification of at least 30% of the implant.

29. A method of treating an autoimmune disorder in a patient in need thereof, said method comprising implanting the implant of any one of claims 1-20 in a non-bony tissue of the patient.

30. The method of claim 29 wherein the autoimmune disorder is selected from the group consisting of: rheumatoid arthritis, type 1 diabetes, multiple sclerosis, vasculitis, alopecia areata, lupus, polymyalgia rheumatic, ankylosing spondylitis, temporal arteritis, autoimmune thyroiditis, myasthenia gravis, Sjogren's syndrome, Addison's disease, Crohn's disease, and celiac disease.

31. The method of any one of claims 29-30 wherein the non-bony tissue is a site within the intraperitoneal cavity.

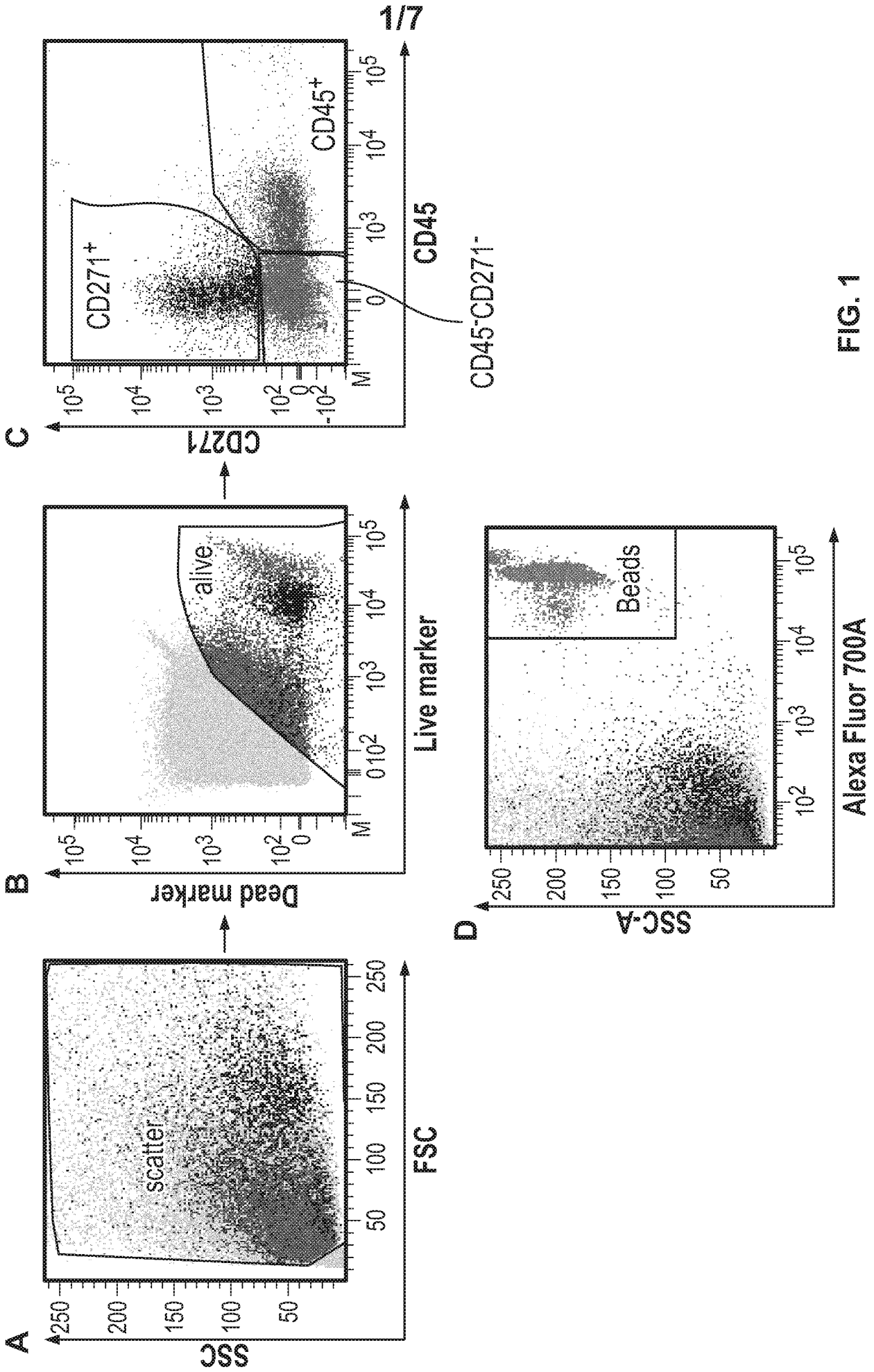
32. The method of any one of claims 29-31, wherein the non-bony tissues is a site proximal to a lymph node.

33. The method of any one of claims 29-32, wherein the non-bony tissue is a site proximal to an organ affected by the immune disorder.

34. The method of any one of claims 29-33, further comprising replacing the implant at a specified time.

35. The method of claim 34, wherein the specified time is a predetermined interval.

36. The method of claim 34, wherein the specified time is a time point where a clinical indication is observed.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B
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FIG. 1

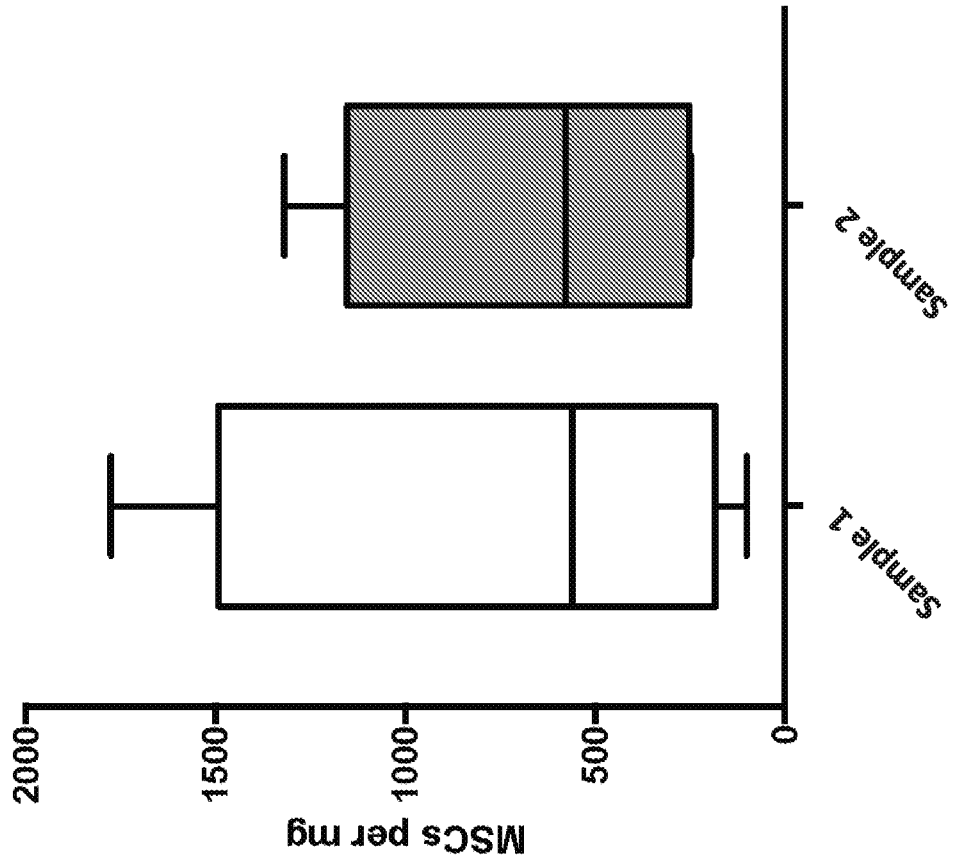
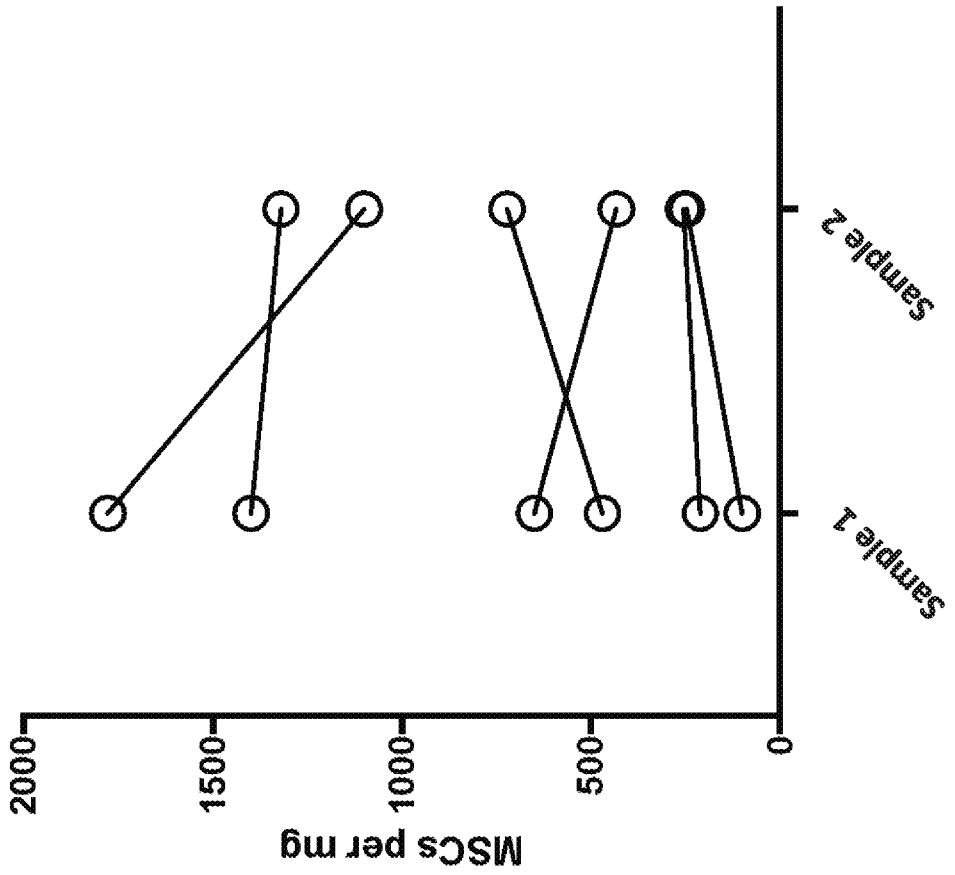
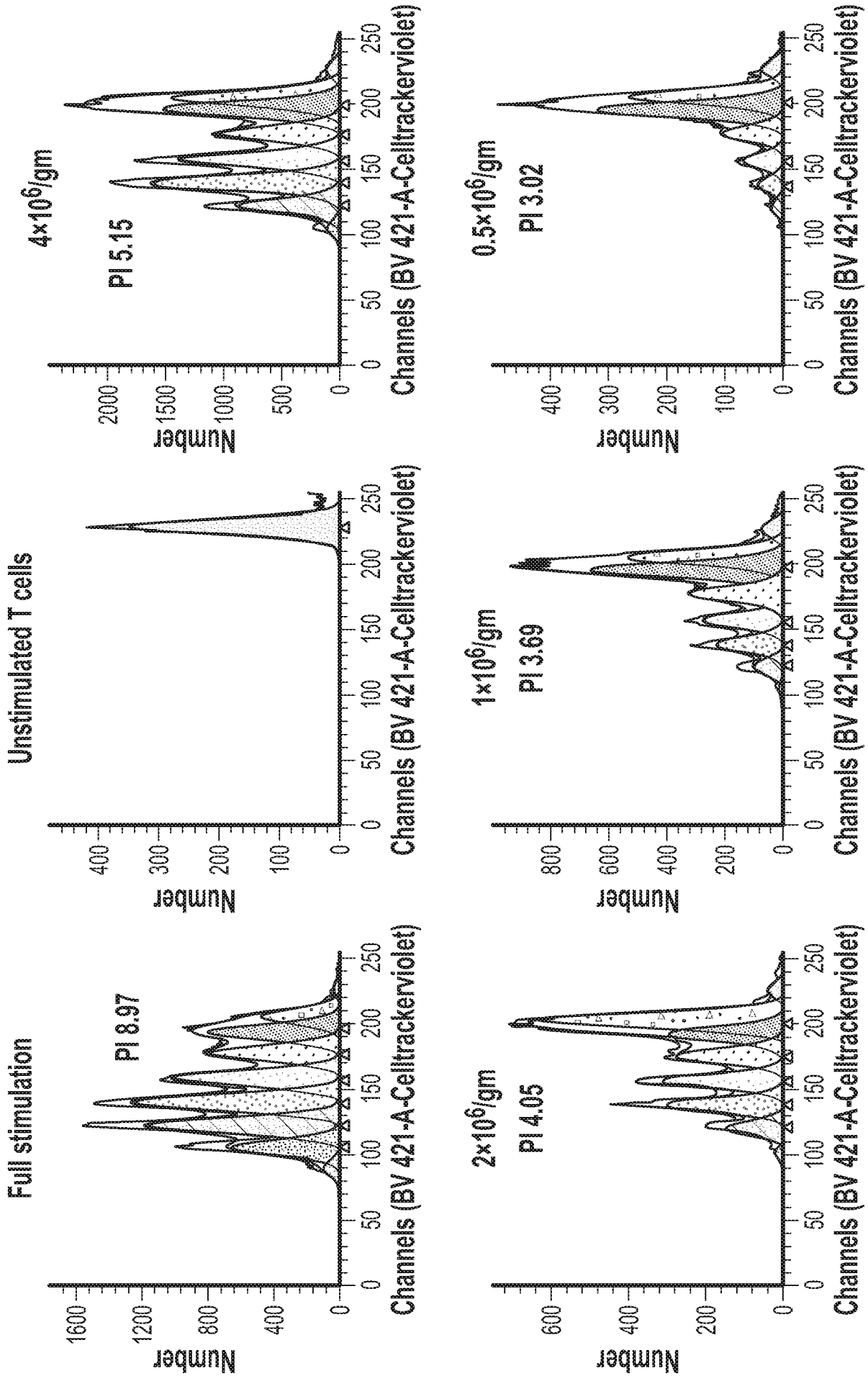


FIG. 2

FIG. 3

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Unstimulated T cells
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PI 5.15
2×10^6/gm
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0.5×10^6/gm
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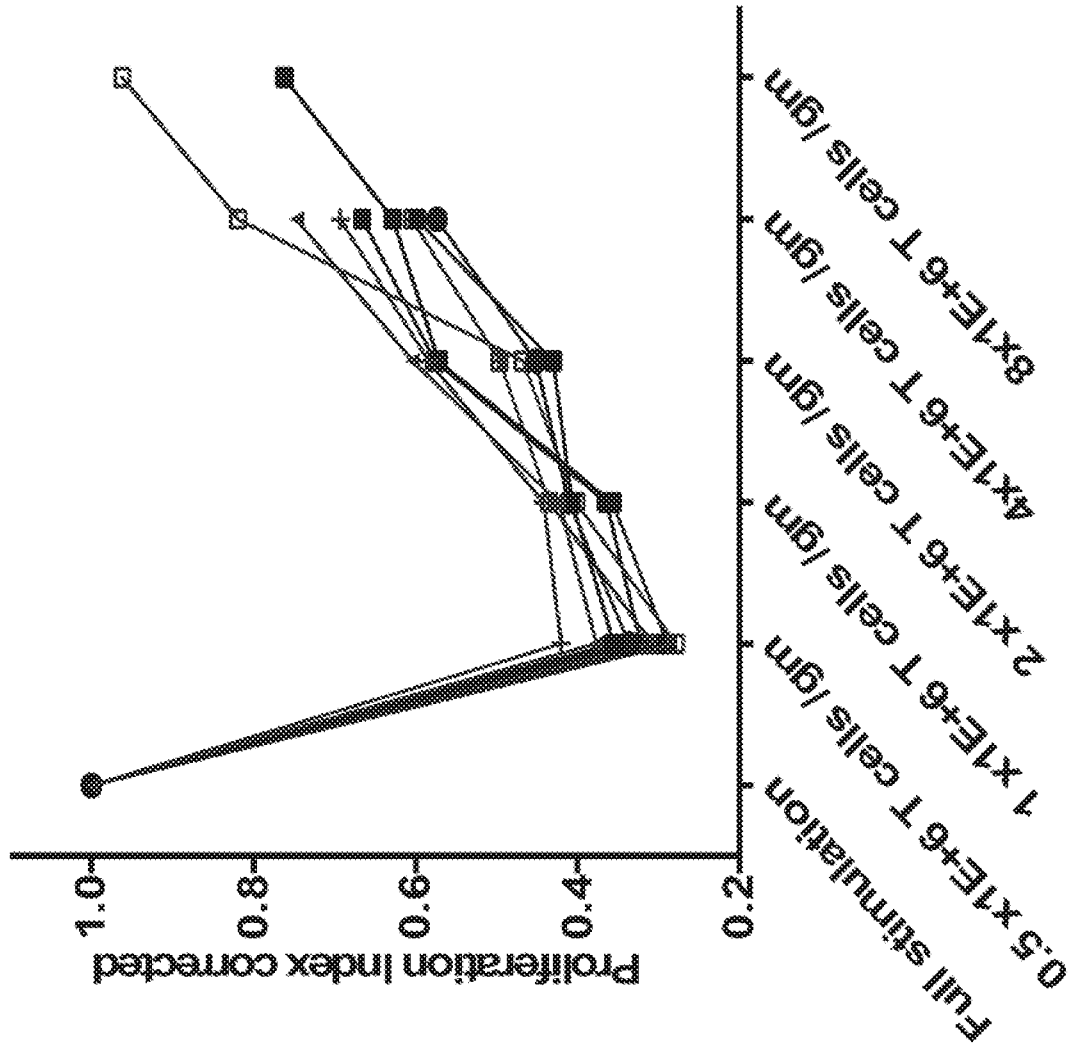
Number
Channels (BV 421-A-Celltrackerviolet)

FIG. 4

FIG. 5


Proliferation Index corrected
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0.8
0.6
0.4
0.2
Full stimulation
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2 x1E+6 T cells /gm
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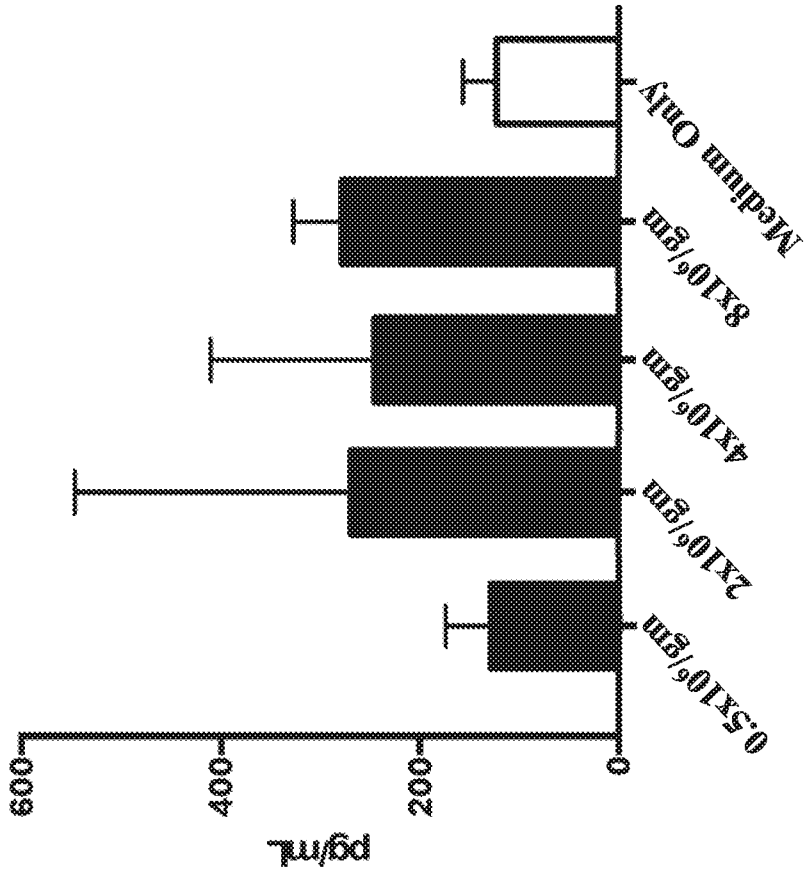
8x1E+6 T cells /gm

FIG. 6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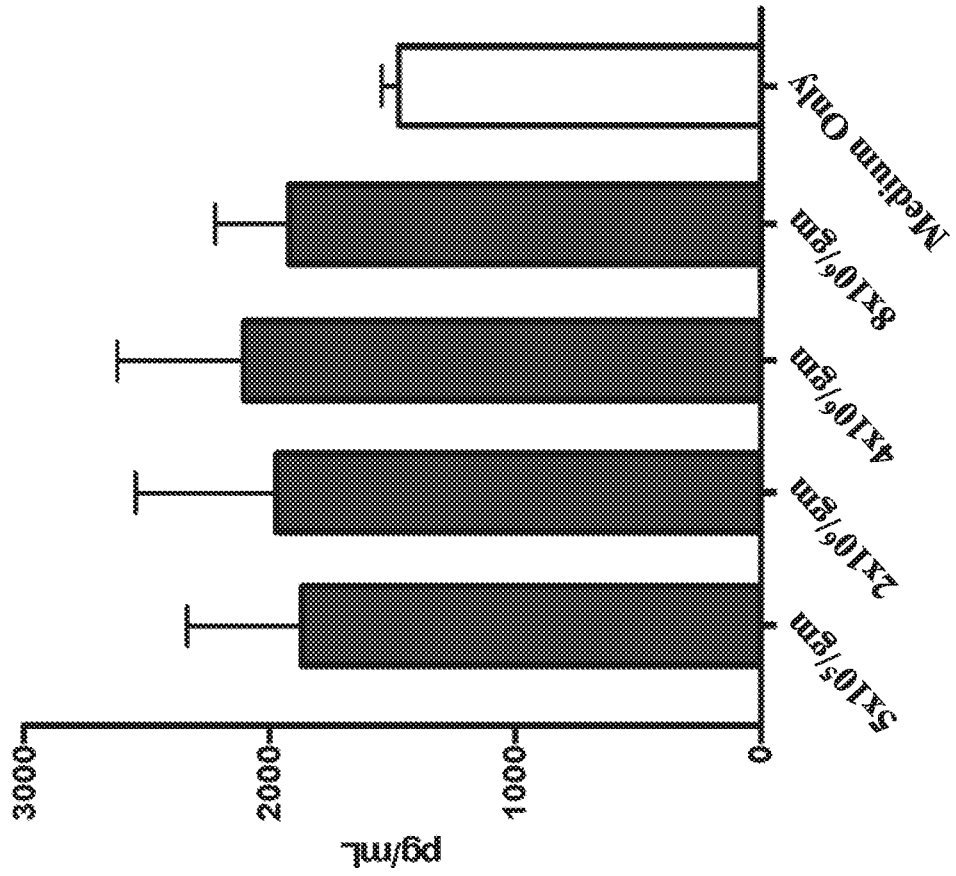
Medium Only

FIG. 7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Medium Only

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/65414

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/65414

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61F 2/28; A61L 27/40, 27/54, 27/56 (2017.01)
CPC - A61F 2/28; A61L 27/00, 27/50

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)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Rfifi Sf arc:h Histnry document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0253470 A1 (GUZE, K et al) October 4, 2012; abstract; claims 1-2, 5, 7, 11	1
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Y		2-3, 4/1, 4/2, 4/3
Y	SKOVRLJ, B et al. Cellular bone matrices; viable stem cell-containing bone graft substitutes. The Spine Journal, November 1, 2014, Volume 14, Issue 11: pages 2763-2772. Published online 2014 Jun 11. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4402977/ >	2-3, 4/2, 4/3
Y	US 2012/0141557 A1 (MCKAY, WF) June 7, 2012; paragraph [0035]	4/1, 4/2, 4/3
A	US 2003/0206937 A1 (GERTZMAN, AA et al) November 6, 2003; paragraph [0072], [0074]	4/1, 4/2, 4/3

☐ 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
07 February 2017 (07.02.2017)	27 FEB 2017

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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