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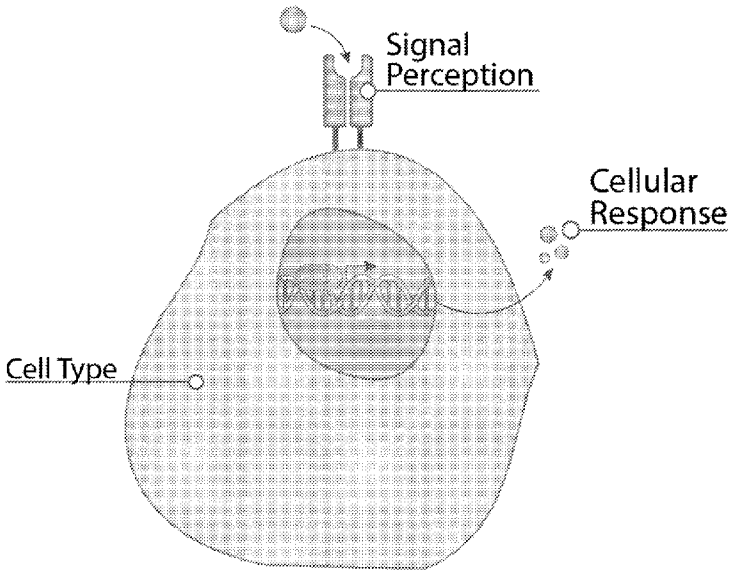

FIG. 1A

(57) Abstract: The present disclosure relates to methods of treating a disease or disorder in the lung or deriving from the lung in a subject with a capsule comprising a plurality of engineered cells.

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ENCAPSULATED CELLS FOR THE TREATMENT OF RESPIRATORY DISEASES AND DISORDERS

CLAIM OF PRIORITY

The instant application claims priority to U.S. Application No. 63/617,707, filed on January 4, 2024; U.S. Application No. 63/617,718, filed on January 4, 2024; U.S. Applicant No. 63/552,882, filed on February 13, 2024; and U.S. Application No. 63/617,724, filed on January 4, 2024. The contents of the foregoing applications are incorporated herein by reference in their entirety.

GOVERNMENT INTERESTS

This invention was made with government support under Grant Nos. R01HL163258 and R01HL174616 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Respiratory diseases and disorders can lead to significant morbidity, with current treatment modalities limited to a small armamentarium of immunosuppressive and anti-inflammatory regimens, among other treatments. Respiratory diseases and disorders are among the most ubiquitously encountered illnesses, which can manifest in clinical presentation from very mild to severe. Despite their prevalence, there is a dearth of efficacious treatment modalities to address respiratory dysregulation in severe clinical presentations, such as pneumonia and COVID-19, leading to significant morbidity and fatality. Certain respiratory diseases and disorders can be characterized by a chronic inflammatory response mediated by a dysregulation and hyperactivity in pro-inflammatory markers, e.g., cytokines and chemokines. Current treatments for these diseases and disorders are not ideal, and thus, there exists a need for improvement in methods of treatment.

SUMMARY

The present disclosure relates to methods of treating respiratory diseases and disorders, such as an autoimmune disease or disorder, a respiratory infection, or an inflammatory disease or

disorder in a subject featuring providing a plurality of encapsulated engineered cells. In particular, one or more engineered cells in the plurality are capable of producing a therapeutic agent, such as a protein, e.g., an antibody, a hormone, or a cytokine. In an embodiment, the methods described herein feature encapsulated engineered cells, wherein at least 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30% or more of the engineered cells in the plurality produce a therapeutic agent, e.g., an antibody, hormone, or cytokine. In an embodiment, the capsules comprise a plurality of engineered cells that produce one or more than one therapeutic agent, e.g., more than one protein, e.g., more than one cytokine.

The cytokine may be an anti-inflammatory cytokine. The cytokine may be a pro-inflammatory cytokine. In particular, the cytokine may comprise IL-10 (e.g., the cytokine is IL-10).

In an aspect, the present disclosure features methods for the treatment of an autoinflammatory disease or disorder, an autoimmune disease or disorder, a respiratory infection, or an inflammatory disease or disorder in a subject through administration of a capsule that is degradable. The capsule may comprise a polymer. The polymer may be a naturally occurring polymer. The polymer may be a synthetic polymer. The polymer may be a polysaccharide (e.g., an alginate).

In an aspect, the present disclosure features methods for the treatment of an autoinflammatory disease or disorder, an autoimmune disease, a respiratory infection, or an inflammatory disease or disorder featuring an encapsulated engineered cell. The engineered cell may be an epithelial cell. The engineered cell may be selected from a Chinese hamster ovary (CHO) cell, retinal pigment epithelial (ARPE-19) cell, human mammary epithelial (MCF-10a and MCF-7) cell, human embryonic kidney (HEK) cell, mesenchymal stem cell (MSC), human umbilical vein endothelial cell (HUVEC), NIH/3T3 cell, BJ fibroblast, and human renal mix epithelial cell (HREC).

The engineered cell may be engineered for regulatable expression of a cytokine. The engineered cell may be engineered for regulatable expression of an antibody, e.g., anti-TNFα, anti-IFNγ, and the like.

In an aspect, the present disclosure features methods wherein the therapeutic agent is selected from interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4),

interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17), and a combination thereof.

In an embodiment, the therapeutic agent is an anti-inflammatory antibody, e.g., an antibody that binds to a cytokine.

In an aspect, the the present disclosure features methods wherein the therapeutic agent is selected from IL-1RA, IL-4, IL-10, IL-11, IL-13, and IL-35, and a combination thereof. In some embodiments, the therapeutic agent comprises IL-1Ra.

In an aspect, the present disclosure features methods wherein the therapeutic agent is an anti-cytokine antibody or an anti-chemokine antibody, e.g., anti-IL-1, anti-IL-1α, anti-IL-1β, anti-IL-1RA, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-7, anti-IL-8, anti-IL-9, anti-IL-10, anti-IL-11, anti-IL-12, anti-IL-12α, anti-IL-12β, anti-IL-13, anti-IL-14, anti-IL-15, anti-IL-16, anti-IL-17, anti-IL-20, anti-IL-22, anti-IFN-α, anti-IFN-β, anti-IFN-γ, anti-TNF-α, anti-TNF-β, anti-TGF-β, anti-CCL1, anti-CCL2, anti-CCL3, anti-CCL4, anti-CCL5, anti-CCL6, anti-CCL7, anti-CCL8, anti-CCL9, anti-CCL10, anti-CCL11, anti-CCL12, anti-CCL13, anti-CCL14, anti-CCL15, anti-CCL16, anti-CCL17, anti-CCL18, anti-CCL19, anti-CCL20, anti-CCL21, anti-CCL22, anti-CCL23, anti-CCL24, ant-CCL25, anti-CCL26, anti-CCL27, anti-CCL28, anti-CXCL1, anti-CXCL2, anti-CXCL3, anti-CXCL4, anti-CXCL5, anti-CXCL6, anti-CXCL7, anti-CXCL8, anti-CXCL9, anti-CXCL10, anti-CXCL11, anti-CXCL12, anti-CXCL13, anti-CXCL14, anti-CXCL15, anti-CXCL16, and anti-CXCL17, and a combination thereof.

The methods as described herein may feature treating a respiratory disease, disorder, or condition. In some embodiments, the respiratory disease, disorder, or condition is selected from anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, and sarcoidosis.

In an aspect, the respiratory infection is be caused by a pathogen. In some embodiments, the pathogen is a bacterium, a fungus, a virus, a protozoan, a nematode, or a flatworm. The virus may be a coronavirus. In particular, the virus may be SARS-CoV2.

The present disclosure features methods for treating a respiratory infection selected from acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis, and a combination thereof.

The inflammatory disease or disorder may be a local inflammatory disease or disorder or a systemic inflammatory disease or disorder. In an embodiment, the inflammatory disease is a local inflammatory disease or disorder. In an embodiment, the inflammatory disease is a systemic inflammatory disease or disorder. In an embodiment, the inflammatory disease or disorder is selected from endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, and Gillian Barre syndrome, and a combination thereof. Preferably, the inflammatory disease or disorder is endometriosis.

In an aspect, the methods as described herein feature administering capsules via local delivery or delivery via inhalation. In particular, the administering may feature disposing, e.g., implanting, e.g., surgically implanting, capsules in a cellular compartment of the subject. The cellular compartment may be an organ, a cavity, or a serous membrane. In particular, the cavity is selected from intraperitoneal cavity (e.g., adjacent to the peritoneum), abdomino-pelvic cavity, cranial cavity, dorsal cavity, ventral cavity, and thoracic cavity. Preferably, the cavity is the intraperitoneal cavity. The local delivery may comprise delivery via instillation, e.g., with a bronchoscope or via bronchoalveolar lavage (BAL). The delivery via inhalation may also comprise delivery via an inhalation device (e.g., inhaler).

In an aspect, the methods as described herein feature administering capsules via implantation, e.g., surgical implantation into a cavity, e.g., the thoracic cavity.

In an aspect, the methods described herein feature a subject, wherein the subject is a mammal (e.g., a human).

The capsule may be formulated as a pharmaceutical composition, optionally comprising a pH modifier, a tonicity agent, a viscosity modifier, a carrier or diluent (e.g., a pharmaceutically acceptable carrier or diluent), a preservative, a surfactant, or a polymer, or a combination thereof.

The present disclosure also features methods of treating a respiratory infection, disease, disorder or condition, or an inflammatory disease or disorder in a subject, wherein the method comprises providing a capsule comprising a plurality of encapsulated engineered retinal pigmented epithelial (RPE) cells expressing a cytokine to a subject and administering the capsule to the subject via local delivery (e.g., delivery about 1 mm, 5 nm, 10 mm, 25 mm, 50 mm, 100 mm, or further from the lung or pleural cavity).

Additional embodiments of the present disclosure are described in further detail herein in the **Drawings**, **Description**, **Examples**, and **Claims**.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A-1B are schematic illustrations of an engineered cell and various cellular compartments which may be targeted by the composition by external regulation, e.g., by a small molecule inducer or via inflammatory induction. **FIG. 1A** is a schematic illustration of an engineered cell wherein perception of an external signal initiates a cellular response. **FIG. 1B** is a schematic of engineered cells illustrating that the stimulus may be a small molecule inducer or via an inflammatory marker.

FIG. 2 is a schematic illustrating an alternative delivery mode comprising instillation of capsules into the airway and delivery of anti-inflammatory proteins to the alveolar tissue in the lungs, enabling superior localization of the therapeutic agent to targeted tissue.

FIGS. 3A-3D demonstrate that capsules of various diameters (e.g., 150μm-1700μm) can be reproducibly generated for delivery of therapeutic agents to a rat lung via intratracheal instillation. **FIG. 3A** shows representative brightfield images of alginate hydrogels with diameter of 300 μm (top) and cell-laden 300 μm hydrogels (bottom). **FIG. 3B** shows that capsules having diameters of 150, 300, 600, 800, 1200, and 1700 μm may be synthesized via the methods of making described herein. **FIG. 3C** is a series of micrographs of capsules measuring 300, 600,

800 and 900 μm. **FIG. 3D** is a series of micrographs of capsules measuring 300, 500, 800, and 1000 μm.

FIGS. 4A-4C demonstrate that the synthesis of capsules is size-controlled with the target size closely aligning with the actually measured capsule diameter. **FIG. 4A** is a bar graph showing the reproducible, size-controlled synthesis of capsules of various target sizes and actual diameters of ~150, 300, 600, 800, 1200 and 1700 μm. **FIG. 4B** is a bar graph showing the reproducible, size-controlled synthesis of capsules of various target sizes and actual diameters of ~300, 600, 800, and 900 μm. **FIG. 4C** is a bar graph showing the reproducible, size-controlled synthesis of capsules of various target sizes and actual diameters of ~300, 600, 800, and 1200 μm.

FIGS. 5A-5D demonstrate that cell viability of the encapsulated engineered cells and the cytokine concentration are substantially independent of capsule diameter. **FIG. 5A** is a bar graph illustrating that the cell viability of the encapsulated engineered cells in capsules of 150, 300, 800 and 1200 μm are all greater than 80% and are not statistically different. **FIG. 5B** is a bar graph showing that the cell viability of 300 μm-diameter capsules is greater than 90% (left), and In vivo bioluminescent imaging confirming the presence and distribution of the capsules within the lung tissue (right). **FIG. 5C** is a bar graph showing that the cell dose is maintained at approximately $2*10^6$ cells in capsules of 150, 300, 800 and 1200μm. **FIG. 5D** is a bar graph showing that Rat IL-1Ra production is approximately $2*10^6$ pg/mL/day across all capsule diameters of 150, 300, 800 and 1200 μm. ns = not statistically significant.

FIG. 6 is a graph of cytokine production (namely, IL-13, FGF21 and IL-1Ra) over time in encapsulated engineered ARPE-19 cells as measured by protein-specific ELISAs. Cytokine productions appears to plateau in approximately 5-10 hours.

FIGS. 7A-7B show delivery and retrieval of the capsules comprising the encapsulated cell therapies intratracheally instilled in the lungs. **FIG. 7A** is a series of images of 100, 200, and 300 μl volumes of capsules delivered by intratracheal instillation to rat lungs and recovered by bronchoalveolar lavage (BAL). **FIG. 7B** is an image of a fixed and stained rat lung section showing 100, 200, and 300 μl samples of the capsules localized to the targeted alveolar tissue.

FIGS. 8A-8B demonstrate that instillation of capsules to the lungs results in local delivery of therapeutic cytokines. **FIG. 8A** is a bar graph showing that capsules administered via intubation result in a 10,000-fold increase in the local versus systemic concentrations of IL-1Ra,

i.e., in the fraction recovered by bronchoalveolar lavage (BAL) compared with the fraction recovered from the pleural cavity and the plasma IL-1Ra. **FIG. 8B** is a bar graph demonstrating that in a lipopolysaccharide (LPS)-induced lung injury model of inflammation, there is significantly less IL-17 in the bronchoalveolar lavage fluid after 24 h with locally delivered capsules secreting IL-1Ra.

FIGS. 9A-9C show a reduction in neutrophil infiltration at 24-h post-treatment with capsules comprising engineered cells. Instillation of 200-μm, IL-1Ra-producing capsules results in significantly less immune filtration at 24 hours after lipopolysaccharide (LPS)-induced lung injury treatment compared to untreated control rats administered vehicle. **FIG 9A** is an image of a histological section of a rat lung after lipopolysaccharide (LPS)-induced lung injury treatment only. **FIG 9B** is an image of a histological section of a rat after lung lipopolysaccharide (LPS)-induced lung injury treatment and IL-1Ra capsule instillation. **FIG 9C** is an image of a histological section of a healthy rat lung as a positive control.

FIG. 10 Illustrates the distribution of fluorescent capsules loaded with FITC dextran are distributed in instilled rat lungs as imaged using a Leica microscope in the GFP channel. Bright spots indicate the presence of capsules.

FIG. 11 is a schematic illustration of transposing therapeutic sequences comprising IL-10, 1L-1Ra, IL-13, IL-4, and FG-21 with a PiggyBac transposon employing a cut-and-paste method into genomic DNA of an engineered cell line for expression of a therapeutic agent.

FIG. 12 is a graph showing FGF-21 concentration (ng/mL) over time in a therapeutic cell, wherein the FGF-21 gene was pasted into the genomic DNA using a PiggyBac mobile element transposition.

FIGS. 13A-13E demonstrate the load and distribution of capsules for instillation in rodent alveolar tissue. **FIG. 13A** is a representative micrograph of the 300 μm-diameter cell-laden capsules loaded with FITC dextran. **FIG. 13B** is a representative IVIS (In Vivo Imaging System) image of rat lungs after instillation of 300 μm capsules comprising GFP-expressing cells which show their uniformity in rat alveolae. **FIGS. 13C-D** are representative *ex vivo* fluorescent images of lungs of instillation with dextran capsules which demonstrate their homogeneous distribution in rat alveolar tissue. **FIG. 13E** is a micrograph of 300 μm-diameter capsules comprising cells capable of expressing the firefly luciferase protein, indicating that the cell-laden capsules are all apportioned a similar amount of cells, e.g., about 10,000 cells.

FIG. 14A-14C demonstrate the distribution and longevity of capsules instilled in rat lungs. **FIG. 14A** is a graph depicting the average radiance of rats instilled with either 300 μm capsules comprising a plurality of firefly-luciferase cells or free cells over a 1 week period. **FIG. 14B** is a series of representative images of rats that were instilled 300 μm-diameter capsules comprising a plurality of firefly-luciferase expressing cells. **FIG. 14C** is a graph demonstrating *in vivo* bioluminescent total flux analysis demonstrating capsule retention for up to three days versus one day in unencapsulated cells (left), *ex vivo* fluorescent images of lungs after instillation with fluorescent dextran-loaded capsules (middle panel), and *in vivo* bioluminescent imaging of *ex vivo* lung one day after capsule instillation (top right panel) and unencapsulated cells (lower right panel) instillation.

FIGS. 15A-15E show the amounts of various cytokines (IL-10, IL-1Ra, FGF21, and IL-4) measured in the bronchoalveolar lavage (BAL) fluid and plasma of rodents 24 h after instillation of capsules containing engineered ARPE-19 cells capable of expressing IL-10, IL-1Ra, FGF21, and IL-4. **FIG. 15A** is a bar graph of IL-10 concentration (ng/mL) in BAL and plasma 24 h after instillation with capsules containing engineered ARPE-19 cells capable of IL-10 expression. **FIG. 15B** is a bar graph of IL-1Ra concentration (ng/mL) in BAL and plasma 24 h after instillation with capsules containing engineered ARPE-19 cells capable of IL-1Ra expression. **FIG. 15C** is a bar graph of FGF21 concentration (ng/mL) in BAL and plasma 24 h after instillation with capsules containing engineered ARPE-19 cells capable of FGF21 expression. **FIG. 15D** is a bar graph of IL-13 concentration (ng/mL) in BAL and plasma 24 h after instillation with capsules containing engineered ARPE-19 cells capable of IL-13 expression. **FIG. 15E** is a bar graph of IL-4 concentration (ng/mL) in BAL and plasma 24 h after instillation with capsules containing engineered ARPE-19 cells capable of IL-4 expression. Note that the concentration is more than one magnitude higher in BAL relative to plasma for IL-10, IL-1Ra, FGF21, IL-13, and IL-4, respectively.

FIGS. 16A-16H demonstrate dose escalation and pharmacokinetics of instilled capsules delivered intratracheally to rat lungs. **FIG. 16A** is a bar graph showing IL-10 protein levels in bronchoalveolar lavage (BAL) and plasma from rats instilled 300 μm-diameter capsules at increasing volumes, i.e., 0, 50, 100, and 150 μl. **FIG. 16B** is a bar graph illustrating the relationship between increasing cell density per capsule (cell concentration per mL alginate) and rat IL-10 production as measured in bronchoalveolar lavage (BAL) and plasma. **FIG. 16C** is a bar graph of

BAL and plasma concentrations of IL-10 24 hours post-instillation with 100 µL of RPE-IL-10 capsules and upon repeat dose at 30 days. Results show no significant difference in local concentrations between day 1 and day 31, indicating no anti-drug antibody development. **FIG. 16D** is a graph quantifying rat IL-10 levels in bronchoalveolar lavage (BAL) and plasma over the duration of 14 days post-instillation with rat IL-10-producing capsules. **FIG. 16E** are graphs of in vitro concentrations of IL-1Ra, FGF-21, IL-13, and IL-4 24 hours after administration of 100 µl of 300 µm capsules. **FIG. 16F** is a bar graph showing the IL-10 levels in the post caval, middle, superior, inferior, and left lobes of lungs of rats 24 hours after instillation with IL-10 producing capsules. **FIG. 16G** is a bar graph of Rat IL-10 concentration in bronchoalveolar lavage (BAL) and plasma at Day 100, representing a return to baseline levels. **FIG. 16H** is a series of histological sections of rat lung (alveolar) tissue corresponding to the kinetic timepoints (Day 1, Day 2, Day 3, Day 4, Day 7, and Day 14).

FIGS. 17A-17C are histological sections of fixed and stained rat alveolar tissue after instillation with capsules expressing anti-inflammatory mediators. **FIG. 17A** shows a histological section of rat alveolar tissue one day after instillation of the capsules. **FIG. 17B** shows a histological section of rat alveolar tissue seven days after instillation of the capsules. **FIG. 17C** shows a histological section of rat alveolar tissue 14 days after instillation of the capsules.

FIGS. 18A-18G illustrate capsule administration in porcine lungs via bronchoscopy. **FIG. 18A**is a representative image of blue-dyed capsules instilled into porcine lungs via bronchoscope. **FIG. 18B** shows IL-10 and IL-1Ra concentrations in BAL and plasma on Day 0, Day 2 and Day 28. **FIG. 18C** is a bar graph of human IL-1Ra (hIL-1Ra) levels in bronchoalveolar lavage (BAL) and plasma prior to and two days after instillation of hIL-1Ra-secreting capsules. **FIG. 18D** is a bar graph of human IL-10 (hIL-10) levels in bronchoalveolar lavage (BAL) and plasma prior to and two days after instillation of hIL-10-secreting capsules. **FIG. 18E** presents the general health of pigs prior to, two days after, and twenty-eight days after instillation of IL-10 and IL-1Ra producing capsules as characterized by triglycerides, total cholesterol and glucose. **FIG. 18F** shows any changes in liver function of pigs prior to, two days after, and twenty-eight days after instillation of hIL-1Ra- and hIL-10 producing capsules as characterized by albumin concentration. **FIG. 18G** are graphs showing spO_2 and $EtCO_2$ levels in pigs throughout the duration of the procedure.

FIGS. 19A-19G describe the establishment of an LPS model of ARDS. **FIG. 19A** summaries the study design: on Day 0, animals receive 200 mg/k LPS or volume-matched saline. Levels of various inflammatory markers, e.g., TNF-α, IL-1α, MCP1, MIP2, in addition to total cell count from lung homogenate were measured in bronchoalveolar lavage (BAL) fluid on Day 2 after instillation. **FIG. 19B** is a bar graph showing that BAL levels of TNF-α on Day 2 post administration of 20 mg/kg LPS are between about 100 and 1000 pg/mL compared to about 1 to about 10 pg/mL for the saline group. **FIG. 19C** is a bar graph showing that BAL levels of IL-1α on Day 2 post-administration of 20 mg/kg LPS are about 1000 pg/mL compared to about 10 pg/mL for the saline group. **FIG 19D** is a bar graph showing that BAL levels of MCP1 on Day 2 post-administration of 20 mg/kg LPS are about 10^5 pg/mL compared to about 10 pg/mL for the saline group. **FIG. 19E** is a bar graph showing that BAL levels of MIP2 on Day 2 post-administration of 20 mg/kg LPS is between about 10^3 and about 10^4 pg/mL compared to between about 10 to about 100 pg/mL for the saline group. **FIG. 19F** is a bar graph showing that BAL levels of total cell count derived lung homogenate are about $4*10^7$ cells compared to about $1*10^7$ to about $2*10^7$ cells for the saline group. **FIG. 19G** shows representative images from a homogenized post caval left lung lobe.

FIG. 20 is a series of images showing the localization of capsules fabricated with a fluorescent dye, which is excitable at a wavelength of 680 nm, instilled in rat lungs.

FIG. 21 shows, in the image on the left, the distribution of fluorescently labelled FITC dextran capsules instilled in the lungs of rats. To the right is an image of LIVE/DEAD staining of cells encapsulated within 300 μm capsules indicating that the cells are alive (live channel has signal and dead channel has no signal).

FIGS. 22A-22B demonstrate the response of engineered ARPE-19 cells to pro-inflammatory cytokines. **FIG. 22A** shows the plasmid used to engineer the ARPE-19 cells to upregulate production of a transgene in response to pro-inflammatory cytokines. **FIG. 22B** is a graph of the relative luminescence of transgene expression in engineered ARPE-19 cells with exposure to the cytokines IFN-γ (10 ng/mL), IL-1β (15 ng/mL), and TNF-α (15 ng/mL), and no exposure to cytokines (Control). RLU: relative luminescence units.

FIG. 23A-23C show results of IL-10 and IL-1Ra combination therapy in an LPS model of ARDS. **FIG. 23A** are graphs showing concentrations of rat IL-1Ra and Rat IL-10 in pleural fluid and plasma over 28 days after intratracheal instillation with LPS and implantation of ~15

μg/day IL-1Ra and ~3 μg/day IL-10 in the pleural cavity. **FIG. 23B** are microscopy images of explanted capsules on Day 1, 3, 7, and 28 demonstrating minimal fibrotic overgrowth. **FIG. 23C** is a graph showing lung histology score for the LPS only and LPS + IL-10 + IL-Ra capsules.

FIG. 24A-24B show the distribution and kinetics of capsules instilled into the lungs of mice. **FIG. 24A** is a series of IVIS images showing the distribution and kinetics of localization of luminescent RPE -Fluc capsules in the lungs of mice over 11 days. **FIG. 24B** is a graph quantifying the total flux of RPE-Fluc capsules in the lungs of mice over 11 days.

FIG. 25 is a series of fluorescence microscopy and fluorescent IVIS images of rat lungs instilled with 100 μl of 300μm green fluorescent protein (GFP) capsules.

FIGS. 26A-26C show results of an LPS model of ARDS. **FIG. 26A** shows images of histological sections of lungs treated with LPS with our without IL-10 producing capsules 24 hours after treatment. **FIG. 26B** show graphs showing total cell counts collected 24 hours after LPS instillation and treatment with saline, blank capsules and IL-10 producing capsules, with healthy rats serving as control (left), and IL1a BAL concentrations 24 hours after LPS and capsule treatment (right). **FIG. 26C** show graphs of IL1b and MCP-1 BAL concentrations 24 hours after LPS and capsule treatment.

FIGS. 27A-27B demonstrate the therapeutic efficacy of IL-10 and IL-1RA combination therapy in an ARDS model. **FIG. 27A** shows total cell counts collected 12 and 24 hours after LPS instillation (left), IL-1a (middle) and TNFa (right) BAL concentrations 12 and 24 days after LPS treatment and administration of IL-10/IL-1a producing capsules. **FIG. 27B** show MCP1 (left), IL-1b concentrations 12 and 24 days after LPS treatment and administration of IL-10/IL-1a producing capsules and perivascular lymphoid cuffing on day 28 for LPS only and LPS/IL1Ra+IL-10 producing capsule group.

FIGS. 28A-28E demonstrate the therapeutic efficacy of capsules in an LPS model of ARDS. **FIG. 28A** shows total cell counts collected 24 hours after LPS instillation and treatment with saline, blank capsules and IL-10 producing capsules, with healthy rats serving as control (left), and IL1a (middle) and TNFa (right) BAL concentrations 24 hours after LPS and capsule treatment. **FIG. 28B** are graphs of IL1b and MCP-1 BAL concentrations 24 hours after LPS and capsule treatment. **FIG. 28C** are graphs showing histological scores of inflammation over time as a factor of neutrophil density, distribution, and immune consolidations (left) and of regulatory response following treatment as a factor of macrophage, fibroblast, and chronic inflammatory

cells. **FIG. 28D** is graph quantifying perivascular lymphoid cuffing over time, which is representative of unresolved inflammation. **FIG. 28E** is a representative histological scan of the right lung 1, 7, 14, and 21 days after LPS with or without capsule treatment. Inset is a 20x magnification of the same image showing the immune phenotypes quantified in the histological scoring (i.e. neutrophils, regulatory cells, and perivascular cuffing).

FIGS. 29A-29C are bar graphs illustrating that ARPE-19 cells can be engineered to constitutively produce different therapeutic agents according to the piggyback transposase system. **FIG. 29A** is a bar graph showing constitutive Anti-IL8 production from ARPE-19 cells. **FIG. 29B** is a bar graph showing constitutive IL-10 production from ARPE-19 cells. **FIG. 29C** is a bar graph showing constitutive IL-1RA production from ARPE-19 cells.

FIG. 30 is a bar graph showing Constitutive Anti-IL6 production from ARPE-19 cells.

FIGS. 31A-31C demonstrate that ARPE-19 cells can be engineered to produce therapeutic agents in response to inflammation. **FIG. 31A** is a bar graph showing anti-IL-8 production in response to pro-inflammatory cytokines TNF-α and IL-1β with ARPE-19 cells engineered with plasmids encoding ant-IL-8 under the control of a NF-κB-responsive promoter. **FIG. 31B** is a bar graph showing IL-10 production in response to pro-inflammatory cytokines TNF-α and IL-1β with ARPE-19 cells engineered with plasmids encoding IL-10 under the control of a NF-κB-responsive promoter. **FIG. 31C** is a bar graph showing IL-1Ra production in response to pro-inflammatory cytokines TNF-α and IL-1β with ARPE-19 cells engineered with plasmids encoding IL-1RA under the control of a NF-κB-responsive promoter.

FIG. 32 is a bar graph showing luciferase production in response to pro inflammatory cytokines from ARPE-19 cells engineered with NFkB-luciferase.

FIG. 33 is a bar graph showing luciferase production from ARPE-19 cells engineered with NFkB-luciferase in response to different forms and concentrations of IL1 (mIL1a is mouse IL1a; hIL1a is human IL1a).

FIGS. 34A-34B demonstrate that cytokine production from engineered ARPE-19 cells is dose-responsive. **FIG. 34A** is a graph showing that engineered ARPE-19 cells produce IL-1RA in a dose-dependent manner when treated with IL-1β and TNF-α. The ARPE-19 cells were engineered to produce IL-1RA by stable transfection with a plasmid containing a NFκB-responsive promoter. **FIG. 34B** is graph illustrating IL-10 production from ARPE-19 cells engineered with NFkB-mIL10. Response to 15mg/mL or 15pg/mL of IL1b over time.

FIGS. 35A-C illustrate the engineering of ARPE-19 cells expressing anti-inflammatory cytokines, their subsequent encapsulation in hydrogel capsules, and finally local delivery to affected tissue to modulate the immune response. **FIG. 35A** is a schematic illustrating the piggyback transposase system for engineering cells capable of expressing anti-inflammatory cytokines. **FIG. 35B** is a schematic illustrating the encapsulation of engineered cells in hydrogel to isolate the cells from an immune response. **FIG. 35C** is a schematic of the implantation of capsules in a rodent model to locally modulate inflammation at the site of endometrial lesions.

FIG. 36A-36D demonstrate that ARPE-19 cells maintain viability and productivity after encapsulated in alginate capsules. **FIG. 36A** is a brightfield microscopy image of capsules encapsulated with ARPE-19 cells capable of expressing IL-10 under control of a NF-κB-responsive promoter. **FIG. 36B** is a microscopy image of Calcein AM-stained capsules encapsulated with ARPE-19 cells capable of expressing IL-10 under control of a NF-κB-responsive promoter. The color green indicates viability. **FIG. 36C** is a microscopy image of ethidium homodimer-stained capsules encapsulated with ARPE-19 cells capable of expressing IL-10 under control of a NF-κB-responsive promoter. Note the lack of staining indicating that all cells are apparently viable. **FIG. 36D** is a bar graph showing IL-10 production responsive to the pro-inflammatory markers IL-1β and TNF-α. The capsules contain ARPE-19 cells capable of producing IL-10 under the control of a NF-κB-responsive promoter.

FIGS. 37A-37B demonstrate that production of therapeutic agents *in vivo* is localized. **FIG. 37A** is a bar graph of anti-IL-8 concentration in the intraperitoneal fluid (IP fluid) and plasma from ARPE-19 cells engineered to constitutively produce anti-IL-8 and nonengineered ARPE-19 cells implanted in the peritoneal cavity of healthy mice. **FIG. 37B** is a bar graph of IL-1RA concentration in the intraperitoneal fluid (IP fluid) and plasma from ARPE-19 cells engineered to constitutively produce IL-1RA and nonengineered ARPE-19 cells implanted in the peritoneal cavity of healthy mice.

DETAILED DESCRIPTION

Definitions

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of

"one or more," "at least one," and "one or more than one." The word "about" means plus or minus 5% of the stated number.

The terms "subject" or "patient," as used herein, refer to an individual receiving the inhalable formulation described herein. The subject may include a human (i.e., a male or female of any age group, e.g., a pediatric subject (e.g., infant, child, adolescent) or adult subject (e.g., young adult, middle–aged adult, or senior adult)) and/or other non–human animals, for example, mammals (e.g., primates (e.g., cynomolgus monkeys, rhesus monkeys); commercially relevant mammals such as cattle, pigs, horses, sheep, goats, cats, and/or dogs) and birds (e.g., commercially relevant birds such as chickens, ducks, geese, and/or turkeys). In certain embodiments, the animal is a mammal. The animal may be a male or female and at any stage of development. A non–human animal may be a transgenic animal.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting of one or more of a symptom, manifestation, or underlying cause of, e.g., a disease, disorder or condition for which the composition is administered.

As used herein, the terms, "delivery" and "delivering" refer to administering a composition, e.g., a pharmaceutical composition, to a subject, e.g., a mammal, e.g., a human. As used herein the terms "local delivery," "delivering locally" and associated terms refer to administering a composition, e.g., a pharmaceutical composition to a particular cellular compartment, organ, tissue, membrane, or cavity of a subject. E.g., "local delivery" may comprise disposing, implanting (e.g., surgically implanting) the composition in a cellular compartment, tissue, serous membrane, or cavity in a subject.

As used herein, the terms "inhale," and "inhalable" and associated terms "inhalable delivery" and "inhalable composition" refer to the act of inspiring, being inspirable, or capable of being inspirable, e.g., to the act of breathing in (e.g., into the lungs or other compartments of the respiratory system), being breathable (e.g., into the lungs or other compartments of the respiratory system), or capable of being breathable (e.g., into the lungs or other compartments of the respiratory system) by a subject, e.g., a subject being administered the capsules of the present disclosure or otherwise in need thereof.

As used herein, the terms "instill" and instillation" refers to administering gradually, e.g., administering gradually a pharmaceutical composition or a medicament, e.g., administering

gradually the composition comprising a capsule featuring a polymer and an plurality of engineered cells of the present disclosure, to a subject, e.g., to a subject in need thereof. Instillation of said capsules may be performed with a device, e.g., with a bronchoscope (e.g., a rigid bronchoscope or a flexible bronchoscope) or with a catheter.

As used herein, the term "inflammatory disease" refers to a disease characterized by an acute or chronic inflammatory state in a subject, e.g., a dysregulation or hyperactivity in pro-inflammatory markers, e.g., cytokines and chemokines. A "local inflammatory disease," as used herein, is characterized by a localized, chronic inflammatory response mediated by a dysregulation and hyperactivity in pro-inflammatory markers, e.g., cytokines and chemokines, e.g., wherein the hyperactivity or dysregulation is confined to a particular cellular compartment or tissue. A "systemic inflammatory disease," as used herein, is characterized by a global, chronic inflammatory response mediated by a dysregulation and hyperactivity in pro-inflammatory markers, e.g., cytokines and chemokines, e.g., wherein the hyperactivity or dysregulation is not confined to a particular cellular compartment, or tissue.

As used herein, the term "autoinflammatory disease," "autoinflammatory disorder," or "autoinflammatory disease or disorder," as well as associated terms, refer to diseases or disorders indicative of, manifesting from, or symptomatic of a dysregulation of the innate immune system and the inflammatory response, wherein the subject mistakenly generates an aberrant inflammatory response against himself, herself, or itself, e.g., a disease or disorder arising from inflammatory hyperactivity and/or dysregulation stemming from defects in Interleukin-1β, NF-κB, Interferon 1 (IFN-1), Interleukin-1 Receptor (IL-1Ra), Interleukin-10 Receptor (IL-10R), Interleukin-36 Receptor (IL-36Ra), *inter alia*.

As used herein, the term "autoimmune disease," "autoimmune disorder," or "autoimmune disease or disorder," as well as associated terms, refer to diseases or disorders indicative of, manifesting from, or symptomatic of a dysregulation of the adaptive immune system, wherein the subject develops an adaptive immune response against himself, herself, or itself.

As used herein, a "pathogen" is a disease-causing agent (i.e., an agent causing an infection) e.g., a microorganism, e.g., a virus, a bacterium, a protozoan, a fungus, a flatworm, a nematode, and the like, leading to the genesis of an infection in a subject, e.g., a mammal, e.g., a human.

As used herein, a "respiratory infection" is an infection of at least a part of the respiratory system of a subject, e.g., a mammal, e.g., a human, arising from a pathogen, e.g., a virus, a bacterium, a protozoan, a fungus, a flatworm, a nematode, and the like.

Chemical Definitions

Definitions of specific functional groups are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in Thomas Sorrell, *Organic Chemistry*, University Science Books, Sausalito, 1999; Smith and March, *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical and biological arts. Also, all publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example, "C_1-C_6 alkyl" is intended to encompass, C_1, C_2, C_3, C_4, C_5, C_6, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, C_1-C_2, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_3-C_6, C_3-C_5, C_3-C_4, C_4-C_6, C_4-C_5, and C_5-C_6 alkyl.

The compounds disclosed herein may possess one or more chiral centers and so exist in a number of stereoisomeric forms. All stereoisomers and mixtures thereof are included in the scope of the present disclosure. Racemic compounds may either be separated using preparative HPLC and a column with a chiral stationary phase or resolved to yield individual enantiomers utilizing methods known to those skilled in the art. In addition, chiral intermediate compounds may be resolved and used to prepare chiral compounds of the disclosure.

The compounds disclosed herein may also comprise one or more isotopic substitutions. For example, H may be in any isotopic form, including ^1H, ^2H (D or deuterium), and ^3H (T or

tritium); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

"Alkyl" refers to a hydrocarbon group containing one or more carbon atoms, where multiple carbon atoms if present are joined by single bonds. The alkyl hydrocarbon group may be straight-chain or contain one or more branches or cyclic groups having from 1 to 24 carbon atoms ("C_1-C_{24} alkyl"). In some embodiments, an alkyl group has 1 to 12 carbon atoms ("C_1-C_{12} alkyl"), 1 to 10 carbon atoms ("C_1-C_{12} alkyl"), 1 to 8 carbon atoms ("C_1-C_8 alkyl"), 1 to 6 carbon atoms ("C_1-C_6 alkyl"), 1 to 5 carbon atoms ("C_1-C_5 alkyl"), 1 to 4 carbon atoms ("C_1-C_4alkyl"), 1 to 3 carbon atoms ("C_1-C_3 alkyl"), 1 to 2 carbon atoms ("C_1-C_2 alkyl"), or 1 carbon atom ("C_1 alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("C_2-C_6 alkyl"). Examples of C_1-C_6 alkyl groups include methyl (C_1), ethyl (C_2), n–propyl (C_3), isopropyl (C_3), n–butyl (C_4), tert–butyl (C_4), sec–butyl (C_4), iso–butyl (C_4), n–pentyl (C_5), 3–pentanyl (C_5), amyl (C_5), neopentyl (C_5), 3–methyl–2–butanyl (C_5), tertiary amyl (C_5), and n–hexyl (C_6). Additional examples of alkyl groups include n–heptyl (C_7), n–octyl (C_8) and the like. Each instance of an alkyl group may be independently optionally substituted, i.e., unsubstituted (an "unsubstituted alkyl") or substituted (a "substituted alkyl") with one or more substituents; e.g., for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent.

"Haloalkyl" refers to an alkyl, wherein one or more hydrogen atoms of the hydrocarbon group are replaced with a halogen, i.e., fluorine, chlorine, bromine, and iodine. "Fluoroalkyl" refers to an alkyl, wherein one or more hydrogen atoms of the hydrocarbon group are replaced with a fluorine. "Chloroalkyl" refers to an alkyl, wherein one or more hydrogen atoms of the hydrocarbon group are replaced with a chlorine. "Bromoalkyl" refers to an alkyl, wherein one or more hydrogen atoms of the hydrocarbon group are replaced with a bromine. "Iodoalkyl" refers to an alkyl, wherein one or more hydrogen atoms of the hydrocarbon group are replaced with a iodine.

The term "halo" encompasses fluoro, chloro, bromo, and iodo.

As used herein, "alkenyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 24 carbon atoms, one or more carbon–carbon double bonds, and no triple bonds ("C_2-C_{24} alkenyl"). In some embodiments, an alkenyl group has 2 to 12 carbon atoms ("C_2-C_{12} alkenyl"), 2 to 10 carbon atoms ("C_2-C_{10} alkenyl"), 2 to 8 carbon atoms ("C_2-C_8 alkenyl"), 2 to 6 carbon atoms ("C_2-C_6 alkenyl"), 2 to 5 carbon atoms ("C_2-C_5 alkenyl"), 2 to 4

carbon atoms ("C_2-C_4 alkenyl"), 2 to 3 carbon atoms ("C_2-C_3 alkenyl"), or 2 carbon atoms ("C_2 alkenyl"). The one or more carbon–carbon double bonds can be internal (such as in 2–butenyl) or terminal (such as in 1–butenyl). Examples of C_2-C_4 alkenyl groups include ethenyl (C_2), 1–propenyl (C_3), 2–propenyl (C_3), 1–butenyl (C_4), 2–butenyl (C_4), butadienyl (C_4), and the like. Examples of C_2-C_6 alkenyl groups include the aforementioned C_{2-4} alkenyl groups as well as pentenyl (C_5), pentadienyl (C_5), hexenyl (C_6), and the like. Each instance of an alkenyl group may be independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents *e.g.,* for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent.

As used herein, the term "alkynyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 24 carbon atoms, one or more carbon–carbon triple bonds ("C_2-C_{24} alkenyl"). In some embodiments, an alkynyl group has 2 to 12 carbon atoms ("C_2-C_{10} alkynyl"), 2 to 10 carbon atoms ("C_2-C_{10} alkynyl"), 2 to 8 carbon atoms ("C_2-C_8 alkynyl"), 2 to 6 carbon atoms ("C_2-C_6 alkynyl"), 2 to 5 carbon atoms ("C_2-C_5 alkynyl"), 2 to 4 carbon atoms ("C_2-C_4 alkynyl"), 2 to 3 carbon atoms ("C_2-C_3 alkynyl"), or 2 carbon atoms ("C_2 alkynyl"). The one or more carbon–carbon triple bonds can be internal (such as in 2–butynyl) or terminal (such as in 1–butynyl). Examples of C_2-C_4 alkynyl groups include ethynyl (C_2), 1–propynyl (C_3), 2–propynyl (C_3), 1–butynyl (C_4), 2–butynyl (C_4), and the like. Each instance of an alkynyl group may be independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents *e.g.,* for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent.

As used herein, the term "heteroalkyl," refers to a non-cyclic stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom selected from the group consisting of O, N, P, Si, and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P, S, and Si may be placed at any position of the heteroalkyl group. Exemplary heteroalkyl groups include, but are not limited to: $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2$, $-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, $-CH=CH-N(CH_3)-CH_3$, $-O-CH_3$, and $-O-CH_2-CH_3$. Up to two or three heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. Where "heteroalkyl" is recited, followed by recitations of specific

heteroalkyl groups, such as $-CH_2O$, $-NR^CR^D$, or the like, it will be understood that the terms heteroalkyl and $-CH_2O$ or $-NR^CR^D$ are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as $-CH_2O$, $-NR^CR^D$, or the like. Each instance of a heteroalkyl group may be independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted heteroalkyl") or substituted (a "substituted heteroalkyl") with one or more substituents *e.g.*, for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent.

As used herein, "cycloalkyl" refers to a radical of a non–aromatic cyclic hydrocarbon group having from 3 to 10 ring carbon atoms ("C_3-C_{10} cycloalkyl") and zero heteroatoms in the non–aromatic ring system. In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms ("C_3-C_8cycloalkyl"), 3 to 6 ring carbon atoms ("C_3-C_6 cycloalkyl"), or 5 to 10 ring carbon atoms ("C_5-C_{10} cycloalkyl"). A cycloalkyl group may be described as, e.g., a C_4-C_7-membered cycloalkyl, wherein the term "membered" refers to the non-hydrogen ring atoms within the moiety. Exemplary C_3-C_6 cycloalkyl groups include, without limitation, cyclopropyl (C_3), cyclopropenyl (C_3), cyclobutyl (C_4), cyclobutenyl (C_4), cyclopentyl (C_5), cyclopentenyl (C_5), cyclohexyl (C_6), cyclohexenyl (C_6), cyclohexadienyl (C_6), and the like. Exemplary C_3-C_8 cycloalkyl groups include, without limitation, the aforementioned C_3-C_6 cycloalkyl groups as well as cycloheptyl (C_7), cycloheptenyl (C_7), cycloheptadienyl (C_7), cycloheptatrienyl (C_7), cyclooctyl (C_8), cyclooctenyl (C_8), cubanyl (C_8), bicyclo[1.1.1]pentanyl (C_5), bicyclo[2.2.2]octanyl (C_8), bicyclo[2.1.1]hexanyl (C_6), bicyclo[3.1.1]heptanyl (C_7), and the like. Exemplary C_3-C_{10} cycloalkyl groups include, without limitation, the aforementioned C_3-C_8 cycloalkyl groups as well as cyclononyl (C_9), cyclononenyl (C_9), cyclodecyl (C_{10}), cyclodecenyl (C_{10}), octahydro–1*H*–indenyl (C_9), decahydronaphthalenyl (C_{10}), spiro [4.5] decanyl (C_{10}), and the like. As the foregoing examples illustrate, in certain embodiments, the cycloalkyl group is either monocyclic ("monocyclic cycloalkyl") or contain a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic cycloalkyl") and can be saturated or can be partially unsaturated. "Cycloalkyl" also includes ring systems wherein the cycloalkyl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is on the cycloalkyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the cycloalkyl ring system. Each instance of a cycloalkyl group may be independently

optionally substituted, *i.e.*, unsubstituted (an "unsubstituted cycloalkyl") or substituted (a "substituted cycloalkyl") with one or more substituents.

"Heterocyclyl" as used herein refers to a radical of a 3– to 10–membered non–aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon ("3–10 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl"), and can be saturated or can be partially unsaturated. Heterocyclyl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more cycloalkyl groups wherein the point of attachment is either on the cycloalkyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. A heterocyclyl group may be described as, e.g., a 3-7-membered heterocyclyl, wherein the term "membered" refers to the non-hydrogen ring atoms, i.e., carbon, nitrogen, oxygen, sulfur, boron, phosphorus, and silicon, within the moiety. Each instance of heterocyclyl may be independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is unsubstituted 3–10 membered heterocyclyl. In certain embodiments, the heterocyclyl group is substituted 3–10 membered heterocyclyl.

As used herein, "hydroxy" refers to the radical –OH.

Alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, and heterocyclyl groups, as defined herein, are optionally substituted (*e.g.,* "substituted" or "unsubstituted" alkyl, "substituted" or "unsubstituted" alkenyl, "substituted" or "unsubstituted" alkynyl, "substituted" or "unsubstituted" heteroalkyl, "substituted" or "unsubstituted" cycloalkyl, "substituted" or "unsubstituted" heterocyclyl, "substituted" or "unsubstituted" aryl or "substituted" or "unsubstituted" heteroaryl group). In general, the term "substituted", whether preceded by the term "optionally" or not, means that at least one hydrogen present on a group (*e.g.,* a carbon or

nitrogen atom) is replaced with a permissible substituent, *e.g.*, a substituent which upon substitution results in a stable compound, *e.g.*, a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a "substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term "substituted" is contemplated to include substitution with all permissible substituents of organic compounds, such as any of the substituents described herein that result in the formation of a stable compound. The present disclosure contemplates any and all such combinations to arrive at a stable compound. For purposes of this disclosure, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

Autoinflammatory and Autoimmune Diseases or Conditions

Autoinflammatory conditions arise as a result of a failure of the innate immune system, which regulates the inflammatory response, activates the complement system, and recruits and modulates the activity of white blood cells in response to potential pathogens, e.g., viruses and bacteria. Inflammatory hyperactivity and/or dysregulation may stem from congenital defects in inflammatory mediators such as Interleukin-1β, NF-κB, Interferon 1 (IFN-1), Interleukin-1 Receptor (IL-1Ra), Interleukin-10 Receptor (IL-10R), Interleukin-36 Receptor (IL-36Ra), *inter alia*. Other genetic abnormalities may induce dysregulation of white blood cells, leading to the destruction of healthy tissue and a chronic inflammatory state.

Autoimmune diseases arise as a result of a failure of the adaptive immune system, which eliminates potential threats by the generation of antibodies capable of identifying antigens associated with a pathogen. Dysregulation of the adaptive immune system may have deleterious consequences in the immune response of a patient, having potentially cascading effects on the innate immune system as well, resulting in an aberrant inflammatory state in response to an autoantigen the patient's immune system has mistakenly identified as a threat.

Respiratory diseases or conditions which arise from a proinflammatory stimulus, autoinflammatory, or autoimmune condition are myriad and include: anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia,

bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, sarcoidosis, and the like.

Potential treatment modalities for autoinflammatory, or autoimmune conditions may be addressable with the inventive composition as described herein via modulation of the inflammatory response via release of a therapeutic agent from the inventive composition as described herein.

Respiratory Infections

Respiratory infection are among the most ubiquitously encountered illnesses, which can manifest in clinical presentation from very mild to severe. Respiratory infections are broadly categorized by the location of infection, i.e., as an upper respiratory infection, e.g., a common cold; or as a lower respiratory infection, e.g., pneumonia, e.g., acute respiratory distress syndrome (ARDS) caused by, e.g., the SARS-CoV-2 coronavirus (COVID-19).

Non-limiting examples of respiratory infections include: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

In certain instances, respiratory infections may be characterized by inflammatory dysregulation, e.g., the inflammatory hyperactivity referred to as a "cytokine storm" in the

evolution of severe COVID-19 pneumonia and ARDS. Thus, the class of respiratory infections are promising targets for the inventive capsules capable of releasing a therapeutic agent, e.g., a protein such as a cytokine, as described herein.

Capsules

A capsule described herein comprises a material that reduces or inhibits a reaction (*e.g.*, such as an immunomodulatory reaction) with an engineered cell disposed within. For example, a capsule may comprise a material that shields the engineered cell from exposure to the surrounding milieu, such as host tissue, host cells, or host cell products. In an embodiment, a capsule minimizes the effect of a host response (*e.g.*, an immune response) directed at an engineered cell disposed within, *e.g.*, as compared with a similar cell that is not disposed within an capsule.

The capsule make take any shape. The surface may be flat surface or a curved surface, and can take a variety of more complex forms such a sphere, a tube (*e.g.*, inside or outside of the tube), a bead, a rod, a wire, or even more complex 3-D structures such as medical devices.

The capsule may comprise a permeable, semi-permeable, or impermeable material to, for example, control the flow of solution in and out of the capsule and/or adopt the shape or size of its surroundings. For example, the material may be permeable or semi-permeable to allow free passage of small molecules, such as nutrients and waste products, in and out of the construct. In addition, the material may be permeable or semi-permeable to allow the transport of an cytokine, out of the capsule. Exemplary materials include polymers, metals, ceramics, and combinations thereof.

In an embodiment, the capsule comprises a polymer (*e.g.*, a naturally occurring polymer or a synthetic polymer). For example, a polymer may comprise polystyrene, polyester, polycarbonate, polyethylene, polypropylene, polyfluorocarbon, nylon, polyacetylene, polyvinyl chloride (PVC), polyolefin, polyurethane, polyacrylate, polymethacrylate, polyacrylamide, polymethacrylamide, polymethyl methacrylate, poly(2-hydroxyethyl methacrylate), polysiloxane, polydimethylsiloxane (PDMS), polyhydroxyalkanoate, PEEK®, polytetrafluoroethylene, polyethylene glycol, polysulfone, polyacrylonitrile, collagen, cellulose, cellulosic polymers, polysaccharides, polyglycolic acid, poly(L-lactic acid) (PLLA), poly(lactic glycolic acid) (PLGA), polydioxanone (PDA), poly(lactic acid), hyaluronic acid, agarose, alginate, chitosan, or a blend or copolymer thereof. In an embodiment, the capsule comprises a polysaccharide (*e.g.*, alginate,

cellulose, hyaluronic acid, or chitosan). In an embodiment, the capsule comprises hyaluronic acid. In an embodiment, the capsule comprises alginate. In some embodiments, the average molecular weight of the polymer is from about 2 kDa to about 500 kDa (*e.g.*, from about 2.5 kDa to about 175 kDa, from about 5 kDa about 150 kDa, from about 10 kDa to about 125 kDa, from about 12.5 kDa to about 100 kDa, from about 15 kDa to about 90 kDa, from about 17.5 kDa to about 80 kDa, from about 20 kDa to about 70 kDa, from about 22.5 kDa to about 60 kDa, or from about 25 kDa to about 50 kDa). The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of a polymer, *e.g.*, a polymer described herein.

In an embodiment, the capsule comprises a polysaccharide, *e.g.*, hyaluronic acid or an alginate. Alginate is a naturally occurring polymer comprising β-(1-4)-linked mannuronic acid and guluronic acid residues, and as a result of its high density of negatively charged carboxylates, may be cross-linked with certain cations to form a larger structure, such as a hydrogel. Alginate polymers described herein may have an average molecular weight from about 2 kDa to about 500 kDa (*e.g.*, from about 2.5 kDa to about 175 kDa, from about 5 kDa about 150 kDa, from about 10 kDa to about 125 kDa, from about 12.5 kDa to about 100 kDa, from about 15 kDa to about 90 kDa, from about 17.5 kDa to about 80 kDa, from about 20 kDa to about 70 kDa, from about 22.5 kDa to about 60 kDa, or from about 25 kDa to about 50 kDa). In an embodiment, the capsule comprises at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of an alginate polymer. In an embodiment, the alginate is an ultrapure alginate (*e.g.*, SLG20 alginate).

In an embodiment, the capsule comprises a metal or a metallic alloy. Exemplary metals or metallic alloys include titanium (*e.g.*, nitinol, nickel titanium alloys, thermo-memory alloy materials), platinum, platinum group alloys, stainless steel, tantalum, palladium, zirconium, niobium, molybdenum, nickel-chrome, cobalt, tantalum, chromium molybdenum alloys, nickel-titanium alloys, and cobalt chromium alloys. In an embodiment, the capsule comprises stainless steel grade. The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of a metal or metallic alloy, *e.g.*, a metal or metallic alloy described herein.

In an embodiment, the capsule comprises a ceramic. Exemplary ceramics include carbide, nitride, silica, or oxide materials (*e.g.*, titanium oxides, hafnium oxides, iridium oxides, chromium

oxides, aluminum oxides, and zirconium oxides). The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of a ceramic, *e.g.*, a ceramic described herein.

In an embodiment, the capsule may comprise glass. The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more glass.

A material within the capsule may be further modified, for example, with a chemical modification. For example, a material may be coated or derivatized with a chemical modification that provides a specific feature, such as an immunomodulatory or antifibrotic feature. Exemplary chemical modifications include small molecules, peptides, proteins, nucleic acids, lipids, or oligosaccharides. The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of a material that is chemically modified, *e.g.*, with a chemical modification described herein.

In some embodiments, the material is chemically modified with a specific density of modifications. The specific density of chemical modifications may be described as the average number of attached chemical modifications per given area. For example, the density of a chemical modification on a material in, on, or within an capsule described herein may be 0.01, 0.1, 0.5, 1, 5, 10, 15, 20, 50, 75, 100, 200, 400, 500, 750, 1,000, 2,500, or 5,000 chemical modifications per square μm or square mm.

In an embodiment, the chemical modification of a material may include a linker or other attachment moiety. These linkers may include a cross-linker, an amine-containing linker, an ester-containing linker, a photolabile linker, a peptide-containing linker, a disulfide-containing linker, an amide-containing linker, a phosphoryl-containing linker, or a combination thereof. A linker may be labile (*e.g.*, hydrolysable). Exemplary linkers or other attachment moieties is summarized in *Bioconjugate Techniques* (3rd ed, Greg T. Hermanson, Waltham, MA: Elsevier, Inc, 2013), which is incorporated herein by reference in its entirety.

In some embodiments, capsules comprising a polymer and a plurality of cells have been engineered to deliver a therapeutic agent, e.g., a protein, e.g., a cytokine. In some embodiments, the polymer forms a hydrogel, or is capable of forming a hydrogel. In some embodiments, the polymer forms a hydrogel, or is capable of forming a hydrogel, that encapsulates a plurality of engineered cells.

A hydrogel is a crosslinked structure of polymers comprising a substantial amount of water, with a myriad of applications in drug delivery, biomedical devices, sensors, tissue engineering, and semiconductors due to its unique combination of physical and mechanical properties as varied as biodegradability, biocompatibility, stimuli-responsiveness (e.g., thermo- or pH responsiveness), non-Newtonian behavior, viscoelasticity, and its ability to self-heal. Hydrogels may comprise a natural polymer or a synthetic polymer. In some embodiments, the hydrogel comprises a natural polymer, wherein the natural polymer is a polypeptide. Exemplary polymers and copolymers capable of forming a hydrogel include, without limitation, alginate, alginate-polyethylene glycol (alginate-PEG), alginate-polyacrylamide (alginate-PAAm), α-cyclodextrin, α-cyclodextrin-polyethylene glycol (α-cyclodextrin-PEG), chitosan, collagen, fibrin, heparin, hyaluronic acid (HA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyacrylamide-ferrocene (PAAm-Fc), poly(L-lysine)/polyacrylic acid (PLL/PAA), poly(N-isopropylacylamide) (PNIPAAM), poly((N-isopropylacrylamide)-co-(sodium acrylic acid)) (P(NIPAAM-co-AAcNa)), polyvinyl alcohol-polyacrylamide (PVA-PAAm), and poly(vinyl pyrrolidine) (PVP). In some embodiments, the hydrogel polymer comprises any of the hydrogel-forming polymers disclosed in Zhang, Y.S. *Science*. 2017, 356, 6337, eaaf3627.

The capsule may comprise a polymer which is degradable, e.g., biodegradable. In some embodiments, the polymer is degradable or capable of being degraded. In some embodiments, the polymer is biodegradable or capable of being biodegraded. In some embodiments, the polymer is degradable, or capable of being degraded, and hydrogel-forming, or capable of forming a hydrogel. In some embodiments, the polymer is biodegradable, or capable of being biodegraded, and hydrogel-forming, or capable of forming a hydrogel. In some embodiments, the biodegradable polymer is a naturally derived polymer. In some embodiments, the biodegradable polymer is a synthetic polymer.

The biodegradable polymer may be a natural polymer. Exemplary natural biodegradable polymers include alginic acid and pharmaceutically acceptable salts thereof (e.g., barium alginate calcium alginate, sodium alginate, potassium alginate and the like), cellulose, cellulose ethers (e.g., cellulose hydrocolloids), chitosan, chondroitin sulphate, starch, collagen, hyaluronic acid (HA), polyphosphosphate, and poly(hydroxybutyrate) (PHB).

The biodegradable polymer may be a synthetic polymer. Exemplary synthetic biodegradable polymers include polycaprolactone (PCL), poly(hydroxybutyrate-co-hydroxyvalerate), poly(γ-glutamic acid), polyglycolic acid (PGA), polylactic acid (PLA), poly(L-lactic acid) (PLLA), and poly(lactic-co-glycolic acid) (PLGA).

The biodegradable polymer may be a poly(amino acid). Exemplary biodegradable amino acids include polylysine, e.g., poly(D/L-lysine), poly(L-lysine), and poly(D-lysine).

The biodegradable polymer may comprise a polyethylene glycol copolymer. Exemplary biodegradable polyethylene glycol copolymers include poly(ethylene glycol)-*b*-hyaluronic acid (PEG-HA), poly(ethylene glycol)-*b*-polycaprolactone (PEG-PCL), poly(ethylene glycol)-*b*-poly(glutamic acid) (PEG-PGA), poly(ethylene glycol)-*b*-polylactic acid (PEG-PLA), poly(ethylene glycol)-*b*-poly(glycolic-co-lactic acid) (PEG-PLGA), poly(ethylene glycol)-*b*-poly(L-lactic acid) (PEG-PLLA), and polyethylene glycol-*b*-poly(ethyleneimine) (PEG-PEI).

The biodegradable polymer may be a polyanhydride. Exemplary biodegradable polyanhydrides include poly(adipic anhydride), poly(azelaic anhydride), poly(dodecanedioc anhydride), poly(dodecane carboxylic acid), poly(fumaric anhydride), poly(fumaric anhydride-sebacic anhydride), poly(furmaric anhydride dodecanedioc anhydride) poly(fumaric anhydride adipic anhydride), poly(hexadecenoic anhydride), poly(isophthalic anhydride), poly(pimelic anhydride), poly(sebacic anhydride), poly(sebacic acid-co-1,3-bis(p-carboxyphenoxy)propane (P(CPP-SA)), poly(suberic anhydride), and poly(terephthalic anhydride) or a copolymer thereof. In some embodiments, the biodegradable polyanhydride polymer comprises any of the polyanhydrides disclosed in Reddy, B.G. *Biomacromolecules*. 2022, 23, 12, 4959-4984.

The biodegradable polymer may be a poly(diol citrate), e.g., a poly(diol citrate) elastomer as disclosed in U.S. Patent No. 8,911,720, which is herein incorporated by reference in its entirety.

The biodegradable polymer may be a polyphosphazene or a derivative thereof, e.g., a polyphosphazene disclosed in U.S. Patent Nos. 11,584,828; 5,562,909; 5,562,099; and 5,500,161, which are herein incorporated by reference in their entirety.

The biodegradable polymer may be a polyurethane, e.g., a biodegradable polyurethane or derivative thereof as described in U.S. Patent No. 9,540,478, which is herein incorporated by reference in its entirety.

The biodegradable polymer may be a polycarbonate, e.g., a biodegradable polycarbonate, copolymer, or derivative thereof, as disclosed in U.S. Patent No. 9,901,649, which is herein incorporated by reference in its entirety.

The biodegradable polymer may be a polyorthoester, e.g., a biodegradable polyorthoester or derivative thereof, as disclosed in U.S. Patent No. 11,413,350, which is herein incorporated by reference in its entirety.

In some embodiments the biodegradable polymer comprises any of the biodegradable polymers disclosed in Doppalapudi S. *et al. Polym. Adv. Technol.* 2014, 25, 5, 427-435.

Fabrication of the capsules allows for diameters between about 5 μm to about 3000 μm for delivery of the therapeutic agent, e.g., a protein, e.g., a cytokine, a chemokine, or secondary polypeptide therapeutic. In some embodiments, the capsule diameter is between about 5 μm to about 3000 μm, about 6 μm to about 3000 μm, about 7 μm to about 3000 μm, about 8 μm to about 3000 μm, about 9 μm to about 3000 μm, about 10 μm to about 3000 μm, about 20 μm to about 3000 μm, about 30 μm to about 3000 μm, about 40 μm to about 3000 μm, about 50 μm to about 3000 μm, about 100 μm to about 3000 μm, about 200 μm to about 3000 μm, about 300 μm to about 3000 μm, about 400 μm to about 3000 μm, about 500 μm to about 3000 μm, about 1000 μm to about 3000 μm, or about 2000 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 6 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 7 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 8 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 9 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 10 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 20 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 30 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 40 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 50 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 100 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 200 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 300 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 400 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 500 μm to about 3000 μm. In some

embodiments, the capsule diameter is between about 1000 μm to about 3000 μm. In some embodiments, the capsule diameter is between about 2000 μm to about 3000 μm.

In some embodiments, the capsule diameter is about 150 μm, about 300 μm, about 600 μm, about 800 μm, about 1200 μm, or about 1700 μm. In some embodiments, the capsule diameter is about 150 μm. In some embodiments, the capsule diameter is about 300 μm. In some embodiments, the capsule diameter is about 600 μm. In some embodiments, the capsule diameter is about 800 μm. In some embodiments, the capsule diameter is about 1200 μm. In some embodiments, the capsule diameter is about 1700 μm.

In some embodiments, the capsule diameter is greater than about 100 μm. In some embodiments, the capsule diameter is greater than about 150 μm, about 200 μm, about 300 μm, about 400 μm, about 500 μm, about 600 μm, about 700 μm, about 800 μm, about 900 μm, about 1000 μm, about 1100 μm, about 1200 μm, about 1300 μm, about 1400 μm, about 1500 μm, about 1600 μm, about 1700 μm, about 1800 μm, about 1900 μm, or about 2000 μm. In some embodiments, the capsule diameter is greater than about 150 μm. In some embodiments, the capsule diameter is greater than about 200 μm. In some embodiments, the capsule diameter is greater than about 300 μm. In some embodiments, the capsule diameter is greater than about 400 μm. In some embodiments, the capsule diameter is greater than about 500 μm. In some embodiments, the capsule diameter is greater than about 600 μm. In some embodiments, the capsule diameter is greater than about 700 μm. In some embodiments, the capsule diameter is greater than about 800 μm. In some embodiments, the capsule diameter is greater than about 900 μm. In some embodiments, the capsule diameter is greater than about 1000 μm. In some embodiments, the capsule diameter is greater than about 1100 μm. In some embodiments, the capsule diameter is greater than about 1200 μm. In some embodiments, the capsule diameter is greater than about 1300 μm. In some embodiments, the capsule diameter is greater than about 1400 μm. In some embodiments, the capsule diameter is greater than about 1500 μm. In some embodiments, the capsule diameter is greater than about 1600 μm. In some embodiments, the capsule diameter is greater than about 1700 μm. In some embodiments, the capsule diameter is greater than about 1800 μm. In some embodiments, the capsule diameter is greater than about 1900 μm. In some embodiments, the capsule diameter is greater than about 2000 μm.

In some embodiments, the capsule diameter is between about 100 μm and 2000 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1900 μm, about

100 μm and about 1800 μm, about 100 μm and about 1700 μm, about 100 μm and about 1600 μm, about 100 μm and about 1500 μm, about 100 μm and about 1400 μm, about 100 μm and about 1300 μm, about 100 μm and about 1200 μm, about 100 μm and about 1100 μm, about 100 μm and about 1000 μm, about 100 μm and about 900 μm, about 100 μm and about 800 μm, about 100 μm and about 700 μm, about 100 μm and about 600 μm, about 100 μm and about 500 μm, about 100 μm and about 400 μm, about 100 μm and about 300 μm, or about 100 μm and about 200 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1600 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1500 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1400 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1300 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1200 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1100 μm. In some embodiments, the capsule diameter is between about 100 μm and about 1000 μm. In some embodiments, the capsule diameter is between about 100 μm and about 900 μm. In some embodiments, the capsule diameter is between about 100 μm and about 800 μm. In some embodiments, the capsule diameter is between about 100 μm and about 700 μm. In some embodiments, the capsule diameter is between about 100 μm and about 600 μm. In some embodiments, the capsule diameter is between about 100 μm and about 500 μm. In some embodiments, the capsule diameter is between about 100 μm and about 400 μm. In some embodiments, the capsule diameter is between about 100 μm and about 300 μm. In some embodiments, the capsule diameter is between about 100 μm and about 200 μm.

In some embodiments, the capsule diameter is about 150 μm.

In some embodiments, the capsule diameter is between about 200 μm and 2000 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1900 μm, about 200 μm and about 1800 μm, about 200 μm and about 1700 μm, about 200 μm and about 1600 μm, about 200 μm and about 1500 μm, about 200 μm and about 1400 μm, about 200 μm and about 1300 μm, about 200 μm and about 1200 μm, about 200 μm and about 1100 μm, about 200 μm and about 1000 μm, about 200 μm and about 900 μm, about 200 μm and about 800 μm,

about 200 μm and about 700 μm, about 200 μm and about 600 μm, about 200 μm and about 500 μm, about 200 μm and about 400 μm, or about 200 μm and about 300 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1600 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1500 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1400 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1300 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1200 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1100 μm. In some embodiments, the capsule diameter is between about 200 μm and about 1000 μm. In some embodiments, the capsule diameter is between about 200 μm and about 900 μm. In some embodiments, the capsule diameter is between about 200 μm and about 800 μm. In some embodiments, the capsule diameter is between about 200 μm and about 700 μm. In some embodiments, the capsule diameter is between about 200 μm and about 600 μm. In some embodiments, the capsule diameter is between about 200 μm and about 500 μm. In some embodiments, the capsule diameter is between about 200 μm and about 400 μm. In some embodiments, the capsule diameter is between about 200 μm and about 300 μm.

In some embodiments, the capsule diameter is about 300 μm.

In some embodiments, the capsule diameter is between about 500 μm and 2000 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1900 μm, about 500 μm and about 1800 μm, about 500 μm and about 1700 μm, about 500 μm and about 1600 μm, about 500 μm and about 1500 μm, about 500 μm and about 1400 μm, about 500 μm and about 1300 μm, about 500 μm and about 1200 μm, about 500 μm and about 1100 μm, about 500 μm and about 1000 μm, about 500 μm and about 900 μm, about 500 μm and about 800 μm, about 500 μm and about 700 μm, or about 500 μm and about 600 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1600 μm. In some embodiments, the

capsule diameter is between about 500 μm and about 1500 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1400 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1300 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1200 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1100 μm. In some embodiments, the capsule diameter is between about 500 μm and about 1000 μm. In some embodiments, the capsule diameter is between about 500 μm and about 900 μm. In some embodiments, the capsule diameter is between about 500 μm and about 800 μm. In some embodiments, the capsule diameter is between about 500 μm and about 700 μm. In some embodiments, the capsule diameter is between about 500 μm and about 600 μm.

In some embodiments, the capsule diameter is about 600 μm.

In some embodiments, the capsule diameter is between about 700 μm and 2000 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1900 μm, about 700 μm and about 1800 μm, about 700 μm and about 1700 μm, about 700 μm and about 1600 μm, about 700 μm and about 1500 μm, about 700 μm and about 1400 μm, about 700 μm and about 1300 μm, about 700 μm and about 1200 μm, about 700 μm and about 1100 μm, about 700 μm and about 1000 μm, about 700 μm and about 900 μm, or about 700 μm and about 800 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1600 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1500 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1400 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1300 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1200 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1100 μm. In some embodiments, the capsule diameter is between about 700 μm and about 1000 μm. In some embodiments, the capsule diameter is between about 700 μm and about 900 μm. In some embodiments, the capsule diameter is between about 700 μm and about 800 μm.

In some embodiments, the capsule diameter is about 800 μm.

In some embodiments, the capsule diameter is between about 1100 μm and 2000 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1900 μm, about 1100 μm and about 1800 μm, about 1100 μm and about 1700 μm, about 1100 μm and about 1600 μm, about 1100 μm and about 1500 μm, about 1100 μm and about 1400 μm, about 1100 μm and about 1300 μm, or about 1100 μm and about 1200 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1600 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1500 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1400 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1300 μm. In some embodiments, the capsule diameter is between about 1100 μm and about 1200 μm.

In some embodiments, the capsule diameter is about 1200 μm.

In some embodiments, the capsule diameter is between about 1600 μm and 2000 μm. In some embodiments, the capsule diameter is between about 1600 μm and about 1900 μm, about 1600 μm and about 1800 μm, or about 1600 μm and about 1700 μm. In some embodiments, the capsule diameter is between about 1600 μm and about 1900 μm. In some embodiments, the capsule diameter is between about 1600 μm and about 1800 μm. In some embodiments, the capsule diameter is between about 1600 μm and about 1700 μm.

In some embodiments, the capsule diameter is about 1700 μm.

In some embodiments, the capsule diameter is less than about 2000 μm. In some embodiments, the capsule diameter is less than about 1900 μm, about 1800 μm, about 1700 μm, about 1600 μm, about 1500 μm, about 1400 μm, about 1300 μm, about 1200 μm, about 1100 μm, about 1000 μm, about 900 μm, about 800 μm, about 700 μm, about 600 μm, about 500 μm, about 400 μm, about 300 μm, about 200 μm, or about 100 μm. In some embodiments, the capsule diameter is less than about 1900 μm. In some embodiments, the capsule diameter is less than about 1800 μm. In some embodiments, the capsule diameter is less than about 1700 μm. In some embodiments, the capsule diameter is less than about 1600 μm. In some embodiments, the capsule diameter is less than about 1500 μm. In some embodiments, the capsule diameter is less than about 1400 μm. In some embodiments, the capsule diameter is less than about 1300 μm. In

some embodiments, the capsule diameter is less than about 1200 μm. In some embodiments, the capsule diameter is less than about 1100 μm. In some embodiments, the capsule diameter is less than about 1000 μm. In some embodiments, the capsule diameter is less than about 900 μm. In some embodiments, the capsule diameter is less than about 800 μm. In some embodiments, the capsule diameter is less than about 700 μm. In some embodiments, the capsule diameter is less than about 600 μm. In some embodiments, the capsule diameter is less than about 500 μm. In some embodiments, the capsule diameter is less than about 400 μm. In some embodiments, the capsule diameter is less than about 300 μm. In some embodiments, the capsule diameter is less than about 200 μm. In some embodiments, the capsule diameter is less than about 100 μm.

Cells

The capsules comprise a plurality of cells that are engineered to deliver a therapeutic agent, e.g., a protein, e.g., a cytokine. Preferably, cells are capable of expressing the therapeutic agent constitutively and/or inducibly (e.g., responsive to a stimulus, e.g., responsive to secretion of a marker, e.g., a pro-inflammatory or anti-inflammatory marker) after transfection with a nucleic acid vector encoding for the therapeutic agent, e.g., transfection with a DNA plasmid using Lipofectamine™ 3000 (commercially available from ThermoFisher Scientific). The engineered cells for encapsulation may be derived from an immortalized cell line. The engineered cells for encapsulation may be derived and cultured *ex vivo* from a living organism, e.g., a human. The engineered cells may be derived from any histology of a living organism, e.g., derived from the connective tissue, the epithelium, the muscle tissue and the central or peripheral nervous system. The engineered cells for encapsulation may be an undifferentiated cell. The engineered cells for encapsulation may comprise an undifferentiated cell that is a unipotent cell or a pluripotent cell, e.g., a stem a cell. Exemplary cell lines include Chinese hamster ovary (CHO), retinal pigment epithelial (ARPE-19), human mammary epithelial (MCF-10a and MCF-7), Human embryonic kidney (HEK), mesenchymal stem cells (MSC), Human umbilical vein endothelial cells (HUVEC), NIH/3T3 cells, BJ fibroblasts, Human renal mix epithelial cells (HREC) and Human Pluripotent Stem Cells (HPSCs). In a preferred embodiment, the cell line is ARPE-19.

The engineered cells are contemplated to deliver a therapeutic agent comprising, e.g., a protein, e.g., a cytokine and/or a chemokine. Exemplary cytokines and chemokines include

interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

The therapeutic agent may be a cytokine selected from IL-1RA, IL-4, IL-10, IL-11, IL-13, and IL-35, and a combination thereof. In some embodiments, the therapeutic agent comprises IL-1Ra.

The therapeutic agent may be an anti-cytokine antibody or an anti-chemokine antibody, anti-IL-1, anti-IL-1α, anti-IL-1β, anti-IL-1RA, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-7, anti-IL-8, anti-IL-9, anti-IL-10, anti-IL-11, anti-IL-12, anti-IL-12α, anti-IL-12β, anti-IL-13, anti-IL-14, anti-IL-15, anti-IL-16, anti-IL-17, anti-IL-20, anti-IL-22, anti-IFN-α, anti-IFN-β, anti-IFN-γ, anti-TNF-α, anti-TNF-β, anti-TGF-β, anti-CCL1, anti-CCL2, anti-CCL3, anti-CCL4, anti-CCL5, anti-CCL6, anti-CCL7, anti-CCL8, anti-CCL9, anti-CCL10, anti-CCL11, anti-CCL12, anti-CCL13, anti-CCL14, anti-CCL15, anti-CCL16, anti-CCL17, anti-CCL18, anti-CCL19, anti-CCL20, anti-CCL21, anti-CCL22, anti-CCL23, anti-CCL24, ant-CCL25, anti-CCL26, anti-CCL27, anti-CCL28, anti-CXCL1, anti-CXCL2, anti-CXCL3, anti-CXCL4, anti-CXCL5, anti-CXCL6, anti-CXCL7, anti-CXCL8, anti-CXCL9, anti-CXCL10, anti-CXCL11, anti-CXCL12, anti-CXCL13, anti-CXCL14, anti-CXCL15, anti-CXCL16, and anti-CXCL17, and a combination thereof.The cells may be engineered to produce a cytokine and a secondary polypeptide therapeutic. In some embodiments, the secondary polypeptide therapeutic is an antibody, e.g., a monoclonal antibody, a polyclonal antibody, an immunoglobulin, or a functional antibody fragment capable of binding to an epitope of an antigen. In some embodiments, the secondary polypeptide therapeutic features an antibody selected from anti-interleukin 6 (anti-IL-6) and anti-interleukin 8 (anti-IL-8). In some embodiments, the secondary polypeptide therapeutic features a receptor agonist or antagonist, e.g., interleukin-1 receptor antagonist protein (IL-1RN). In some embodiments, the secondary polypeptide therapeutic features an enzyme, e.g., aromatase.

The cells may be engineered to produce a cytokine and a secondary polypeptide therapeutic. In some embodiments, the secondary polypeptide therapeutic is an antibody, e.g., a monoclonal antibody, a polyclonal antibody, an immunoglobulin, or a functional antibody fragment capable of binding to an epitope of antigen. In some embodiments, the secondary polypeptide therapeutic features an antibody selected from anti-interleukin 6 (anti-IL-6) and anti-interleukin 8 (anti-IL-8). In some embodiments, the secondary polypeptide therapeutic features a receptor agonist or antagonist, e.g., interleukin-1 receptor antagonist protein (IL-1RN). In some embodiments, the secondary polypeptide therapeutic features an enzyme, e.g., aromatase.

In an aspect, the cells may be engineered to produce an antibody, e.g., an a monoclonal antibody, a polyclonal antibody, an immunoglobulin, or a or a functional antibody fragment capable of binding to an epitope of an antigen. In some embodiments, the therapeutic agent comprises a monoclonal antibody. In some embodiments, the therapeutic agent comprises a polyclonal antibody. In some embodiments, the therapeutic agent comprises an immunoglobulin. In some embodiments, the therapeutic agent comprises a functional antibody fragment capable of binding to an epitope of antigen.

Capsules described herein may contain a plurality of cells, for example, a plurality of engineered cells. A cell may be derived from any mammalian organ or tissue, including the brain, nerves, ganglia, spine, eye, heart, liver, kidney, lung, spleen, bone, thymus, lymphatic system, skin, muscle, pancreas, stomach, intestine, blood, ovary, uterus, or testes.

A cell may be derived from a donor (*e.g.*, an allogeneic cell), derived from a subject (*e.g.*, an autologous cell), or from another species (*e.g.*, a xenogeneic cell). In an embodiment, a cell can be grown in cell culture, or prepared from an established cell culture line, or derived from a donor (*e.g.*, a living donor or a cadaver). In an embodiment, a cell is genetically engineered. In another embodiment, a cell is not genetically engineered. A cell may include a stem cell, such as a reprogrammed stem cell, or an induced pluripotent cell. Exemplary cells include mesenchymal stem cells (MSCs), fibroblasts (*e.g.*, primary fibroblasts). HEK cells (*e.g.*, HEK293T), Jurkat cells, HeLa cells, retinal pigment epithelial (RPE) cells, HUVEC cells, NIH3T3 cells, CHO-K1 cells, COS-1 cells, COS-7 cells, PC-3 cells, HCT 116 cells, A549MCF-7 cells, HuH-7 cells, U-2 OS cells, HepG2 cells, Neuro-2a cells, and SF9 cells. In an embodiment, a cell for use in an capsule is an RPE cell.

A cell included in a capsule may produce or secrete a therapeutic agent. In an embodiment, a cell included in an capsule may produce or secrete a single type of therapeutic agent or a plurality of therapeutic agents. In an embodiment, a capsule may comprise a cell that is transduced or transfected with a nucleic acid (*e.g.*, a vector) comprising an expression sequence of a therapeutic agent. For example, a cell may be transduced or transfected with a lentivirus. A nucleic acid introduced into a cell (*e.g.*, by transduction or transfection) may be incorporated into a nucleic acid delivery system, such as a plasmid, or may be delivered directly. In an embodiment, a nucleic acid introduced into a cell (*e.g.*, as part of a plasmid) may include a region to enhance expression of the therapeutic agent and/or to direct targeting or secretion, for example, a promoter sequence, an

activator sequence, or a cell-signaling peptide, or a cell export peptide. Exemplary promoters include EF-1a, CMV, Ubc, hPGK, VMD2, and CAG. Exemplary activators include the TET1 catalytic domain, P300 core, VPR, rTETR, Cas9 (*e.g.*, from *S. pyogenes* or *S. aureus*), and Cpf1 (*e.g.*, from *L. bacterium*).

A capsule described herein may comprise a cell or a plurality of cells. In the case of a plurality of cells, the concentration and total cell number may be varied depending on a number of factors, such as cell type, implantation location, and expected lifetime of the capsule. In an embodiment, the total number of cells included in a capsule is greater than about 2, 4, 6, 8, 10, 20, 30, 40, 50, 75, 100, 200, 250, 500, 750, 1000, 1500, 2000, 5000, 10000, or more. In an embodiment, the total number of cells included in an capsule is greater than about 1.0×10^2, 1.0×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, 1.0×10^8, 1.0×10^9, 1.0×10^{10}, or more. In an embodiment, the total number of cells included in a capsule is less than about than about 10000, 5000, 2500, 2000, 1500, 1000, 750, 500, 250, 200, 100, 75, 50, 40, 30, 20, 10, 8, 6, 4, 2, or less. In an embodiment, the total number of cells included in a capsule is less than about 1.0×10^{10}, 1.0×10^9, 1.0×10^8, 1.0×10^7, 1.0×10^6, 1.0×10^5, 1.0×10^4, 1.0×10^3, 1.0×10^2, or less. In an embodiment, a plurality of cells is present as an aggregate. In an embodiment, a plurality of cells is present as a cell dispersion.

Specific features of a cell contained within a capsule may be determined, *e.g.*, prior to and/or after incorporation into the capsule. For example, cell viability, cell density, or cell expression level may be assessed. In an embodiment, cell viability, cell density, and cell expression level may be determined using standard techniques, such as cell microscopy, fluorescence microscopy, histology, or biochemical assay.

Engineered Cells

In an embodiment, the capsule comprises a cell or a plurality of cells that are genetically engineered to produce or secrete a therapeutic agent. In an embodiment, the capsule comprises a cell producing or secreting a protein. The protein may be of any size, *e.g.*, greater than about 100 Da, 200 Da, 250 Da, 500 Da, 750 Da, 1 KDa, 1.5 kDa, 2 kDa, 2.5 kDa, 3 kDa, 4 kDa, 5 kDa, 6 kDa, 7 kDa, 8 kDa, 9 kDa, 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, 35 kDa, 40 kDa, 45 kDa, 50 kDa, 55 kDa, 60 kDa, 65 kDa, 70 kDa, 75 kDa, 80 kDa, 85 kDa, 90 kDa, 95 kDa, 100 kDa, 125 kDa, 150 kDa, 200 kDa, 200 kDa, 250 kDa, 300 kDa, 400 kDa, 500 kDa, 600 kDa, 700 kDa, 800

Da, 900 kDa, or more. In an embodiment, the protein is composed of a single subunit or multiple subunits (*e.g.*, a dimer, trimer, tetramer, *etc.*). A protein produced or secreted by a cell may be modified, for example, by glycosylation, methylation, or other known natural or synthetic protein modification. A protein may be produced or secreted as a pre-protein or in an inactive form and may require further modification to convert it into an active form.

Proteins produced or secreted by a cell may include antibodies or antibody fragments, for example, an Fc region or variable region of an antibody. Exemplary antibodies include anti-PD-1, anti-PD-L1, anti-CTLA4, anti-TNFα, and anti-VEGF antibodies. An antibody may be monoclonal or polyclonal. Other exemplary proteins include a lipoprotein, an adhesion protein, blood clotting factor (*e.g.*, Factor VII, Factor VIII, Factor IX, GCG, or VWF), hemoglobin, enzymes, proenkephalin, a growth factor (*e.g.*, EGF, IGF-1, VEGF alpha, HGF, TGF beta, bFGF), or a cytokine.

A protein produced or secreted by a cell may include a hormone. Exemplary hormones include growth hormone, growth hormone releasing hormone, prolactin, lutenizing hormone (LH), anti-diuretic hormone (ADH), oxytocin, thyroid stimulating hormone (TSH), thyrotropin-release hormone (TRH), adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), thyroxine, calcitonin, parathyroid hormone, aldosterone, cortisol, epinephrine, glucagon, insulin, estrogen, progesterone, and testosterone.

A protein produced or secreted by a cell may include a cytokine. A cytokine may be a pro-inflammatory cytokine or an anti-inflammatory cytokine. Example of cytokines include IL-1, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-12a, IL-12b, IL-13, IL-14, IL-16, IL-17, G-CSF, GM-CSF, IL-20, IFN-α, IFN-β, IFN-γ, CD154, LT-β, CD70, CD153, CD178, TRAIL, TNF-α, TNF-β, SCF, M-CSF, MSP, 4-1BBL, LIF, OSM, and others. For example, a cytokine may include any cytokine described in M.J. Cameron and D.J. Kelvin, *Cytokines, Chemokines, and Their Receptors* (2013), Landes Biosciences, which is incorporated herein by reference in its entirety.

A capsule may comprise a cell expressing a single type of therapeutic agent, *e.g.*, a single protein or nucleic acid, or may express more than one type of therapeutic agent, *e.g.*, a plurality of proteins or nucleic acids. In an embodiment, a capsule comprises a cell expressing two types of therapeutic agents (*e.g.*, two types of proteins or nucleic acids). In an embodiment, a capsule comprises a cell expressing three types of therapeutic agents (*e.g.*, three types of proteins or nucleic

acids). In an embodiment, a capsule comprises a cell expressing four types of therapeutic agents (*e.g.*, four types of proteins or nucleic acids).

In an embodiment, a capsule comprises a cell expressing a single type of nucleic acid (*e.g.*, DNA or RNA) or may express more than one type of nucleic acid, *e.g.*, a plurality of nucleic acid (*e.g.*, DNA or RNA). In an embodiment, a capsule comprises a cell expressing two types of nucleic acids (*e.g.*, DNA or RNA). In an embodiment, a capsule comprises a cell expressing three types of nucleic acids (*e.g.*, DNA or RNA). In an embodiment, a capsule comprises a cell expressing four types of nucleic acids (*e.g.*, DNA or RNA).

In an embodiment, a capsule comprises a cell expressing a single type of protein, or may express more than one type of protein, *e.g.*, a plurality of proteins. In an embodiment, a capsule comprises a cell expressing two types of proteins. In an embodiment, a capsule comprises a cell expressing three types of proteins. In an embodiment, a capsule comprises a cell expressing four types of proteins.

In an embodiment, a capsule comprises a cell expressing a single type of enzyme, or may express more than one type of enzyme, *e.g.*, a plurality of enzymes. In an embodiment, a capsule comprises a cell expressing two types of enzymes. In an embodiment, a capsule comprises a cell expressing three types of enzymes. In an embodiment, a capsule comprises a cell expressing four types of enzymes.

In an embodiment, a capsule comprises a cell expressing a single type of antibody or antibody fragment or may express more than one type of antibody or antibody fragment, *e.g.*, a plurality of antibodies or antibody fragments. In an embodiment, a capsule comprises a cell expressing two types of antibodies or antibody fragments. In an embodiment, a capsule comprises a cell expressing three types of antibodies or antibody fragments. In an embodiment, a capsule comprises a cell expressing four types of antibodies or antibody fragments.

In an embodiment, a capsule comprises a cell expressing a single type of hormone, or may express more than one type of hormone, *e.g.*, a plurality of hormones. In an embodiment, a capsule comprises a cell expressing two types of hormones. In an embodiment, a capsule comprises a cell expressing three types of hormones. In an embodiment, a capsule comprises a cell expressing four types of hormones.

In an embodiment, a capsule comprises a cell expressing a single type of enzyme, or may express more than one type of enzyme, *e.g.*, a plurality of enzymes. In an embodiment, a capsule

comprises a cell expressing two types of enzymes. In an embodiment, a capsule comprises a cell expressing three types of enzymes. In an embodiment, a capsule comprises a cell expressing four types of enzymes.

In an embodiment, a capsule comprises a cell expressing a single type of cytokine or may express more than one type of cytokine, *e.g.*, a plurality of cytokines. In an embodiment, a capsule comprises a cell expressing two types of cytokines. In an embodiment, a capsule comprises a cell expressing three types of cytokines. In an embodiment, a capsule comprises a cell expressing four types of cytokines.

Gene Autoregulation of Therapeutic Agent Expression

It is contemplated that in instances where the engineered cell expresses a therapeutic agent, e.g., a cytokine, it is desirable that the level of production of the therapeutic agent be auto regulated in order to prevent secretion of toxic levels of the cytokine. One way to accomplish this is to introduce an operator site into the DNA region between the cytokine gene and its promoter in a first ORF. A second ORF is used that encodes a transcriptional repressor that binds to the operator site under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. In this way, the cells can sense the cytokine in their environment and reduce their production of the cytokine when there is sufficient cytokine already present.

Another possible strategy is to introduce a sequence that forms a higher-order structure into the 5' untranslated region (5' UTR) of the cytokine gene. Then a second ORF is used that encodes an RNA-binding protein that binds to the higher-order structure, and suppresses translation, under the control of a promoter that is activated as a result of signaling through the cytokine's receptor.

Another possible strategy is to introduce several repeats of a synthetic microRNA (miRNA) target site into the 3' untranslated region (3' UTR) of the cytokine gene. Then a second ORF is used that encodes the miRNA under the control of a promoter that is activated as a result of signaling through the cytokine's receptor.

Another possible strategy is to use a second ORF encoding a synthetic ubiquitin ligase that targets the cytokine, and leads to ubiquitin-mediated proteolysis, under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. For example, if the cytokine is IL-2, then the promoter controlling the expression of the ubiquitin ligase could

be a STAT transcription factor (FIG. 8). In this case, the cytokine gene may be modified to include additional protein domains if doing so is necessary in order to make the cytokine recognizable by the synthetic ubiquitin ligase. Ideally, the addition of any additional protein domains will not alter the cytokine's immunological functions.

A further strategy is to incorporate a mechanism where the therapeutic agent is under control of a promoter, e.g., a synthetic promoter or a natural promoter), which binds to a native transcription factor whose activation is downstream of a receptor, e.g., a cytokine receptor, e.g., NF-κB or NFAT.

These self-regulated control strategies could be combined with small molecule-based strategies to provide an additional level of control to the cytokine production. Using a small molecule-activated promoter (such as the TRE/tetracycline system) to drive expression of the cytokine would allow for external regulation of the cytokine production by the administration of the small molecule. Post-transcriptional control of the cytokine expression is also possible using small molecule-dependent riboswitches - a short sequence could be added to the 5' or 3' UTR of the cytokine gene that forms a small molecule-dependent functional higher-order structure, such as a frame-shifting aptamer or a mRNA-cleaving aptazyme, allowing for similar external control of the cytokine production, since there are examples of these systems that turn on frame-shifting or cleavage upon the addition of a small molecule and examples that turn off in the presence of the small molecule. This type of control is also possible at the protein level by adding the sequence for a destabilization domain that can be stabilized by a small molecule to the beginning or end of the gene for the cytokine, which would lead to targeted degradation of the cytokine whenever the small molecule is not present. The reverse is also possible by augmenting the gene for the cytokine with the sequence for a small molecule-assisted shutoff (SMASh) system, which includes a destabilization domain and a non-mammalian protease that cleaves the destabilization domain from the cytokine except in the presence of a small molecule protease inhibitor that would prevent cleavage and lead to degradation of the cytokine. All these modifications to the protein structure could also be done indirectly by instead modifying a synthetic transcription factor that activates the promoter controlling expression of the cytokine, which would ensure that all these protein modifications stay within the therapeutic cells instead of being secreted and potentially generating an immune response to these unnatural protein domains. One possible synthetic transcription factor to use for this purpose is a fusion between

the transcriptional activators VP64, p65, and Rta (VPR) and catalytically inactivated Cas9 (dCas9), which when coexpressed with a guide RNA (gRNA) will localize the VPR complex to the synthetic promoter with complementarity to the gRNA in order to activate transcription of the cytokine gene.

Controlling Drug Delivery and Release Kinetics

The vector systems contemplated in the engineered cells may further comprise a kill switch to arrest the therapy, similar to the kill switch designed for CAR T cells. For example, two engineered proteins may be located inside the encapsulated cells, that dimerize when exposed to a small molecule drug called rimiducid. This drug activates a protein called caspase-9, which induces cell death.

Chemical Induction of Dimerization (CID) with small molecules is an effective technology used to generate switches of protein function to alter cell physiology. A high specificity, efficient dimerizer is rimiducid (AP1903), which has two identical, protein-binding surfaces arranged tail-to-tail, each with high affinity and specificity for a mutant or variant of FKBP12: FKBP12(F36V) (FKBP12v36, FV36 or FV). Attachment of one or more FV domains onto one or more cell signaling molecules that normally rely on homodimerization can convert that protein to rimiducid control. For example, a molecular switch is provided that provides the option to activate a pro-apoptotic polypeptide, such as, for example, Caspase-9, with rimiducid, wherein the chimeric pro-apoptotic polypeptide comprises a rimiducid-induced switch.

In one embodiment of the switch technology, a homodimerizer, such as AP1903 (rimiducid), activates a safety switch, causing apoptosis of the modified cell. In this embodiment, for example, a chimeric pro-apoptotic polypeptide, such as, for example, Caspase-9, comprising a FKBP12 multimerizing region is expressed in a cell. Upon contacting the cell with a dimerizer that binds to the Fv regions, the chimeric polypeptide dimerizes or multimerizes, and activates the cell. The cell may, for example, be an engineered cell that expresses a protein, e.g., a cytokine.

Furthermore, a transmembrane sensor can be engineered into the engineered cells to create a feedback loop to regulate output of the therapeutic agent. The transmembrane sensor responds to varying concentrations of the protein of interest and uses a negative feedback loop to

suppress the transcription of the therapeutic agent of interest, , e.g., cytokine or chemokine, with the help of an inducible promoter. This allows fine-tuning of the localized delivery of the protein of interest and ensures that there is no over-expression of the protein of interest. The alginate biomaterial used allows for rapid diffusion across the inner and outer shell to give real-time feedback to this sense-and-respond genetic cellular circuit.

Features of Capsules

The capsule described herein may take any suitable shape or morphology. For example, n capsule may be a sphere, spheroid, tube, cord, string, ellipsoid, disk, cylinder, sheet, torus, cube, stadiumoid, cone, pyramid, triangle, rectangle, square, or rod. A capsule may comprise a curved or flat section. In an embodiment, the capsule may be prepared through the use of a mold, resulting in a custom shape.

The capsule may vary in size, depending, for example, on the use or site of implantation. For example, the capsule may have a mean diameter or size greater than 0.1 mm, *e.g.*, greater than 0.25 mm, 0.5 mm, 0.75, 1 mm, 1.5 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, 20 mm, 30 mm, 40 mm, 50 mm, or more. In an embodiment, the capsule may have a section or region with a mean diameter or size greater than 0.1 mm, *e.g.*, greater than 0.25 mm, 0.5 mm, 0.75, 1 mm, 1.5 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, 20 mm, 30 mm, 40 mm, 50 mm, or more. In an embodiment, the capsule may have a mean diameter or size less than 1 cm, *e.g.*, less 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 7.5 mm, 5 mm, 2.5 mm, 1 mm, 0.5 mm, or smaller. In an embodiment, the capsule may have a section or region with a mean diameter or size less than 1 cm, *e.g.*, less 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 7.5 mm, 5 mm, 2.5 mm, 1 mm, 0.5 mm, or smaller.

In an embodiment, the capsule comprises a pore or opening to permit passage of an object, such as a small molecule (*e.g.*, nutrients or waste), a protein, or a nucleic acid. For example, a pore in or on a capsule may be greater than 0.1 nm and less than 10 μm. In an embodiment, the capsule comprises a pore or opening with a size range of 0.1 μm to 10 μm, 0.1 μm to 9 μm, 0.1 μm to 8 μm, 0.1 μm to 7 μm, 0.1 μm to 6 μm, 0.1 μm to 5 μm, 0.1 μm to 4 μm, 0.1 μm to 3 μm, 0.1 μm to 2 μm.

The capsule described herein may comprise a chemical modification in or on any enclosed material. Exemplary chemical modifications include small molecules, peptides, proteins, nucleic

acids, lipids, or oligosaccharides. The capsule may comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or more of a material that is chemically modified, *e.g.*, with a chemical modification described herein. A capsule may be partially coated with a chemical modification, *e.g.*, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 99.9% coated with a chemical modification.

In some embodiments, the capsule is chemically modified with a specific density of modifications. The specific density of chemical modifications may be described as the average number of attached chemical modifications per given area. For example, the density of a chemical modification on or in an capsule may be 0.01, 0.1, 0.5, 1, 5, 10, 15, 20, 50, 75, 100, 200, 400, 500, 750, 1,000, 2,500, or 5,000 chemical modifications per square μm or square mm.

A capsule may be formulated or configured for implantation in any organ, tissue, cell, or part of a subject. For example, the capsule may be implanted or disposed into the intraperitoneal space of a subject. An capsule may be implanted in or disposed on a tumor or other growth in a subject, or be implanted in or disposed about 0.1 mm, 0.5 mm, 1 mm, 0.25 mm, 0.5 mm, 0.75, 1 mm, 1.5 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, 20 mm, 30 mm, 40 mm, 50 mm, 1 cm, 5, cm, 10 cm, or further from a tumor or other growth in a subject. A capsule may be configured for implantation, or implanted, or disposed on or in the skin, a mucosal surface, a body cavity, the central nervous system (*e.g.*, the brain or spinal cord), an organ (*e.g.*, the heart, eye, liver, kidney, spleen, lung, ovary, breast, uterus), the lymphatic system, vasculature, oral cavity, nasal cavity, gastrointestinal tract, bone, muscle, adipose tissue, skin, or other area.

A capsule may be formulated for use for any period of time. For example, a capsule may be used for 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 1 day, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, or longer. A capsule can be configured for limited exposure (*e.g.*, less than 2 days, *e.g.*, less than 2 days, 1 day, 24 hours, 20 hours, 16 hours, 12 hours, 10 hours, 8 hours, 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, 1 hour or less). A capsule can be configured for prolonged exposure (*e.g.*, at least 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24

months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years, 4 years or more). An capsule can be configured for permanent exposure (*e.g.*, at least 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years, 4 years or more).

Pharmaceutical Compositions

The capsules may be formulated to provide a therapeutic agent, e.g., a protein such as a cytokine to the lungs or other adjacent tissues of the respiratory system, directly or indirectly as a pharmaceutical composition or a pharmaceutical product. In some embodiments, the capsules may be formulated to provide a therapeutic agent, e.g., a protein such as a cytokine, to the lungs or other adjacent tissues of the respiratory system, directly or indirectly as a pharmaceutical composition or a pharmaceutical product for the treatment of a disease, e.g., an immune disease or disorder, e.g., an autoimmune disease or disorder, or an inflammatory disorder, e.g., an autoinflammatory disease or disorder. In some embodiments, the capsule comprising a plurality of engineered cells is formulated as an inhalable composition. In some embodiments, the capsule is formulated for local delivery, e.g., via intratracheal instillation or intubation, e.g., with a bronchoscope or a catheter. The composition may further comprise a pH modifier, a tonicity agent, a viscosity modifier, a carrier or diluent (e.g., a pharmaceutically acceptable carrier or diluent), a preservative, a surfactant, or a polymer.

In some embodiments, the composition comprises a pH modifier. A function of the pH modifier is to maintain the pH of the composition within a predetermined range, which is optimal for administration of the composition, efficacious for delivery of the therapeutic agent (e.g., a protein such as a cytokine) ensures the stability or prevents the degradation or hydrolysis of the therapeutic agent, and the like. Suitable pH modifiers include pharmaceutically acceptable buffering agents, e.g., a combination of a weak Lewis acid and/or its conjugate base. Exemplary pH modifiers featured in the composition include, without limitation, adipic acid, ammonium bicarbonate, sodium hydrogen carbonate, L-tartaric acid, and potassium citrate monohydrate.

The composition features a pH modifier such that the pH of the composition is maintained within a predetermined range. In some embodiments, the pH of the composition is between about 3 to about 10, about 4 to about 9, about 5 to about 8, or about 6 to about 7. In

some embodiments, the pH of the composition is between about 3 to about 10. In some embodiments, the pH of the composition is between about 4 to about 9. In some embodiments, the pH of the composition is between about 5 to about 8. In some embodiments, the pH of the composition is between about 6 to about 7.

In some embodiments, the composition comprises a tonicity agent. Exemplary tonicity agents include dextrose, glycerin, mannitol, potassium chloride, sodium chloride, and the like.

In some embodiments, the composition may comprise between about 0.1% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 2% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 7.5% w/w to about 20% w/w of the tonicity agent. In some embodiments, the capsule comprises between about 10% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the tonicity agent. In some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the

tonicity agent. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the tonicity agent.

In some embodiments, the composition comprises a viscosity modifier. Suitable pharmaceutically acceptable viscosity modifiers include guar gum, xanthan gum, gellan gum, dextran, pullulan, guar gum, acacia gum, carrageenan, pectin, starch or modified starch derivatives, cellulose, carboxymethylcellulose, chitosan, gelatin, hydroxypropyl methylcellulose, methyl hydroxypropyl cellulose, methyl hydroxyethyl cellulose, hydroxypropyl cellulose, nanocellulose, and the like.

The composition may comprise between about 0.1% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition between about 2% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 7.5% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 10% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the viscosity modifier. In

some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the viscosity modifier. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the viscosity modifier.

In some embodiments, the composition comprises a carrier or diluent, e.g., a pharmaceutically acceptable carrier or diluent. Exemplary pharmaceutically acceptable carriers or diluents include lactose (e.g., lactose monohydrate), microcrystalline cellulose, native (uncooked starch), pregelatinized starch, calcium phosphate, calcium carbonate, sucrose, maltodextrin, D-mannitol, sorbitol, and sodium chloride.

The composition may comprise between about 0.1% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 2% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition

comprises between about 7.5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 10% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the pharmaceutically acceptable carrier or diluent.

In some embodiments, the composition comprises a preservative. In some embodiments, the composition comprises a preservative with bactericidal, bacteriostatic, anti-fungal, or anti-protozoal activity. In some embodiments, the preservative is a bactericide. In some embodiments, the preservative is an anti-fungal agent. Exemplary preservatives include benzoic acid, benzyl alcohol, benzalkonium chloride, benzethonium chloride, bronidol, butylatehydroxytoluene (BHT), butyl paraben, chlorobutanol, chlorocresol, meta cresol, methyl paraben, phenyl ethyl alcohol, propyl paraben, phenol, propyl gallate, propylene glycol, sodium benzoate, sodium calcium edetate, sorbic acid, thiomersal, Vitamin C, and Vitamin E.

The composition may comprise between about 0.1% w/w to about 20% w/w of the preservative. In some embodiments, the capsule comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 2% w/w to about 20% w/w of the preservative. In

some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 7.5% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 10% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the preservative. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the preservative.

In some embodiments, the composition features a surfactant. In some embodiments, the composition comprises an anionic surfactant. In some embodiments, the anionic surfactant is a (C_{10}-C_{20}) carboxylate salt, e.g., a salt of capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid , margaric acid, stearic acid, nonadecylic acid, or arachidinic acid, wherein the counterion is selected from an alkali metal cation, e.g., sodium. In some embodiments, the anionic surfactant is an alkyl (poly)ether sulfate. Exemplary alkyl (poly)ether sulfates include ammonium lauryl sulfate, sodium dodecyl sulfate, sodium lauryl sulfate, and sodium pareth sulfate.

In some embodiments, the composition comprises a cationic surfactant. In some embodiments, the cationic surfactant is an alkyl benzalkonium chloride.

In some embodiments, the composition comprises a zwitterionic (i.e., an amphoteric) surfactant. In some embodiments, the zwitterionic (i.e., amphoteric) surfactant is a zwitterionic phospholipid, e.g., phosphatidylcholine, e.g., lecithin, e.g., soy lecithin or egg lecithin. In some embodiments, the composition comprising a polymer an plurality of engineered cells comprises a zwitterionic (i.e., an amphoteric) surfactant, wherein the zwitterionic (i.e., amphoteric) surfactant is cocamidopropyl betaine, or cocamidopropyl hydroxysultaine.

In some embodiments, the composition comprises a nonionic surfactant. In some embodiments, the nonionic surfactant is an alcohol ethoxylate, a polyethoxylated glycol ether, a polysorbate, a sorbitan ester, or poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO).

In some embodiments, the composition comprises a surfactant selected from any of the pharmaceutically relevant surfactants disclosed in Tadros, T.F. *Applied surfactants: principles and applications*. 2006: John Wiley & Sons.

The composition may comprise between about 0.1% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 2% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 7.5% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 10% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the surfactant. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the surfactant.

In some embodiments, the composition features a polymer. Exemplary polymers include polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) , polylactic acid (PLA), poly(ε-caprolactone) (PCL), and any copolymer thereof.

The composition may comprise between about 0.1% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 0.1% w/w to about 20% w/w, about 0.2% w/w to about 20% w/w, about 0.3% w/w to about 20% w/w, about 0.4% w/w to about 20% w/w, about 0.5% w/w to about 20% w/w, about 1% w/w to about 20% w/w, about 2% w/w to about 20% w/w, about 3% w/w to about 20% w/w, about 4% w/w to about 20% w/w, about 5% w/w to about 20% w/w, about 7.5% w/w to about 20% w/w, about 10% w/w to about 20% w/w, about 12.5% w/w to about 20% w/w, about 15% w/w to about 20% w/w, or about 17.5% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 0.2% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 0.3% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 0.4% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 0.5% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 1% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 2% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 3% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 4% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 5% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 7.5% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 10% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 12.5% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 15% w/w to about 20% w/w of the polymer. In some embodiments, the composition comprises between about 17.5% w/w to about 20% w/w of the polymer.

Delivery Modes

Local Delivery

The present disclosure provides modes for local delivery of a composition featuring capsules comprising a plurality of engineered cells.

The composition may be disposed in a cellular compartment, e.g., an organ, or a particular region of an organ. In some embodiments, the composition is disposed in the respiratory system, e.g., the nose, the paranasal sinuses, the pharynx, the larynx, the trachea, the left bronchus, the right bronchus, the left lung, and/or the right lung. In some embodiments, the composition is disposed in the nose, e.g., the nasal cavity, the nasal conchae, or the nasal vestibule. In some embodiments, capsule is disposed in the paranasal sinuses, e.g., the frontal paranasal sinuses or the sphenoid nasal sinuses. In some embodiments, the composition is disposed in the pharynx or the oral cavity. In some embodiments, the composition is disposed in the larynx. In some embodiments, the composition is disposed in the trachea, e.g., adjacent to the carina of trachea. In some embodiments, the composition is disposed in the left bronchus. In some embodiments, the composition is disposed in the right bronchus. In some embodiments, the composition is disposed in the left lung, e.g., the inferior lobe, the middle lobe, or the superior lobe. In some embodiments, the composition is disposed in the left lung, e.g., the left bronchiole, the alveolar duct, the alveolar sac, or the alveolus. In some embodiments, the composition is disposed in the right lung, e.g., the inferior lobe, the middle lobe, or the superior lobe. In some embodiments, the composition is disposed in the right lung, e.g., the right bronchiole, the alveolar duct, the alveolar sac, or the alveolus.

The composition may be disposed in a cellular compartment, e.g., a cavity, e.g., the pleural cavity or the thoracic cavity. In some embodiments, the composition is disposed in the pleural cavity. In some embodiments, the capsule is disposed in the diaphragm.

In an aspect, the composition is provided as an implantable drug delivery system, e.g., the implantable drug delivery system is provided to a subject via surgical implantation.

In some embodiments, the composition is characterized by an immediate release pharmacokinetic profile, e.g., a “burst” release profile, of the therapeutic agent.

In some embodiments, the composition features a drug depot, wherein the pharmacokinetic profile is characterized by a sustained release pharmacokinetic profile.

In some embodiments, the composition features a drug depot, wherein a therapeutic agent is released responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the therapeutic agent is released responsive to a cytokine. In some embodiments, the therapeutic agent is released responsive to a chemokine. In some embodiments, the therapeutic agent is

released responsive to an antigen. In some embodiments, the therapeutic agent is released responsive to an antibody. In some embodiments, the therapeutic agent is released responsive to a pathogen.

In some embodiments, the therapeutic agent is released constitutively and/or responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the constitutive release of the therapeutic agent is characterized by a sustained release pharmacokinetic profile. In some embodiments, the constitutive release of the therapeutic agent is characterized by an immediate "burst" release pharmacokinetic profile. In some embodiments, additional therapeutic agent is released responsive to the stimulus, characterized in that the additional release of the therapeutic agent is additive to the constitutive release of the therapeutic agent.

In some embodiments, the stimulus is a cytokine and/or chemokine selected from one or more of: interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-b), interferon-gamma gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22

(CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

In some embodiments, the stimulus is an anti-inflammatory cytokine selected from IL-1RA, IL-4, IL-10, IL-11, IL-13, and IL-35, and a combination thereof. In some embodiments, the stimulus comprises IL-1Ra.

In some embodiments, the stimulus is an anti-cytokine (auto)antibody or anti-chemokine (auto)antibody, e.g., anti-IL-1, anti-IL-1α, anti-IL-1β, anti-IL-1RA, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-7, anti-IL-8, anti-IL-9, anti-IL-10, anti-IL-11, anti-IL-12, anti-IL-12α, anti-IL-12β, anti-IL-13, anti-IL-14, anti-IL-15, anti-IL-16, anti-IL-17, anti-IL-20, anti-IL-22, anti-IFN-α, anti-IFN-β, anti-IFN-γ, anti-TNF-α, anti-TNF-β, anti-TGF-β, anti-CCL1, anti-CCL2, anti-CCL3, anti-CCL4, anti-CCL5, anti-CCL6, anti-CCL7, anti-CCL8, anti-CCL9, anti-CCL10, anti-CCL11, anti-CCL12, anti-CCL13, anti-CCL14, anti-CCL15, anti-CCL16, anti-CCL17, anti-CCL18, anti-CCL19, anti-CCL20, anti-CCL21, anti-CCL22, anti-CCL23, anti-CCL24, ant-CCL25, anti-CCL26, anti-CCL27, anti-CCL28, anti-CXCL1, anti-CXCL2, anti-CXCL3, anti-CXCL4, anti-CXCL5, anti-CXCL6, anti-CXCL7, anti-CXCL8, anti-CXCL9, anti-CXCL10, anti-CXCL11, anti-CXCL12, anti-CXCL13, anti-CXCL14, anti-CXCL15, anti-CXCL16, and anti-CXCL17, and a combination thereof.

In some embodiments, the composition is provided as an implantable drug delivery system, wherein the drug delivery system is a drug depot. A drug depot as contemplated herein is a local delivery system with a matrix comprised of polymers, hydrogels, or phospholipids and the like, allowing for local delivery of a therapeutic agent. In some embodiments, the drug depot

features a polymer or copolymer selected from one or more of polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(hydroxybutyrate-co-hydroxyvalerate), poly(γ-glutamic acid), polyglycolic acid (PGA), polylactic acid (PLA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol)-*b*-hyaluronic acid (PEG-HA), poly(ethylene glycol)-*b*-polycaprolactone (PEG-PCL), poly(ethylene glycol)-*b*-poly(glutamic acid) (PEG-PGA), poly(ethylene glycol)-*b*-polylactic acid (PEG-PLA), poly(ethylene glycol)-*b*-poly(glycolic-co-lactic acid) (PEG-PLGA), poly(ethylene glycol)-*b*-poly(L-lactic acid) (PEG-PLLA), and poly(ethylene glycol)-*b*-poly(ethyleneimine) (PEG-PEI). In some embodiments, the drug depot features a phospholipid selected from one or more of a phosphatidylcholine, a phosphatidylserine, a phosphatidylinositol, a phosphatidylglycerol, or a phosphatidic acid. In some embodiments, the drug depot features a phosphatidylcholine, e.g., lecithin (e.g., soy lecithin or egg lecithin), hydrogenated phosphatidylcholine (HPC), or 1,2-dipalmitoyl-*sn*-glycero-3-phosphcholine (DPPC). In some embodiments, the drug depot features a polyethylene glycol-conjugated phospholipid, e.g., 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG). In some embodiments, the drug depot features a hydrogel-forming polymer selected from one or more of alginate, alginate-polyethylene glycol (alginate-PEG), alginate-polyacrylamide (alginate-PAAm), α-cyclodextrin, α-cyclodextrin-polyethylene glycol (α-cyclodextrin-PEG), chitosan, collagen, fibrin, heparin, hyaluronic acid (HA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyacrylamide-ferrocene (PAAm-Fc), poly(L-lysine)/polyacrylic acid (PLL/PAA), poly(N-isopropylacylamide) (PNIPAAM), poly((N-isopropylacrylamide)-co-(sodium acrylic acid)) (P(NIPAAM-co-AAcNa)), polyvinyl alcohol-polyacrylamide (PVA-PAAm), and poly(vinyl pyrrolidine) (PVP).

In an aspect, the composition may be delivered with a device. In some embodiments, the device is bronchoscope. In some embodiments, the composition is delivered with a rigid bronchoscope. In some embodiments, the composition is delivered with a flexible bronchoscope.

In some embodiments, the composition is delivered as part of an endoscopic procedure. In some embodiments, the composition is delivered through the mouth, e.g., the oral cavity. In some embodiments, the composition is delivered through the nose. In some embodiments, the capsule is delivered through a tracheostomy. In some embodiments, the composition is delivered through the mouth and disposed in the bronchi, the bronchioles, or various parts of the lung. In some embodiments, the composition is delivered through the nose and disposed in the bronchi,

the bronchioles, or various parts of the lung. In some embodiments, the composition is delivered through a tracheostomy and disposed in the bronchi, the bronchioles, or various parts of the lung. In some embodiments, the composition is delivered as part of a diagnostic procedure. In some embodiments, the capsule is delivered as part of a therapeutic procedure.

In an aspect, the composition is provided, e.g., administered, by injection, e.g., intramuscular injection or subcutaneous injection. In some embodiments, the composition is administered by intramuscular injection. In some embodiments, the composition is administered by subcutaneous injection. The composition provided for injection may be formulated as a solid, e.g., a lyophilized powder that may be reconstituted before or at the time of administration. The composition provided for injection may be formulated as a liquid. The composition provided for injection may be formulated as a semi-solid preparation, e.g., a gel, a hydrogel, and the like. The composition provided for injection may be formulated as a drug depot.

In some embodiments, the composition provided for injection features a drug depot, wherein a therapeutic agent is released responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the therapeutic agent is released responsive to a cytokine. In some embodiments, the therapeutic agent is released responsive to a chemokine. In some embodiments, the therapeutic agent is released responsive to an antigen. In some embodiments, the therapeutic agent is released responsive to an antibody. In some embodiments, the therapeutic agent is released responsive to a pathogen.

In some embodiments, the composition provided for injection features a drug depot, wherein a therapeutic agent is released constitutively and/or responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the constitutive release of the therapeutic agent is characterized by a sustained release pharmacokinetic profile. In some embodiments, the constitutive release of the therapeutic agent is characterized by an immediate "burst" release pharmacokinetic profile. In some embodiments, additional therapeutic agent is released responsive to the stimulus, characterized in that the additional release of the therapeutic agent is additive to the constitutive release of the therapeutic agent.

In some embodiments, the stimulus is a cytokine and/or chemokine selected from one or more of: interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β),

interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-b), interferon-gamma gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

In some embodiments, the stimulus is an anti-inflammatory cytokine selected from IL-1RA, IL-4, IL-10, IL-11, IL-13, and IL-35, and a combination thereof. In some embodiments, the stimulus comprises IL-1Ra.

In some embodiments, the stimulus is an anti-cytokine (auto)antibody or anti-cytokine (auto)antibody, e.g., anti-IL-1, anti-IL-1α, anti-IL-1β, anti-IL-1RA, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-7, anti-IL-8, anti-IL-9, anti-IL-10, anti-IL-11, anti-IL-12, anti-IL-12α, anti-IL-12β, anti-IL-13, anti-IL-14, anti-IL-15, anti-IL-16, anti-IL-17, anti-IL-20, anti-IL-22, anti-IFN-α, anti-IFN-β, anti-IFN-γ, anti-TNF-α, anti-TNF-β, anti-TGF-β, anti-CCL1, anti-CCL2, anti-CCL3, anti-CCL4, anti-CCL5, anti-CCL6, anti-CCL7, anti-CCL8, anti-CCL9, anti-CCL10, anti-CCL11, anti-CCL12, anti-CCL13, anti-CCL14, anti-CCL15, anti-CCL16, anti-CCL17, anti-CCL18, anti-CCL19, anti-CCL20, anti-CCL21, anti-CCL22, anti-CCL23, anti-CCL24, ant-CCL25, anti-CCL26, anti-CCL27, anti-CCL28, anti-CXCL1, anti-CXCL2, anti-CXCL3, anti-CXCL4, anti-CXCL5, anti-CXCL6, anti-CXCL7, anti-CXCL8, anti-CXCL9, anti-CXCL10, anti-CXCL11, anti-CXCL12, anti-CXCL13, anti-CXCL14, anti-CXCL15, anti-CXCL16, and anti-CXCL17, and a combination thereof.

In some embodiments, the composition provided for injection features a drug delivery system, wherein the drug delivery system is a drug depot. A drug depot as contemplated herein is a local delivery system with a matrix comprised of polymers, hydrogels, or phospholipids and the like, allowing for local delivery of a therapeutic agent. In some embodiments, the drug depot features a polymer or copolymer selected from one or more of polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(hydroxybutyrate-co-hydroxyvalerate), poly(γ-glutamic acid), polyglycolic acid (PGA), polylactic acid (PLA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol)-*b*-hyaluronic acid (PEG-HA), poly(ethylene glycol)-*b*-polycaprolactone (PEG-PCL), poly(ethylene glycol)-*b*-poly(glutamic acid) (PEG-PGA), poly(ethylene glycol)-*b*-polylactic acid (PEG-PLA), poly(ethylene glycol)-*b*-poly(glycolic-co-lactic acid) (PEG-PLGA), poly(ethylene glycol)-*b*-poly(L-lactic acid) (PEG-PLLA), and poly(ethylene glycol)-*b*-poly(ethyleneimine) (PEG-PEI). In some embodiments, the drug depot features a phospholipid selected from one or more of a phosphatidylcholine, a phosphatidylserine, a phosphatidylinositol, a phosphatidylglycerol, or a phosphatidic acid. In some embodiments, the drug depot features a phosphatidylcholine, e.g., lecithin (e.g., soy lecithin or egg lecithin), hydrogenated phosphatidylcholine (HPC), or 1,2-dipalmitoyl-*sn*-

glycero-3-phosphcholine (DPPC). In some embodiments, the drug depot features a polyethylene glycol-conjugated phospholipid, e.g., phosph1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG). In some embodiments, the drug depot features a hydrogel-forming polymer selected from one or more of alginate, alginate-polyethylene glycol (alginate-PEG), alginate-polyacrylamide (alginate-PAAm), α-cyclodextrin, α-cyclodextrin-polyethylene glycol (α-cyclodextrin-PEG), chitosan, collagen, fibrin, heparin, hyaluronic acid (HA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyacrylamide-ferrocene (PAAm-Fc), poly(L-lysine)/polyacrylic acid (PLL/PAA), poly(N-isopropylacylamide) (PNIPAAM), poly((N-isopropylacrylamide)-co-(sodium acrylic acid)) (P(NIPAAM-co-AAcNa)), polyvinyl alcohol-polyacrylamide (PVA-PAAm), and poly(vinyl pyrrolidine) (PVP).

Systemic Delivery

The present disclosure provides modes for systemic delivery of a therapeutic agent, e.g., a protein such as a cytokine, by administering a composition featuring capsules comprising a plurality of engineered cells.

The composition may be disposed in a cellular compartment, e.g., an organ, a cavity or a serous membrane. In some embodiments, the capsule is disposed in the abdomino-pelvic cavity, cranial cavity, dorsal cavity, ventral cavity, or thoracic cavity. In a preferred embodiment, the composition is disposed in the intraperitoneal cavity or adjacent to the peritoneum.

In an aspect, the composition is provided as an implantable drug delivery system, e.g., the implantable drug delivery system is provided to a subject via surgical implantation. In some embodiments, the composition is implanted in a cellular compartment, e.g., a cavity or serous membrane. In some embodiments, the composition is implanted in the abdomino-pelvic cavity, cranial cavity, dorsal cavity, ventral cavity, or thoracic cavity. In a preferred embodiment, the composition is implanted in the intraperitoneal cavity or adjacent to the peritoneum.

In some embodiments, the composition is characterized by an immediate release pharmacokinetic profile, e.g., a “burst” release profile, of the therapeutic agent. In some embodiments, the pharmacokinetic profile is characterized by a sustained release pharmacokinetic profile.

In some embodiments, the therapeutic agent is released responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an

antibody, or a pathogen. In some embodiments, the therapeutic agent is released responsive to a cytokine. In some embodiments, the agent is released responsive to a chemokine. In some embodiments, the therapeutic agent is released responsive to an antigen. In some embodiments, the therapeutic agent is released responsive to an antibody. In some embodiments, the therapeutic agent is released responsive to a pathogen.

In some embodiments, the therapeutic agent is released constitutively and/or responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the constitutive release of the therapeutic agent is characterized by a sustained release pharmacokinetic profile. In some embodiments, the constitutive release of the therapeutic agent is characterized by an immediate "burst" release pharmacokinetic profile. In some embodiments, additional therapeutic agent is released responsive to the stimulus, characterized in that the additional release of the therapeutic agent is additive to the constitutive release of the therapeutic agent.

In some embodiments, the stimulus is a cytokine and/or chemokine selected from one or more of: interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interferon-alpha (IFN-α), interferon-beta (IFN-b), interferon-gamma gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif ligand 18 (CCL18), chemokine (C-C motif) ligand 19

(CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

In some embodiments, the composition featuring the capsules is characterized as a drug depot. A drug depot as contemplated herein is a systemic delivery system with a matrix comprised of polymers, hydrogels, or phospholipids and the like, allowing for local or systemic delivery of a therapeutic agent. In some embodiments, the drug depot features a polymer or copolymer selected from one or more of polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(hydroxybutyrate-co-hydroxyvalerate), poly(γ-glutamic acid), polyglycolic acid (PGA), polylactic acid (PLA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol)-*b*-hyaluronic acid (PEG-HA), poly(ethylene glycol)-*b*-polycaprolactone (PEG-PCL), poly(ethylene glycol)-*b*-poly(glutamic acid) (PEG-PGA), poly(ethylene glycol)-*b*-polylactic acid (PEG-PLA), poly(ethylene glycol)-*b*-poly(glycolic-co-lactic acid) (PEG-PLGA), poly(ethylene glycol)-*b*-poly(L-lactic acid) (PEG-PLLA), and poly(ethylene glycol)-*b*-poly(ethyleneimine) (PEG-PEI). In some embodiments, the drug depot features a phospholipid selected from one or more of a phosphatidylcholine, a phosphatidylserine, a phosphatidylinositol, a phosphatidylglycerol, or a phosphatidic acid. In some embodiments, the drug depot features a phosphatidylcholine, e.g., lecithin (e.g., soy lecithin or egg lecithin), hydrogenated phosphatidylcholine (HPC), or 1,2-dipalmitoyl-*sn*-glycero-3-phosphcholine (DPPC). In some embodiments, the drug depot features a polyethylene glycol-conjugated phospholipid, e.g., 1,2-

distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG). In some embodiments, the drug depot features a hydrogel-forming polymer selected from one or more of alginate, alginate-polyethylene glycol (alginate-PEG), alginate-polyacrylamide (alginate-PAAm), α-cyclodextrin, α-cyclodextrin-polyethylene glycol (α-cyclodextrin-PEG), chitosan, collagen, fibrin, heparin, hyaluronic acid (HA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyacrylamide-ferrocene (PAAm-Fc), poly(L-lysine)/polyacrylic acid (PLL/PAA), poly(N-isopropylacylamide) (PNIPAAM), poly((N-isopropylacrylamide)-co-(sodium acrylic acid)) (P(NIPAAM-co-AAcNa)), polyvinyl alcohol-polyacrylamide (PVA-PAAm), and poly(vinyl pyrrolidine) (PVP).

In an aspect, the composition featuring the capsules comprising a plurality of engineered cells may be delivered with a device.

In an aspect, the composition is provided, e.g., administered, by injection, e.g., intramuscular injection or subcutaneous injection. In some embodiments, the composition is administered by intramuscular injection. In some embodiments, the composition is administered by subcutaneous injection. The composition may be formulated as a solid, e.g., a lyophilized powder that may be reconstituted before or at the time of administration. The composition may be formulated as a liquid. The composition may be formulated as a semi-solid preparation, e.g., a gel, a hydrogel, and the like. The composition be formulated as a drug depot.

In some embodiments, the composition provided for injection features a drug depot, wherein a therapeutic agent is released responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the therapeutic agent is released responsive to a cytokine. In some embodiments, the therapeutic agent is released responsive to a chemokine. In some embodiments, the therapeutic agent is released responsive to an antigen. In some embodiments, the therapeutic agent is released responsive to an antibody. In some embodiments, the therapeutic agent is released responsive to a pathogen.

In some embodiments, the therapeutic agent is released constitutively and/or responsive to a stimulus, e.g., responsive to a disease or inflammatory marker, e.g., a cytokine, a chemokine, an antigen, an antibody, or a pathogen. In some embodiments, the constitutive release of the therapeutic agent is characterized by a sustained release pharmacokinetic profile. In some embodiments, the constitutive release of the therapeutic agent is characterized by an immediate

"burst" release pharmacokinetic profile. In some embodiments, additional therapeutic agent is released responsive to the stimulus, characterized in that the additional release of the therapeutic agent is additive to the constitutive release of the therapeutic agent.

In some embodiments, the stimulus is a cytokine and/or chemokine selected from one or more of: interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interferon-alpha (IFN-α), interferon-beta (IFN-b), interferon-gamma gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11),

chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

In some embodiments, the composition is provided for injection, wherein the drug delivery system is a drug depot. A drug depot as contemplated herein is a local delivery system with a matrix comprised of polymers, hydrogels, or phospholipids and the like, allowing for local delivery of a therapeutic agent. In some embodiments, the drug depot features a polymer or copolymer selected from one or more of polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(hydroxybutyrate-co-hydroxyvalerate), poly(γ-glutamic acid), polyglycolic acid (PGA), polylactic acid (PLA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol)-*b*-hyaluronic acid (PEG-HA), poly(ethylene glycol)-*b*-polycaprolactone (PEG-PCL), poly(ethylene glycol)-*b*-poly(glutamic acid) (PEG-PGA), poly(ethylene glycol)-*b*-polylactic acid (PEG-PLA), poly(ethylene glycol)-*b*-poly(glycolic-co-lactic acid) (PEG-PLGA), poly(ethylene glycol)-*b*-poly(L-lactic acid) (PEG-PLLA), and poly(ethylene glycol)-*b*-poly(ethyleneimine) (PEG-PEI). In some embodiments, the drug depot features a phospholipid selected from one or more of a phosphatidylcholine, a phosphatidylserine, a phosphatidylinositol, a phosphatidylglycerol, or a phosphatidic acid. In some embodiments, the drug depot features a phosphatidylcholine, e.g., lecithin (e.g., soy lecithin or egg lecithin), hydrogenated phosphatidylcholine (HPC), or 1,2-dipalmitoyl-*sn*-glycero-3-phosphcholine (DPPC). In some embodiments, the drug depot features a polyethylene glycol-conjugated phospholipid, e.g., phosph1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG). In some embodiments, the drug depot features a hydrogel-forming polymer selected from one or more of alginate, alginate-polyethylene glycol (alginate-PEG), alginate-polyacrylamide (alginate-PAAm), α-cyclodextrin, α-cyclodextrin-polyethylene glycol (α-cyclodextrin-PEG), chitosan, collagen, fibrin, heparin, hyaluronic acid (HA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyacrylamide-ferrocene (PAAm-Fc), poly(L-lysine)/polyacrylic acid (PLL/PAA), poly(N-isopropylacylamide) (PNIPAAM), poly((N-isopropylacrylamide)-co-(sodium acrylic acid)) (P(NIPAAM-co-AAcNa)), polyvinyl alcohol-polyacrylamide (PVA-PAAm), and poly(vinyl pyrrolidine) (PVP).

Inhalable Delivery

The present disclosure provides modes for inhalable delivery of a composition featuring capsules comprising a plurality of engineered cells, e.g., with a device.

It is contemplated in certain aspects of the invention that the device providing a mode for inhalable delivery of the composition features:

(i) a first compartment, wherein the composition is disposed in said first compartment;

(ii) a second compartment, fixedly connected to the first compartment, wherein the composition is disposed in said second compartment, and wherein the composition comprises aerosolized capsules;

(iii) an actuator, capable of providing means for aerosolizing/atomizing said composition;

(iv) an ejector, capable of providing means for dispersing said aerosolized composition; and

(v) an orifice, providing for ejection of said aerosolized composition;

thereby providing a composition featuring capsules comprising a plurality of engineered cells.

In some embodiments, the device is a nebulizer, e.g., a jet nebulizer, a soft mist nebulizer, an ultrasonic nebulizer, or an ultrasonic vibrating mesh nebulizer, or an inhaler, e.g., a metered-dose inhaler, a dry powder inhaler, or a rotary inhaler.

In some embodiments, the device is a spray bottle. In some embodiments, the device is a spray can. In some embodiments, the device is an atomizer. In some embodiments, the device is a vaporizer.

In some embodiments, the device forms an aerosol comprising a plurality of liquid droplets comprising capsules encapsulating a plurality of engineered cells for inhalable delivery. In some embodiments, the device is a nebulizer. In some embodiments, the device is an ultrasonic nebulizer, wherein the ultrasonic nebulizer forms an aerosol comprising a plurality of liquid droplets via piezoelectric actuation. In some embodiments, the device is an ultrasonic vibrating mesh nebulizer, wherein the ultrasonic vibrating mesh nebulizer forms an aerosol comprising a plurality of liquid droplets via the ultrasonic vibration of a membrane. In some embodiments, the device is a jet nebulizer or an atomizer, wherein the jet nebulizer forms an aerosol comprising a plurality of liquid droplets via an actuation facilitated or enabled by a compressed gas. In some embodiments, the nebulizer forms an aerosol comprising a plurality of liquid droplets via an actuation facilitated or enabled, i.e., at least in part, by the force of the subject's respiration. In some embodiments, the device is a metered-dose inhaler. In some

embodiments, the metered-dose inhaler forms an aerosol comprising liquid droplets via an actuation facilitated or enabled by a propellant or compressed gas. In some embodiments, the device is a metered-dose inhaler. In some embodiments, the metered-dose inhaler forms an aerosol comprising liquid droplets via an actuation facilitated or enabled, i.e., at least in part, by the force of the subject's respiration.

In some embodiments, the device forms an aerosol comprising a plurality of solid particles comprising polymeric capsules encapsulating a cell or plurality of engineered cells for inhalable delivery. In some embodiments, the device is an inhaler. In some embodiments, the device is a dry-powder inhaler or a rotary inhaler. In some embodiments, the dry-powder inhaler forms an aerosol comprising solid particles via an actuation facilitated or enabled, i.e., at least in part, by the force of the subject's respiration.

Dosages

The present disclosure provides methods for delivery of the composition featuring capsules and associated dosages, e.g., a predetermined, e.g., a prescribed dosage, or dosing schedule. The capsule may be delivered via inhalation with a device, e.g., a nebulizer, e.g., a jet nebulizer, a soft mist nebulizer, an ultrasonic nebulizer, or an ultrasonic vibrating mesh nebulizer, or an inhaler, a metered-dose inhaler, a dry powder inhaler or rotary inhaler and the like at a predetermined dosage. In some embodiments, the capsule for inhalable delivery may be delivered with a spray bottle, a spray can, an atomizer, or a vaporizer. Alternatively, the capsule may be delivered locally, e.g., via surgical implantation, e.g., as a drug depot, or via intubation or instillation, e.g., intratracheal instillation, optionally with a device, e.g., a bronchoscope (e.g., a rigid bronchoscope or a flexible bronchoscope) or a catheter at a predetermined dosage.

The composition may be provided at a predetermined dosage of the therapeutic agent of between about 10 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 10 ng to about 10 mg, about 20 ng to about 10 mg, about 30 ng to about 10 mg, about 40 ng to about 10 mg, about 50 ng to about 10 mg about 100 ng to about 10 mg, about 200 ng to about 10 mg, 300 ng to about 10 mg, 400 ng to about 10 mg, 500 ng to about 10 mg, 1 μg to about 10 mg, 2 μg to about 10 mg, 3 μg to about 10 mg, 4 μg to about 10 mg, 5 μg to about 10 mg, 10 μg to about 10 mg, 20 μg to about 10 mg, 30 μg to about 10 mg, 40 μg to about 10 mg, 50 μg to about 10 mg, 100 μg to about 10 mg, 200 μg to

about 10 mg, 300 μg to about 10 mg, 400 μg to about 10 mg, 500 μg to about 10 mg, 1 mg to about 10 mg, 2 mg to about 10 mg, 3 mg to about 10 mg, 4 mg to about 10 mg, or 5 mg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 20 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 30 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 40 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 50 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 100 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 200 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 300 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 400 ng to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 500 ng to about 10 mg. In some embodiments, the composition featuring the capsules comprising a polymer and an plurality of engineered cells is provided at a dosage of the therapeutic agent of between about 1 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 2 μg to about 10 mg. In some embodiments, the capsule is provided at a dosage of the therapeutic agent of between about 3 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 4 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 5 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 10 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 20 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 30 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 40 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 50 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 100 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 200 μg to

about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 300 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 400 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 500 μg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 1 mg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 2 mg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 3 mg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 4 mg to about 10 mg. In some embodiments, the composition is provided at a dosage of the therapeutic agent of between about 5 mg to about 10 mg.

In some embodiments, the therapeutic agent comprises a protein such as a cytokine, a chemokine, and the like. In some embodiments, the cytokine and/or chemokine is selected from one or more of: interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20),

chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17).

In some embodiments, the therapeutic agent is selected from IL-1RA, IL-4, IL-10, IL-11, IL-13, and IL-35, and a combination thereof. In some embodiments, the therapeutic agent comprises IL-1Ra.

In some embodiments, the therapeutic agent is an anti-cytokine antibody or an anti-chemokine antibody, e.g., anti-IL-1, anti-IL-1α, anti-IL-1β, anti-IL-1RA, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-7, anti-IL-8, anti-IL-9, anti-IL-10, anti-IL-11, anti-IL-12, anti-IL-12α, anti-IL-12β, anti-IL-13, anti-IL-14, anti-IL-15, anti-IL-16, anti-IL-17, anti-IL-20, anti-IL-22, anti-IFN-α, anti-IFN-β, anti-IFN-γ, anti-TNF-α, anti-TNF-β, anti-TGF-β, anti-CCL1, anti-CCL2, anti-CCL3, anti-CCL4, anti-CCL5, anti-CCL6, anti-CCL7, anti-CCL8, anti-CCL9, anti-CCL10, anti-CCL11, anti-CCL12, anti-CCL13, anti-CCL14, anti-CCL15, anti-CCL16, anti-CCL17, anti-CCL18, anti-CCL19, anti-CCL20, anti-CCL21, anti-CCL22, anti-CCL23, anti-CCL24, ant-CCL25, anti-CCL26, anti-CCL27, anti-CCL28, anti-CXCL1, anti-CXCL2, anti-CXCL3, anti-CXCL4, anti-CXCL5, anti-CXCL6, anti-CXCL7, anti-CXCL8, anti-CXCL9, anti-CXCL10, anti-CXCL11, anti-CXCL12, anti-CXCL13, anti-CXCL14, anti-CXCL15, anti-CXCL16, and anti-CXCL17, and a combination thereof.

The composition may be provided at a predetermined dosing schedule, e.g., to maintain a therapeutic concentration of the therapeutic agent. In some embodiments, the composition is provided between four times per day to once per month. In some embodiments, the composition

is provided four times per day, three times per day, twice per day, once per day, once every other day, twice per week, once per week, once every two weeks, once every three weeks, once every four weeks, or once per month. In some embodiments, the composition is provided four times per day. In some embodiments, the composition is provided three times per day. In some embodiments, the composition is provided twice per day. In some embodiments, the composition is provided once per day. In some embodiments, the capsule is provided once every other day. In some embodiments, the composition is provided twice per week. In some embodiments, the composition is provided once per week. In some embodiments, the composition is provided once every two weeks. In some embodiments, the composition is provided once every three weeks. In some embodiments, the composition is provided once every four weeks. In some embodiments, the composition is provided once per month.

The composition may be provided at a predetermined time of the day. In some embodiments, the composition is provided in the morning, e.g., about 6:00 AM, about 7:00 AM, about 8:00 AM, about 9:00 AM, about 10:00 AM, or about 11:00 AM. In some embodiments, the composition is provided in the afternoon, e.g., about 12:00 PM, about 1:00 PM, about 2:00 PM, about 3:00 PM, about 4:00 PM, or about 5:00 PM. In some embodiments, the composition is provided in the evening, e.g., about 6:00 PM, about 7:00 PM, about 8:00 PM, or about 9:00 PM. In some embodiments, the composition is provided at night, e.g., about 10:00 PM, about 11:00 PM, about 12:00 AM, or later.

Pathogens

The present disclosure provides methods of treating a respiratory infection, comprising administering a composition featuring capsules comprising a plurality of engineered cells, wherein the engineered cells release, either constitutively and/or responsive to a stimulus, a therapeutic agent. In some embodiments, the therapeutic agent is for treating a respiratory infection, wherein the disease or disorder is caused by a pathogen, e.g., a virus, a bacterium, a fungus, a protozoan, a nematode, a flatworm, and the like.

In some embodiments, the capsule releases a therapeutic agent for treating a respiratory disease or disorder caused by a virus. In some embodiments, the virus is selected from one or more of an adenovirus, a coronavirus, a coxsackievirus, a cytomegalovirus, a herpes virus, an

influenza virus, a morbillivirus, an orthohantavirus, an orthopneumovirus, a parainfluenza virus, and a rhinovirus.

In some embodiments, the disease or disorder is caused by an adenovirus. Exemplary adenovirus species include human adenovirus B, e.g., serotype 14, and human adenovirus C.

In some embodiments, the disease or disorder is caused by a coxsackievirus. Exemplary coxsackievirus species include Coxsackie A virus.

In some embodiments, the disease or disorder is caused by a cytomegalovirus. Exemplary cytomegalovirus species include human cytomegalovirus (i.e., human betaherpesvirus 5).

In some embodiments, the disease or disorder is caused by a coronavirus. Exemplary coronaviruses include severe acute human coronavirus 229E, human coronavirus NL63 (HCoV-NL63), human coronavirus HKU1 (HCoV-HKU1), human coronavirus OC43, Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

In some embodiments, the disease or disorder is caused by a herpes virus. Exemplary herpes virus species include herpes simplex virus 1.

In some embodiments, the disease or disorder is caused by an influenza virus. Exemplary influenza viruses include influenza A virus, influenza B virus, and influenza C virus. Exemplary serotypes of influenza A include H1N1 , H1N2, H2N2, H3N2, H5N1, and H7N9. Exemplary serotypes of influenza B include the Victoria and Yamagata subtypes.

In some embodiments, the disease or disorder is caused by a morbillivirus. Exemplary morbillivirus species include measles morbillivirus, the causative pathogen of measles.

In some embodiments, the disease or disorder is caused by an orthohantavirus. Exemplary orthohantavirus species include black creek canal virus, Monongahela virus, new York orthohantavirus, and sin nombre orthohantavirus.

In some embodiments, the disease or disorder is caused by an orthopneumovirus. Exemplary orthopneumovirus species include human respiratory syncytial virus.

In some embodiments, the disease or disorder is caused by a parainfluenza virus. Exemplary parainfluenza species include human parainfluenza virus type 3.

In some embodiments, the disease or disorder is caused by a rhinovirus. Exemplary rhinoviruses include any of the infectious serotypes encompassed by human rhinovirus A, human rhinovirus B, and human rhinovirus C.

In some embodiments, the disease or disorder is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

In some embodiments, the disease or disorder is caused by a virus, wherein the virus causes an opportunistic infection. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a virus, wherein the subject is immuno-compromised. In some embodiments, the capsule releases a therapeutic agent wherein for treating a disease or disorder caused by a virus that is characterized as a hospital-acquired infection.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a bacterium. In some embodiments, the disease or disorder is a bacteria selected from *Chlamydia psittaci*, *Chlamydia pneumoniae, Chlamydia trachomatis*, *Corynebacterium diptheriae, Corynebacterium haemolyticum, Coxiella burnetti*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Mycoplasma hominis*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoaea*, *Neisseria meningiditis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus* pyogenes, *Yersinia pestis Xanthomonas pseudomallei*, or a combination thereof. In some embodiments, the disease or disorder is caused by *C. albicans*. In some embodiments, the disease or disorder is caused by *K. pneumoniae*. In some embodiments, the disease or disorder is caused by *L. pneumophila*. In some embodiments, the disease or disorder is caused by *P. aeruginosa*. In some embodiments, the disease or disorder is caused by *S. aureus*, e.g., methicillin-resistant *S. aureus* (MRSA). In some embodiments, the disease or disorder is caused by *S. pyogenes*.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a bacterium, wherein the bacterium causes an opportunistic infection. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a bacterium, wherein the subject is immuno-compromised. In some embodiments, capsules release a therapeutic agent for treating a

respiratory infection, wherein the caused by a bacterium that is characterized as a hospital-acquired infection.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a fungal pathogen, e.g., a fungal pathogen selected from one or more of *Aspergillus fumigatus*, *Candida albicans*, *Coccidioides immitis*, *Coccidioides posadasii*, *Cryptococcus gatti*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis jiroveci*, and *Sporothrix schenckii*.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a fungus, wherein the fungus causes an opportunistic infection. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a fungus, wherein the subject is immuno-compromised. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a fungus, that is characterized as a hospital-acquired infection.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a protozoan, e.g., wherein the protozoan is selected from one or more of *Babesia divergens*, *Babesia microti*, *Entamoeba histolytica*, *Leishmania donovani*, *Plasmodium falciparum*, and *Toxoplasma gondii*.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a protozoan, wherein the protozoan causes an opportunistic infection. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a protozoan, wherein the subject is immuno-compromised. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a protozoan, that is characterized as a hospital-acquired infection.

In some embodiments, capsules a therapeutic agent for treating a respiratory infection caused by a nematode, e.g., wherein the nematode is selected from *Ascaris lumbricoides*, *Brugia malayi*, *Dirofilarasis immitis*, *Strongyloides stercoralis*, *Toxocara canis*, *Toxocara cati*, *Trichinella spiralis*, and *Wuchereria bancrofti*.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a nematode, wherein the nematode causes an opportunistic infection. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a nematode, wherein the subject is immuno-

compromised. In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by a nematode, that is characterized as a hospital-acquired infection.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection, wherein the disease or disorder is caused by a flatworm, e.g., wherein the flatworm is selected from one or more of *Echinococcus granulosus*, *Echinococcus multilocularis*, *Paragnimiasis westermani*, *Schistosoma haemotobium*, *Schistosoma intercalatum*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma mekongi*,

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by *Rhinosporidium seeberi*.

In some embodiments, capsules release a therapeutic agent for treating a respiratory infection caused by any of the parasitic organisms disclosed in Khemasuwan, D. *et al. Diseases of the Central Airways*, 2016, 231-253.

Patient Selection

The present disclosure provides capsules comprising a plurality of engineered cells for administration of a therapeutic agent to a subject in need thereof. In some embodiments, the capsule is formulated for delivery via inhalation to a subject in need thereof. In some embodiments, the capsule is formulated for local delivery to a subject in need thereof, e.g., via surgical implantation, or intubation or instillation, e.g., intratracheal instillation, e.g., with a device, e.g., a bronchoscope (e.g., a rigid bronchoscope or flexible bronchoscope) or a catheter. The subject may include a human. In some embodiments, the subject is a female. In some embodiments, the subject is a male. In some embodiments, the subject is aged 18 years or older. In some embodiments, the subject is less than 18 years of age.

The subject may be selected based on being at risk of developing one or more of an autoinflammatory condition or disorder or an autoimmune condition or disorder. In some embodiments, the subject is at risk of developing one or more of the following: anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis,

lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, sarcoidosis, and the like.

The subject may be selected based on having one or more symptoms of one or more of an autoinflammatory condition or disorder or an autoimmune condition or disorder. In some embodiments, the subject has one or more symptoms of one or more of the following: anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, sarcoidosis, and the like.

The subject may be selected based on having had one or more of an autoinflammatory condition or disorder or an autoimmune condition or disorder. In some embodiments, the subject has had one or more of the following: anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, sarcoidosis, and the like.

The subject may be selected based on having one or more of a respiratory condition or disorder. In some embodiments, the subject has one or more of the following: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

The subject may be selected based on being at risk of developing one or more of a respiratory condition or disorder. In some embodiments, the subject is at risk of developing one or more of the following: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

The subject may be selected based on having one or more symptoms of one or more of a respiratory condition or disorder. In some embodiments, the subject has one or more symptoms of one or more of the following: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

The subject may be selected based on having had one or more of a pulmonary condition or disorder. In some embodiments, the subject has had one or more of the following: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

The subject may be selected based on having one or more of an inflammatory condition, disease, or disorder. In some embodiments, the subject has one or more of the following: endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, or Gillian Barre syndrome.

The subject may be selected based on being at risk of developing one or more of an inflammatory condition, disease, or disorder. In some embodiments, the subject is at risk of developing one or more of the following: endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, or Gillian Barre syndrome.

The subject may be selected based on having one or more symptoms of one or more of an inflammatory condition, disease or disorder. In some embodiments, the subject has one or more symptoms of one or more of the following: endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis

(biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, or Gillian Barre syndrome.

The subject may be selected based on having had one or more of an inflammatory condition, disease or disorder. In some embodiments, the subject has had one or more of the following: endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, or Gillian Barre syndrome.

Combination Therapy

The present disclosure provides compositions featuring capsules comprising a plurality of engineered cells, which may be concomitantly administered with an additional therapeutic regimen. It is contemplated that encompassed within certain modes of the invention as described herein, the composition is administered, e.g., co-administered, with an additional therapy. In some embodiments, the composition is administered for the treatment of an immune disease or condition or an autoimmune disease or condition.

In some embodiments, the subject is administered an additional therapy, wherein the additional therapy is useful in the treatment of an autoinflammatory disease or condition or autoimmune disease or condition associated with the lungs, respiratory system, and/or the pleura or pleural cavity. In some embodiments the autoinflammatory disease or condition or autoimmune disease or condition associated with the lungs, respiratory system, and/or the pleura or pleural cavity is selected from one or more of: anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary

hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, sarcoidosis, and the like.

In some embodiments, the subject is administered an additional therapy, wherein the additional therapy is useful in the treatment of an autoinflammatory disease or condition or an autoimmune disease or condition. In some embodiments, the additional therapy is an antibiotic, e.g., amoxicillin, azithromycin, doxycycline, and levofloxacin. In some embodiments, the additional therapy is an antiviral, e.g., ensitrelvir, molnupiravir, oseltamivir, remdesivir, and zanamivir. In some embodiments, the additional therapy is an analgesic, e.g., aspirin, buprenorphine, celecoxib, codeine, dihydromorphine, etoricoxib, hydrocodone, ibuprofen, morphine, naproxen, oxycodone, paracetamol, pethidine, rofecoxib, tapentadol, and tramadol. In some embodiments, the additional therapy is an antipyretic, e.g., aspirin, celecoxib, clonidine, diclofenac, etoricoxib, flurbiprofen, ibuprofen, ketoprofen, magnesium salicylate, naproxen, nimesulide, paracetamol rofecoxib, and sodium salicylate. In some embodiments, the additional therapy is a cough suppressant. In some embodiments, the additional therapy is a decongestant, e.g., beclomethasone dipropionate, budesonide, ciclesonide, dexamethasone, ephedrine, flunisolide, fluticasone, fluticasone furoate, fluticasone propionate, levomethamphetamine, mometasone furoate naphazoline, oxymetazoline, phenylephrine, phenylpropanolamine, prednisolone, propylhexedrine, pseudoephedrine, synephrine, tetryzoline, tixocortol, tramazoline, triamcinolone, triamcinolone acetonide, and xylometazoline. In some embodiments, the additional therapy is an antihistamine, e.g., brompheniramine, cetirizine, chlorpheniramine, diphendramine, fexofenadine, levocetirizine, and loratadine. In some embodiments, the additional therapy is an expectorant or a mucolytic, e.g., acetylcysteine, ambroxol, ammonium chloride, bromhexine, carbocisteine, dornase alfa, erdosteine, guaifenesin, mecysteine, potassium citrate, potassium iodide, and sodium citrate.

In some embodiments, the subject is administered an additional therapy, wherein the additional therapy is useful in the treatment of a respiratory disease or condition. In some embodiments the respiratory disease or condition is selected from one or more of: acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung

disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis.

In some embodiments, the subject is administered an additional therapy useful in the treatment of a pulmonary disease or condition. In some embodiments, the additional therapy is an antibiotic, e.g., amoxicillin, azithromycin, doxycycline, and levofloxacin. In some embodiments, the additional therapy is an antiviral, e.g., ensitrelvir, molnupiravir, oseltamivir, remdesivir, and zanamivir. In some embodiments, the additional therapy is an analgesic, e.g., aspirin, buprenorphine, celecoxib, codeine, dihydromorphine, etoricoxib, hydrocodone, ibuprofen, morphine, naproxen, oxycodone, paracetamol, pethidine, rofecoxib, tapentadol, and tramadol. In some embodiments, the additional therapy is an antipyretic, e.g., aspirin, celecoxib, clonidine, diclofenac, etoricoxib, flurbiprofen, ibuprofen, ketoprofen, magnesium salicylate, naproxen, nimesulide, paracetamol rofecoxib, and sodium salicylate. In some embodiments, the additional therapy is a cough suppressant. In some embodiments, the additional therapy is a decongestant, e.g., beclomethasone dipropionate, budesonide, ciclesonide, dexamethasone, ephedrine, flunisolide, fluticasone, fluticasone furoate, fluticasone propionate, levomethamphetamine, mometasone furoate naphazoline, oxymetazoline, phenylephrine, phenylpropanolamine, prednisolone, propylhexedrine, pseudoephedrine, synephrine, tetryzoline, tixocortol, tramazoline, triamcinolone, triamcinolone acetonide, and xylometazoline. In some embodiments, the additional therapy is an antihistamine, e.g., brompheniramine, cetirizine, chlorpheniramine, diphendramine, fexofenadine, levocetirizine, and loratadine. In some embodiments, the additional therapy is an expectorant or a mucolytic, e.g., acetylcysteine, ambroxol, ammonium chloride, bromhexine, carbocisteine, dornase alfa, erdosteine, guaifenesin, mecysteine, potassium citrate, potassium iodide, and sodium citrate.

In some embodiments, the subject is administered an additional therapy that is useful in the treatment of an inflammatory disease, disorder or condition. In some embodiments the disease, disorder, or condition affects the uterus, adrenal glands, liver, gastrointestinal tract, pancreas, thyroid, nervous system, stomach, or muscles. In some embodiments the disease, disorder, or condition is selected from one or more of: endometriosis, arthritis, psoriasis, alopecia areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's

disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, or Gillian Barre syndrome.

In some embodiments, the additional therapy is useful in the treatment of an inflammatory condition, disease, or disorder. In some embodiments, the additional therapy is an antibiotic, e.g., amoxicillin, azithromycin, doxycycline, and levofloxacin. In some embodiments, the additional therapy is an antiviral, e.g., ensitrelvir, molnupiravir, oseltamivir, remdesivir, and zanamivir. In some embodiments, the additional therapy is an analgesic, e.g., aspirin, buprenorphine, celecoxib, codeine, dihydromorphine, etoricoxib, hydrocodone, ibuprofen, morphine, naproxen, oxycodone, paracetamol, pethidine, rofecoxib, tapentadol, and tramadol. In some embodiments, the additional therapy is an antipyretic, e.g., aspirin, celecoxib, clonidine, diclofenac, etoricoxib, flurbiprofen, ibuprofen, ketoprofen, magnesium salicylate, naproxen, nimesulide, paracetamol rofecoxib, and sodium salicylate. In some embodiments, the additional therapy is an antihistamine, e.g., brompheniramine, cetirizine, chlorpheniramine, diphendramine, fexofenadine, levocetirizine, and loratadine.

In some embodiments, the subject is administered an additional therapy, wherein the additional therapy is an anti-inflammatory agent. In some embodiments, the anti-inflammatory agent is a corticosteroid, e.g., beclomethasone dipropionate, budesonide, ciclesonide, cortisone, dexamethasone, flunisolide, fluticasone furoate, fluticasone propionate, hydrocortisone, methylprednisolone, mometasone furoate, prednisone and triamcinolone acetonide.

In some embodiments, the subject is administered an additional therapy, wherein the additional therapy is an immunomodulatory or immunosuppressive agent, e.g., azathioprine, cyclophosamide, methotrexate, mycophenolate mofetil, obinutuzumab, ocrelizumab, ofatumumab, and rituximab.

Methods of Making

The present disclosure provides methods for making capsules comprising a plurality of engineered cells, for delivery of a therapeutic agent, e.g., a protein, e.g., a cytokine.

The present disclosure further provides devices to fabricate said capsules. In some embodiments, the method features a device, e.g., an electrostatic spraying device. In an

embodiment, the method of making features a device that is a custom-built, coaxial electrostatic spraying device. The custom-built device comprises a voltage-generator that is fixedly connected to a tip of a coaxial needle and grounded to a bath comprising reagents for crosslinking or forming a hydrogel from a hydrogel-forming polymer. The co-axial needles contain the polymer capable of forming a hydrogel responsive to contacting with a crosslinker, e.g., a barium or calcium salt, e.g., $BaCl_2$.

In some embodiments, the method of making the capsules features the following steps: (i) fixedly connecting a voltage generator to a tip of a 30 g needle of an, electrostatic spraying device, wherein the tip of the needle is grounded to a 1:4 $BaCl_2$:mannitol cross-linking bath ; (ii) combining a mixture comprising 1.4% w/v SLG20 sodium alginate diluted in 0.9%w/v saline with a cell suspension of engineered cells, e.g., ARPE-19 cells, capable of constitutively or inducibly expressing a therapeutic agent, e.g., a protein, e.g., a cytokine, thereby forming an alginate-cell mixture; (iii) loading the alginate-cell mixture of step (ii) into the 30 g needle (iv) contacting the alginate-cell mixture with the $BaCl_2$:mannitol bath, e.g., via applying pressure, e.g., manually or mechanically, to extrude the alginate-cell mixture from the needle into the $BaCl_2$:mannitol bath; and (v) responsive to the contacting, crosslinking the alginate-cell mixture, thereby forming barium alginate hydrogel capsules comprising a plurality of engineered cells.

In some embodiments, the method of making the capsules features the following steps: (i) fixedly connecting a voltage generator to a tip of a coaxial needle of a co-axial, electrostatic spraying device, wherein the tip of the coaxial needle is grounded to a 1:4 $BaCl_2$:mannitol cross-linking bath ; (ii) combining a mixture comprising 1.4% w/v SLG20 sodium alginate diluted in 0.9%w/v saline with a cell suspension of engineered cells, e.g., ARPE-19 cells, capable of constitutively or inducibly expressing a therapeutic agent, e.g., a protein such as, thereby forming an alginate-cell mixture; (iii) loading the alginate-cell mixture of step (ii) into the interior a plurality of the coaxial needles (e.g., 2 coaxial needles); (iv) contacting the alginate-cell mixture with the $BaCl_2$:mannitol bath, e.g., via applying pressure, e.g., manually or mechanically, to extrude the alginate-cell mixture from the interior of the plurality of the coaxial needles into the $BaCl_2$:mannitol bath; and (v) responsive to the contacting, crosslinking the alginate-cell mixture, thereby forming barium alginate hydrogel capsules comprising an plurality of engineered cells.

The fabrication technique provides the ability to modulate the diameter of the capsules to between about 50 μm to about 3000μm. The fabrication technique further allows for making capsules encapsulating between about 250 to about 1.86e6 cells.

In another aspect, the method of making comprises the use of a BUCHI Encapsulator (e.g., models B-390™, B-395™ and the like, commercially available form BUCHI). In some embodiments, the capsules are fabricated according to the manufacturer's instructions for the formation of microcapsules.

In an embodiment, the method of making capsules allows for the capsules to comprise between about 250 to about 1.86e6 cells, e.g., engineered cells, e.g., ARPE-19 cells. In an embodiment, the method of making capsules allows for a capsule diameter of between about 50 μm to about 3 mm.

In an embodiment, the method of making capsules allows for the target size of capsules to be approximately equivalent to the actually measured capsule diameter when the capsule diameter is 150, 300, 600, 800, 1200, or 1700 μm and when the number of capsules is at least between one and five.

In an embodiment, the viability of encapsulated engineered cells, e.g., ARPE-19 cells, in alginate capsules is greater than 80%, wherein the capsule diameter is 150, 300, 800, or 1200 μm. In an embodiment, the viability of encapsulated engineered cells, e.g., ARPE-19 cells, in alginate capsules is greater than 90%, wherein the capsule diameter is 300 μm. In an embodiment, the cell count of encapsulated engineered cells, e.g., ARPE-19 cells, in alginate capsules is between about $2*10^6$ and $3*10^6$ cells, wherein the capsule diameter is 150, 300, 800, or 1200 μm. In an embodiment, rat IL-1Ra production is about $2*10^6$ pg/mL/day for capsules having a capsule diameter of 150, 300, 800, and 1200 μm. In an embodiment, rat IL-1Ra production is about $2*10^6$ pg/mL/day and is not a function of capsule diameter between about 150 and about 1200 μm.

In an embodiment, the production of IL-13 attains a maximum concentration of 10^3 ng/mL between about 12 and 24 h after instillation as measured by a protein-specific ELISA. In an embodiment, the production of FGF21 attains a maximum concentration of 10^3 ng/mL between about 12 and 24 h after instillation as measured by a protein-specific ELISA. In an embodiment, the production of IL-1Ra attains a maximum concentration of 10^3 ng/mL between about 12 and 24 h after instillation as measured by a protein-specific ELISA.

In an embodiment, capsules comprising a plurality of engineered cells, e.g., ARPE-19 cells, localize adjacent to or in the vicinity of alveolar tissue, e.g., rodent alveolar tissue, when the capsule dose is 100, 200 or 300 µl. In an embodiment, capsules comprising a plurality of engineered cells, e.g., ARPE-19 cells, localize adjacent to or in the vicinity of alveolar tissue, e.g., rodent alveolar tissue, irrespective of the particular capsule dose when the capsule dose is between about 100 and about 300 µl.

In an embodiment, the administration of capsules via intubation is sufficient for a 10,000-fold increase in the local IL-1Ra concentration in the bronchoalveolar lavage (BAL) fluid obtained from the lungs relative to the systemic, i.e., blood plasma and pleural, IL-1Ra concentrations. In an embodiment, the administration of capsules via intubation is sufficient for a reduction of IL-17 concentration of about 20 pg/mL as measured in bronchoalveolar lavage fluid (BALF) in a LPS injury rodent model. In an embodiment, the administration of capsules via intubation is sufficient for the IL-17 concentration in BALF to decrease from about 30 pg/mL to about 10 pg/mL in an LPS injury rodent model.

In an embodiment, the administration of capsules via intubation is sufficient for a measurable reduction in neutrophil recruitment to the injured lung tissue in an LPS injury rodent model, characterized in that the LPS samples treated with instilled capsules comprising IL-1Ra-producing cells, e.g., ARPE-19 cells, regains or partially regains the healthy histological phenotype after 24 h.

In an embodiment, the distribution of capsules comprising a plurality of engineered cells fluorescently labeled with FITC dextran are homogenously distributed in rodent lung tissue after administration, e.g., instillation.

In an embodiment, the production of FGF21 attains a maximum concentration of between about 10^2 and about 10^3 ng/mL between about 12 and 24 h after instillation as measured by a protein-specific ELISA.

In an embodiment, the administration of capsules via intubation is sufficient for a measurable reduction in neutrophil recruitment to the injured lung tissue in an LPS injury rodent model, characterized in that the LPS samples treated with instilled capsules comprising IL-1Ra-producing cells, e.g., ARPE-19 cells, regains or partially regains the healthy histological phenotype after 1 day, 7 days, or 14 days.

In an embodiment, luciferase production of engineered cells, e.g., ARPE-19 cells, stably transfected with a plasmid encoding for a luciferase enzyme (Luc) with a NF-κB-responsive promoter (NFkB TRE) and a polyadenylation (PA) signal treated with no cytokines for 24 h is less than 10,000 RLU. In an embodiment, luciferase production of engineered cells, e.g., ARPE-19 cells, stably transfected with a plasmid encoding for a luciferase enzyme (Luc) with a NF-κB-responsive promoter (NFkB TRE) and a polyadenylation (PA) signal treated with IFNγ for 24 h is less than 10,000 RLU. In an embodiment, luciferase production of engineered cells, e.g., ARPE-19 cells, stably transfected with a plasmid encoding for a luciferase enzyme (Luc) with a NF-κB-responsive promoter (NFkB TRE) and a polyadenylation (PA) signal treated with IL-1β for 24 h is less between about 20,000 to about 30000 RLU. In an embodiment, luciferase production of engineered cells, e.g., ARPE-19 cells, stably transfected with a plasmid encoding for a luciferase enzyme (Luc) with a NF-κB-responsive promoter (NFkB TRE) and a polyadenylation (PA) signal treated with TNF-α for 24 h is less between about 20,000 to about 30000 RLU.

In an embodiment, cells, e.g., ARPE-19 cells loaded with FITC dextran and encapsulated in alginate capsules, are homogenously distributed as evaluated by a fluorescence imaging technique. In an embodiment, capsules comprising GFP-expressing cells are uniformly distributed in rat alveolae after instillation as evaluated by In Vivo Imaging System (IVIS).

In an embodiment, the average radiance of firefly luciferase producing capsules reaches a maximum of about 10^4 p/s/cm^2 about one day after encapsulation relative to a maximum radiance of about 10^3 p/s/cm^2 for capsules comprising free cells. In an embodiment, radiance measured in rats instilled with the firefly luciferase expressing capsules was greater than the radiance measured in rats instilled with the free cell containing capsules through seven days post-instillation.

In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats, for 12, 24, 36, 48, 72 h or more as measured by *in vivo* total flux analysis. In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats, for 12 h or more as measured by *in vivo* total flux analysis. In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats, for 24 h or more as measured by *in vivo* total flux analysis. In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats, for 36 h or more as measured by *in vivo* total flux analysis. In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats for 48 h or more as

measured by *in vivo* total flux analysis. In an embodiment, capsules are retained in the lungs, e.g., the lungs of rats for 72 h, or more as measured by *in vivo* total flux analysis.

In an embodiment , capsules are uniformly distributed in the lungs, e.g., the lungs of rats, subsequent to instillation as characterized by ex vivo fluorescent imaging.

In an embodiment, subsequent to instillation of capsules comprising IL-10-producing cells in rat lungs, IL-10 production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 0.1 to 1 ng/mL in plasma. In an embodiment, subsequent to instillation of capsules comprising IL-1Ra-producing cells in rat lungs, IL-1Ra production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma. In an embodiment, subsequent to instillation of capsules comprising FGF21-producing cells in rat lungs, FGF21 production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 1 to 10 ng/mL in plasma. In an embodiment, subsequent to instillation of capsules comprising IL-13-producing cells in rat lungs, IL-13 production is localized to BAL and is about 100 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma. In an embodiment, subsequent to instillation of capsules comprising IL-4-producing cells in rat lungs, IL-4 production is localized to BAL and is between about 1 to about 10 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma.

In an embodiment, in rats administered 300 μm-diameter IL-10-producing capsules at increasing volumes, e.g., 0, 50, 100, and 150 μl, via instillation, the IL-10 concentration increases from about 0.1 ng/mL in BAL to about 100 ng/mL as the capsule dose increases from 0 to 150 μl, whereas the IL-10 concentration in plasma remains relatively constant at about 0.1 ng/mL regardless of capsule dose volume administered. In an embodiment, rats administered 300 μm-diameter IL-10-producing capsules at increasing volumes, e.g., 0, 50, 100, and 150 μl, via instillation, IL-10 in BAL increases from about 0.01 ng/mL to between about 10 to about 100 ng/mL as the cell concentration per mL of alginate increases from 0 to about $1*10^7$ cells/mL, whereas IL-10 in plasma remains relatively constant between about 0.01 to 0.1 ng/mL as the cell concentration per mL of alginate increases from 0 to about $1*10^7$ cells/mL.

In an embodiment, repeat administration of rat IL-10 producing capsules on Day 30 yields an equivalent local concentration of rat IL-10 in bronchoalveolar lavage (BAL) of about 1-100 ng/mL and an equivalent plasma concentration of about 10^{-4} to about 10^{-2} ng/mL compared to a single administration. In an embodiment, the rat IL-10 concentration in BAL is about 10

ng/mL upon repeat administration of 100 µl of RPE IL-10 capsules. In an embodiment, the rat IL-10 concentration in plasma is about 10^{-3} ng/mL upon repeat administration of 100 µl of RPE IL-10 capsules.

In an embodiment, the in vitro concentration of a therapeutic is about 1, about 10, 10^2, 103, 104 ng/mL or greater 24 h after administration of a capsule capable of producing a therapeutic agent. In an embodiment, the therapeutic agent is IL-1Ra, FGF21, IL-13, or IL-4. In some embodiments, the diameter of the capsule is about 150, 300, 800, 1200, or 1700 µm. In an embodiment, 10, 50, 100, 200, 300, 500 µl or more of the capsules are administered. In an embodiment, the IL-1Ra concentration is about 10^3 ng/mL 24 h after administration of a IL-1Ra producing capsule. In an embodiment, the FGF21 concentration is between about 10^3 to about 10^4 ng/mL 24 h after administration of a FGF21 producing capsule. In an embodiment, the IL-13 concentration is between about 10^3 to about 10^4 ng/mL 24 h after administration of a IL-13 producing capsule. In an embodiment, the IL-4 concentration is about 10^2 ng/mL 24 h after administration of a IL-4 producing capsule.

In an embodiment, IL-10 concentration is uniformly distributed across various lobes of the lung, e.g., the post caval, middle, superior, inferior, and left lobes, 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 5, 10, 15, 20, 25 or 30 pg/mg across various lobes of the lung, e.g., the post-caval, middle, superior, inferior, and left lobes, 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 12 pg/mg in the post caval lobe 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 8 pg/mg in the middle lobe 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 12 pg/mg in the middle lobe 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 17 pg/mg in the middle lobe 24 hours after instillation of IL-10 capsules in rats. In an embodiment, the IL-10 concentration is about 14 pg/mg in the middle lobe 24 hours after instillation of IL-10 capsules in rats.

In an embodiment, IL-10 concentrations in bronchoalveolar lavage fluid (BALF) and plasma return to baseline levels 50, 60, 70, 80, 90, 100, 200, 300 days or longer following a single intratracheal instillation of IL-10 producing capsules. In an embodiment, IL-10 concentrations in bronchoalveolar lavage fluid (BALF) and plasma return to baseline levels 100 days following a single intratracheal instillation of 100 µl of 300 µm diameter IL-10 producing capsules.

In an embodiment, in rats administered 300 μm-diameter IL-10-producing capsules, rat IL-10 is elevated in BAL relative to plasma over a 14-day period, attaining a maximum value of between about 10 to about 100 ng/mL in BAL one day post-instillation.

In an embodiment, in rats administered 300 μm-diameter IL-10-producing capsules, rat IL-10 is elevated in BAL relative to plasma over a 14-day period and there is a concomitant progressive diminution in markers of inflammation as indicated by histological sectioning of rat lung tissue.

In an embodiment, in pigs administered human IL-1Ra (hIL-1Ra)-producing capsules via bronchoscope, hIL-1Ra levels in BAL are about 30 ng/mL two days post-installation while remaining undetectable in plasma. In an embodiment, in pigs administered human IL-10 (hIL-10)-producing capsules via bronchoscope, hIL-10 levels in BAL are about 13 ng/mL two days post-installation while remaining undetectable in plasma.

In an embodiment, in pigs administered human IL-1Ra (hIL-1Ra)-producing capsules via bronchoscope, triglycerides decrease from about 27 mg/dl to about 25 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered human IL-1Ra (hIL-1Ra)-producing capsules via bronchoscope, total cholesterol increases from about 80 mg/dl to about 70 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered human IL-1Ra (hIL-1Ra)-producing capsules via bronchoscope, glucose slightly increases from about 80 mg/dl to about 90 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered human IL-1Ra (hIL-1Ra)-producing capsules via bronchoscope, albumin levels do not materially change from about 3.4 g/dl from prior to instillation to two days after instillation of capsules, indicating no deterioration in liver function.

In an embodiment, in pigs administered human IL-10 (hIL-10)-producing capsules via bronchoscope, triglycerides decrease from about 27 mg/dl to about 25 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered human IL-10 (hIL-10)-producing capsules via bronchoscope, total cholesterol increases from about 80 mg/dl to about 70 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered human IL-10 (hIL-10)-producing capsules via bronchoscope, glucose slightly increases from about 80 mg/dl to about 90 mg/dl from prior to instillation to two days after instillation of capsules. In an embodiment, in pigs administered

human IL-10 (hIL-10)-producing capsules via bronchoscope, albumin levels do not materially change from about 3.4 g/dl from prior to instillation to two days after instillation of capsules, indicating no deterioration in liver function.

In an embodiment, local concentrations of cytokines, e.g., human IL-1Ra and human IL-10 are elevated in bronchoalveolar lavage (BAL) fluid relative to plasma for about 7, 14, 21, 28, days or longer following instillation of cytokine-producing capsules in healthy pigs via bronchoscope. In an embodiment, human IL-1Ra concentration in BAL fluid is between about 30 to about 40 ng/mL on Day 2 following instillation of cytokine-producing capsules in healthy pigs via bronchoscope before returning to baseline on Day 28. In an embodiment, human IL-10 concentration in BAL fluid is between about 15 to about 20 ng/mL on Day 2 following instillation of cytokine-producing capsules in healthy pigs via bronchoscope before returning to baseline on Day 28.

In an embodiment, markers of general health in pigs, e.g., plasma triglycerides, total cholesterol, and glucose, do not significantly change over the course of 28 days following instillation of cytokine-producing capsules in healthy pigs via bronchoscope. In an embodiment, plasma triglycerides is between about 10 to about 30 mg/dl prior to instillation, 2 days after, and 28 days after instillation. In an embodiment, total cholesterol is between about 70 to about 110 mg/dl prior to instillation, 2 days after, and 28 days after instillation. In an embodiment, glucose is between about 60 to about 120 mg/dl prior to instillation, 2 days after, and 28 days after instillation.

In an embodiment, markers of liver function in pigs, e.g., albumin, do not significantly change over the course of 28 days following instillation of cytokine-producing capsules in healthy pigs via bronchoscope. In an embodiment, albumin is between about 3 to about 5 g/dl prior to instillation, 2 days after, and 28 days after instillation.

In an embodiment, spO_2 and $EtCO_2$ do not significantly change over the course of 28 days following instillation of cytokine-producing capsules in healthy pigs via bronchoscope. In an embodiment, spO_2 is between about 100 mmHg prior to instillation, 2 days after, and 28 days after instillation. In an embodiment, EtO_2 is between about 40 to about 60 mmHg prior to instillation, 2 days after, and 28 days after instillation.

In an embodiment, the local concentration of a therapeutic agent in the pleural fluid is elevated for 7, 14, 21, 28 days or longer following administration of lipopolysaccharide (LPS)

intratracheally and concomitant implantation into the pleural cavity of rats of capsules capable of producing a therapeutic agent. In an embodiment, the therapeutic agent is IL-1Ra and/or IL-10. In an embodiment, rat IL-1Ra concentration in pleural fluid is about 104 pg/mL over a period of 28 days following administration of lipopolysaccharide (LPS) intratracheally and concomitant implantation into the pleural cavity of IL-1Ra and IL-10 producing capsules. In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-1Ra at a rate of about 15 µg/day. . In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-10 at a rate of about 3 µg/day.

In an embodiment, explanted capsules demonstrate no substantial fibrotic overgrowth for a period of 7, 14, 21, 28 days or longer following administration of lipopolysaccharide (LPS) intratracheally and concomitant implantation into the pleural cavity of rats of capsules capable of producing a therapeutic agent. In an embodiment, the therapeutic agent is IL-1Ra and/or IL-10. In an embodiment, explanted capsules demonstrate no substantial fibrotic overgrowth for a period of 28 days following administration of lipopolysaccharide (LPS) intratracheally and concomitant implantation into the pleural cavity of rats of IL-1Ra and IL-10 producing capsules. In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-1Ra at a rate of about 15 µg/day. . In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-10 at a rate of about 3 µg/day.

In an embodiment, the lung histology score in rats administered LPS and capsules capable of producing a therapeutic agent is less than rats administered LPS only after 7, 14, 21, 28 days or longer following administration. In an embodiment, the therapeutic agent is IL-1Ra and/or IL-10. In an embodiment, the lung histology score in rats of IL-1Ra is about 0, 0.2, 0.4, 0.6, 0.8, or 1 on Day 1, 7, 14, 21, or 28 following administration of lipopolysaccharide (LPS) intratracheally and concomitant implantation into the pleural cavity of rats of IL-1Ra and IL-10 producing capsules. In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-1Ra at a rate of about 15 µg/day. . In an embodiment, IL-1Ra and IL-10 producing capsules produce IL-10 at a rate of about 3 µg/day.

In an embodiment, capsules remain localized to the lungs for about 1, 3, 5, 7, 9, 11 days or longer as measured by fluorescent IVIS imaging. In an embodiment, 50 µl of 300 µm diameter RPE -Fluc capsules instilled into the lungs of mice remain localized to the lungs for about 7 days.

In an embodiment, the fluorescent signal of fluorescent capsules instilled into the lungs is measurable for about 1, 3, 5, 7, 9, 11 days or longer. In an embodiment, the luminescent signal of 50 µl of 300 µm diameter RPE -Fluc capsules instilled into the lungs of mice is measurable for about 11 days.

In an embodiment, fluorescent capsules are uniformly distributed in the lungs of rats following instillation, euthanasia, and harvesting. In an embodiment, 100µl of 300 µm green fluorescent protein (GFP) producing capsules instilled into the lungs of mice are uniformly distributed as characterized by fluorescence microscopy and fluorescent IVIS imaging.

In an embodiment, histological sections of lungs of subjects administered LPS + IL-10 producing capsules intratracheally are characterized by reduced inflammation compared to subject administered LPS only. In an embodiment, histological sections of lungs of rats administered 5 mg/kg LPS and 300 µm IL-10 producing capsules are characterized by reduced inflammation compared to rats administered 5 mg/kg LPS only 24 h after administration. In an embodiment, the BAL total cells collected from subjects administered LPS + IL-10 producing capsules is reduced compared to Blank or LPS only. In an embodiment, the BAL total cells collected from rats administered 5 mg/kg LPS and 300 µm IL-10 producing capsules is about 5 X 10^8 cells compared to about 1.5 X 107 cells for both the LPS only and Blank groups 24 h after administration.

In an embodiment, local concentrations of inflammatory proteins, e.g., IL-1a, IL-1b, TNFa, and MCP1, *inter alia*, are reduced in BAL of subjects administered LPS + IL-10 capsules compared to Blank or LPS only. In an embodiment, the IL1a concentration in BAL is about 0.1 ng/mL compared to about 0.7 ng/mL for LPS only and 0.4 ng/mL for Blank 24 h after administration of 300 µm IL-10 capsules. In an embodiment, the IL1b concentration in BAL is about 3 ng/mL compared to about 6 ng/mL for LPS only and 6 ng/mL for Blank 24 h after administration of 300 µm IL-10 capsules. In an embodiment, the TNFa concentration in BAL is about 0.1 ng/mL compared to about 0.3 ng/mL for LPS only and 0.25 ng/mL for Blank 24 h after administration of 300 µm IL-10 capsules. In an embodiment, the MCP1 concentration in BAL is about 10 ng/mL compared to about 60 ng/mL for LPS only and 80 ng/mL for Blank 24 h after administration of 300 µm IL-10 capsules.

In an embodiment, the BAL total cells collected from subjects administered LPS and IL-10 +IL-1Ra producing capsules is less than about 3 X 10^7, 2 X 10^7, 1 X 10^7 cells or less 12 or 24

h after administration. In an embodiment, the BAL total cells collected from the lungs of rats administered 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules is about 3 X 10^8 cells after about 12 h. In an embodiment, the BAL total cells collected from the lungs of rats administered 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules is about 6 X 10^8 cells after about 12 h.

In an embodiment, local concentrations of inflammatory proteins, e.g., IL-1a, TNFa, and MCP1, IL-1b, *inter alia*, are reduced in BAL of subjects administered LPS and IL-10 + IL-1Ra capsules compared to LPS only. In an embodiment, the IL-1a concentration in BAL is about 0.05 ng/mL 12 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the IL-1a concentration in BAL is about 0.1 ng/mL 24 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the TNFa concentration in BAL is about 0.1 ng/mL 12 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the TNFa concentration in BAL is about 0.1 ng/mL 24 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the MCP1 concentration in BAL is about 2 ng/mL 12 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the MCP1 concentration in BAL is about 25 ng/mL 24 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules.In an embodiment, the IL-1b concentration in BAL is about 2 ng/mL 12 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules. In an embodiment, the IL-1b concentration in BAL is about 3 ng/mL 24 h after administration of 5 mg/kg LPS and 300 μm IL-10 and IL1Ra producing capsules.

In an embodiment, the BAL total cells collected from subjects administered LPS and IL-10 producing capsules is reduced compared to LPS+Blank or LPS only. In an embodiment, the BAL total cells is collected from rats administered LPS and IL-10 producing capsules us about 5 X 10^8 cells 24 h after instillation, compared to between about 1 X 10^7 to about 2 X 10^7 cells for LPS only and LPS only+ Blank capsules. In an embodiment, local concentrations of inflammatory proteins, e.g., IL-1a, TNFa, IL-1b, and MCP1, inter alia, are reduced in BAL of subjects administered LPS+Blank or LPS only. In an embodiment, the IL-1a concentration BAL collected from rats is about 0.1 ng/mL compared to 0.7 ng/mL for LPS only and 0.4 ng/mL for LPS+Blank 24 h after administration of IL-10 producing capsules.I n an embodiment, the TNFa

concentration BAL collected from rats is about 0.1 ng/mL compared to 0.3 ng/mL for LPS only and 0.25 ng/mL for LPS+Blank 24 h after administration of IL-10 producing capsules. In an embodiment, the IL-1b concentration in BAL collected from rats is about 3 ng/mL compared to 6 ng/mL for LPS only and 6 ng/mL for LPS+Blank 24 h after administration of IL-10 producing capsules. In an embodiment, the MCP1 concentration in BAL collected from rats is about 10 ng/mL compared to 60 ng/mL for LPS only and 80 ng/mL for LPS+Blank 24 h after administration of IL-10 producing capsules.

In an embodiment, the histological score of inflammation as a factor of neutrophil density, distribution, and presence of immune consolidations for rats administered LPS and IL-10 producing capsules is less than about 1.5, 1.4, 1.3, 1.2, 1.1, 1.0 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or 0 on Day 1, 7, 14, or 21 post administration. In an embodiment, the inflammation score as a factor of neutrophil density, distribution, and presence of immune consolidations for rats administered LPS and IL-10 producing capsules is about 0 on Day 7, 14, or 21 post administration. In an embodiment, the inflammation score as a factor of neutrophil density, distribution, and presence of immune consolidations for rats administered LPS and IL-10 producing capsules is about 0 on Day 7 post administration. In an embodiment, the inflammation score as a factor of neutrophil density, distribution, and presence of immune consolidations for rats administered LPS and IL-10 producing capsules is about 0 on Day 14 post administration. In an embodiment, the inflammation score as a factor of neutrophil density, distribution, and presence of immune consolidations for rats administered LPS and IL-10 producing capsules is about 0 on Day 21 post administration.

In an embodiment, the histological score of regulatory response as a factor of macrophage, fibroblast, and chronic inflammatory cells for rats administered LPS and IL-10 producing capsules is greater than 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0 on Day 1, 7, 1, or 21 post administration. In an embodiment, the histological score of regulatory response as a factor of macrophage, fibroblast, and chronic inflammatory cells for rats administered LPS and IL-10 producing capsules is between about 1.5 to about 2.0 on Day post administration.

In an embodiment, perivascular lymphoid cuffing of rats administered LPS and IL-10 producing capsules on Day 1, Day 7, Day 14, and Day 21 post administration is reduced compared to rats administered LPS only. perivascular lymphoid cuffing of rats administered LPS

and IL-10 producing capsules on Day 7, Day 14, and Day 21 post administration is between about 2 to about 5 compared to about 18 to about 25 for the LPS only group.

In an embodiment, a histological scan of the lungs of rats administered LPS and IL-10 producing capsules on Day 1, Day 7, Day 14, and Day 21 post administration manifests an improved immune phenotype compared to the LPS+Blank and LPS only groups.

In an embodiment, in an LPS model of ARDS, BAL levels of TNF-α on Day 2 post administration of 20 mg/kg LPS are between about 100 and 1000 pg/mL compared to about 1 to about 10 pg/mL for the saline group. In an embodiment, in an LPS model of ARDS, BAL levels of IL-1α on Day 2 post-administration of 20 mg/kg LPS are about 1000 pg/mL compared to about 10 pg/mL for the saline group. In an embodiment, in an LPS model of ARDS, BAL levels of MCP1 on Day 2 post-administration of 20 mg/kg LPS are about 10^5 pg/mL compared to about 10 pg/mL for the saline group. In an embodiment, in an LPS model of ARDS, BAL levels of MIP2 on Day 2 post-administration of 20 mg/kg LPS is between about 10^3 and about 10^4 pg/mL compared to between about 10 to about 100 pg/mL for the saline group. In an embodiment, in an LPS model of ARDS, BAL levels of total cell count derived lung homogenate are about $4*10^7$ cells compared to about $1*10^7$ to about $2*10^7$ cells for the saline group.

EXAMPLES

Example 1. Fabrication of Alginate Capsules

Provided below is a method to fabricate barium alginate hydrogel capsules utilizing a custom-built, two-fluid co-axial electrostatic spraying device. The custom-built device consists of a voltage generator that is connected to the tip of a co-axial needle and grounded to a 1:4 $BaCl_2$:mannitol cross-linking bath. The co-axial needle is fed by two separate syringes containing 1.4% alginate solutions diluted in 0.9% saline. Engineered cells, e.g., ARPE-19 cells, programmed to constitutively express cytokines are combined with the 1.4% alginate solutions to form cross-linked hydrogel capsules containing the engineered cells. The cytokine dosage may be modulated according to necessary constraints on a per patient basis by altering the number of cells in an individual capsule, or alternatively altering the number of capsules per dose, or a combination thereof. The fabrication technique at least allows for individual capsules to contain between 250 to 1.86e6 cells and for capsule diameters between 50 μm – 3 mm.

Example 2. Direct Instillation of Capsules to the Lungs

The example set forth below describes alternative non-invasive delivery modes for lung delivery. The capsules comprising a polymer and an plurality of engineered cells may be delivered via instillation with a device **(FIG. 2),** e.g., a catheter, or a bronchoscope, e.g., a therapeutic bronchoscope or a diagnostic bronchoscope. The device may extend down to the trachea, e.g., for intratracheal instillation, or deeper into lungs, e.g., the bronchi. The capsules are prepared as a liquid composition which is imparted gradually. At the distal end of the device, the liquid composition is aerosolized, allowing for the subject to inhale the capsules to enable local distribution deep into the lungs and allowing for superior localization of the therapeutic agent in alveolar tissue relative to other delivery modes.

Example 3. Capsules for Delivery of Therapeutic Agents via Instillation

The example set forth below describes methods to reproducibly generate capsules comprising a cell or a plurality of engineered cells of various diameters, e.g., capsules having diameters of 150, 300, 600, 800, 1200, and 1700 μm. The capsules are synthesized as in Example 1 employing a custom-built, two-fluid co-axial electrostatic spraying device. The custom-built device consists of a voltage generator that is connected to the tip of a co-axial needle and grounded to a 1:4 $BaCl_2$:mannitol cross-linking bath. The co-axial needle is fed by two separate syringes containing 1.4% SLG20 sodium alginate solutions diluted in 0.9% saline. Engineered cells, e.g., ARPE-19 cells, programmed to constitutively express cytokines are combined with the 1.4% alginate solutions to form cross-linked hydrogel capsules containing the engineered cells. Multiple syntheses demonstrate that the target size of the capsules closely aligns with the actual measured diameter as shown in the bar graph in **FIG. 4A**. The synthesized capsules may then be administered directly, e.g., intratracheally instilled directly to the lungs with a device, e.g., a bronchoscopic device or a catheter.

Example 4. Viability, Cell Number and Cytokine Concentration Independent of Capsule Diameter

The example set forth below demonstrates that the viability and cell number of engineered ARPE-19 cells encapsulated in the hydrogel capsules is robust and consistent regardless of capsule diameter; moreover, the difference in production of the therapeutic agent

rat IL-1Ra from the capsules of various diameters is not statistically significant. Briefly, barium alginate hydrogel capsules were synthesized according to the methods delineated in Examples 1 and 8 above to fabricate capsules having diameters of 150, 300, 800, and 1200 μm. ARPE-19 cells programmed to constitutively express Rat IL-1Ra were encapsulated in the hydrogel capsules of various diameters. LIVE/DEAD™ cell viability assay (commercially available from ThermoFisher Scientific) was used to assess viability of the engineered ARPE-19 cells and was determined to be greater than 80% for all of the capsule diameters tested, as shown in **FIG. 5A**. Cells were counted on an automatic cell counter. The cell count between capsule samples having a diameter of 150, 300, 800 and 1200 μm was between approximately $2*10^6$ and $3*10^6$ cells, and the difference was determined not to be statistically significant among capsule diameters as shown in **FIG. 5C**. Likewise, Rat IL-1Ra production between capsules having a diameter of 150, 300, 800 and 1200 μm was approximately $2*10^6$ pg/mL/day, and statistically determined to be insignificant among the various capsule diameters as illustrated in the bar graph in **FIG. 5D**.

Example 5. Delivery and Retrieval of Encapsulated Cell Therapies Upon Instillation to the Lungs

The example set forth below describes methods to deliver up to 300 μl of 200 μm-diameter capsules directly to the lungs in a rodent lung injury model. Briefly, barium alginate hydrogel capsules comprising ARPE-19 cells were synthesized according to the scheme as described in Examples 1 and 8. The capsules were suspended in 300 μl liquid buffer and introduced directly into rat lungs via intratracheal instillation with a bronchoscopic device, e.g., a bronchoscope, e.g., a therapeutic bronchoscope or a diagnostic bronchoscope. The bronchoscope was introduced via the mouth or nasal passages of the rodent and directed to a region adjacent to the lungs, e.g., the trachea, for local delivery of the capsules to the lungs. Bronchoalveolar lavage (BAL) was then performed to recover instilled capsules. Rodents were sacrificed and lung tissue was prepared for histological sectioning and staining with hematoxylin and eosin employing methods commonly understood in the art to interrogate capsule localization in the lungs. **FIG. 7B** demonstrate that, independent of the dose of capsules (e.g., 100, 200 or 300μl), capsules were localized adjacent to rodent alveolar tissue.

Example 6. Instillation of Capsules to the Lungs Results in Local Delivery of Therapeutic Cytokines

The example set forth below demonstrates that instillation of capsules comprising engineered ARPE-19 cells results in local delivery of therapeutic cytokines. Briefly, barium alginate hydrogel capsules comprising ARPE-19 cells capable of expressing IL-1Ra were synthesized according to the fabrication methods described in Examples 1 and 8. The capsules were then formulated as a liquid pharmaceutical composition for administration to a rodent lung injury model via intubation. Lipopolysaccharide (LPS) lung injury in rodents was used to induce respiratory inflammation and distress. Briefly, the rodents were anesthetized via intraperitoneal injection. Rodents received LPS in buffered saline, and the remaining rodents received a vehicle control. LPS-treated rodents were further divided into groups with LPS only and LPS with capsule treatment. Capsules were instilled intratracheally and localized to alveolar tissue in the lungs as confirmed by histological sectioning. Bronchoalveolar lavage (BAL) was subsequently performed and the bronchoalveolar lavage fluid (BALF) was collected for analysis. Samples were also collected from the pleural cavity and blood plasma for the detection of cytokines. As shown in **FIG. 8A**, administration of the capsules via intubation resulted in a 10,000-fold increase in the local IL-1Ra concentration in the lungs (BAL) relative to the systemic IL-1Ra concentrations, e.g., the plasma and pleural IL-Ra concentrations. There was a significant reduction in the IL-17 concentration measured in BALF in rodents instilled with capsules capable of expressing IL-1Ra and secreting in lung tissue relative to the IL-17 concentration in BALF of rodents administered vehicle as shown in **FIG. 8B**.

Example 7. Histological Sections Show a Reduction in Neutrophil Infiltration at 24-h Post-Treatment

The example set forth below indicates a reduction in neutrophil infiltration 24 h after administration of capsules comprising ARPE-19 cells capable of expressing IL-1Ra in a rodent model of lung injury. Briefly, barium alginate hydrogel capsules comprising ARPE-19 cells capable of expressing IL-1Ra were synthesized according to the fabrication methods described in Example 1. The capsules were then formulated as a liquid pharmaceutical composition for administration to a rodent lung injury model via intubation. Lipopolysaccharide (LPS) lung injury in rodents was used to induce respiratory inflammation and distress. Briefly, the rodents

were anesthetized via intraperitoneal injection. Rodents received LPS in buffered saline, and the remaining rodents received a vehicle control. LPS-treated rodents were further divided into groups with LPS only and LPS with capsule treatment. The capsules were instilled intratracheally for direct localization of the capsules to alveolar tissue. 24 hours after instillation, rodents were sacrificed and lung tissue was prepared for histological sectioning and staining with hematoxylin and eosin employing methods commonly understood in the art. **FIGS. 9A-C** demonstrate a significant reduction in neutrophil recruitment to the injured lung tissue in LPS samples treated with instilled capsules after 24 h relative to the histology of the LPS-only lung tissue. Capsule instillation significantly abrogates the immunological and inflammatory response to LPS injury relative to health lung tissue.

Example 8. Imaging of Fluorescent Capsules in a Rat Instillation Model

The example set forth below illustrates the distribution of fluorescent capsules in instilled rat lungs. Briefly, fluorescent capsules were fabricated wherein the capsules were loaded with the fluorescent dye FITC dextran. Upon instillation into rat lungs, the rats were euthanized, and the lungs were surgically removed for fluorescence microscopy imaging. As shown in **FIG. 10**, imaging with a Leica microscope in the GFP channel shows the prevalence of the capsules in rat lung tissue, as indicated by the bright spots.

Example 9. PiggyBac Mobile Elements for Incorporating Therapeutic Sequences into Genomic DNA of Engineered Cells

The example set forth below describes a method to incorporate therapeutic sequences into genomic DNA of engineered cell lines employing a PiggyBac transposon. Briefly, A vector comprising therapeutic sequences for IL-10, IL-1Ra, IL-13, IL-4, FGF-21 with a PiggyBac inverted terminal repeat sequences (ITRs) is transfected into an exemplary cell line, as shown in **FIG. 9**. Transposases identify ITRs on the PiggyBac vector, enabling the transposition of therapeutic genes into genomic DNA, thereby creating a genetically engineered therapeutic cell line capable of expressing IL-10, IL-1Ra, IL-13, IL-4, FGF-21. **FIG. 12** illustrates FGF-21 protein expression in the hours following transposition employing the PiggyBac method,

Example 10. Rat IL-10 Production in an LPS-Induced Lung Injury Model

The example set forth below characterizes rat IL-10 production in a rodent model of lung injury. Briefly, barium alginate hydrogel capsules comprising ARPE-19 cells capable of expressing IL-10 were synthesized according to the fabrication methods described in Example 1. The capsules were then formulated as a liquid pharmaceutical composition for administration to a rodent lung injury model via intubation. Lipopolysaccharide (LPS) lung injury in rodents was used to induce respiratory inflammation and distress. Briefly, the rodents were anesthetized via intraperitoneal injection. Rodents received LPS in buffered saline, and the remaining rodents received a vehicle control. LPS-treated rodents were further divided into groups with LPS only and LPS with capsule treatment. The capsules were instilled intratracheally at various doses (0, 50, 100, and 150 µl) for direct localization of the capsules to alveolar tissue. **FIG. 16A** shows rat IL-10 concentration (ng/mL) in plasma and bronchoalveolar lavage fluid at various doses (0, 50, 100, and 150 µl) of the capsules 24 h after instillation. FIG. 12

Example 11. Histological Sections Show a Reduction in Neutrophil Recruitment 1 Day, 7 Days, and 14 Days After Administration

The example set forth below indicates a reduction in neutrophil infiltration 1 day, 7 days, and 14 days after administration of capsules comprising ARPE-19 cells capable of expressing IL-1Ra in a rodent model of lung injury. Briefly, barium alginate hydrogel capsules comprising ARPE-19 cells capable of expressing IL-1Ra were synthesized according to the fabrication methods described in Example1. The capsules were then formulated as a liquid pharmaceutical composition for administration to a rodent lung injury model via intubation. Lipopolysaccharide (LPS) lung injury in rodents was used to induce respiratory inflammation and distress. Briefly, the rodents were anesthetized via intraperitoneal injection. Rodents received LPS in buffered saline, and the remaining rodents received a vehicle control. LPS-treated rodents were further divided into groups with LPS only and LPS with capsule treatment. The capsules were instilled intratracheally for direct localization of the capsules to alveolar tissue. The rodents were sacrificed and lung tissue was prepared for histological sectioning and staining with hematoxylin and eosin 1 day, 7 days and 14 days after administration. **FIGS. 17A-C** demonstrate a significant reduction in neutrophil recruitment to the injured lung tissue in LPS samples treated with instilled capsules after 1, 7 and 14 days relative to the histology of the LPS-only lung tissue.

Capsule instillation significantly abrogates the immunological and inflammatory response to LPS injury relative to health lung tissue.

Example 12. Distribution of Instilled Capsules in Rat Lungs Using IVIS Imaging

The example set forth below demonstrates the distribution of instilled capsules in a rat lung model. Briefly, capsules of various diameters (300, 500, 800, and 1000 μm) were loaded with a fluorescent dye that was excitable in the far red channel. The capsules were subsequently instilled into the rats. The rats were then euthanized and the excised lungs were imaged using IVIS imaging. **FIG. 20** is a representative image of lungs with each representative capsule diameter, demonstrating the distribution by capsule diameter in rat lungs.

Example 13. Cell Viability of Encapsulated Engineered Cells

The example set forth below demonstrates the viability of engineered ARPE-19 cells in capsules of various diameters. Briefly, capsules were fabricated according to the methods outlined in Example 1 encapsulating ARPE-19 cells. A LIVE-DEAD cell assay (commercially available from Thermo Fisher Scientific) was employed to the assess the cell viability after encapsulation following the manufacturer's instructions. **FIG. 21** shows cell viability of capsules comprising a plurality of engineered cells employing a LIVE/DEAD assay, where the live channel has signal and dead channel has no signal.

Example 14. Response of Engineered ARPE-19 Cells to Pro-Inflammatory Cytokines

The example set forth below demonstrates the response of engineered ARPE-19 cells expressing a transgene to pro-inflammatory cytokines. Briefly, cultured ARPE-19 cells were stably transfected with a plasmid encoding for a luciferase enzyme (Luc) with a NF-κB-responsive promoter (NFkB TRE) and a polyadenylation (PA) signal, as shown in **FIG. 22A**, using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions. Luciferase production was characterized by measuring the relative luminescence of engineered ARPE-19 cells in media containing no cytokines (Control), 10 ng/mL IFN-γ, 15 ng/mL IL-1β, and 15 ng/mL TNF-α, as shown in **FIG. 22B**. As demonstrated in the bar graph, luciferase production was upregulated in response to IFN-γ, IL-1β, and TNF-α.

Example 15. Autoregulated Gene System

The example set forth below delineates strategies to autoregulate the genetic system underlying inducible expression of a therapeutic agent, e.g., a protein, e.g., a cytokine in a capsule comprising a plurality of engineered cells. It may be advantageous within certain modes that the level of cytokine production be auto regulated in order to prevent secretion of toxic levels of the cytokine. The first route to accomplish this feedback mechanism is to introduce an operator site into the DNA region between the cytokine gene, e.g., IL-2, IL-10, etc. and its promoter in a first ORF. A second ORF will be used that encodes a transcriptional repressor that binds to the operator site under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. For example, if the cytokine is IL-2, then the promoter controlling the expression of the transcriptional repressor could be a STAT transcription factor. In this way, the cells can sense the cytokine in their environment and reduce their production of the cytokine when there is sufficient cytokine already present.

A second route entails introduction of a sequence that forms a higher-order structure into the 5' untranslated region (5' UTR) of the cytokine gene. Then a second ORF is utilized that encodes an RNA-binding protein, which binds to the higher-order structure, and suppresses translation, under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. For example, if the cytokine is IL-2, then the promoter controlling the expression of the RNA-binding protein could be a STAT transcription factor.

A further strategy to accomplish gene autoregulation and a feedback mechanism for secretion of the therapeutic agent is to introduce several repeats of a synthetic microRNA (miRNA) target site into the 3' untranslated region (3' UTR) of the cytokine gene. Then a second ORF is used that encodes the miRNA under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. For example, if the cytokine is IL-2, then the promoter controlling the expression of the miRNA could be a STAT transcription factor.

Finally, an alternative strategy is to employ a second ORF encoding a synthetic ubiquitin ligase that targets the cytokine, and leads to ubiquitin-mediated proteolysis, under the control of a promoter that is activated as a result of signaling through the cytokine's receptor. For example, if the cytokine is IL-2, then the promoter controlling the expression of the ubiquitin

ligase could be a STAT transcription factor. In this instance, the cytokine gene may be modified to include additional protein domains if doing so is necessary in order to make the cytokine recognizable by the synthetic ubiquitin ligase. Ideally, the addition of any additional protein domains will not alter the cytokine's immunological functions.

Example 16. Capsule Distribution in Lungs Results in a Gradient in Cytokine Concentration between Local and Systemic Compartments

The example set forth below evaluates the distribution and pharmacokinetics of cell-laden capsules capable of secreting various proteins, including the therapeutic agents IL-10, IL-1Ra, FGF21, IL-13, and IL-4. The loading and distribution of 300 μm-diameter capsules in rat after instillation into the lungs was evaluated by various methods. **FIG. 13A** shows a representative micrograph of the 300 μm-diameter cell-laden capsules loaded with FITC dextran. Note that the capsules are uniformly green, indicating that ARPE-19 capsules are homogenously distributed. **FIG. 13B** is a representative IVIS (In Vivo Imaging System) image of rat lungs after instillation of 300 μm capsules comprising GFP-expressing cells which show their uniformity in rat alveolae. **FIGS. 13C-D** are representative *ex vivo* fluorescent images of lungs of instillation with dextran capsules which demonstrate their homogeneous distribution in rat alveolar tissue. **FIG. 13E** is a micrograph of 300 μm-diameter capsules comprising cells capable of expressing the firefly luciferase protein, indicating that the cell-laden capsules are all apportioned a similar amount of cells, e.g., about 10,000 cells. **FIG. 25** shows additional fluorescence microscopy and fluorescent IVIS imaging of rat lungs instilled with 100 μl of 300μm green fluorescent protein (GFP) capsules. **FIG. 14A** is a graph depicting the average radiance of rats instilled with either 300 μm capsules comprising a plurality of firefly-luciferase cells or free cells over a 1 week period. As shown in the graph, the average radiance of the firefly luciferase producing capsules reaches a maximum of about 10^4 p/s/cm^2 about one day after encapsulation relative to a maximum radiance of about 10^3 p/s/cm^2 for capsules comprising free cells. The average radiance of firefly luciferase producing cells is always greater than the average radiance of the capsules comprising a plurality of free cells over the period of one week. **FIG. 14B** shows a series of representative images of rats that were instilled 300 μm-diameter capsules comprising a plurality of firefly-luciferase expressing cells. Corroborating the average radiance data as shown in **FIG. 14A**, the radiance measured in rats instilled with the firefly luciferase expressing capsules was

greater than the radiance measured in rats instilled with the free cell containing capsules through the seven day period post-instillation. The radiance appears to reach its maximum value approximately one day post-instillation for both groups. **FIG. 14C** shows *in vivo* bioluminescent total flux analysis demonstrating capsule retention for up to three days versus one day in unencapsulated cells. *Ex vivo* fluorescent images of lungs after instillation with fluorescent dextran-loaded capsules and *in vivo* bioluminescent imaging of *ex vivo* lung one day after capsule instillation further show retention of capsules in the lungs of rats. **FIG. 15A** illustrating the production and distribution of IL-10 in bronchoalveolar lavage (BAL) and plasma 24 h subsequent to instillation of capsules comprising IL-10-producing cells. As noted in the graph, IL-10 production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 0.1 to 1 ng/mL in plasma. **FIG. 15B** illustrating the production and distribution of IL-1Ra in bronchoalveolar lavage (BAL) and plasma 24 h subsequent to instillation of capsules comprising IL-1Ra-producing cells. As noted in the graph, IL-1Ra production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma. **FIG. 15C** illustrating the production and distribution of FGF21 in bronchoalveolar lavage (BAL) and plasma 24 h subsequent to instillation of capsules comprising FGF21-producing cells. As noted in the graph, FGF21 production is localized to BAL and is between about 10 to about 100 ng/mL compared to about 1 to 10 ng/mL in plasma. **FIG. 15D** illustrating the production and distribution of IL-13 in bronchoalveolar lavage (BAL) and plasma 24 h subsequent to instillation of capsules comprising IL-13-producing cells. As noted in the graph, IL-13 production is localized to BAL and is about 100 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma. **FIG. 15E** illustrating the production and distribution of IL-4 in bronchoalveolar lavage (BAL) and plasma 24 h subsequent to instillation of capsules comprising IL-4-producing cells. As noted in the graph, IL-4 production is localized to BAL and is between about 1 to about 10 ng/mL compared to about 0.01 to 0.1 ng/mL in plasma.

Example 17. Dose Escalation and Pharmacokinetics of Instilled Capsules

The example set forth below illustrates a dose titration and pharmacokinetics study of 300-μm capsules delivered via intratracheal instillation to rat lungs. Briefly, escalating doses (0, 50, 100, and 150 μl) of capsules capable of secreting rat IL-10 were instilled to rat lungs. Rat IL-10 concentrations in BAL and plasma were assessed over time to determine cytokine localization

and pharmacokinetic parameters. Changes in alveolar histology were also assessed to complement the pharmacokinetic analysis. **FIG. 16A** is a bar graph showing IL-10 protein levels in bronchoalveolar lavage (BAL) and plasma from rats instilled 300 μm-diameter capsules at increasing volumes, i.e., 0, 50, 100, and 150 μl. As evidenced, IL-10 is localized to BAL relative to plasma and demonstrates a direct dose-response relationship. IL-10 concentration increases from about 0.1 ng/mL in BAL to about 100 ng/mL as the capsule dose increases from 0 to 150 μl. However, the IL-10 concentration in plasma remains relatively constant at about 0.1 ng/mL regardless of capsule dose volume administered. **FIG. 16B** is a bar graph illustrating the relationship between increasing cell density per capsule (cell concentration per mL alginate) and rat IL-10 production as measured in bronchoalveolar lavage (BAL) and plasma. As indicated in the bar graph, IL-10 in BAL increases from about 0.01 ng/mL to between about 10 to about 100 ng/mL as the cell concentration per mL of alginate increases from 0 to about $1*10^7$ cells/mL. In contrast, IL-10 in plasma remains relatively constant between about 0.01 to 0.1 ng/mL as the cell concentration per mL of alginate increases from 0 to about $1*10^7$ cells/mL. **FIG. 16C** shows BAL and plasma concentrations of IL-10 24 hours post-instillation with 100 μL of RPE-IL-10 capsules and upon repeat dose at 30 days, demonstrating that there is no significant difference in local concentrations between day 1 and day 31, indicating no anti-drug antibody development. **FIG. 16D** is a graph quantifying rat IL-10 levels in bronchoalveolar lavage (BAL) and plasma over the duration of 14 days post-instillation with rat IL-10-producing capsules. As indicated in the graph, rat IL-10 levels are particularly elevated in BAL relative to plasma, peaking between about 10 to about 100 ng/mL about one day after instillation. Rat IL-10 levels in plasma remain relatively constant over the course of 14 days at about 0.1 ng/mL. **FIG. 16E** shows *in vitro* concentrations of IL-1Ra, FGF-21, IL-13, and IL-4 24 hours after administration of 100 μl of 300 μm capsules. **FIG. 16F** demonstrates that IL-10 levels in the post caval, middle, superior, inferior, and left lobes of lungs of rats 24 hours after instillation with IL-10 producing capsules. FIG. 16G shows that Rat IL-10 concentration in bronchoalveolar lavage (BAL) and plasma at Day 100, demonstrating a return to baseline levels. **FIG. 16H** is a series of histological sections of rat lung (alveolar) tissue corresponding to the kinetic timepoints (Day 1, Day 2, Day 3, Day 4, Day 7, and Day 14) as shown in **FIG. 16D**. Scale bar = 200 μm. A progressive diminution in inflammation and improvement in healthy lung tissue is observed, corresponding to the production of rat IL-10 from instilled rat IL-10-producing capsules.

Example 18. Capsule Administration in Porcine Lung via Bronchoscopy

The example set forth below describes a proof-of-concept demonstration of capsule instillation in porcine lungs via bronchoscopy. Briefly, capsules capable of secreting IL-10 and IL-1Ra, respectively, were intratracheally instilled in a pig model using a bronchoscope. **FIG. 18A** shows IL-10 and IL-1Ra concentrations in BAL and plasma on Day 0, Day 2 and Day 28. **FIG. 18B** is a representative image of blue-dyed capsules instilled into porcine lungs via this technique. **FIG. 18C** is a bar graph of human IL-1Ra (hIL-1Ra) levels in bronchoalveolar lavage (BAL) and plasma prior to and two days after instillation of hIL-1Ra-secreting capsules. Human IL-1Ra production is localized to the lungs; hIL-1RA in BAL and plasma is nearly undetectable prior to administration, whereas two days post-instillation hIL-1Ra levels in BAL are about 30 ng/mL while remaining undetectable in plasma. **FIG. 18D** is a bar graph of human IL-10 (hIL-10) levels in bronchoalveolar lavage (BAL) and plasma prior to and two days after instillation of hIL-10-secreting capsules. Human IL-10 production is localized to the lungs; hIL-10 in BAL and plasma is undetectable prior to administration, whereas two days post-instillation hIL-10 levels in BAL are about 13 ng/mL while remaining undetectable in plasma. **FIG. 18E** presents the general health of pigs prior to, two days after, and twenty-eight days after instillation of IL-10 and IL-1Ra producing capsules as characterized by triglycerides, total cholesterol and glucose. **FIG. 18F** shows any changes in liver function of pigs prior to, two days after, and twenty-eight days after instillation of hIL-1Ra- and hIL-10 producing capsules as characterized by albumin concentration. **FIG. 18G** are graphs showing spO_2 and $EtCO_2$ levels in pigs throughout the duration of the procedure. These data demonstrate that hIL-1Ra- and hIL-10-secreting capsules can be successfully delivered to porcine lungs to deliver anti-inflammatory cytokines and improve biomarkers associated with decreased inflammation and improved overall health, e.g., triglycerides, cholesterol, glucose, and liver functioning.

Example 19. Establishment of an LPS model of ARDS

The example set forth below describes the establishment of an lipopolysaccharide (LPS) model of acute respiratory distress syndrome (ARDS). Briefly, animals (e.g., rodents or pigs) arrive seven days prior to the initiation of the LPS investigation. On Day 0, animals receive 200 mg/k LPS or volume-matched saline. Levels of various inflammatory markers, e.g., TNF-α, IL-

1α, MCP1, MIP2, in addition to total cell count from lung homogenate were measured in bronchoalveolar lavage (BAL) fluid on Day 2 after instillation (**FIG. 19A**). BAL levels of TNF-α on Day 2 post administration of 20 mg/kg LPS are between about 100 and 1000 pg/mL compared to about 1 to about 10 pg/mL for the saline group (**FIG. 19B**). BAL levels of IL-1α on Day 2 post-administration of 20 mg/kg LPS are about 1000 pg/mL compared to about 10 pg/mL for the saline group (**FIG. 19C**). BAL levels of MCP1 on Day 2 post-administration of 20 mg/kg LPS are about 10^5 pg/mL compared to about 10 pg/mL for the saline group (**FIG. 19D**). BAL levels of MIP2 on Day 2 post-administration of 20 mg/kg LPS is between about 10^3 and about 10^4 pg/mL compared to between about 10 to about 100 pg/mL for the saline group (**FIG. 19E**). BAL levels of total cell count derived lung homogenate are about $4*10^7$ cells compared to about $1*10^7$ to about $2*10^7$ cells for the saline group (**FIG. 19F**). **FIG. 19G** shows representative images from a homogenized post caval left lung lobe.

Example 20. IL-10 and IL-1Ra Combination Therapy in an LPS Model of ARDS

The example set forth below describes the results of IL-10 and IL-1Ra combination therapy in an LPS model of ARDS in rats. Briefly, rats were administered lipopolysaccharide (LPS) via intratracheal instillation and 300 μm capsules producing ~ 3 μg/day IL-10 and ~15 μg/day IL-1Ra were implanted into the pleural cavity. Local concentrations of IL-10 and I:-1Ra remained elevated for the duration of the experiment. Systemic concentrations were elevated 24 hours after transplantation; however, the systemic concentrations were an order of magnitude less than local concentrations. Capsules explanted on Day 28 also presented minimal fibrotic overgrowth, demonstrating that immune response to the capsules was attenuated. **FIG. 23A** are graphs showing concentrations of rat IL-1Ra and Rat IL-10 in pleural fluid and plasma over 28 days, demonstrating localization of both IL-10 and IL-1Ra in the pleural fluid. **FIG. 23B** are microscopy images of explanted capsules on Day 1, 3, 7, and 28 demonstrating minimal fibrotic overgrowth. **FIG. 23C** is a graph showing lung histology score for the LPS only and LPS + IL-10 + IL-Ra capsules, demonstrating .

In a subsequent study, rats were treated with 5mg/kg LPS intratracheally. 300 μm capsules producing both IL-10 and IL-1Ra were administered concomitantly with LPS. Bronchoalveolar lavage (BAL) fluid and lungs were subsequently collected for analysis. Local concentrations of critical inflammatory proteins in the lungs were significantly reduced by

treatment with combination IL-10 and IL-1Ra capsules. Total cells collected from BAL fluid were also significantly reduced following treatment (**FIG 27A**). At the end of the 28-day period, combination therapy resulted in a reduction is perivascular lymphoid cuffing, which is a marker of unresolved inflammation (**FIG. 27B**).

Example 21. IL-10 Therapy in an LPS Model of LPS

The example set forth below describes the results of IL-10 therapy in an LPS model of ARDS in rats. Briefly, rats were treated with 5mg/kg lipopolysaccharide (LPS) intratracheally. 300 μm diameter capsules producing IL-10 were administer concurrently with LPS. 24 h after instillation, bronchoalveolar lavage (BAL) fluid and were collected for further analysis. Total cells collected from BAL fluid were significantly reduced following treatment. Local concentrations of key inflammatory proteins were significantly reduced by IL-10 treatment but not the RPE capsule group (Control). **FIG. 26A** are images of histological sections of rat lungs treated with LPS only and LPS + IL-10 producing capsules after 24 hours, highlighting the reduced inflammation. **FIG. 26B** shows total cell counts collected 12 and 24 hours after LPS instillation, as well as IL-1a and TNFa BAL concentrations 12 and 24 days after LPS treatment and administration of IL-10/IL-1a producing capsules. **FIG. 26C** show IL1b and MCP-1 BAL concentrations 24 hours after LPS and capsule treatment.

Example 22. Constitutive Production of Anti-IL-8, IL-10, and IL-1RA from ARPE-19 Cells

The example set forth below characterizes the ability of ARPE-19 cells engineered to constitutively express anti-IL-8, IL-10, and IL-1Ra in the absence of stimulus. Briefly, ARPE-19 cells were stably transfected with a plasmid encoding for one of anti-IL-8, IL-10, or IL-1Ra expressed under the CAG promoter using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions and integrated into the cells using a piggybac transposase system. After selection, the engineered cells were plated, allowed to adhere, and incubated with fresh media for 24 h. Constitutive anti-IL-8, IL-10, and IL-1Ra expression was determined to be ~900 ng/mL, ~7,000 pg/mL, and ~27,000 pg/mL, respectively as shown in **FIGS. 29A-C**.

Example 23. Constitutive Production of Anti-IL-6 from ARPE-19 Cells

The example set forth below characterizes the ability of ARPE-19 cells engineered to constitutively express anti-IL-6 in the absence of stimulus. Briefly, ARPE-19 cells were stably transfected with a plasmid encoding for anti-IL-6 under the control of a CAG promoter using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions. and integrated into the cells using a piggybac transposase system. After selection, the engineered cells were plated, allowed to adhere, and incubated with fresh media for 24 h. After selection, media was harvested and constitutive anti-IL-6 expression was determined to be ~500 ng/mL, as shown in the **FIG. 30**.

Example 24. Anti-IL-8, IL-10, and IL-1RA Production in Response to Pro-Inflammatory Cytokines

The example set forth below characterizes therapeutic cell lines engineered to express anti-IL-8, IL-10, or IL-1Ra in response to pro-inflammatory cytokines such as IL-1β and TNF-α under the control of NF-κB promoter expression systems. Briefly, ARPE-19 cells were stably transfected with a plasmid encoding for a NF-κB-responsive promoter and one of anti-IL-8, IL-10 or IL-1Ra using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions and integrated into the cells using a piggybac transposase system. The engineered cells were then plated, allowed to adhere and incubated with fresh media treated with the inflammatory cytokines IL-1β or TNF-α. Production of each of anti-IL-8, IL-10 and IL-1Ra was measured by a protein-specific ELISA. Although there is some background constitutive expression of anti-IL-8, IL-10 and IL-1Ra in the absence of any inflammatory cytokines, anti-IL-8, IL-10, and IL-1Ra production all increase significantly in the presence of IL-1β or TNFα as demonstrated in **FIGS. 31A-C**. As shown in **FIG. 31A**, anti-IL8 production is negligible in the Control sample, whereas the IL-1β and TNF-α-treated samples had anti-IL-8 productions of ~50 ng/mL. As shown in **FIG. 31B**, IL-10 production is minimal in the Control sample, whereas the IL-1β and TNF-α-treated samples had IL-10 productions of ~5000 pg/mL. As shown in **FIG. 31C**, IL-1Ra production is nearly zero in the Control sample, whereas the IL-1β and TNF-α-treated samples had anti-IL-8 productions of ~500 and ~1700 pg/mL, respectively.

Example 25. Characterization of Luciferase Production in Response to Pro-Inflammatory Cell Lines in a NF-κB-Luciferase Cell Line

The example set forth below characterized luciferase production in a NF-κB-luciferase therapeutic cell line, which expresses luciferase in response to pro-inflammatory stimuli. Briefly, ARPE-19 cells were stably transfected with a plasmid encoding for a NF-κB-responsive promoter and luciferase using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions. Luciferase production was measured in engineered cells incubated in media containing no treatment, 10 ng/mL IFNγ, 15 ng/mL IL-1β, and 15 ng/mL TNFα, as shown in **FIG. 32**. Relative fluorescence was negligible in the control and 10 ng/mL IFNγ-treated samples; however, the 15 ng/mL IL-1β-treated sample had ~30,000 RLU and the 15 ng/mL TNF-α-treated sample had ~40,000 RLU.

Example 26. Production of Anti-inflammatory Cytokines from Engineered Cells is Dose Responsive.

The example set forth below demonstrates that the production of IL-1Ra and IL-10 from engineered ARPE-19 cells is dose-responsive. Briefly, ARPE-19 cells were stably transfected with a plasmid encoding for a NF-κB-responsive promoter and one of IL-1Ra or IL-10 using Lipofectamine™ 3000 Transfection Reagent (ThermoFisher Scientific) following manufacturer's instructions. After selection, cells were plated and adhered and incubated for 16 h (IL-1Ra) or 24 h (IL-10). In FIG. 8A, IL-1Ra production follows a sigmoidal dose-response relationship relative to both IL-1β and TNFα. IL-1Ra production plateaus at ~8000 pg/mL for TNF-α between 0.1 and 100 ng/mL and plateaus between 1000 and 2000 pg/mL for IL-1β for concentrations greater than ~1 ng/mL. As shown in **FIG. 34B**, IL-10 production increases relative to both IL-1β and TNF-α does, reaching a plateau near ~5000-12000 pg/mL for 15 pg/mL and 15 ng/mL IL-1β, respectively. Mouse IL-10 production was measured in engineered cells incubated in media containing no treatment, 15 ng/mL IL-1β, and 15 pg/mL IL-1β (as shown in **FIG. 34B**). mIL-10 production follows IL-1β concentration, with higher levels of pro-inflammatory cytokine resulting in higher mIL-10 expression.

Example 27. ARPE-19 Maintain Viability and Productivity Upon Encapsulation

The example set forth below demonstrates that engineered ARPE-19 cells remain highly viable when encapsulated in alginate capsules. Specifically, cells engineered to express IL-10 in response to inflammation maintain function while encapsulated and produce IL-10 in response to IL-1β and TNF-α. Briefly, ARPE-19 cells were engineered to produce IL-10 under the control of an NF-κB-responsive promoter and encapsulated in alginate capsules as previously described. The encapsulated cells were then live/dead stained using Calcein AM and Ethidium Homodimer and imaged (**FIGS. 36A-C**). **FIG. 36A** is a micrograph of capsules containing a number of capsules containing engineered ARPE-19 cells. **FIG. 36B** demonstrates that capsules may be loaded with a number of capsules containing engineered ARPE-19 cells that remain viable. **FIG. 36C** shows that there is a negligible number of dead cells as evidenced by a lack of any significant staining. IL-10 production was evaluated by assaying capsules treated with media containing IL-1β or TNF-α for 16 h. IL-10 production was then determined by protein-specific ELISA as previously described as shown in **FIG. 36D**. IL-10 production in the absence of inflammatory marker was ~1000 pg/mL, whereas in the presence of IL-1β and TNF-α it is ~8000 pg/mL.

Example 28. Cytokine Production is Localized *In Vivo*

The example set forth below demonstrates that encapsulated cells continue to produce therapeutic agents after implantation *in vivo*. Briefly, ARPE-19 cells engineered to constitutively express anti-IL-8 or IL-1Ra were encapsulated into alginate hydrogel capsules and subsequently implanted in the peritoneal cavity of healthy mice. After 24 h blood and intraperitoneal fluid (IP fluid) were collected and anti-IL-8 and IL-1Ra concentrations were measured by protein-specific ELISAs as previously described. As shown in **FIGS. 37A-B**, both anti-IL-8 and IL-1RA are detected in collected, engineered ARPE-19 samples; however, the protein are localized to the IP fluid a the implantation site. Anti-IL-8 is more than 2000 ng/mL in IP fluid (**FIG. 37A**), whereas it is less than 500 ng/mL in plasma. IL-1Ra is nearly 20,000 pg/mL in IP fluid (**FIG. 37B**), whereas it is nearly undetectable in plasma.

CLAIMS

1. A method of treating a disease or disorder in the lung or deriving from the lung, the method comprising:

providing a capsule comprising a plurality of encapsulated engineered cells;

wherein one or more of the encapsulated engineered cells in the plurality produces a therapeutic agent, e.g., a protein, e.g., a cytokine; and

administering the capsule to the subject,

thereby treating the disease or disorder in the lung or deriving from the lung in the subject.

2. The method of claim 1, wherein therapeutic agent is a cytokine or an antibody.

3. The method of claim 2, wherein the cytokine is an anti-inflammatory cytokine or a pro-inflammatory cytokine.

4. The method of claim 2, wherein the cytokine comprises interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), or tumor necrosis factor alpha (TNF-α).

5. The method of claim1, wherein at least 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, or more of the engineered cells in the plurality produce the therapeutic agent, e.g., the cytokine.

6. The method of claim 1, wherein the capsule is degradable.

7. The method of claim 1, wherein the capsule comprises a polymer.

8. The method of claims 7, wherein the polymer is a naturally occurring polymer or a synthetic polymer.

9. The method of claim 7, wherein the polymer is a polysaccharide (e.g., alginate).

10. The method of claim 1, wherein the engineered cell is an epithelial cell.

11. The method of claim 1, wherein the engineered cell is selected from Chinese hamster ovary (CHO) cell, retinal pigment epithelial (ARPE-19) cell, human mammary epithelial (MCF-10a and MCF-7) cell, human embryonic kidney (HEK) cell, a mesenchymal stem cell (MSC), human umbilical vein endothelial cell (HUVEC), NIH/3T3 cell, BJ fibroblast, and human renal mix epithelial cell (HREC).

12. The method of claim 1, wherein the engineered cell is engineered for regulatable expression of said cytokine.

13. The method of claim 1, wherein the therapeutic agent is selected from interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11),

chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23), chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17), and a combination thereof.

14. The method of claim 1, wherein the disease or disorder in the lung or deriving from the lung is a respiratory disease, disorder, or condition.

15. The method of claim 1, wherein the disease or disorder in the lung or deriving from the lung is a respiratory infection, autoimmune disease, or inflammatory disease or condition.

16. The method of claim 14, wherein the respiratory disease, disorder or condition is selected from anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell

histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, and sarcoidosis.

17. The method of claim 14, wherein the respiratory disease, disorder, or condition is selected from anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, and sarcoidosis.

18. The method of claim 14 , wherein the respiratory disease, disorder, or condition is selected from acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD), legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis, and a combination thereof.

19. The method of claim 14, wherein the respiratory disease, disorder or condition is selected from anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic

obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, and sarcoidosis.

20. The method of claim 1, wherein the administering comprises local delivery or delivery via inhalation.

21. The method of claim 20, wherein local delivery comprises delivery via bronchoalveolar lavage (BAL).

22. The method of claim 20, wherein the delivery via inhalation comprises delivery via an inhalation device (e.g., inhaler).

23. The method of claim 1, wherein the subject is a mammal.

24. The method of claim 1, wherein the subject is a human.

25. The method of claim 1, wherein the capsule is formulated as a pharmaceutical composition.

26. The method of claim 25, wherein the pharmaceutical composition further comprises a pH modifier, a tonicity agent, a viscosity modifier, a carrier or diluent (e.g., a pharmaceutically acceptable carrier or diluent), a preservative, a surfactant, or a polymer, or a combination thereof.

27. A method of treating a disease or disorder in the lung or deriving from the lung of claim 14 in a subject, wherein the method comprises:

providing a capsule comprising a plurality of encapsulated engineered cells, wherein plurality of encapsulated engineered cells produces a therapeutic agent, e.g., a protein, e.g., a cytokine to a subject; and

administering the capsule to the subject via local delivery (e.g., delivery about 1 mm, 5 nm, 10 mm, 25 mm, 50 mm, 100 mm, or further from the lung or pleural cavity),

thereby treating said respiratory disease, disorder or condition in the subject.

28. The method of claim 27, wherein the disease or disorder in the lung or deriving from the lung is selected from anti-glomerular basement membrane disease, acute interstitial pneumonitis, asthma, bronchopulmonary dysplasia, bronchiectasis, chemical pneumonitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), conditions with a pleural effusion, cystic fibrosis, hypersensitivity pneumonitis (e.g., due to an environmental allergen, e.g., pollen, mold, dust, dander, and the like), (eosinophilic) granulomatosis with polyangiitis, Langerhans cell histiocytosis, lymphangioleiomyomatosis, infant respiratory distress syndrome (IRDS), pneumoconiosis (e.g., asbestosis), primary ciliary dyskinesia, pulmonary alveolar proteinosis (e.g., of autoimmune etiology), pleural empyema, pleural mesothelioma, pulmonary hamartoma, pulmonary embolism, pulmonary edema, pulmonary hypertension, pulmonary sequestration, radiation pneumonitis or radiation fibrosis, and sarcoidosis.

29. The method of claim 15, wherein the inflammatory disease is selected from endometriosis, arthritis, psoriasis, alopecia, areata, eczema, familial Mediterranean fever, adenomyosis and uterine fibroids, Addison's disease, autoimmune hepatitis, celiac disease, Crohn's disease, type I diabetes, Grave's disease, Hashimoto's thyroiditis, pernicious anemia, multiple sclerosis, primary biliary cholangitis (biliary cirrhosis), sclerosing cholangitis, ulcerative colitis, myasthenia gravis, and Gillian Barre syndrome, and a combination thereof.

30. The method of claim 15, wherein the respiratory infection is selected from acute bronchitis, acute chest syndrome, acute respiratory distress syndrome (ARDS) aspergillosis, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, chronic coughing, coccidioidomycosis, common cold, COVID-19, cryptogenic organizing pneumonia, emphysema, hantavirus pulmonary syndrome, histoplasmosis, human metapneumovirus, influenza, interstitial lung disease (ILD),

legionnaire's disease, *mycobacterium avium* complex disease, middle eastern respiratory syndrome (MERS), nontuberculous mycobacteria lung disease, pertussis, pneumonia, pneumothorax, respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), and tuberculosis and a combination thereof.

31. The method of claim 27, wherein therapeutic agent is a cytokine.

32. The method of claim 31, wherein the cytokine is an anti-inflammatory cytokine or a pro-inflammatory cytokine.

33. The method of claim 27 , wherein the cytokine comprises interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interleukin-22 (IL-22), interleukin-35 (IL-35), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), or tumor necrosis factor alpha (TNF-α).

34. The method of claim 27, wherein at least 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, or more of the engineered cells in the plurality produce the therapeutic agent, e.g., the cytokine.

35. The method of claim 27, wherein the capsule is degradable.

36. The method of claim 27, wherein the capsule comprises a polymer.

37. The method of claim 36, wherein the polymer is a naturally occurring polymer or a synthetic polymer.

38. The method of claim 36, wherein the polymer is a polysaccharide (e.g., alginate).

39. The method of claim 27, wherein the engineered cell is an epithelial cell.

40. The method of claim 27, wherein the engineered cell is selected from Chinese hamster ovary (CHO) cell, retinal pigment epithelial (ARPE-19) cell, human mammary epithelial (MCF-10a and MCF-7) cell, human embryonic kidney (HEK) cell, a mesenchymal stem cell (MSC), human umbilical vein endothelial cell (HUVEC), NIH/3T3 cell, BJ fibroblast, and human renal mix epithelial cell (HREC).

41. The method of claim 27, wherein the engineered cell is engineered for regulatable expression of said cytokine.

42. The method of claim 27, wherein the therapeutic agent is selected from interleukin-1 (IL-1), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist protein (IL-1RA), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11) , interleukin-12 (IL-12), interleukin-12 alpha (IL-12α), interleukin-12 beta (IL-12β), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-20 (IL-20), interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), chemokine (C-C motif) ligand 1 (CCL1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), chemokine (C-C motif) ligand 4 (CCL4), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 6 (CCL6), chemokine (C-C motif) ligand 7 (CCL7), chemokine (C-C motif) ligand 8 (CCL8), chemokine (C-C motif) ligand 9 (CCL9) , chemokine (C-C motif) ligand 10 (CCL10), chemokine (C-C motif) ligand 11 (CCL11), chemokine (C-C motif) ligand 12 (CCL12), chemokine (C-C motif) ligand 13 (CCL13), chemokine (C-C motif) ligand 14 (CCL14), chemokine (C-C motif) ligand 15 (CCL15), chemokine (C-C motif) ligand 16 (CCL16), chemokine (C-C motif) ligand 17 (CCL17), chemokine (C-C motif) ligand 18 (CCL18), chemokine (C-C motif) ligand 19 (CCL19), chemokine (C-C motif) ligand 20 (CCL20), chemokine (C-C motif) ligand 21 (CCL21), chemokine (C-C motif) ligand 22 (CCL22), chemokine (C-C motif) ligand 23 (CCL23),

chemokine (C-C motif) ligand 24 (CCL24), chemokine (C-C motif)ligand 25 (CCL25), chemokine (C-C motif) ligand 26 (CCL26), chemokine (C-C motif) ligand 27 (CCL27), chemokine (C-C motif) ligand 28 (CCL28), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-X-C motif) ligand 3 (CXCL3), chemokine (C-X-C motif) ligand 4 (CXCL4), chemokine (C-X-C motif) ligand 5 (CXCL5), chemokine (C-X-C motif) ligand 6 (CXCL6), chemokine (C-X-C motif) ligand 7 (CXCL7), chemokine (C-X-C motif) ligand 8 (CXCL8), chemokine (C-X-C motif) ligand 9 (CXCL9), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), chemokine (C-X-C motif) ligand 12 (CXCL12), chemokine (C-X-C motif) ligand 13 (CXCL13), chemokine (C-X-C motif) ligand 14 (CXCL14), chemokine (C-X-C motif) ligand 15 (CXCL15), chemokine (C-X-C motif) ligand 16 (CXCL16), and chemokine (C-X-C motif) ligand 17 (CXCL17), and a combination thereof.

43. The method of claim 27, wherein the administering comprises local delivery or delivery via inhalation.

44. The method of claim 27, wherein local delivery comprises delivery via bronchoalveolar lavage (BAL).

45. The method of claim 27, wherein the delivery via inhalation comprises delivery via an inhalation device (e.g., inhaler).

46. The method of claim 27, wherein the capsule is formulated as a pharmaceutical composition.

47. The method of claim 46, wherein the pharmaceutical composition further comprises a pH modifier, a tonicity agent, a viscosity modifier, a carrier or diluent (e.g., a pharmaceutically acceptable carrier or diluent), a preservative, a surfactant, or a polymer, or a combination thereof.

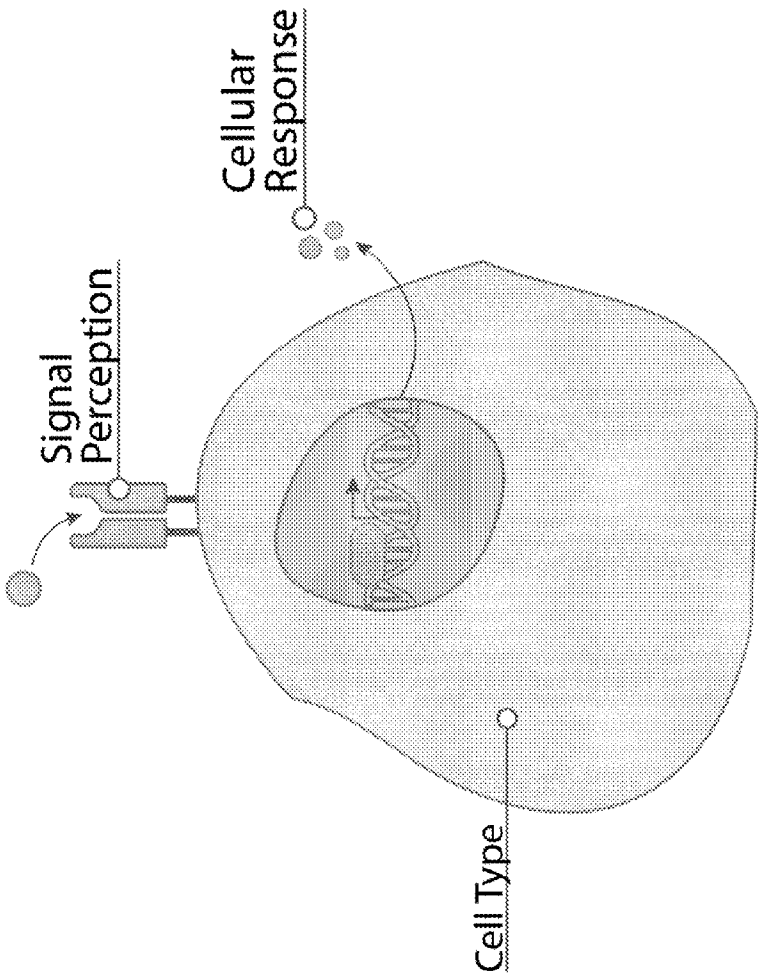

FIG. 1A

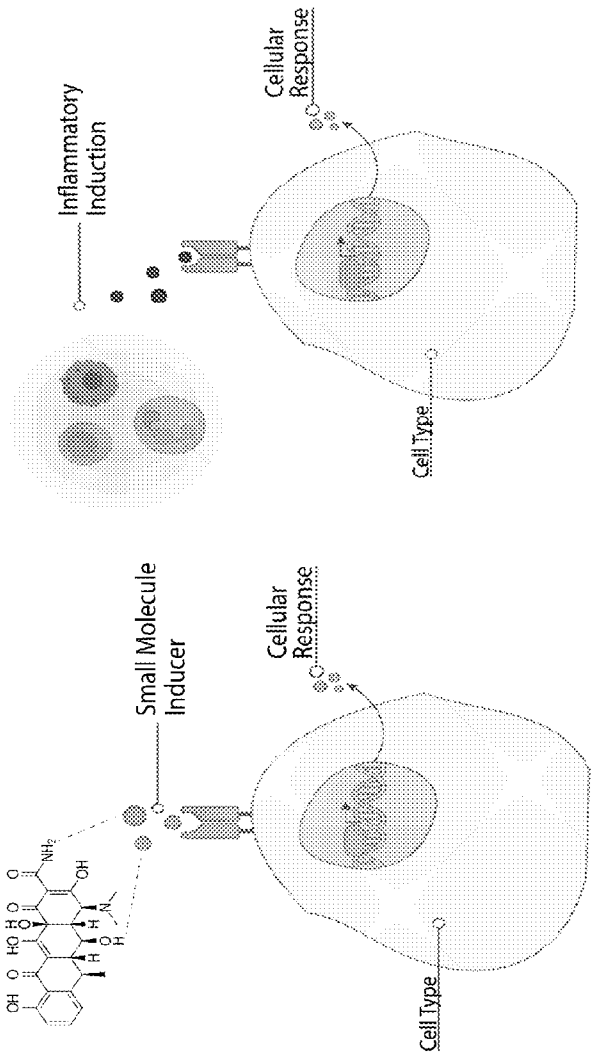
Inflammatory Induction
Cellular Response
Cell Type
Small Molecule Inducer
Cellular Response
Cell Type

FIG. 1B

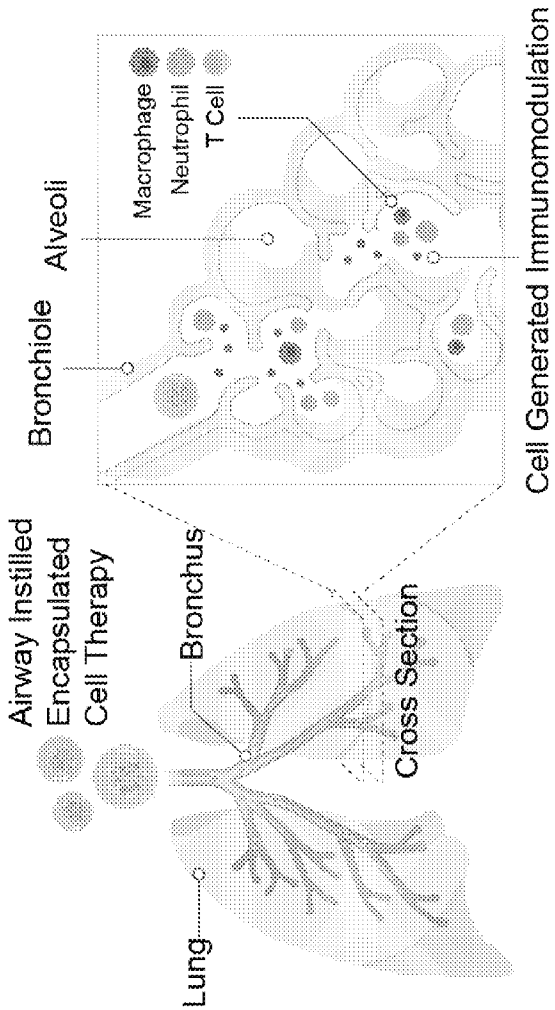
Airway Instilled Encapsulated Cell Therapy
Lung
Bronchus
Cross Section
Bronchiole
Alveoli
Macrophage
Neutrophil
T Cell
Cell Generated Immunomodulation

FIG. 2

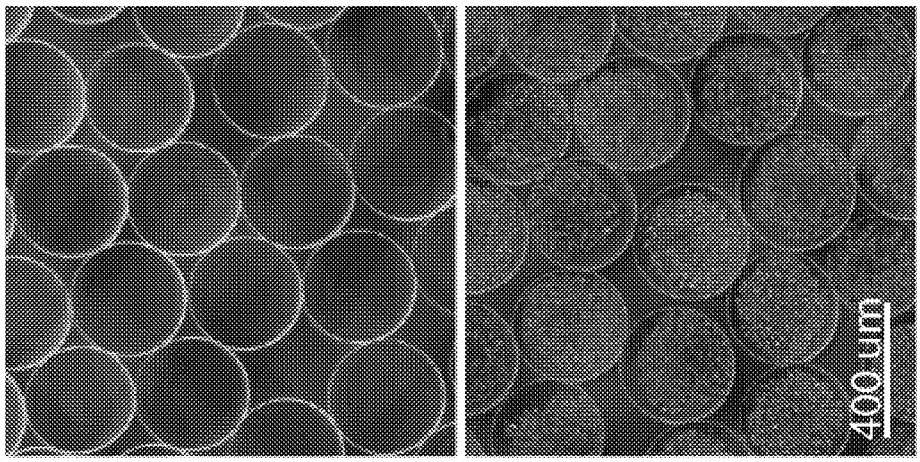

FIG. 3A

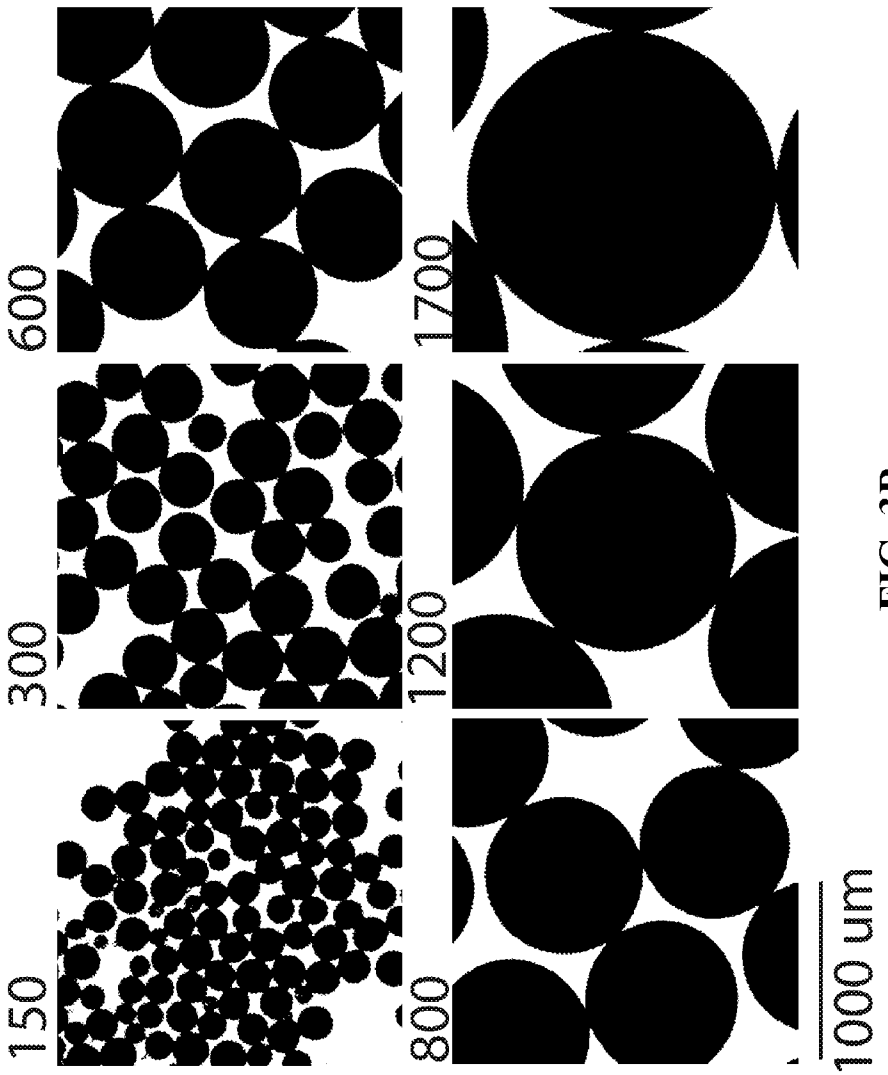

FIG. 3B

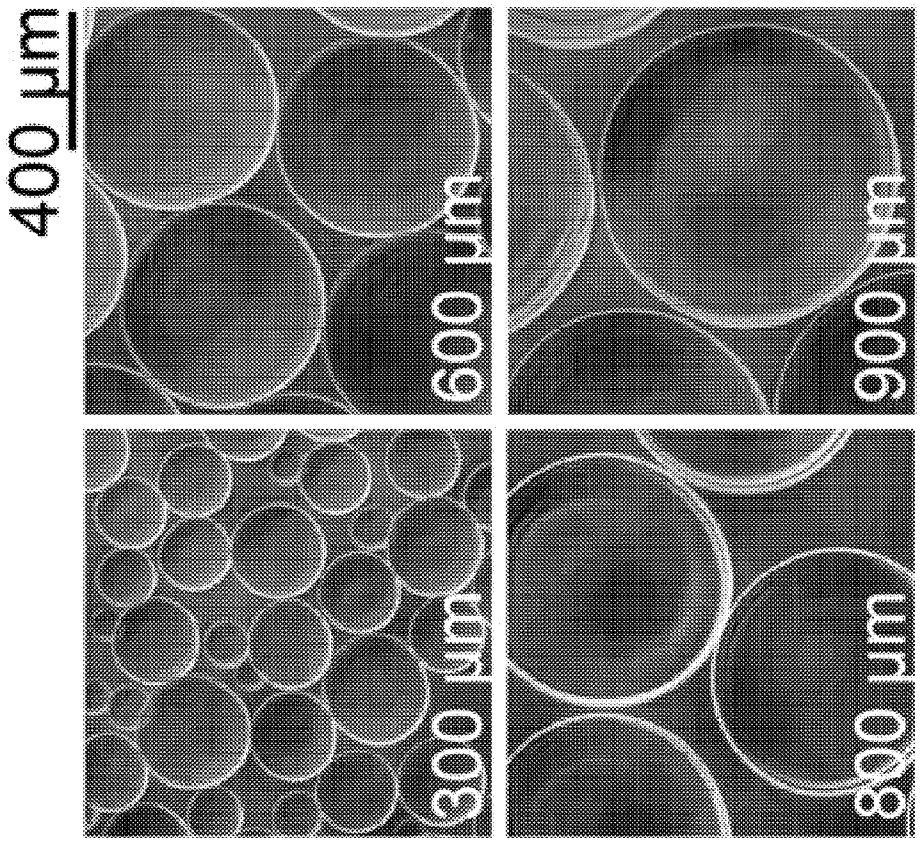

FIG. 3C

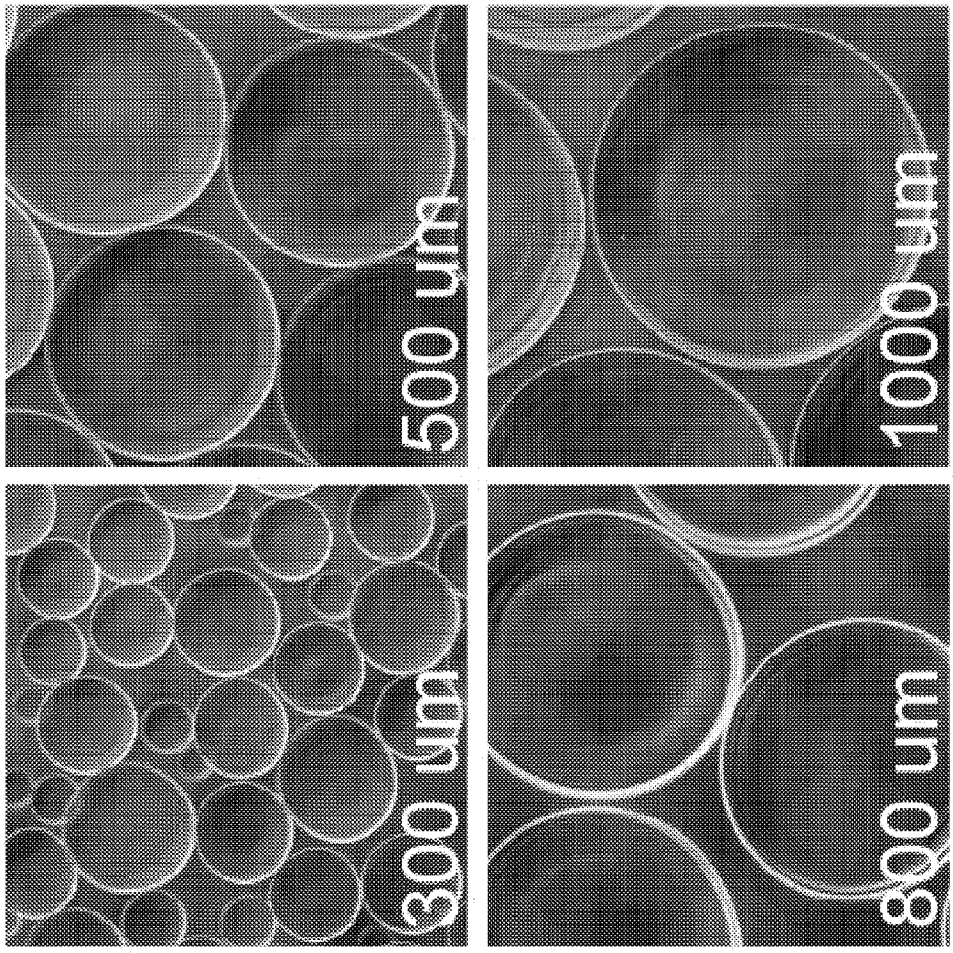

FIG. 3D

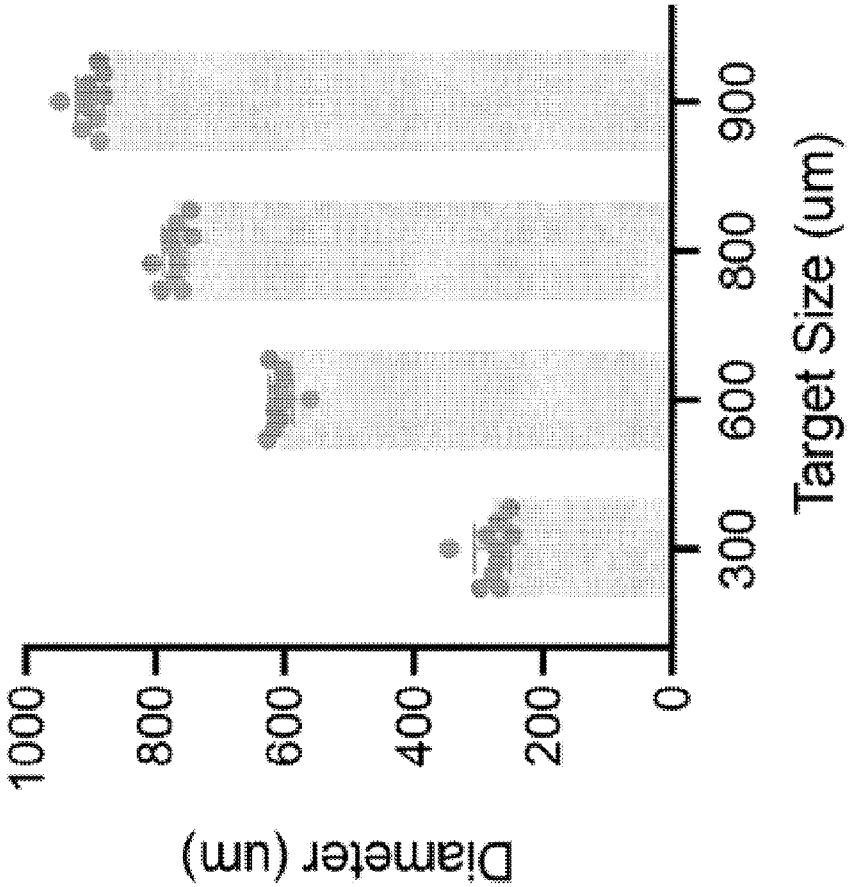
Diameter (um)
0
200
400
600
800
1000
300
600
800
900
Target Size (um)

FIG. 4B

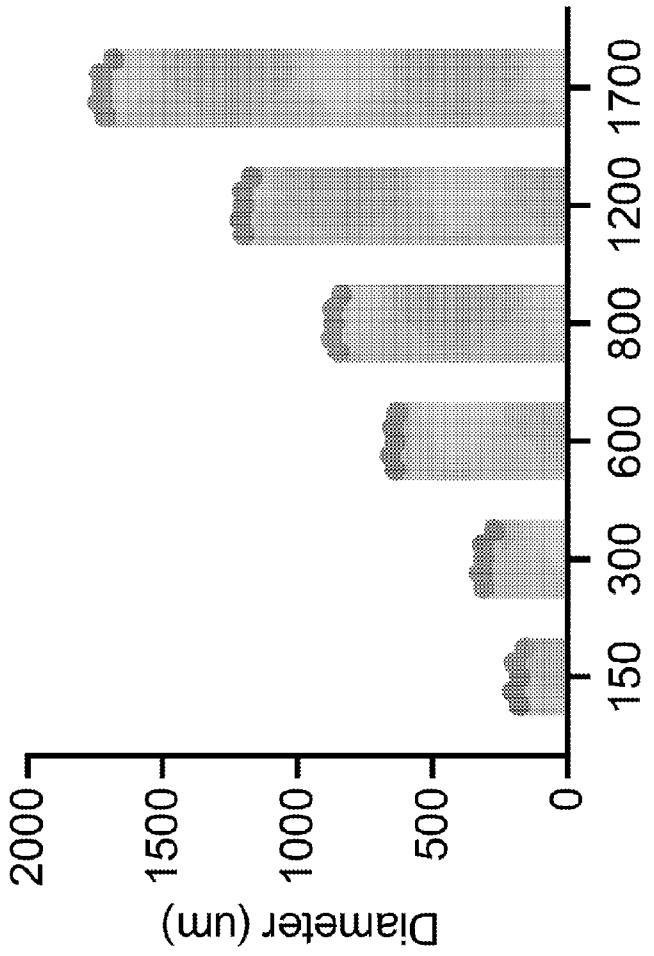
Diameter (um)
0
500
1000
1500
2000
150
300
600
800
1200
1700
Target Size (um)

FIG. 4A

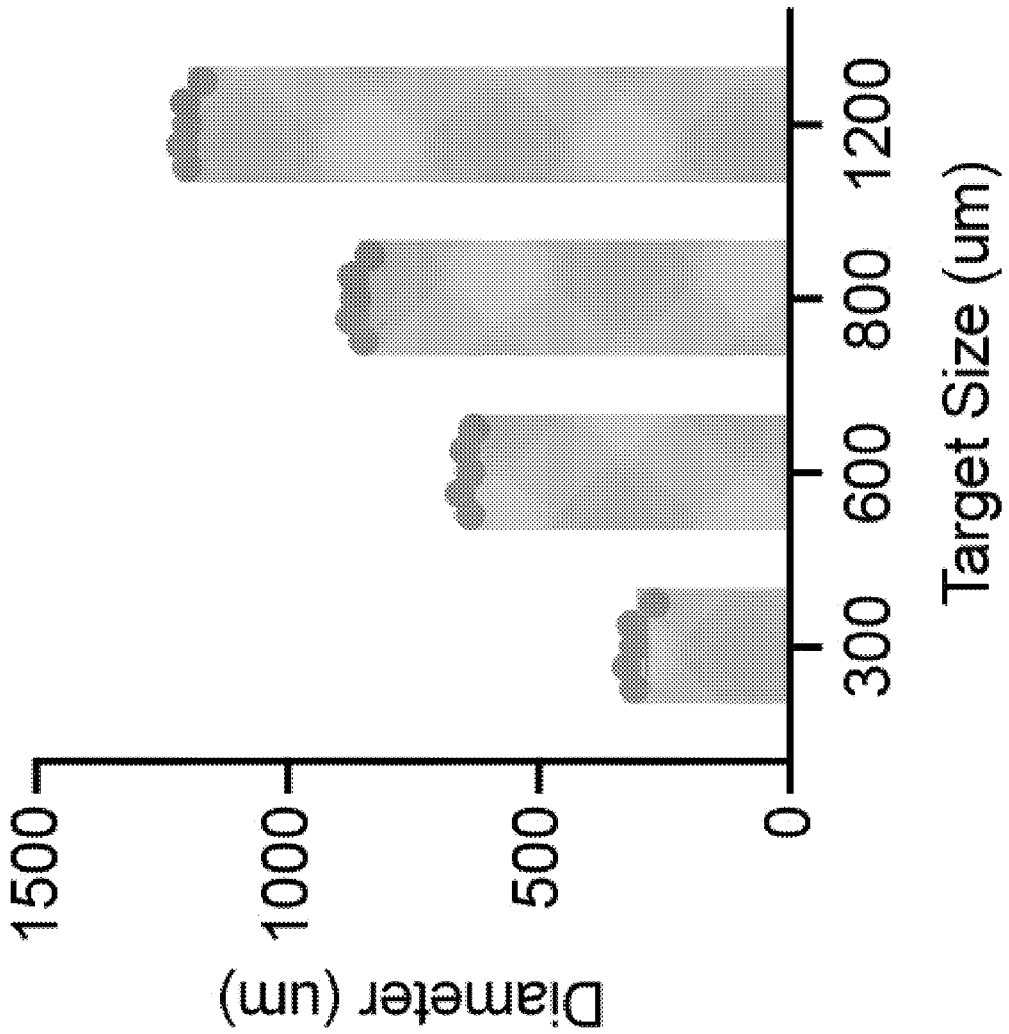

FIG. 4C

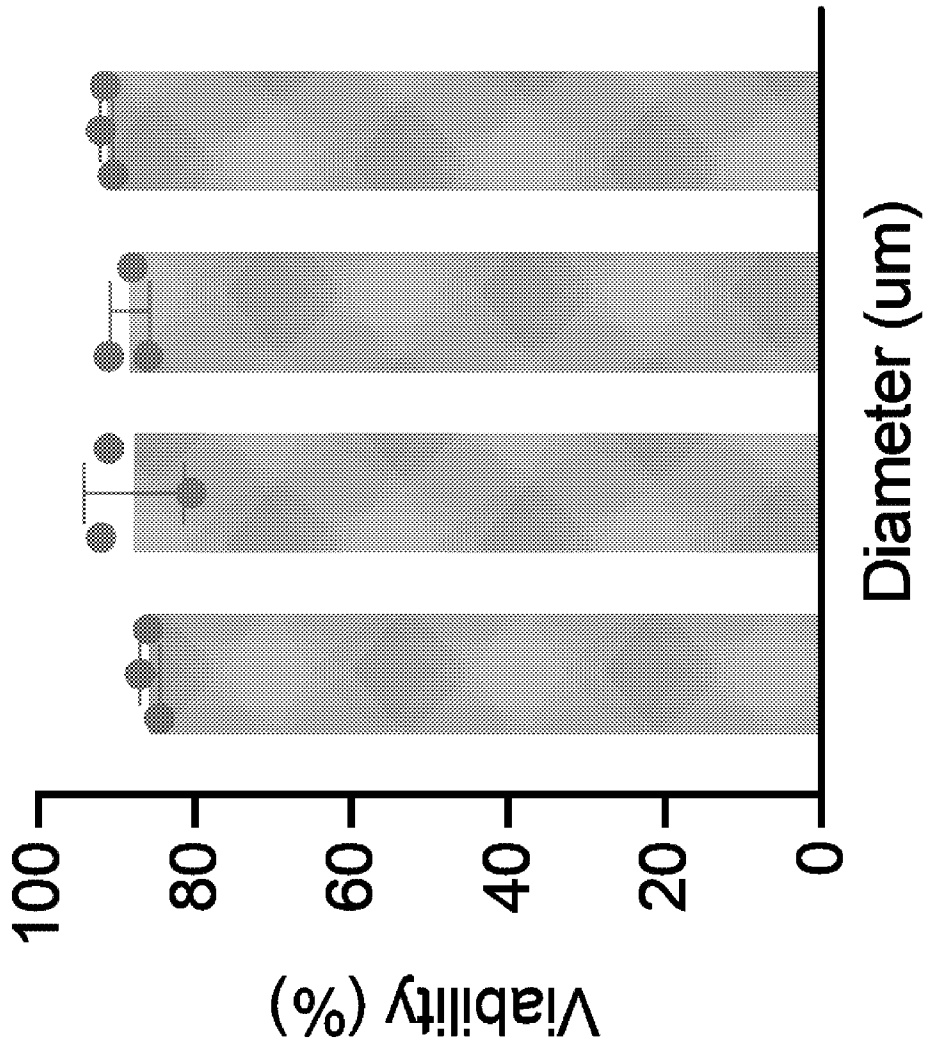

FIG. 5A

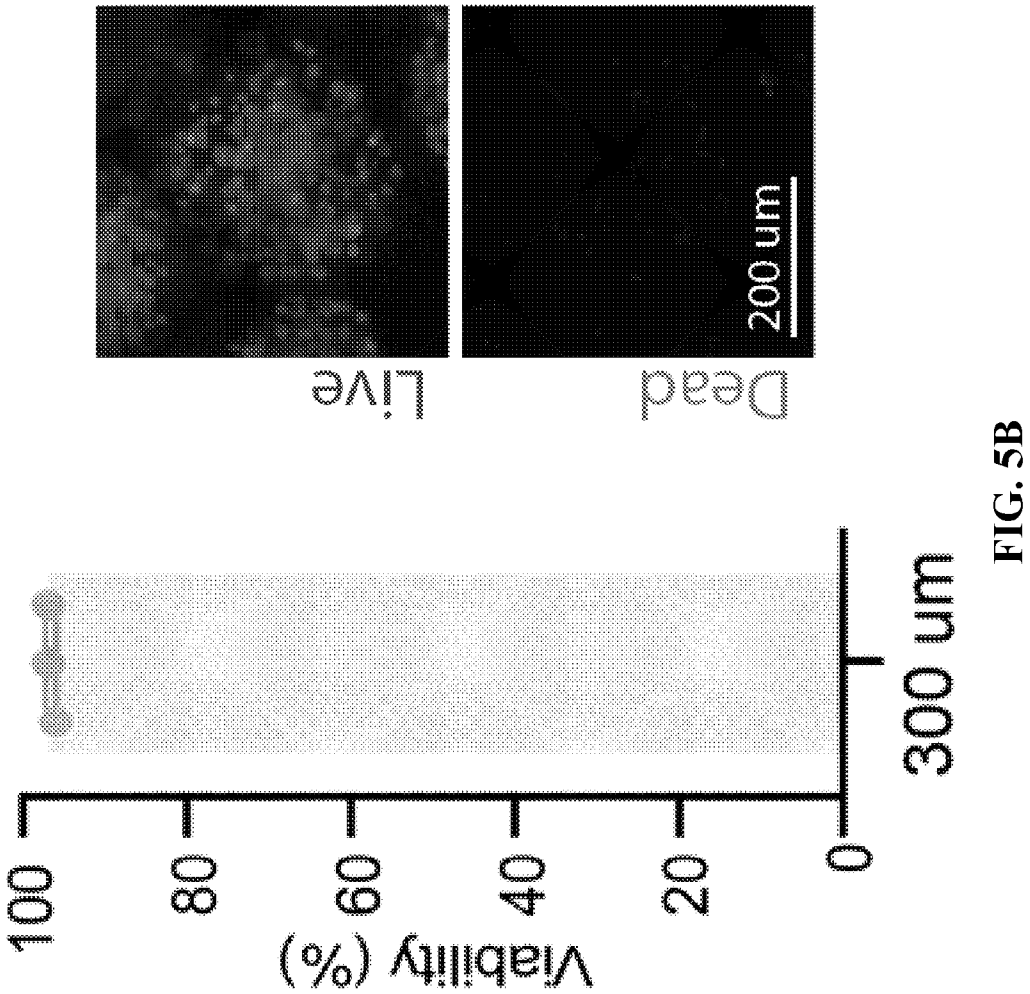

FIG. 5B

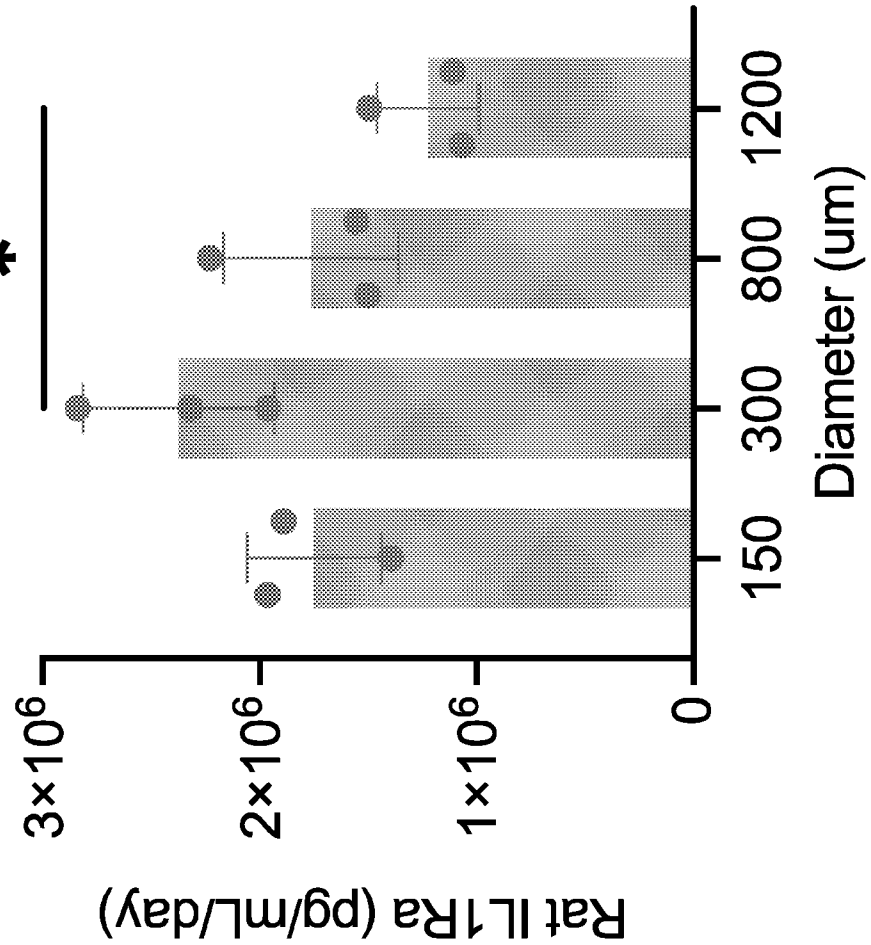
Rat IL1Ra (pg/mL/day)
3×10^6
2×10^6
1×10^6
0
ns
*
150
300
800
1200
Diameter (um)

FIG. 5D

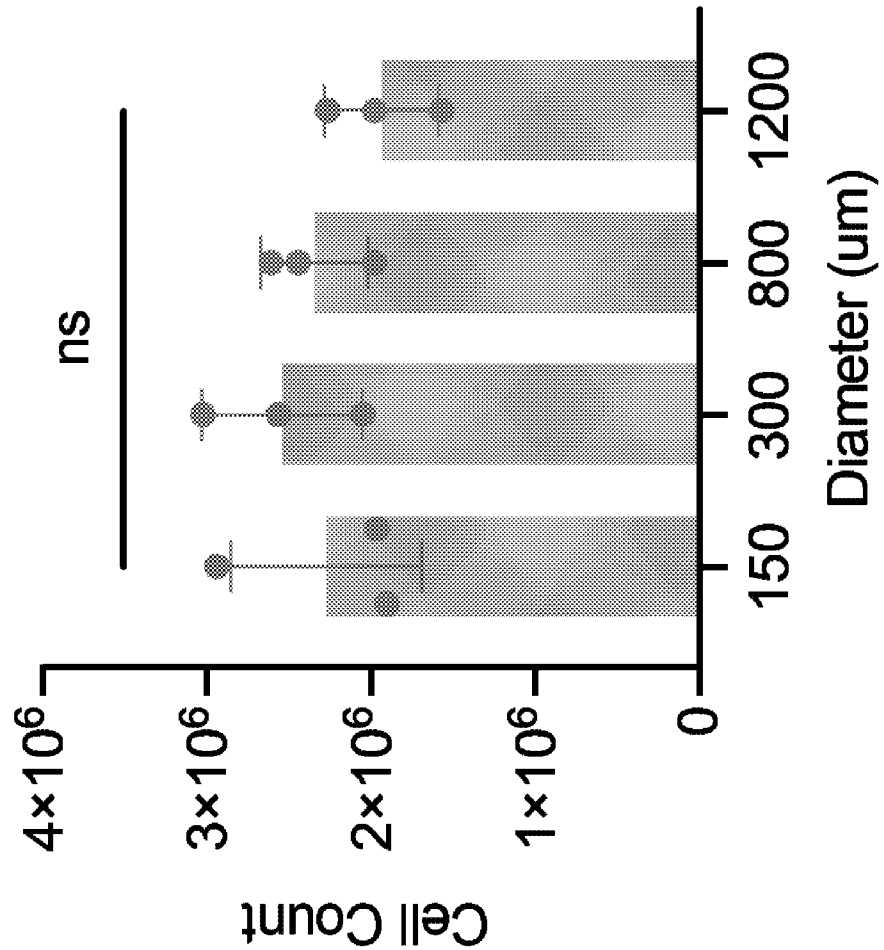
Cell Count
4×10^6
3×10^6
2×10^6
1×10^6
0
ns
150
300
800
1200
Diameter (um)

FIG. 5C

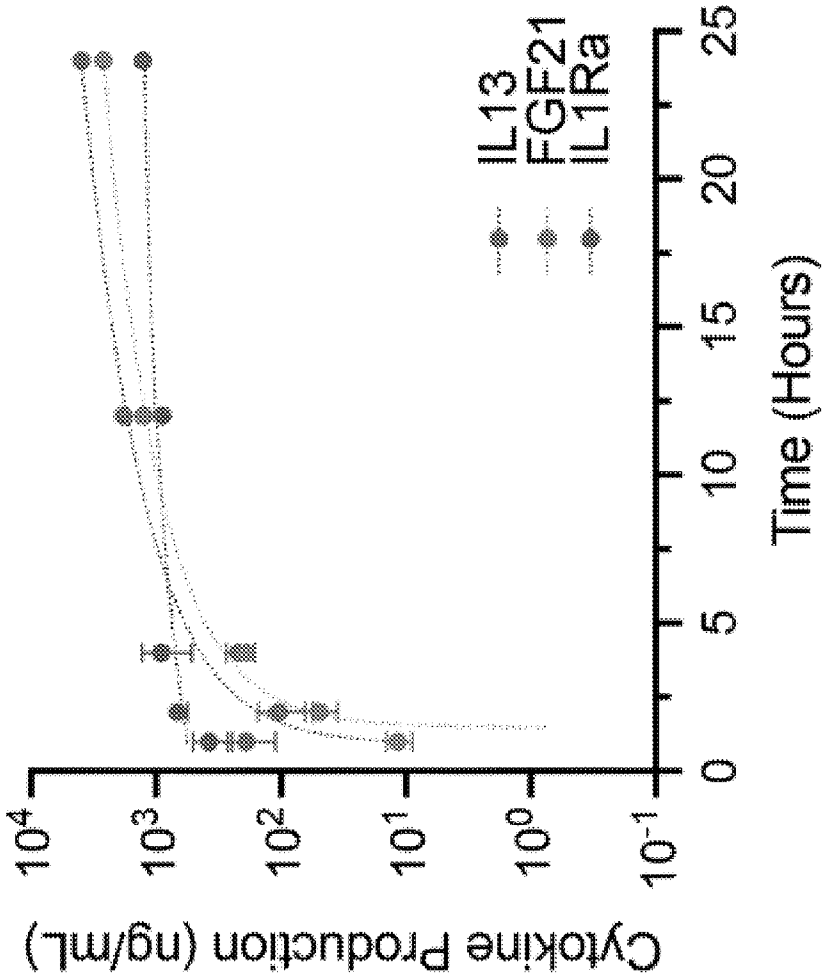
Cytokine Production (ng/mL)
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
0
5
10
15
20
25
Time (Hours)
IL13
FGF21
IL1Ra

FIG. 6

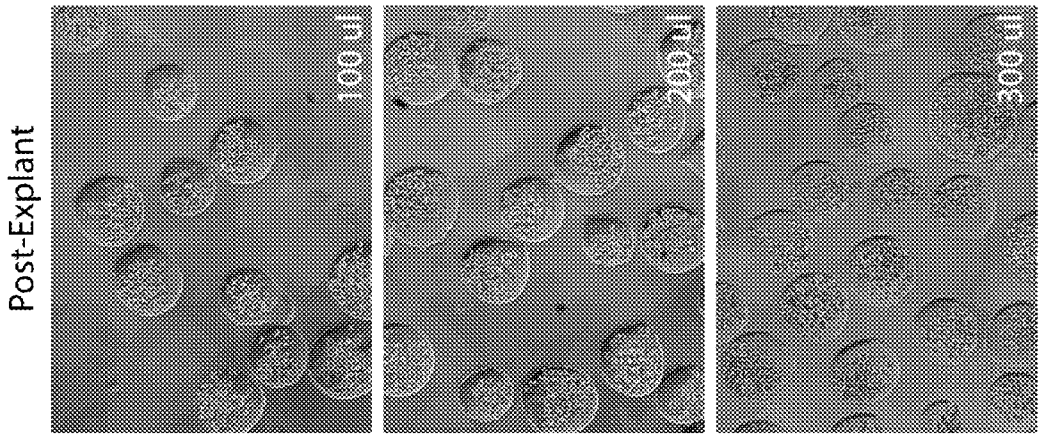

FIG. 7A

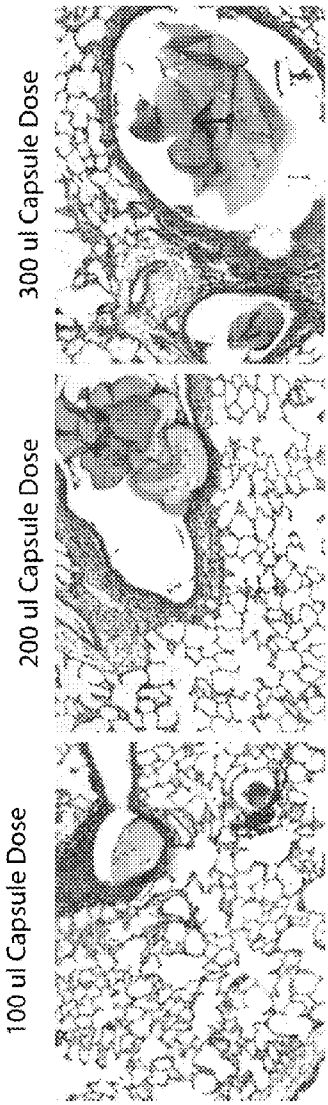

FIG. 7B

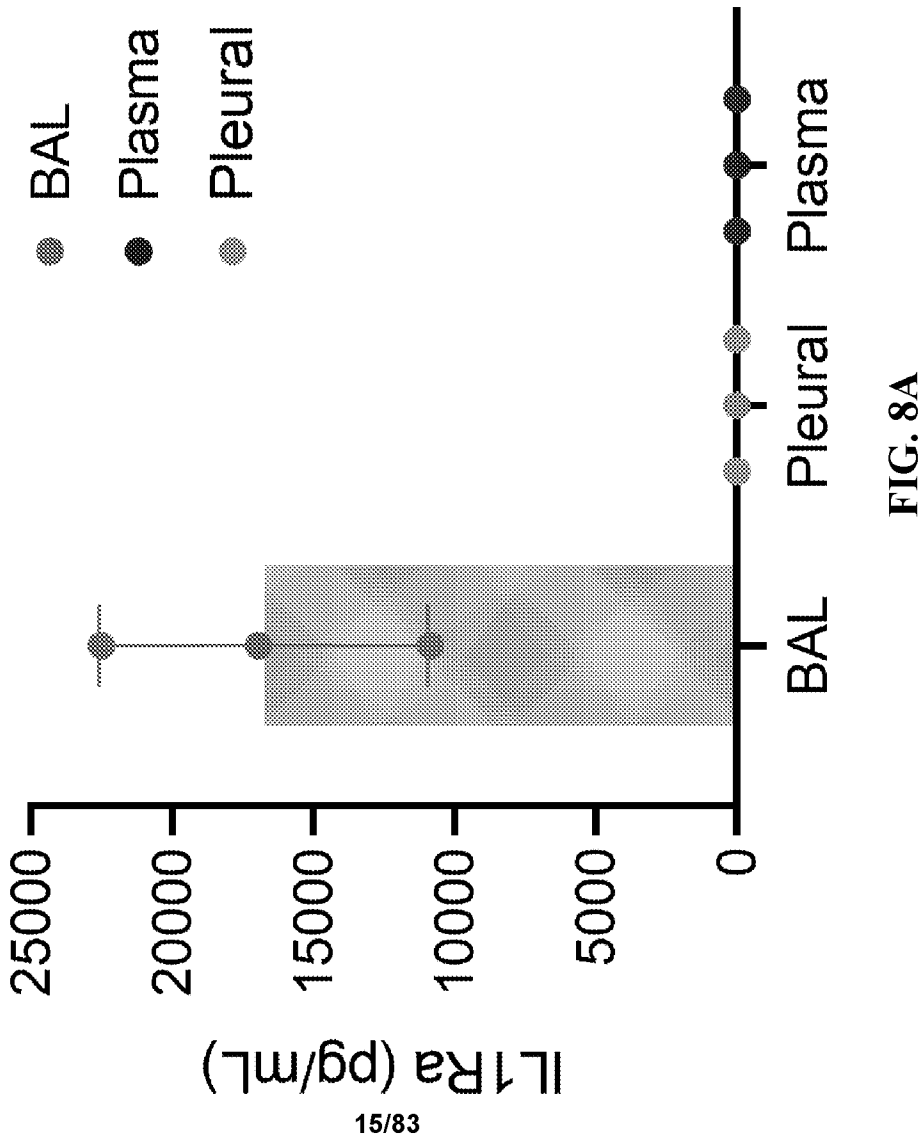
IL1Ra (pg/mL)
25000
20000
15000
10000
5000
0
BAL
Pleural
Plasma
BAL
Plasma
Pleural

FIG. 8A

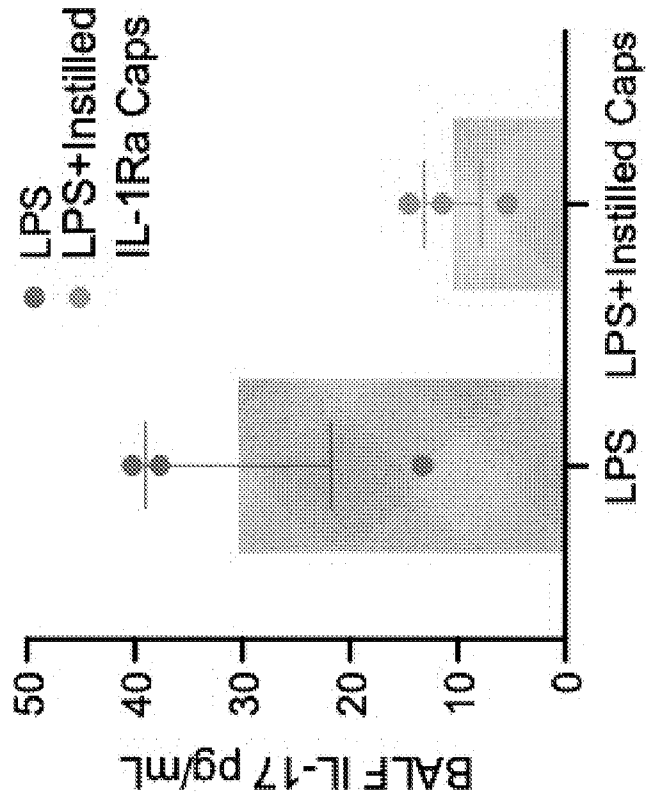
BALF IL-17 pg/mL
50
40
30
20
10
0
LPS
LPS+Instilled Caps
LPS
LPS+Instilled
IL-1Ra Caps

FIG. 8B

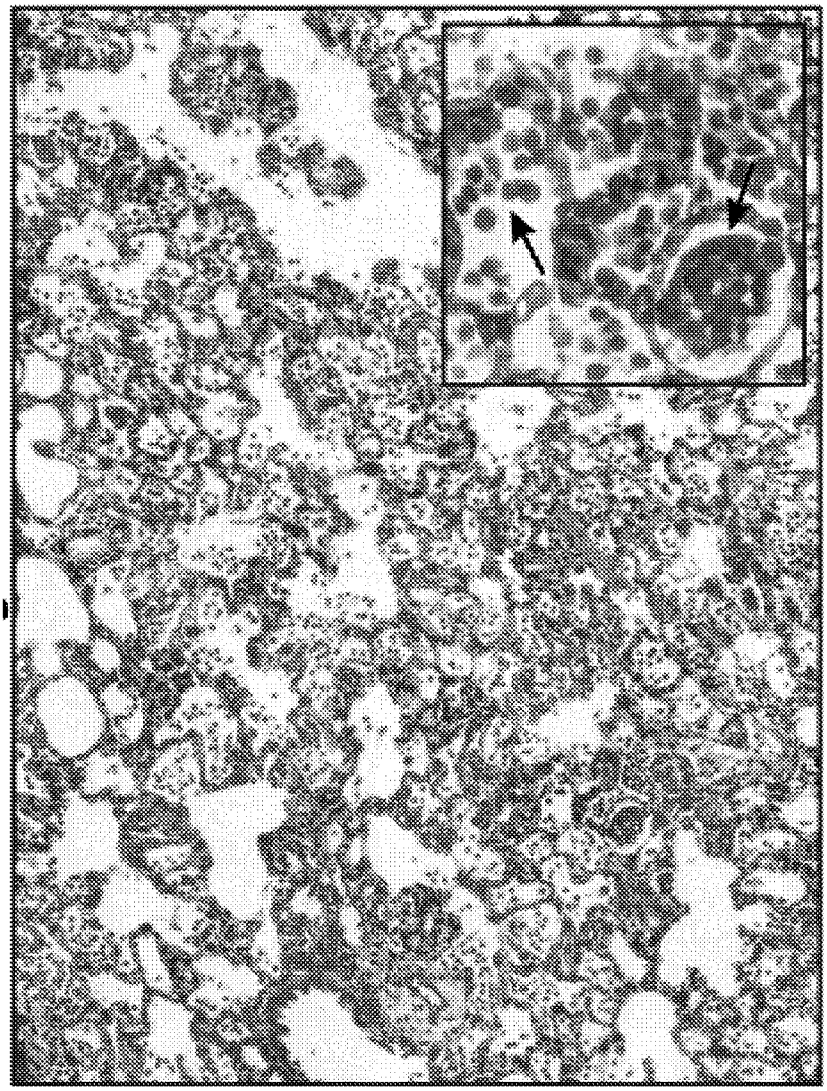

FIG. 9A

LPS + IL1Ra Capsule Instillation

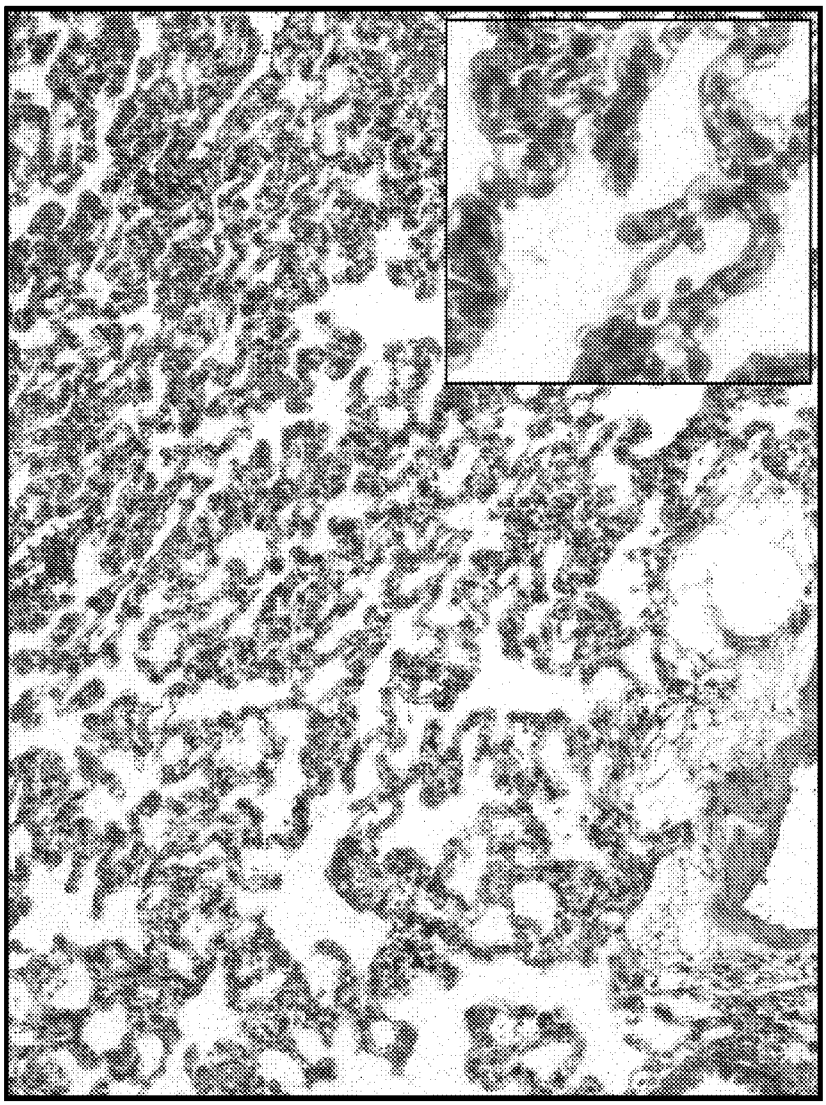

FIG. 9B

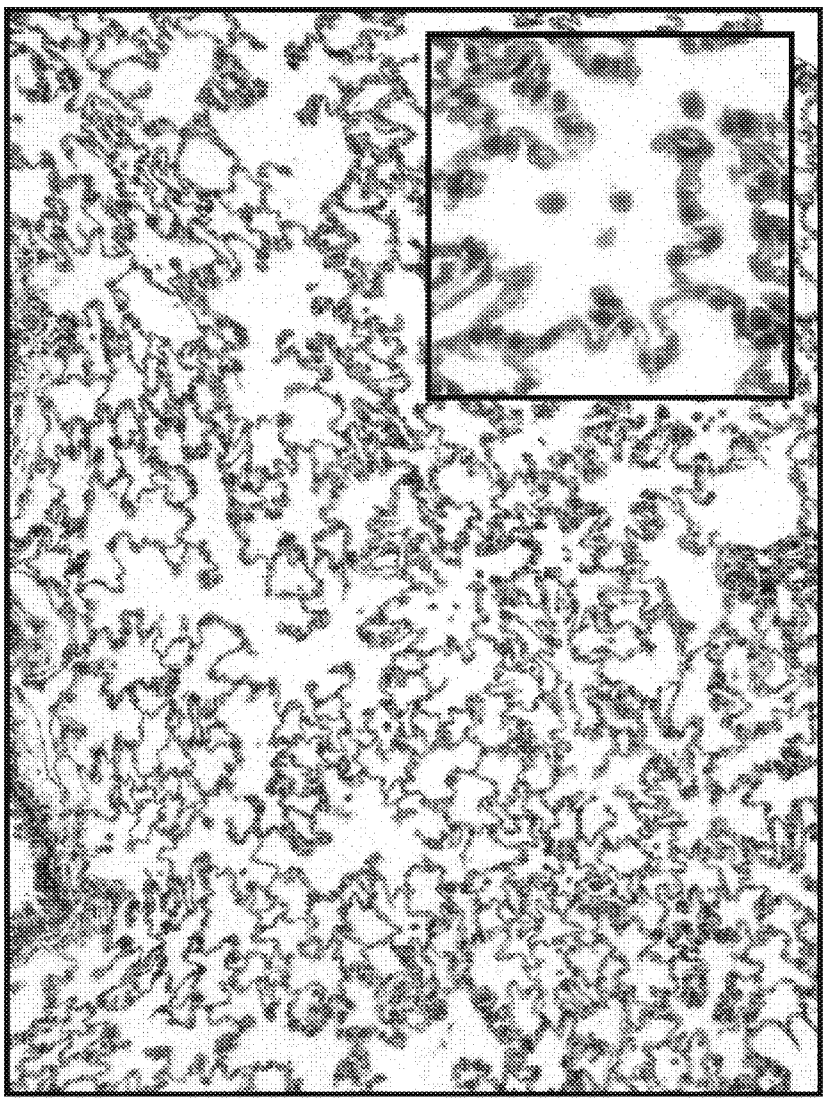

FIG. 9C

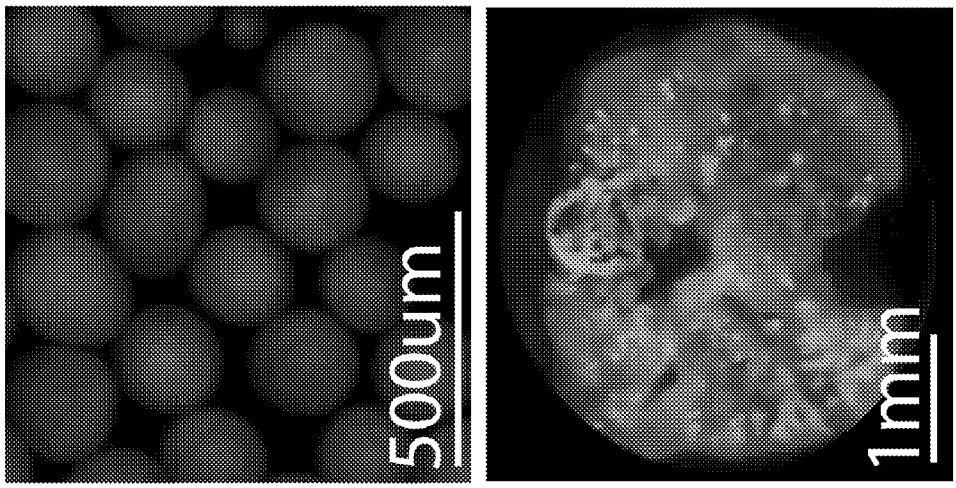

FIG. 10

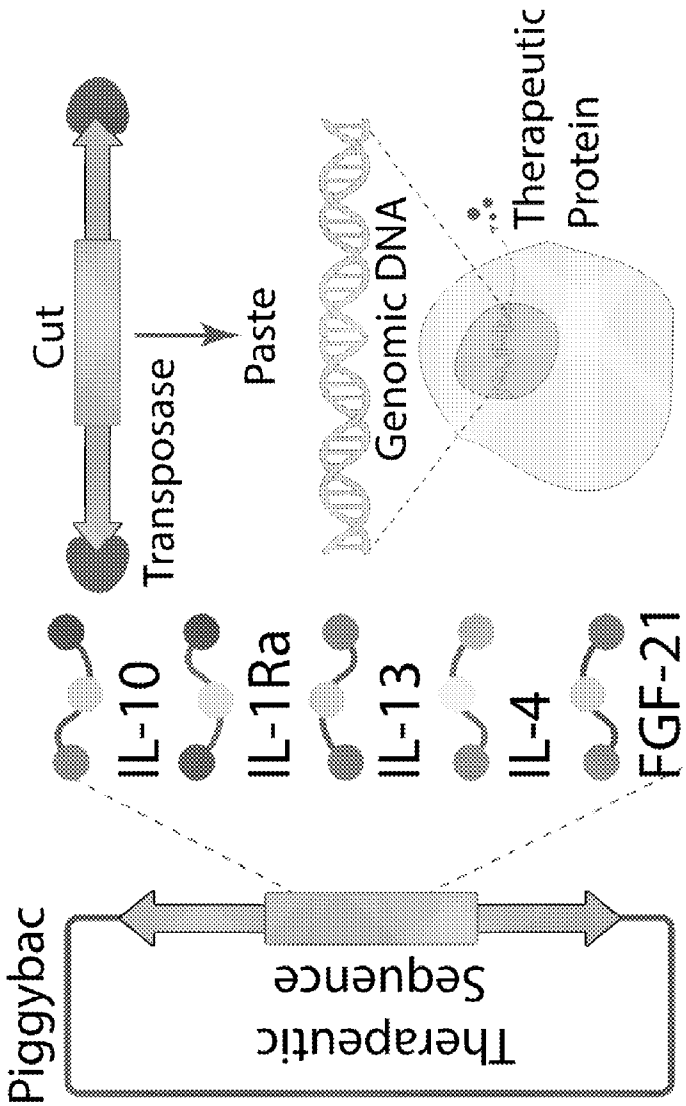
Piggybac
Therapeutic Sequence
IL-10
IL-1Ra
IL-13
IL-4
FGF-21
Cut
Transposase
Paste
Genomic DNA
Therapeutic Protein

FIG. 11

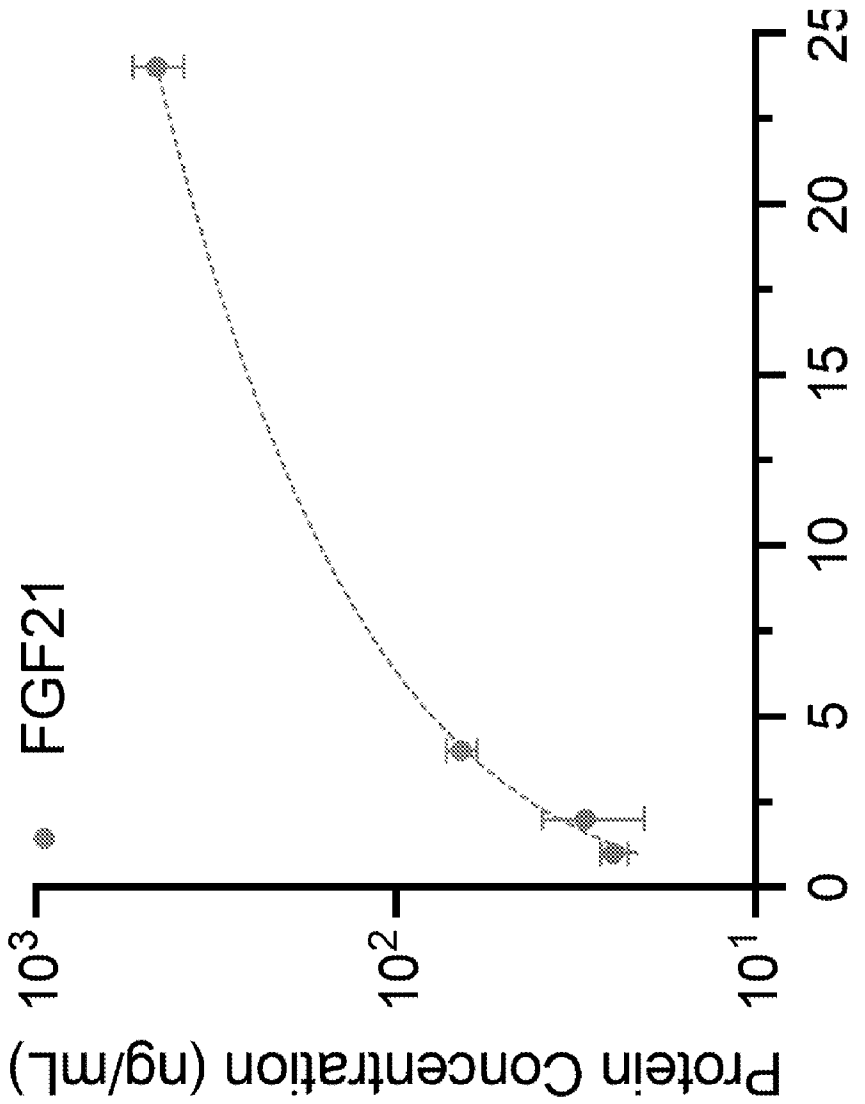
Protein Concentration (ng/mL)
10^3
10^2
10^1
FGF21
0
5
10
15
20
25
Time Hours

FIG. 12

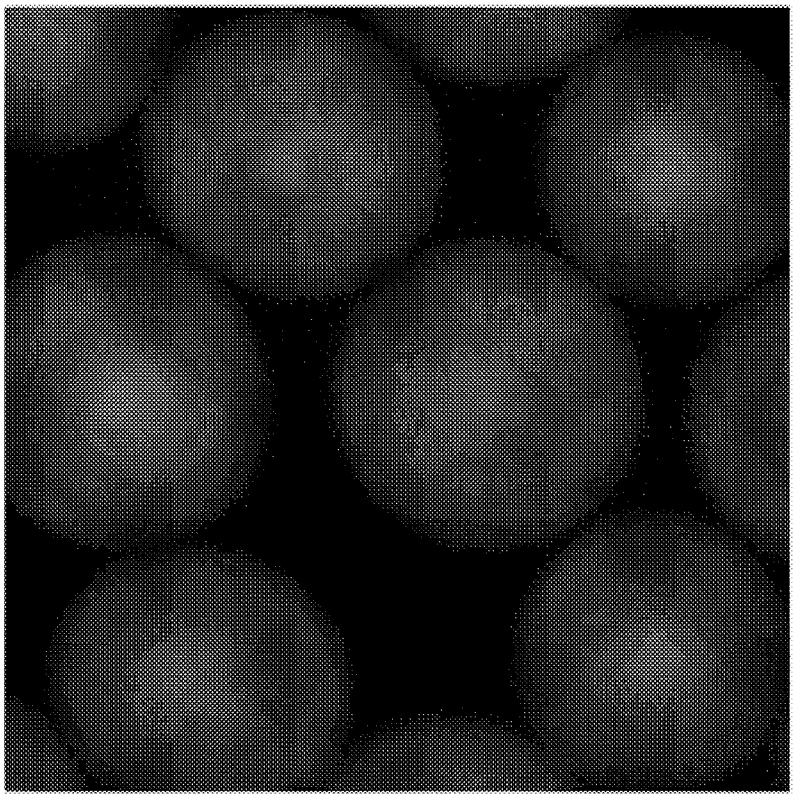

FIG. 13A

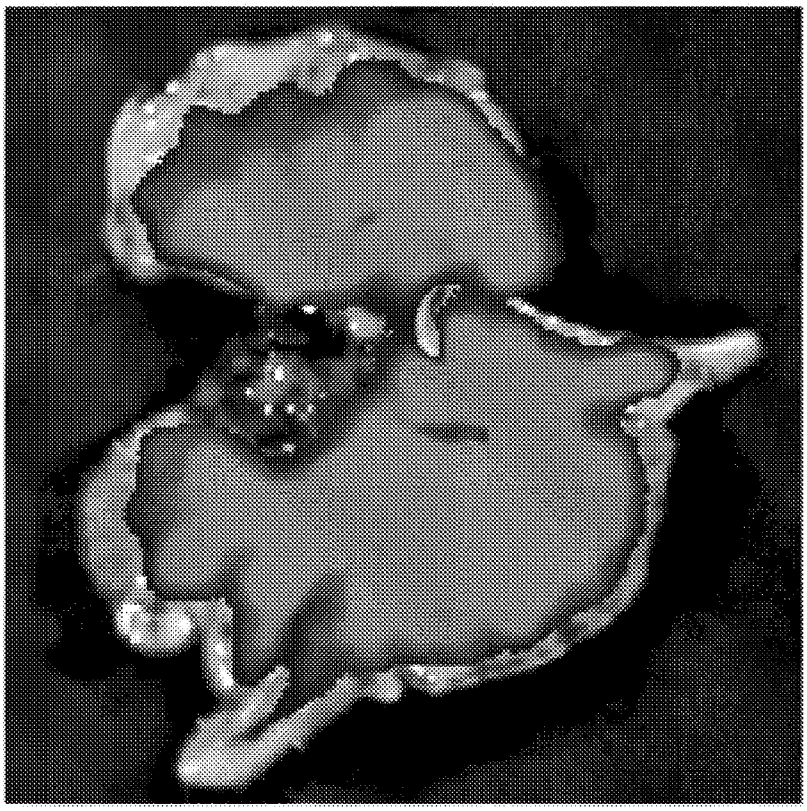

FIG. 13B

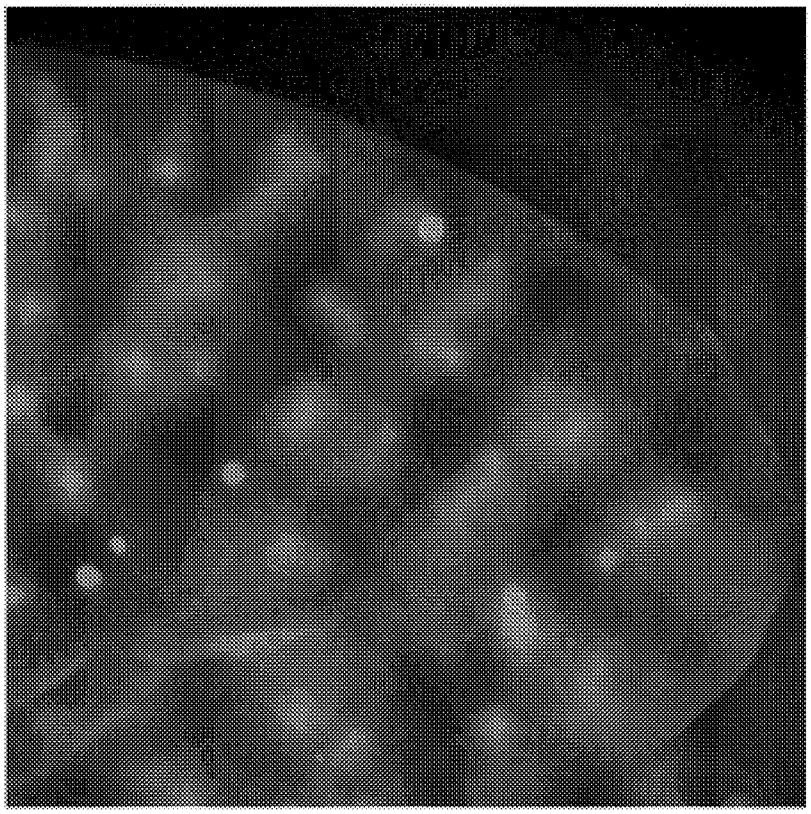

FIG. 13D

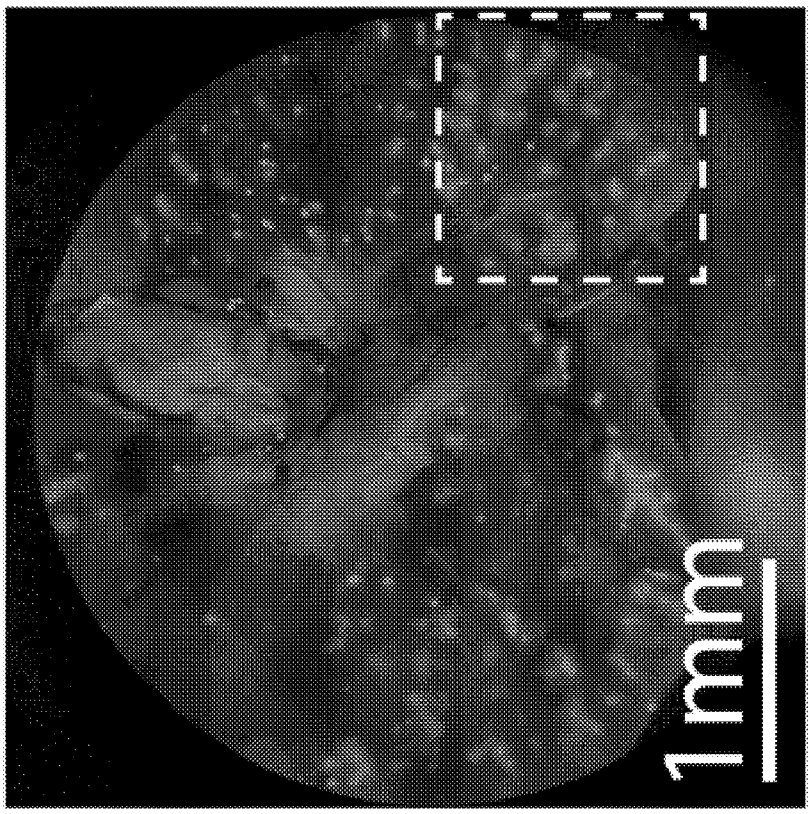

FIG. 13C

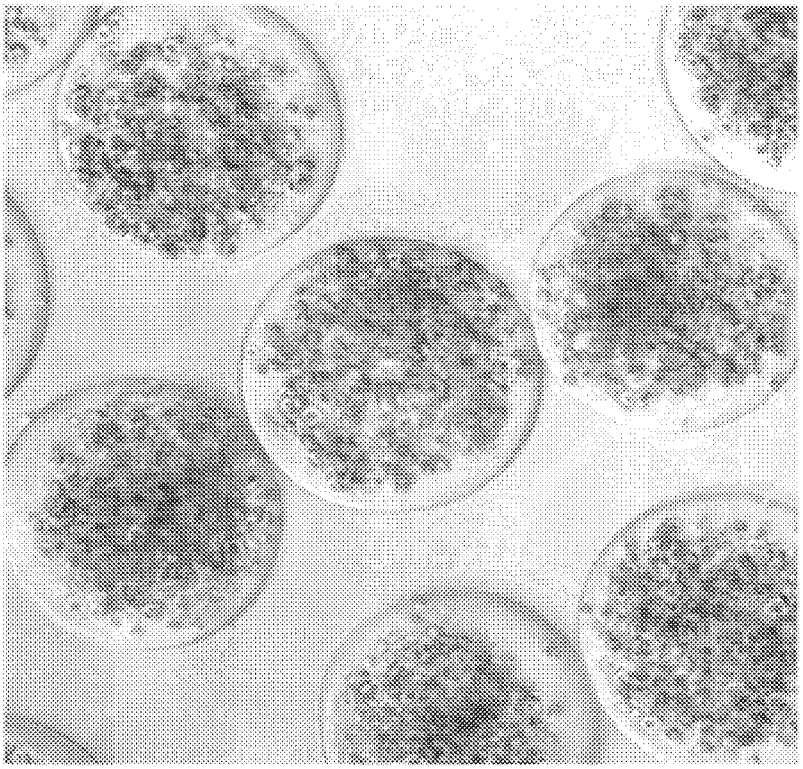

FIG. 13E

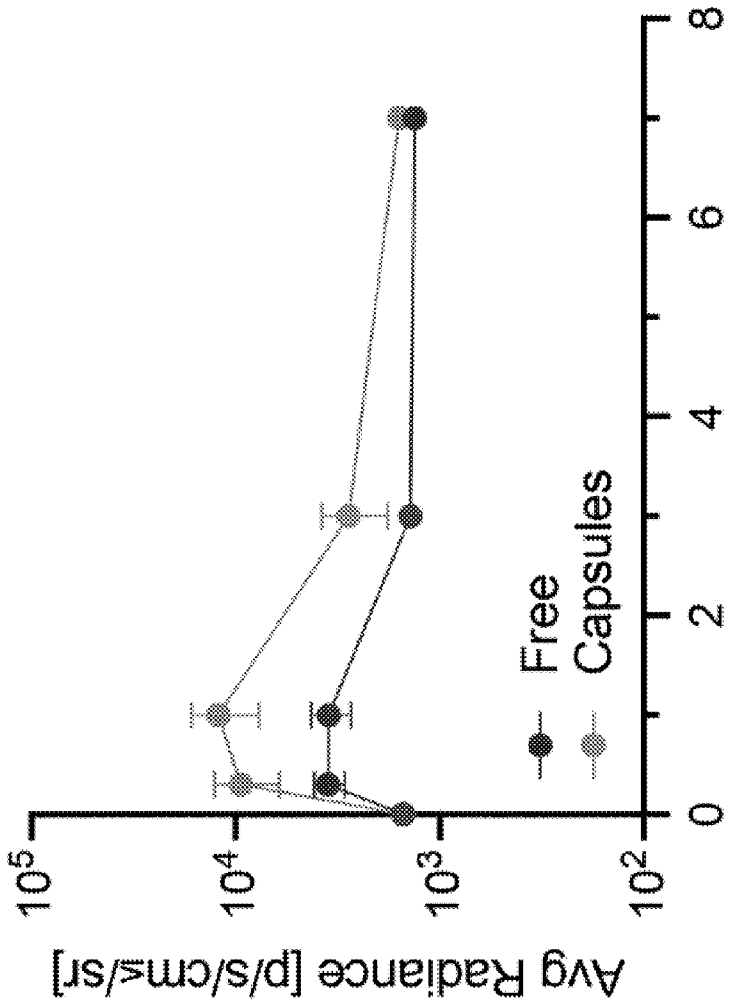
Avg Radiance [p/s/cm²/sr]
10^5
10^4
10^3
10^2
0
2
4
6
8
Time (Days)
Free
Capsules

FIG. 14A

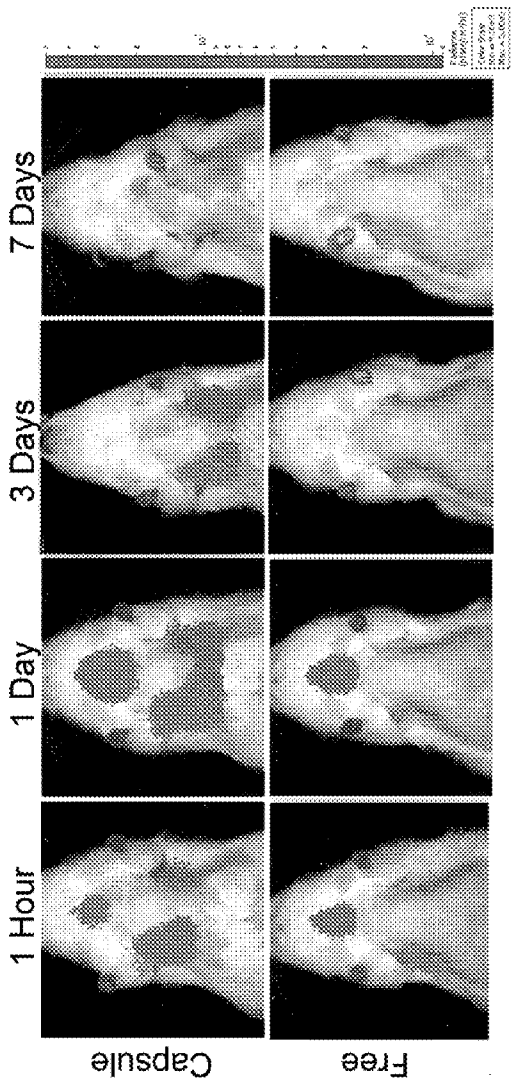

FIG. 14B

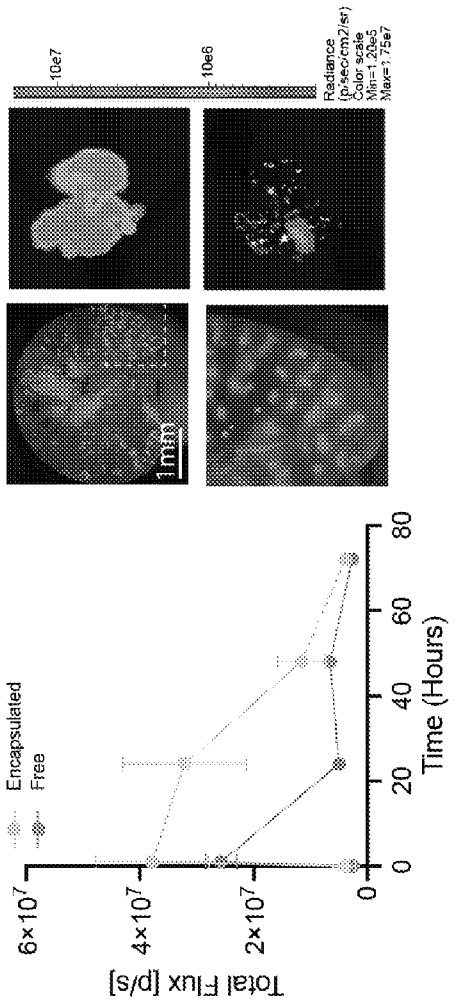
Encapsulated
Free
Total Flux [p/s]
6×10^7
4×10^7
2×10^7
0
0
20
40
60
80
Time (Hours)
1mm
10e7
10e6
Radiance (p/sec/cm2/sr)
Color scale
Min=1.20e5
Max=1.75e7

FIG. 14C

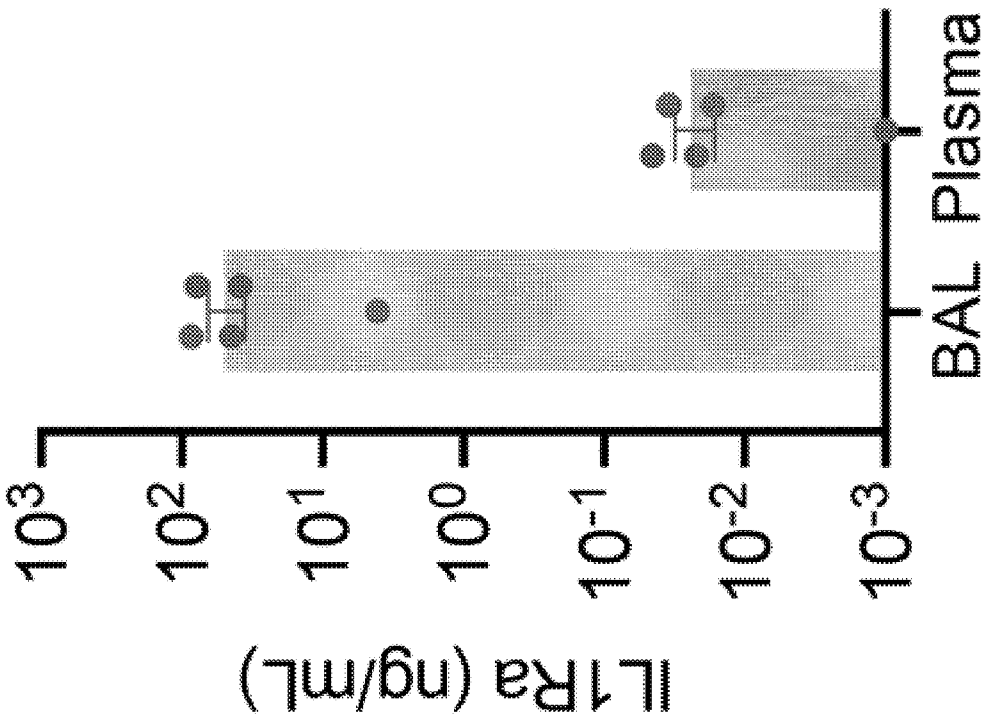

FIG. 15B

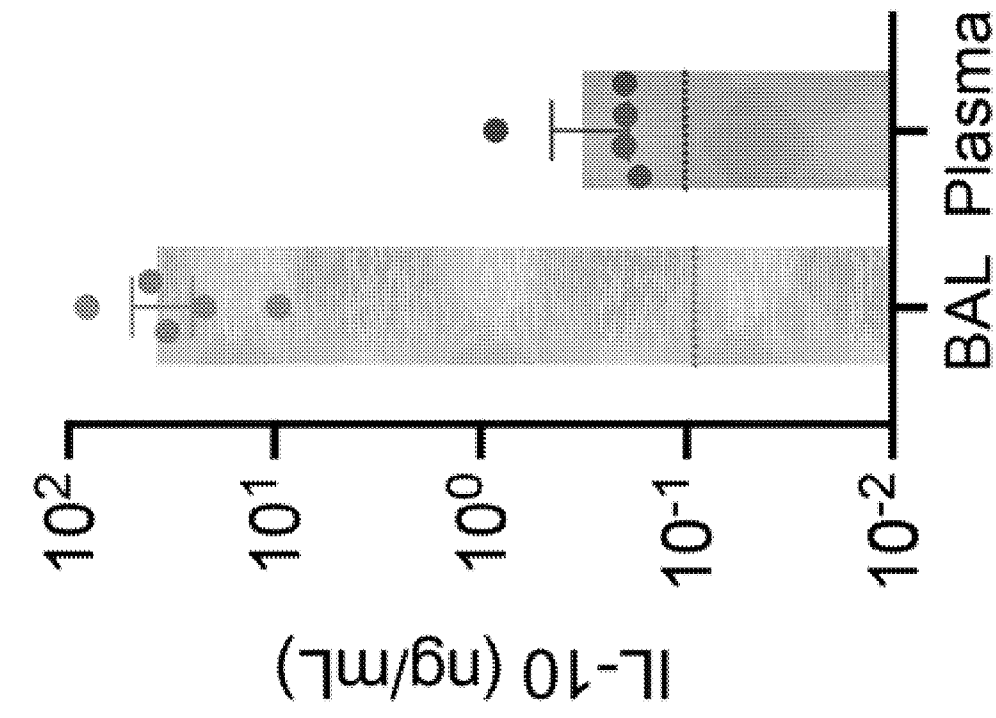

FIG. 15A

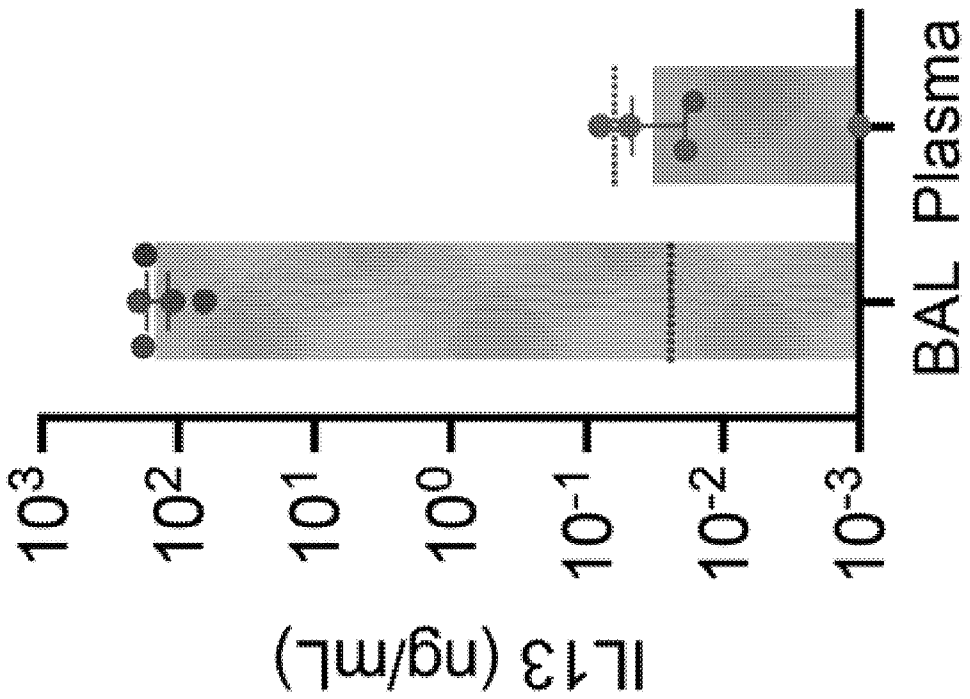

FIG. 15D

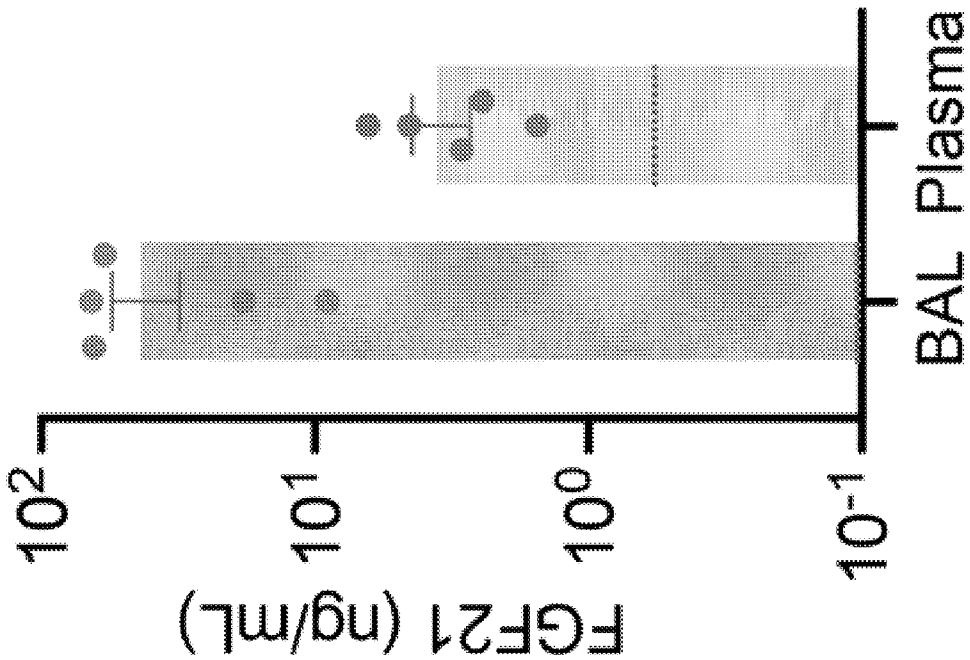

FIG. 15C

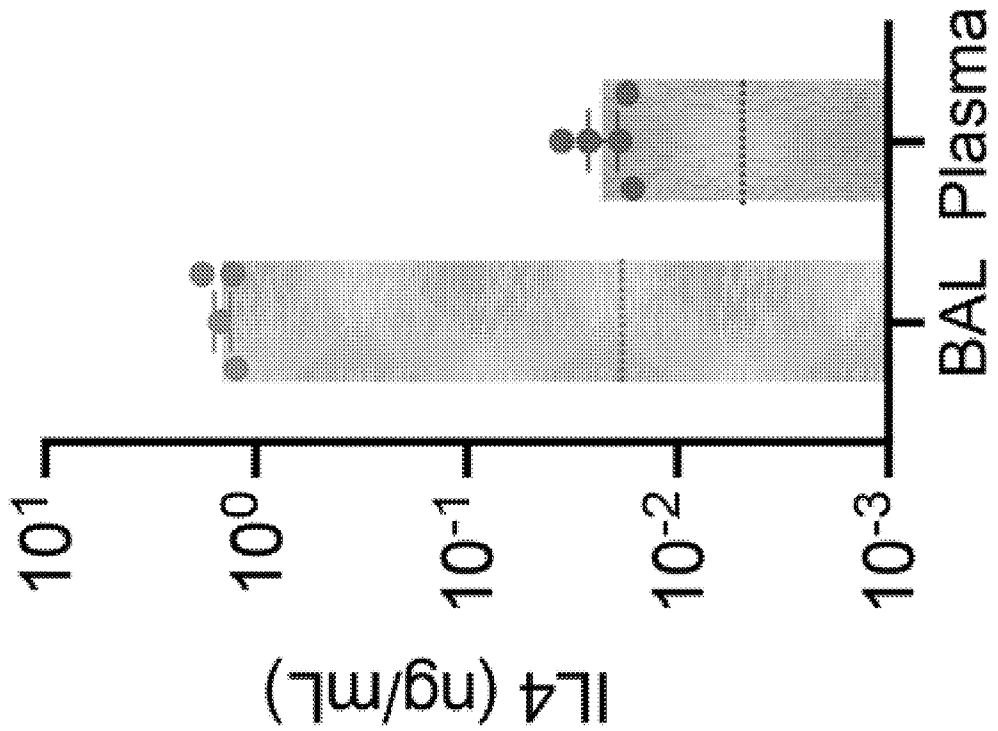

FIG. 15E

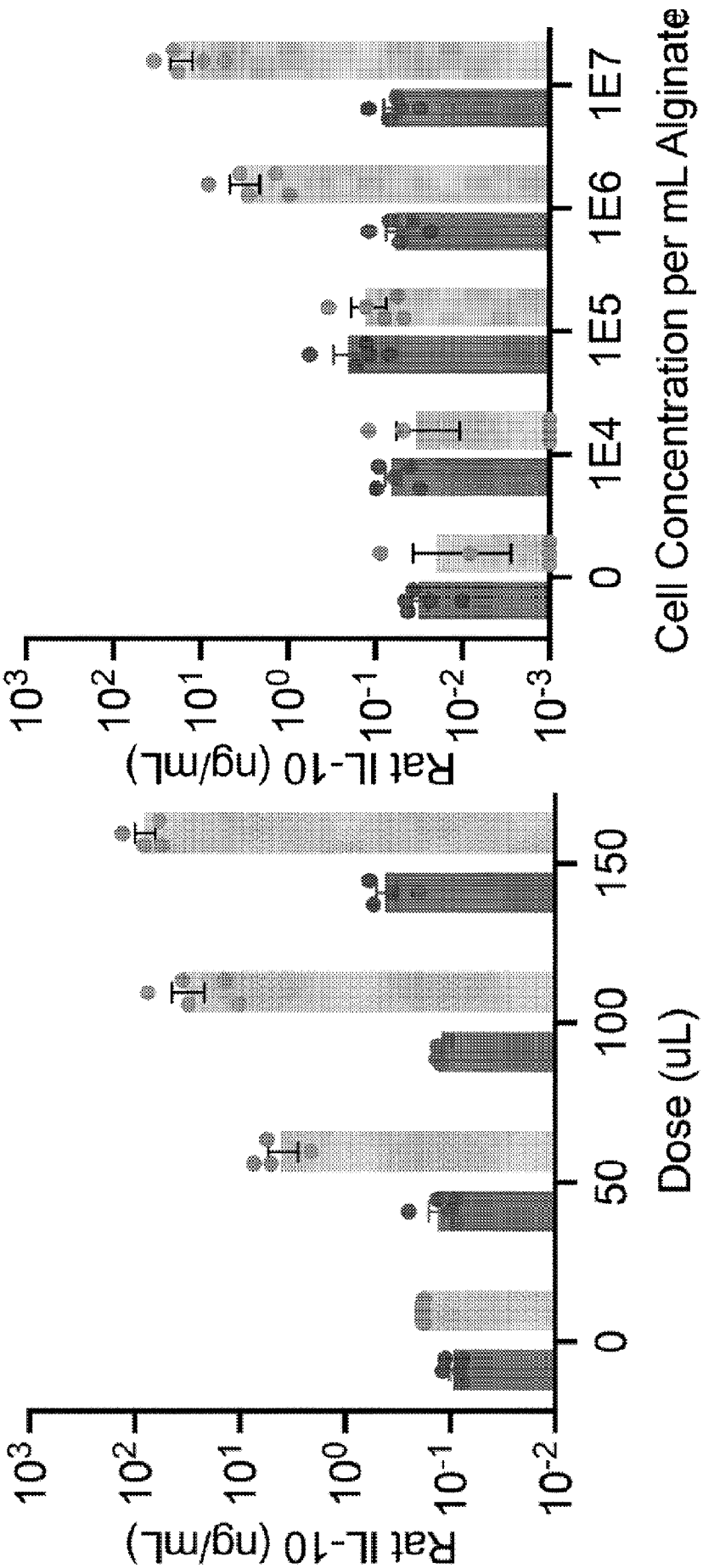

FIG. 16A

FIG. 16B

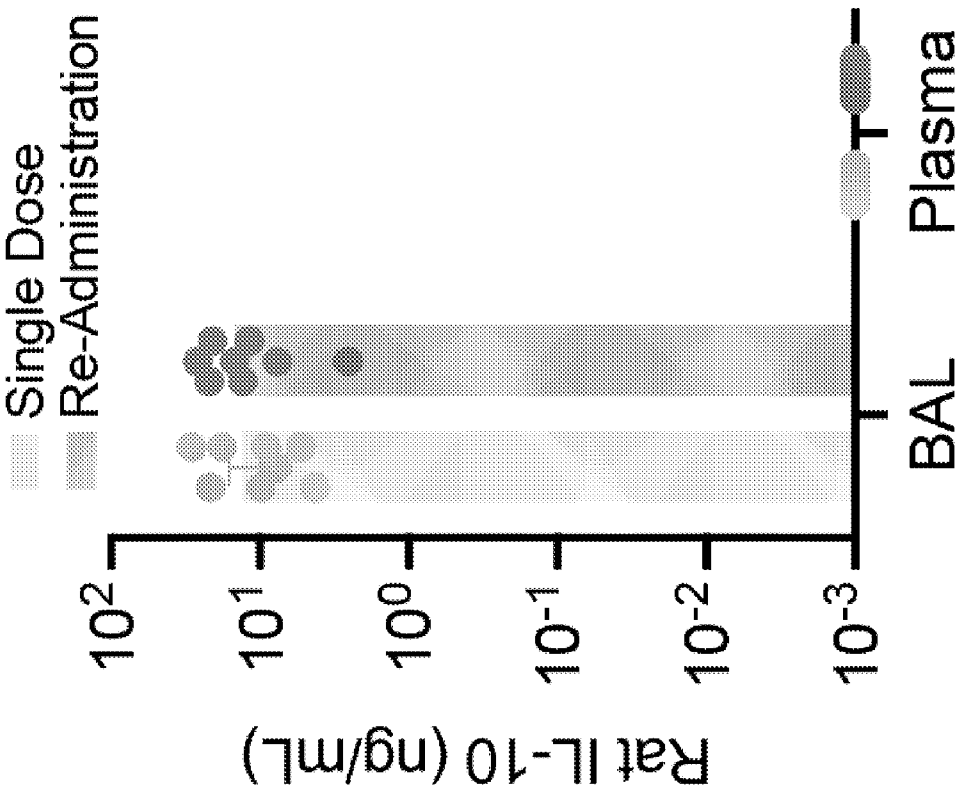
Single Dose
Re-Administration
Rat IL-10 (ng/mL)
10^2
10^1
10^0
10^-1
10^-2
10^-3
BAL
Plasma

FIG. 16C

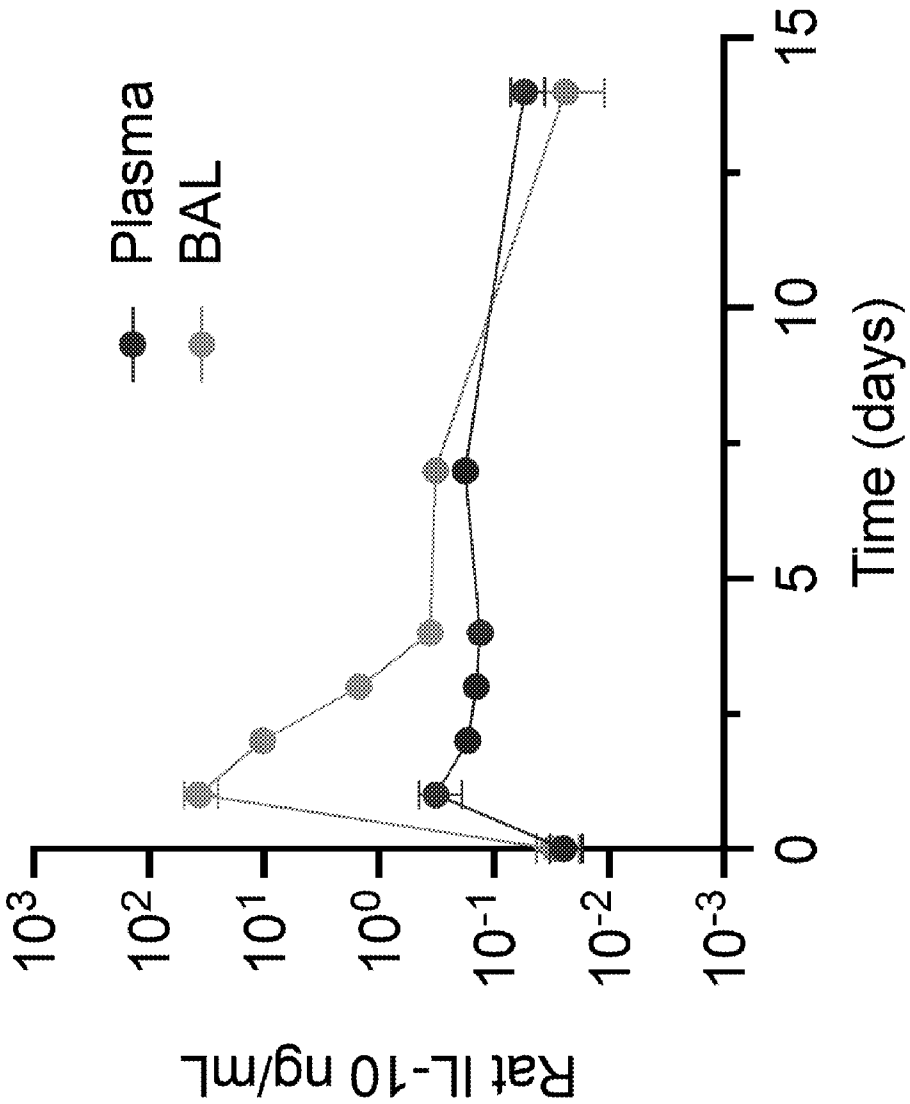
Plasma
BAL
Rat IL-10 ng/mL
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
0
5
10
15
Time (days)

FIG. 16D

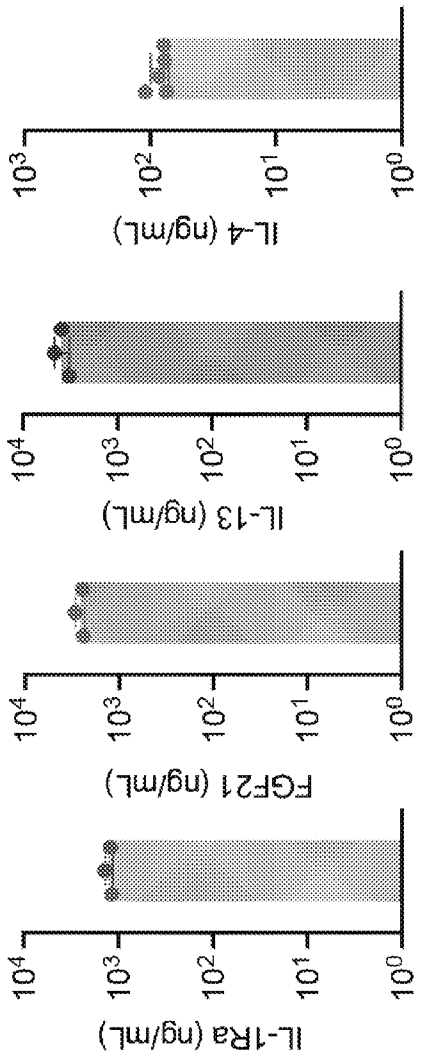

FIG. 16E

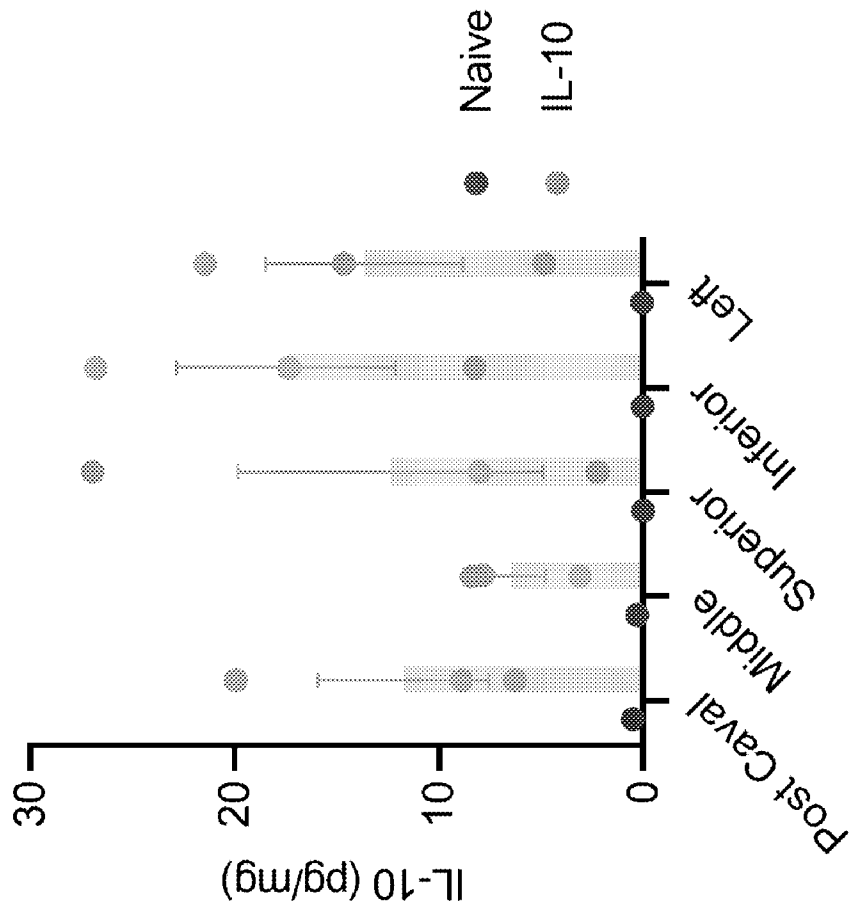
Naive
IL-10
IL-10 (pg/mg)
0
10
20
30
Post Caval
Middle
Superior
Interior
Left
Lung Lobe

FIG. 16F

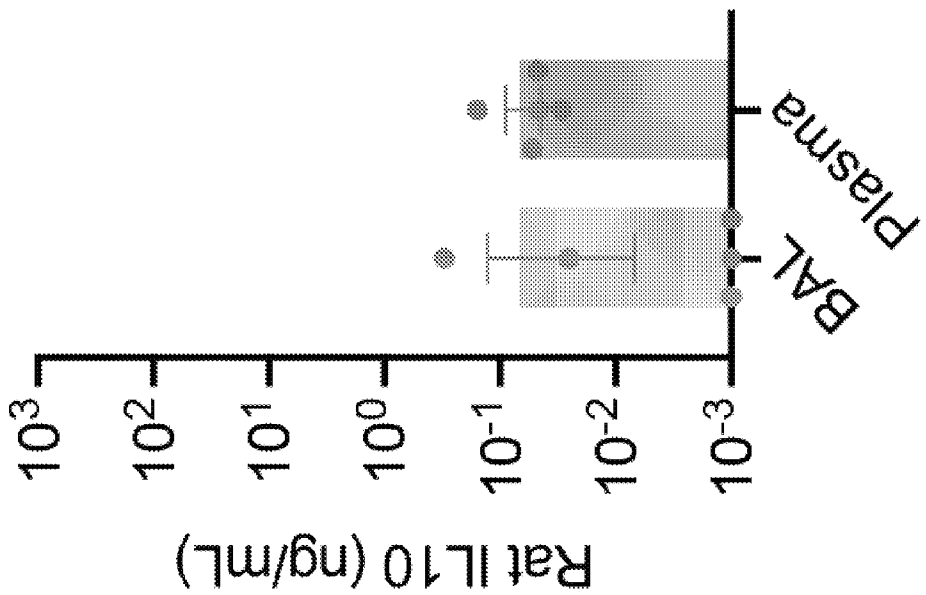

FIG. 16G

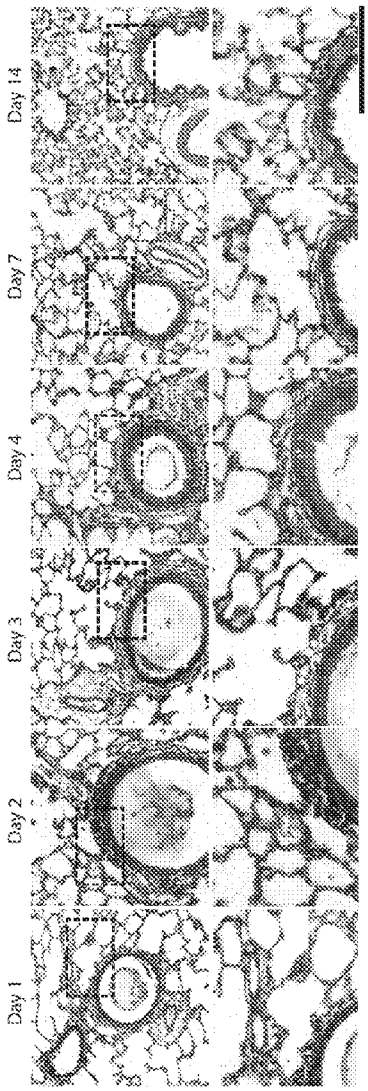

FIG. 16H

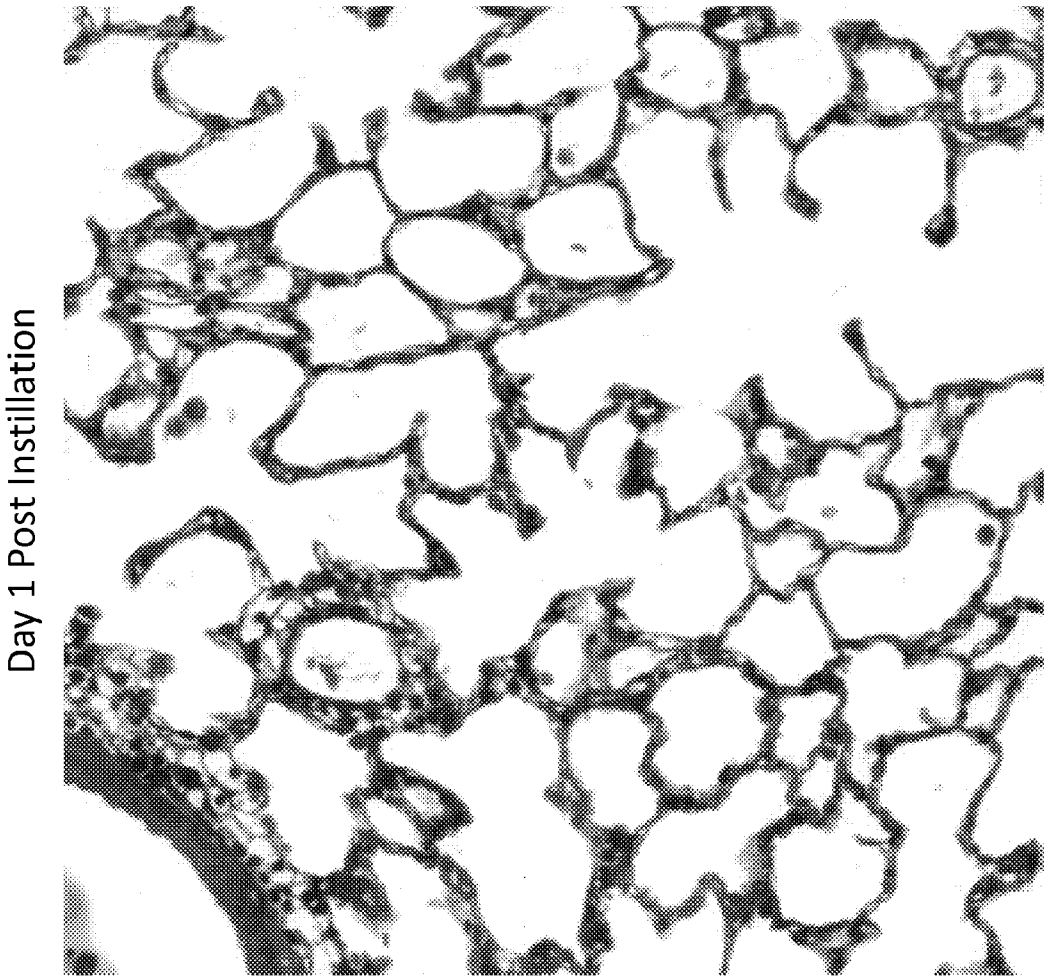

FIG. 17A

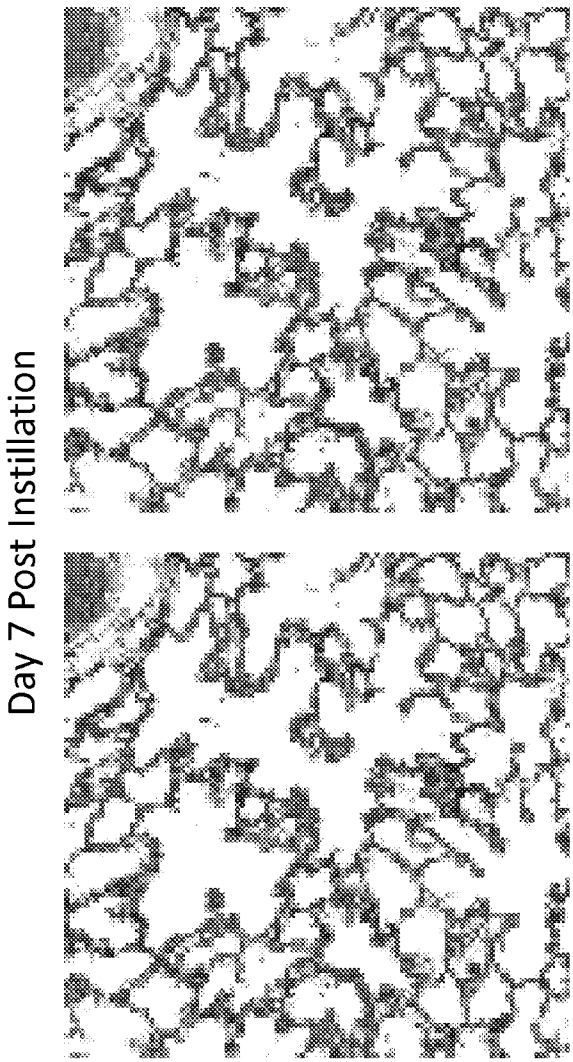

FIG. 17B

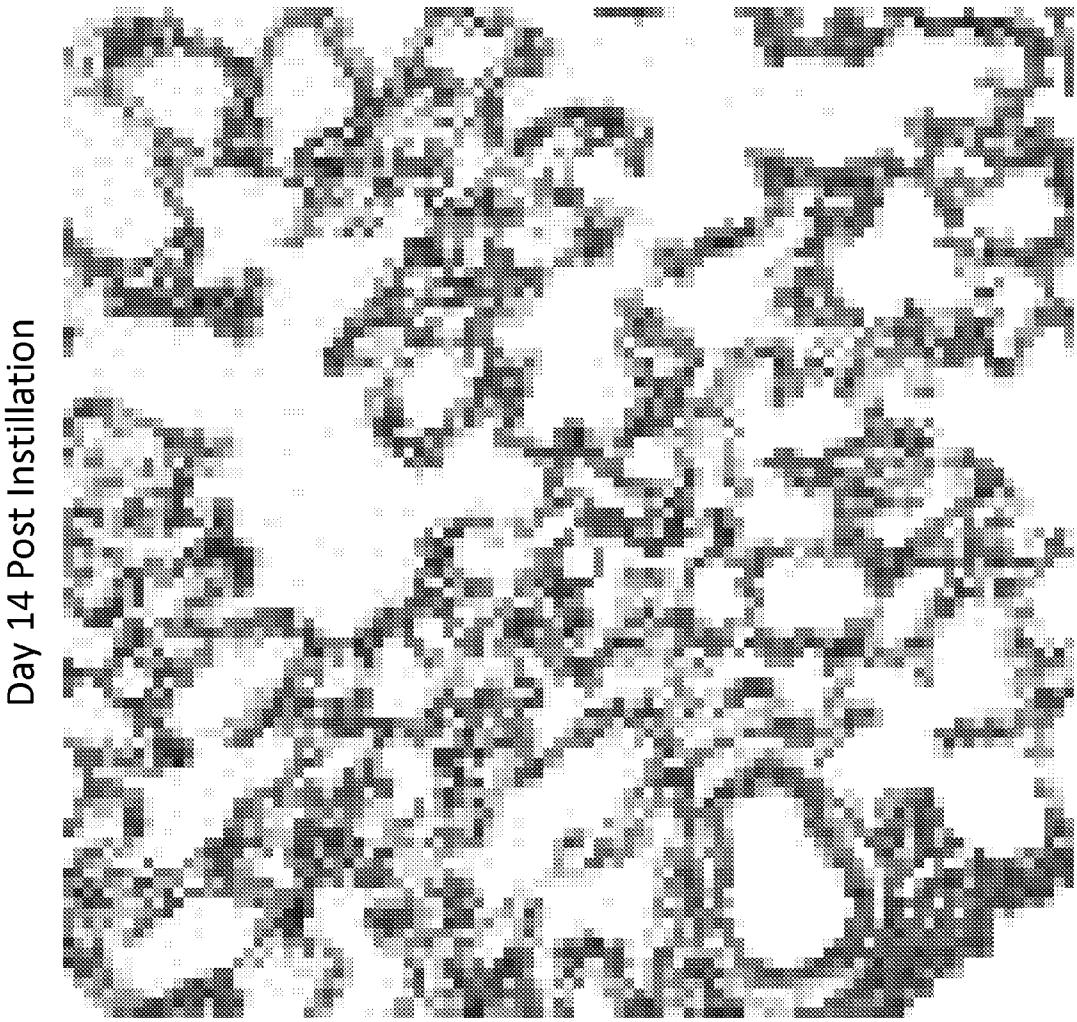

FIG. 17C

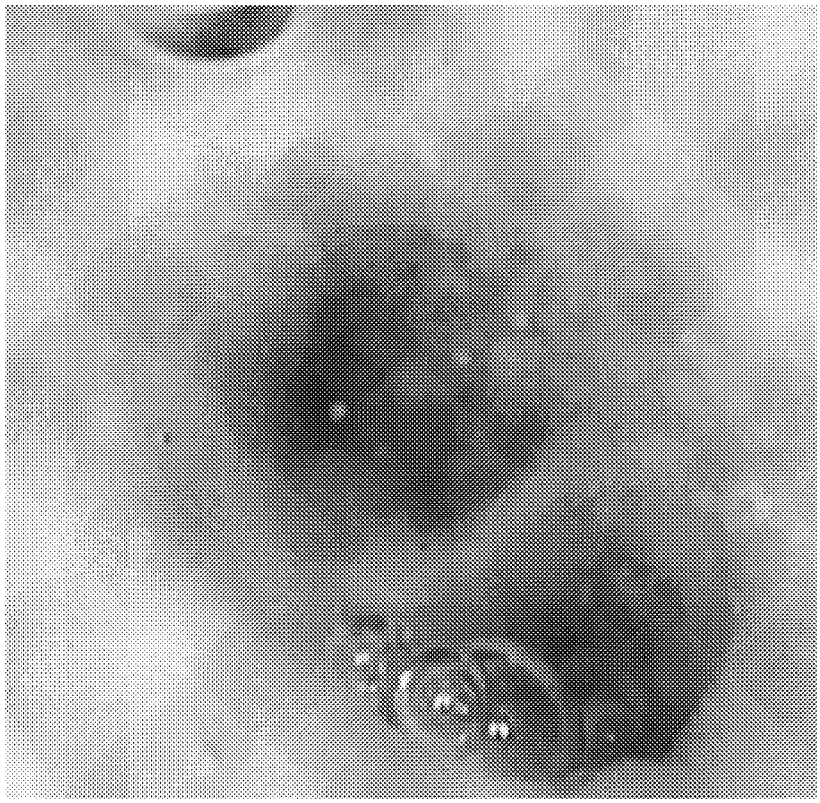

FIG. 18A

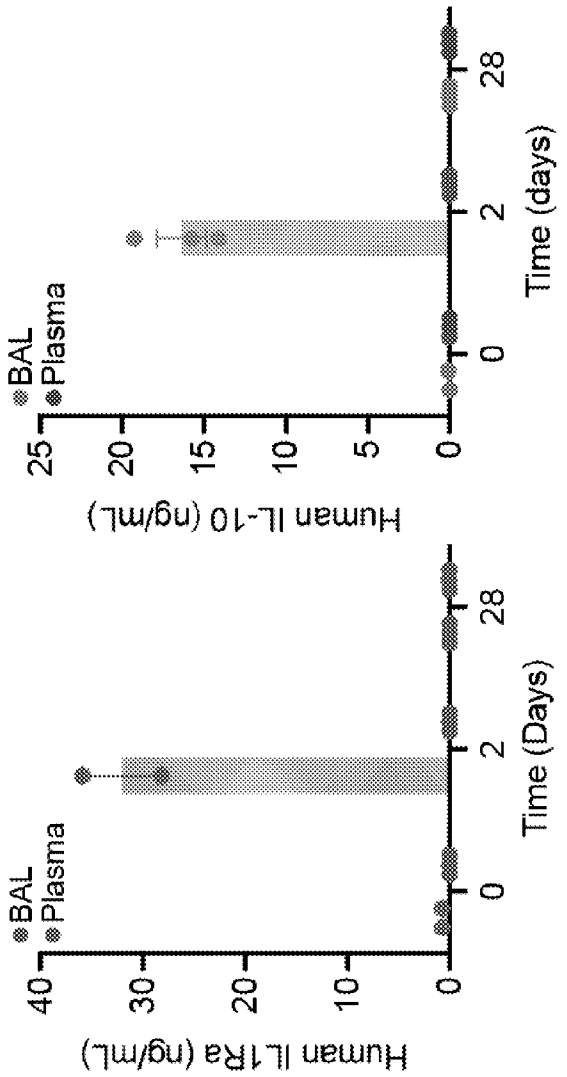
Human IL-10 (ng/mL)
25
20
15
10
5
0
BAL
Plasma
0
2
28
Time (days)
Human IL1Ra (ng/mL)
40
30
20
10
0
BAL
Plasma
0
2
28
Time (Days)

FIG. 18B

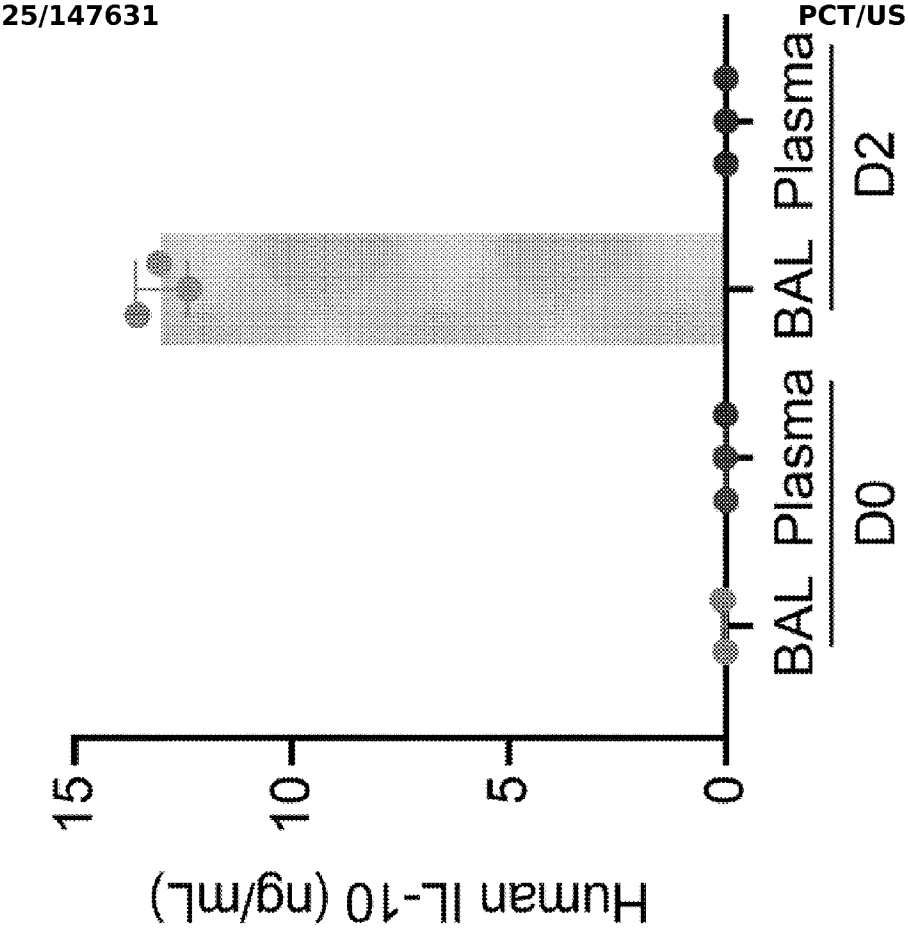

FIG. 18D

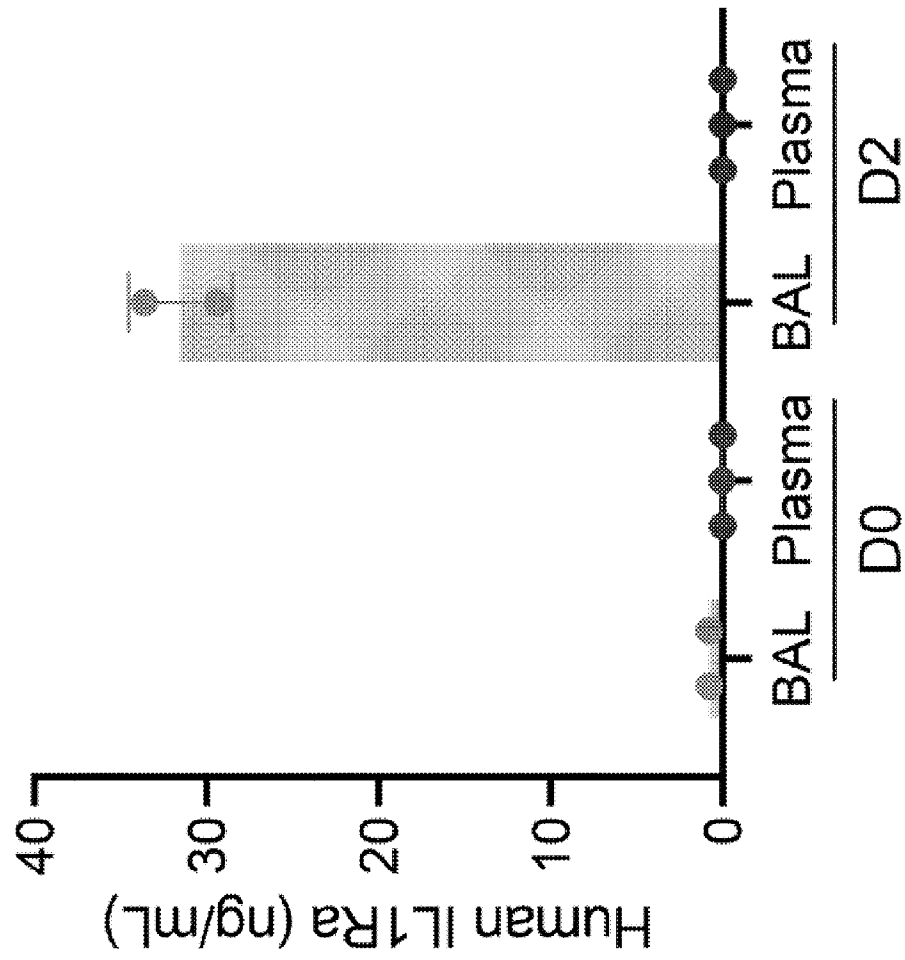

FIG. 18C

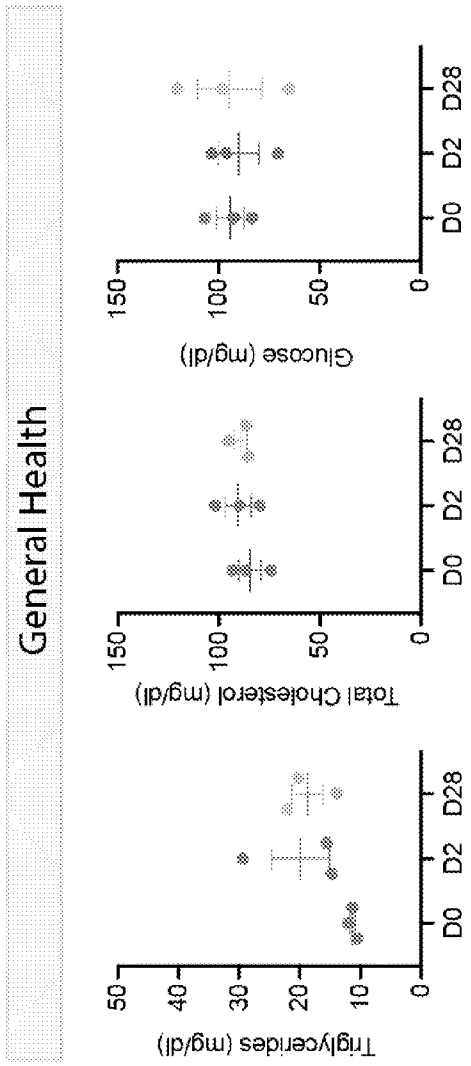
General Health
Triglycerides (mg/dl)
0
10
20
30
40
50
D0
D2
D28
Total Cholesterol (mg/dl)
0
50
100
150
D0
D2
D28
Glucose (mg/dl)
0
50
100
150
D0
D2
D28

FIG. 18E

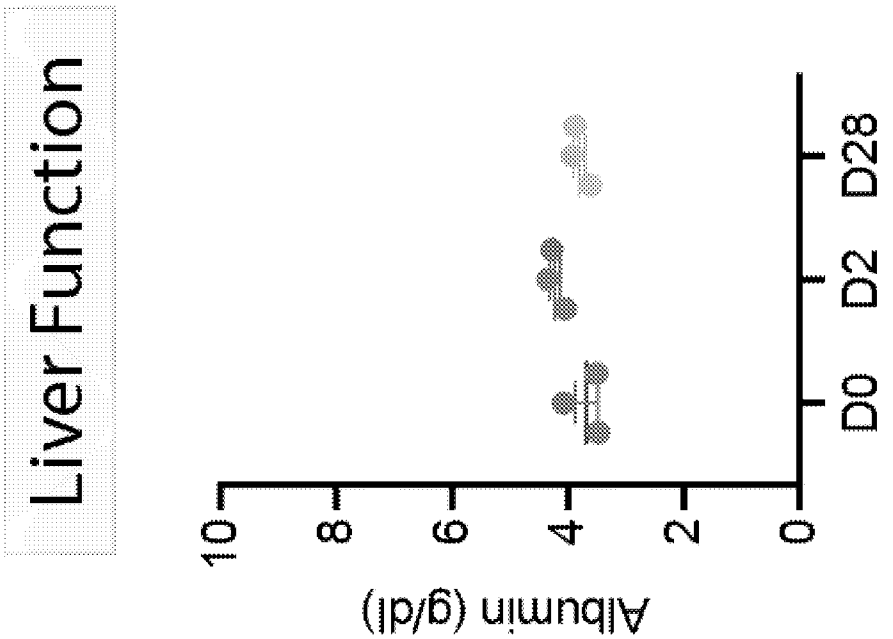

FIG. 18F

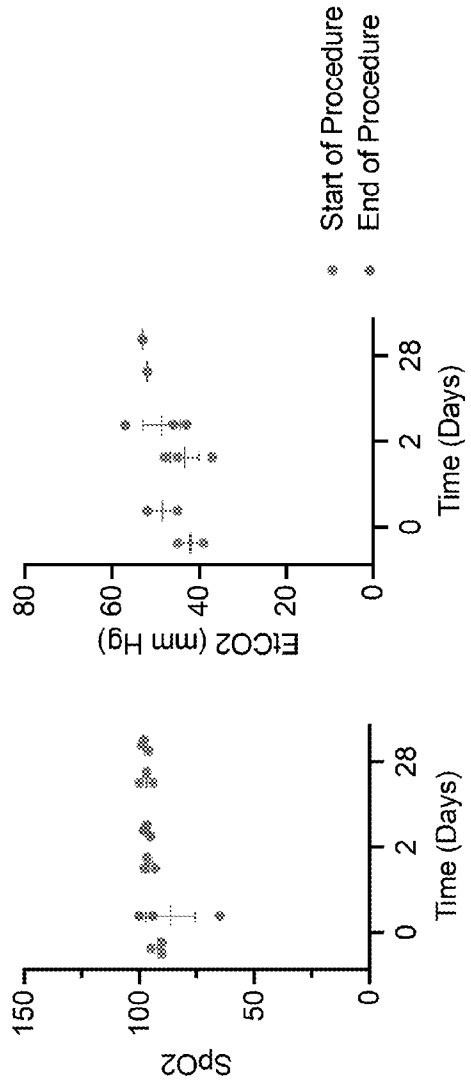
Start of Procedure
End of Procedure
EtCO2 (mm Hg)
80
60
40
20
0
0
2
28
Time (Days)
SpO2
150
100
50
0
0
2
28
Time (Days)

FIG. 18G

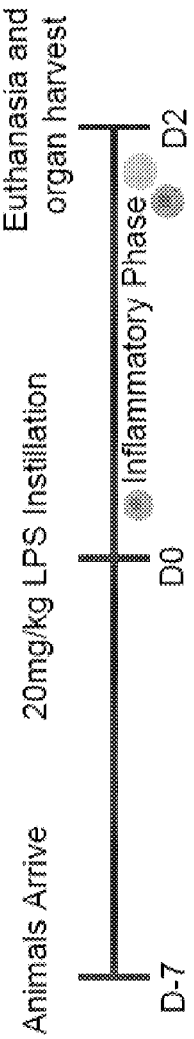
Animals Arrive
20mg/kg LPS Instillation
Euthanasia and organ harvest
Inflammatory Phase
D-7
D0
D2

FIG. 19A

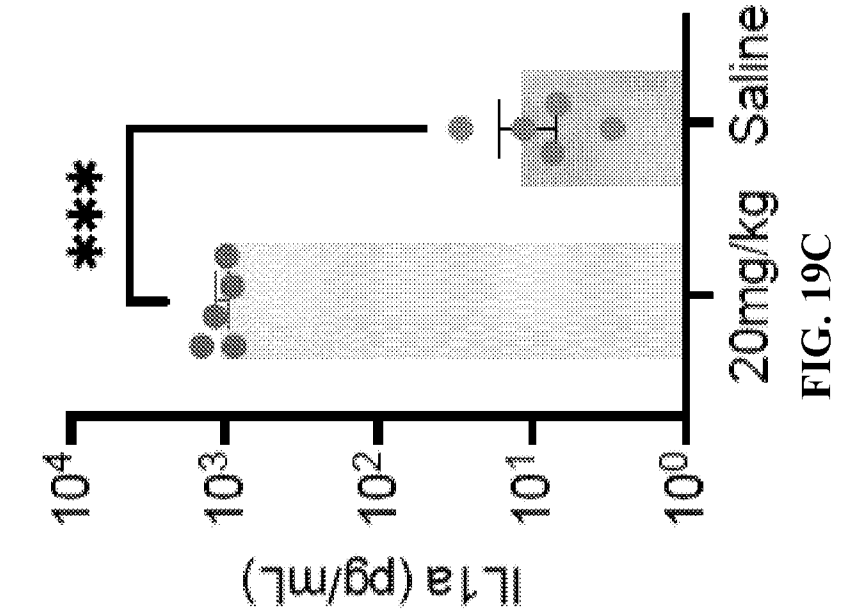

FIG. 19C

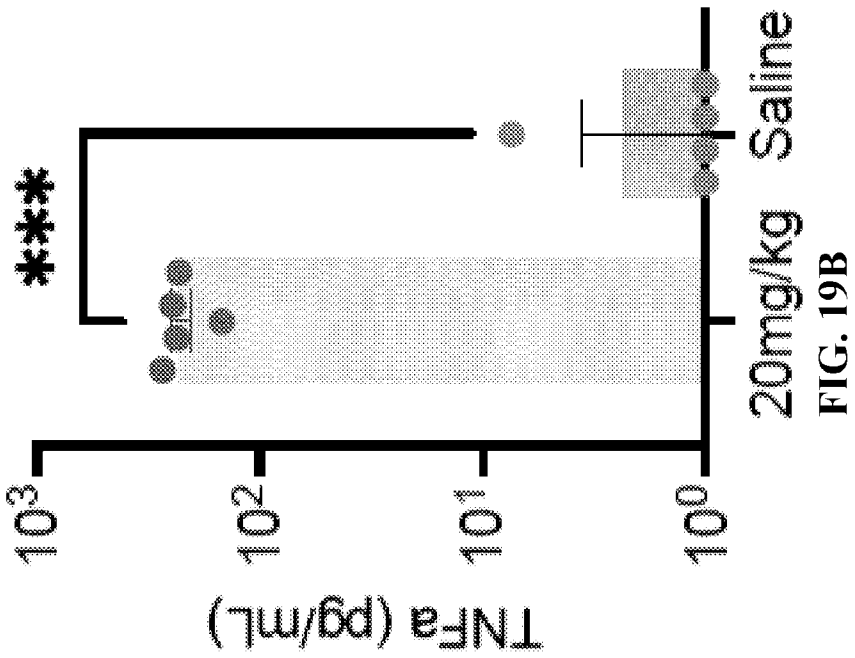

FIG. 19B

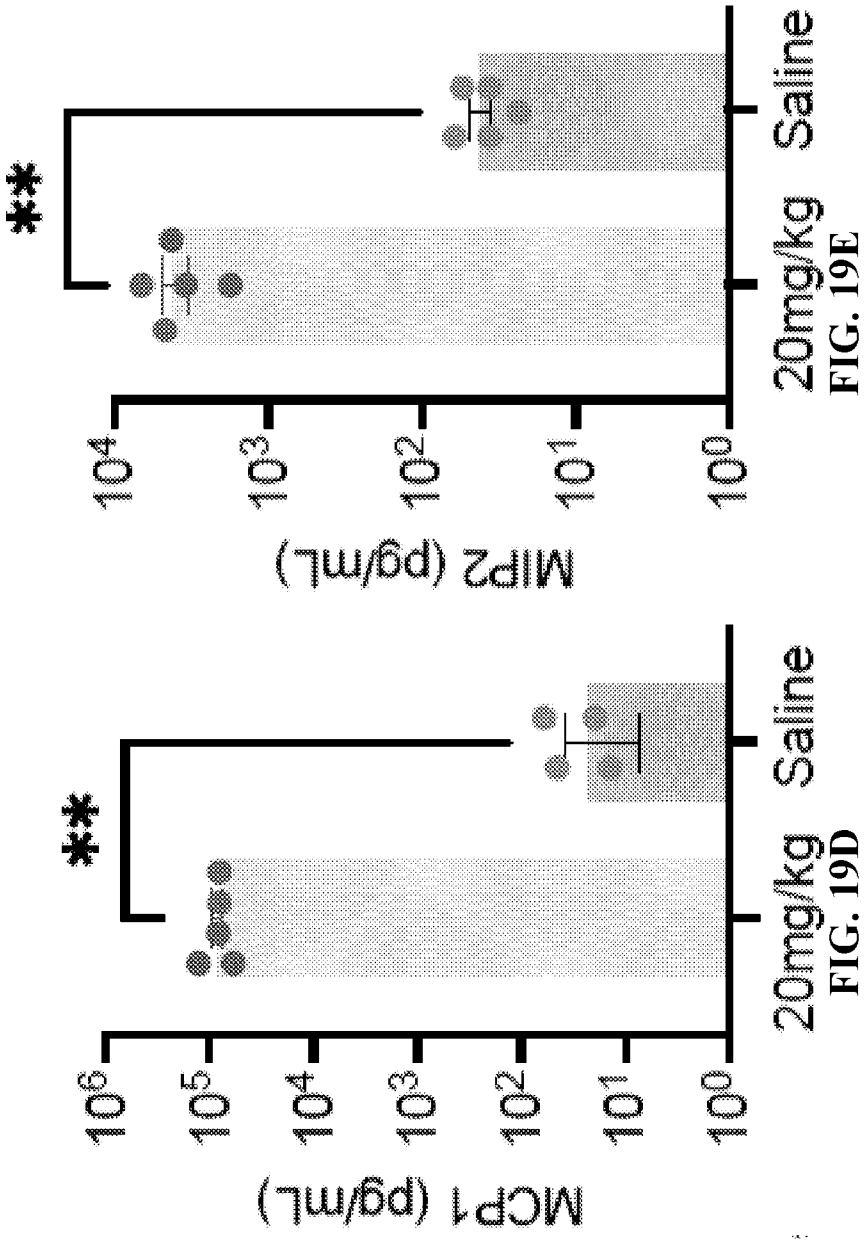

FIG. 19D

FIG. 19E

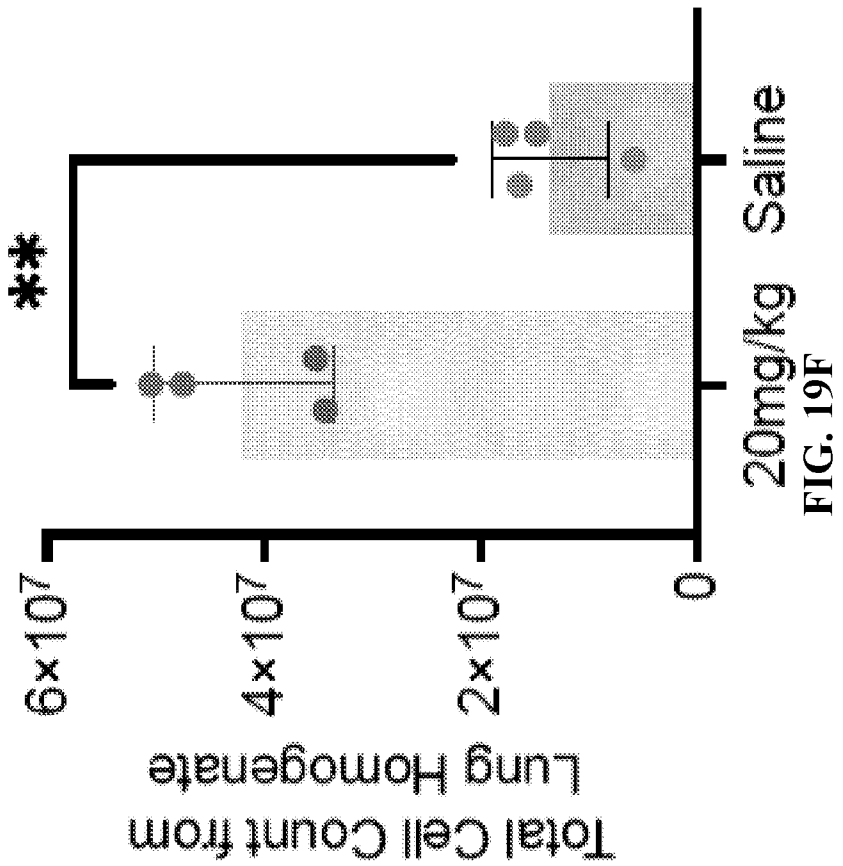
Total Cell Count from
Lung Homogenate
6x10^7
4x10^7
2x10^7
0
**
20mg/kg
Saline

FIG. 19F

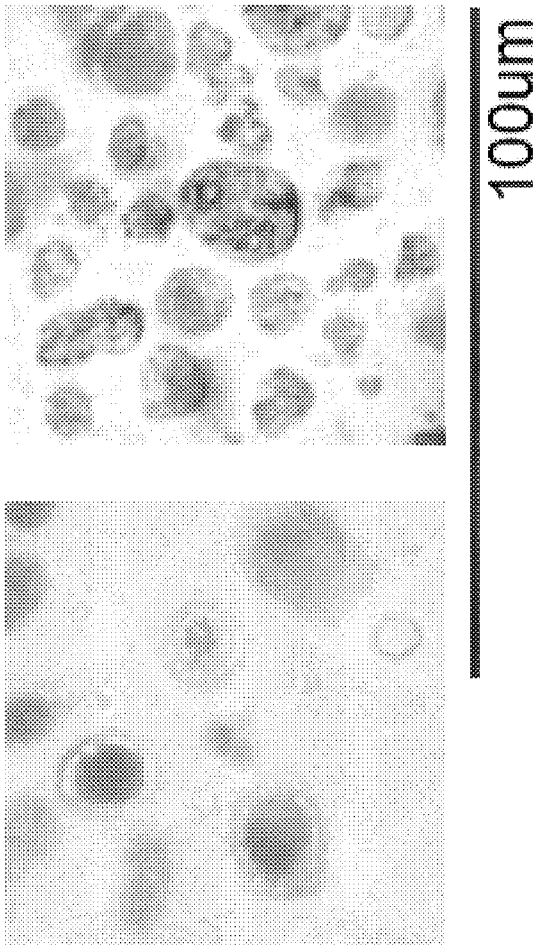

FIG. 19G

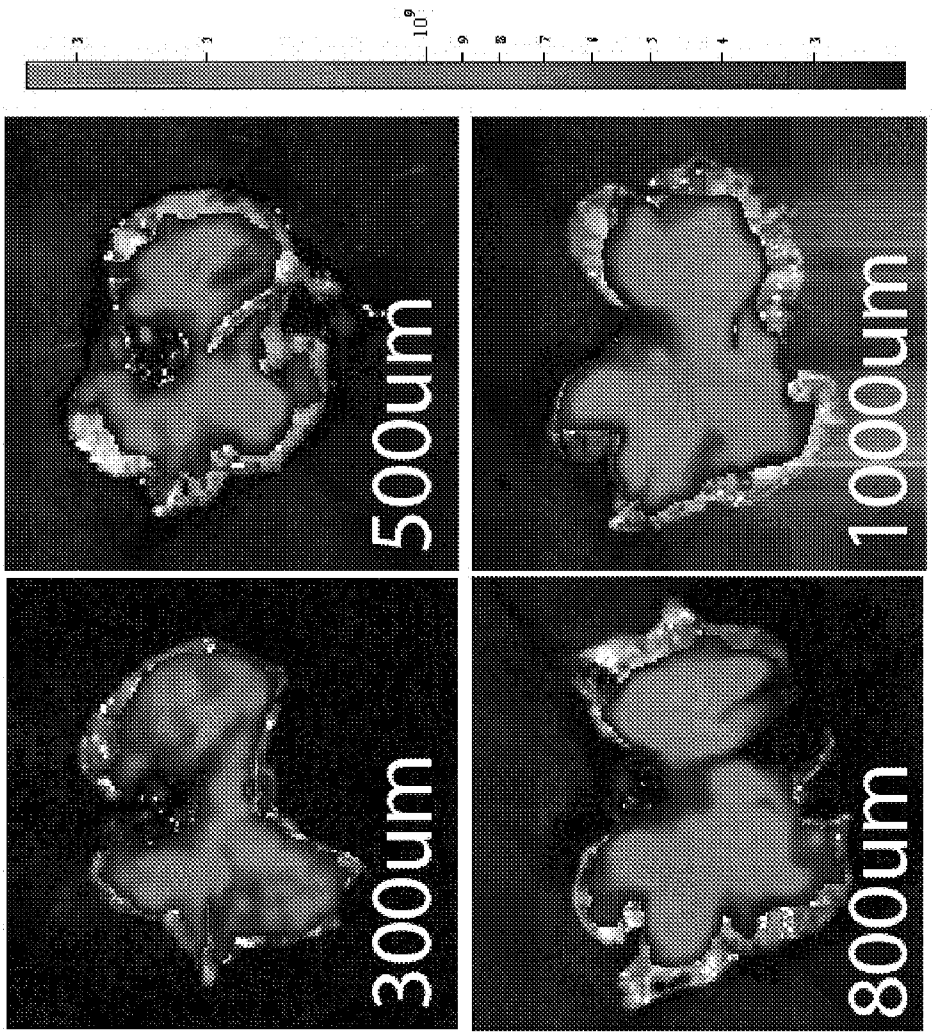
300um
500um
800um
1000um
Radiant Efficiency p/sec/cm²/sr / uW/cm²

FIG. 20

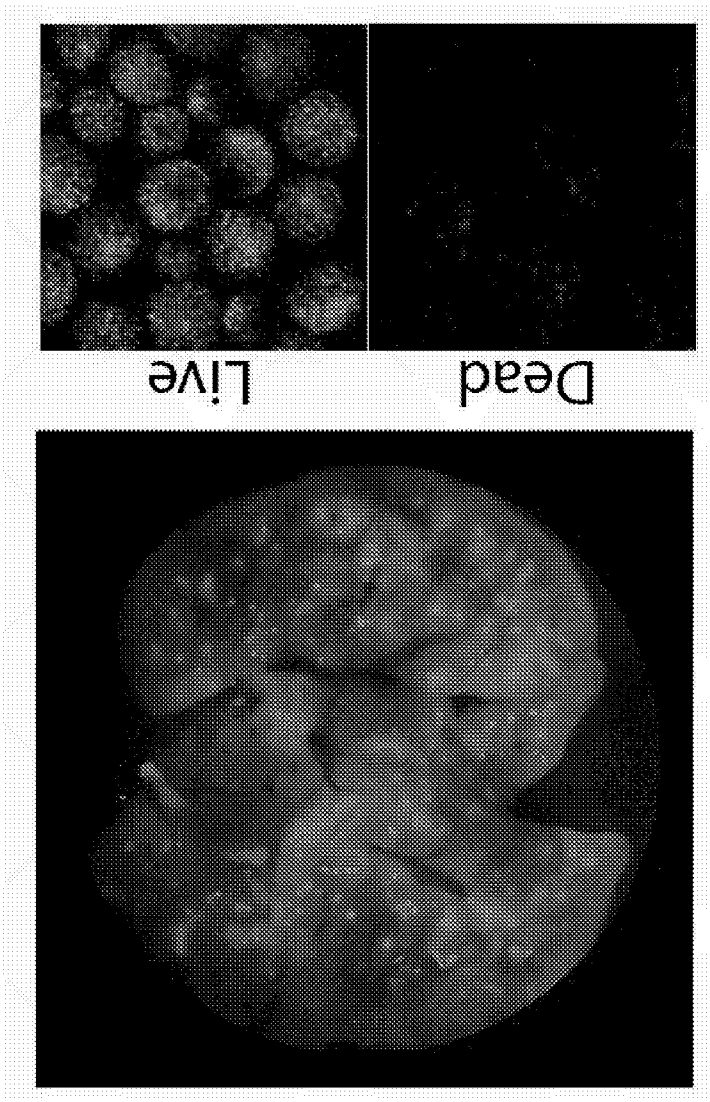

FIG. 21

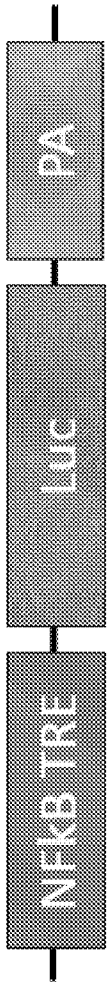

FIG. 22A

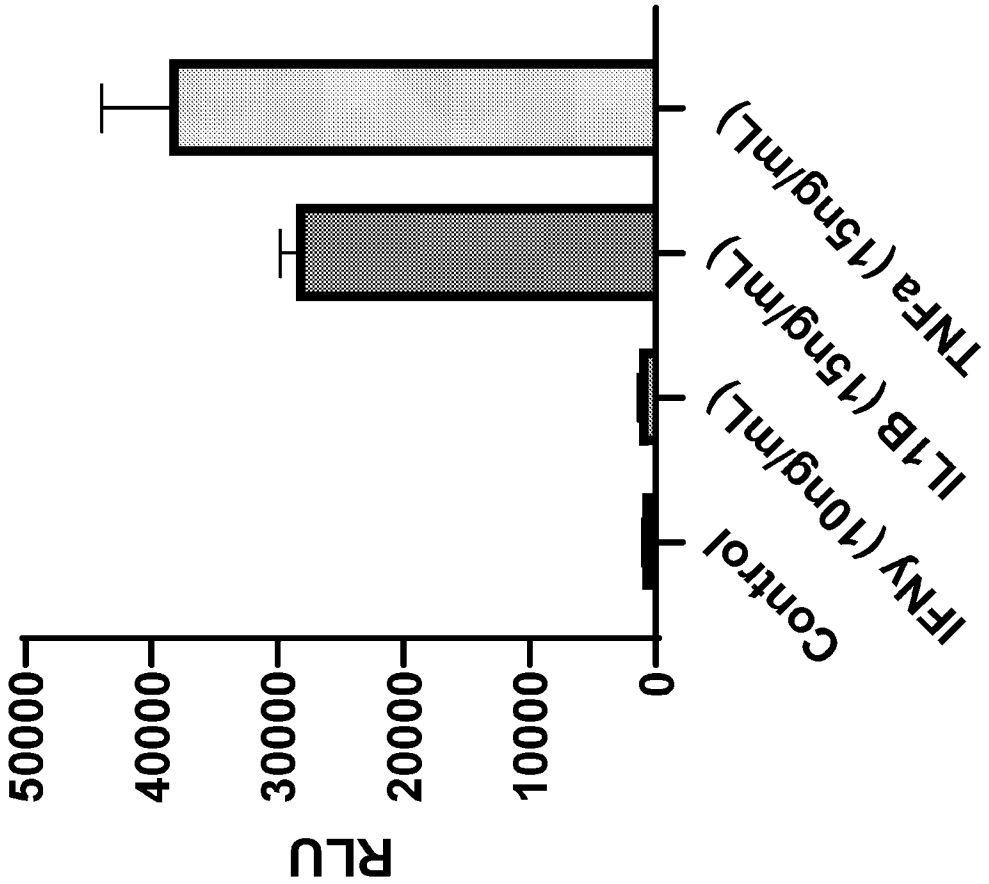
RLU
50000
40000
30000
20000
10000
0
Control
IFNγ (10ng/mL)
IL-1B (15ng/mL)
TNFa (15ng/mL)

FIG. 22B

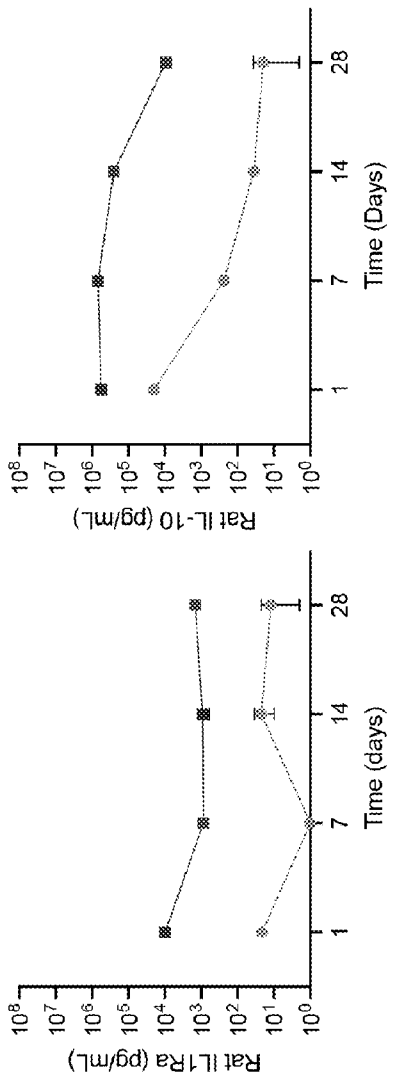

FIG. 23A

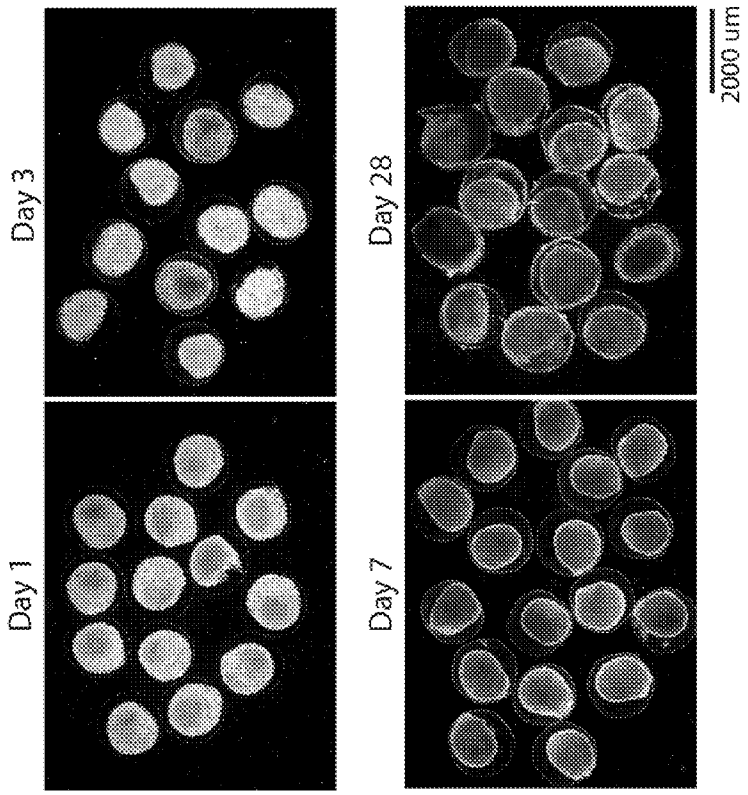

FIG. 23B

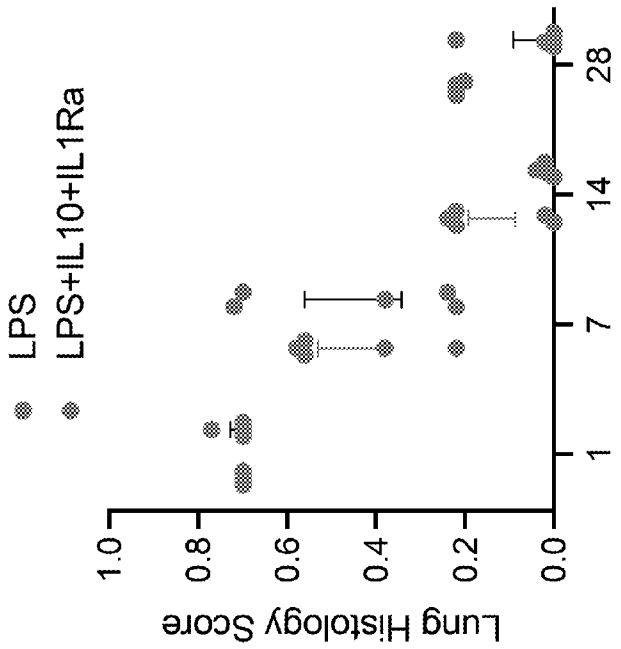
LPS
LPS+IL10+IL1Ra
Lung Histology Score
1.0
0.8
0.6
0.4
0.2
0.0
1
7
14
28
Time (Days)

FIG. 23C

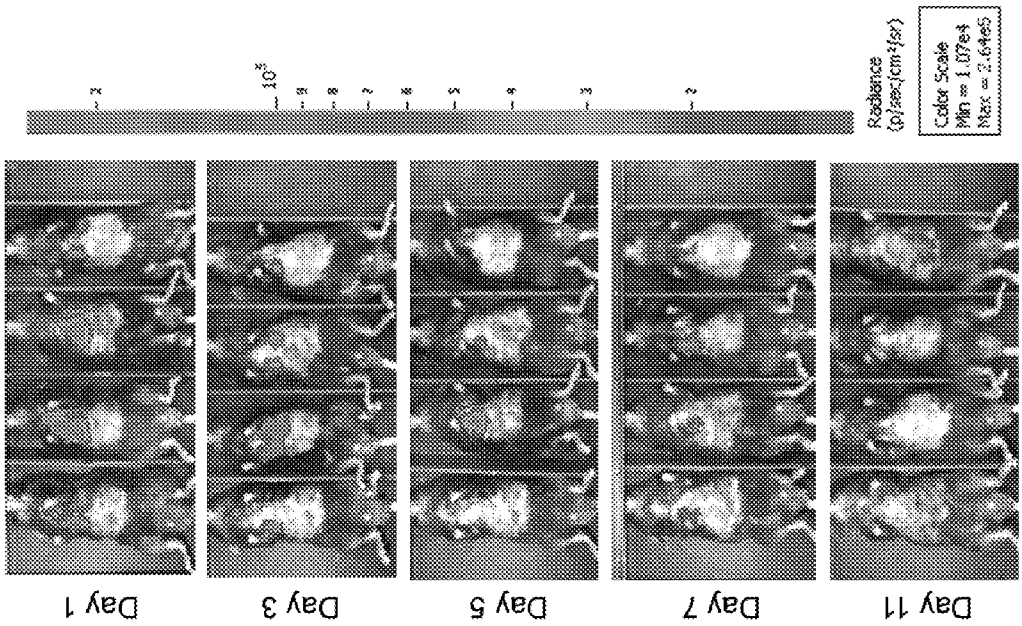
Day 1
Day 3
Day 5
Day 7
Day 11
Radiance (p/sec/cm²/sr)
Color Scale
Min = 1.07e4
Max = 2.64e5

FIG. 24A

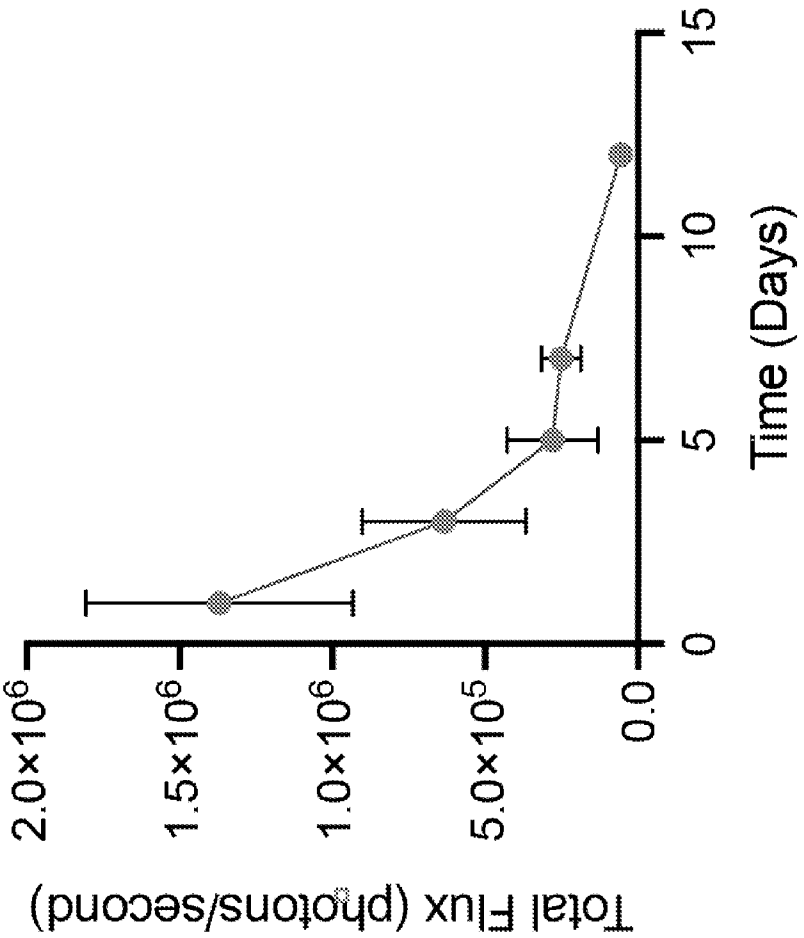
Total Flux (photons/second)
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
0
5
10
15
Time (Days)

FIG. 24B

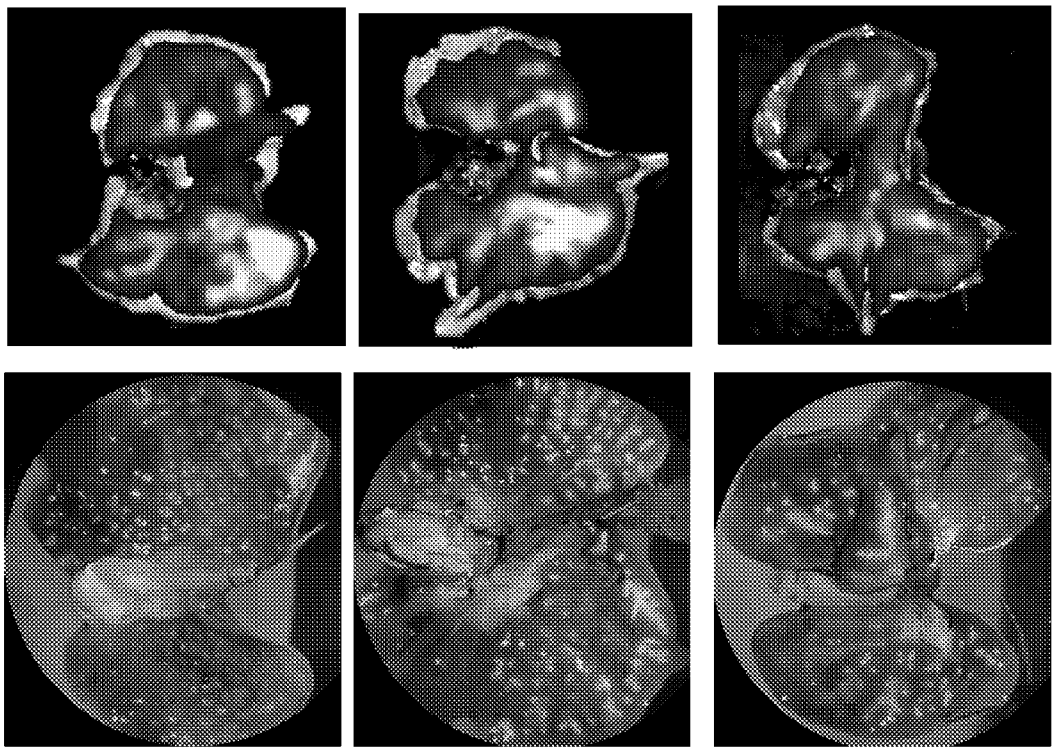

FIG. 25

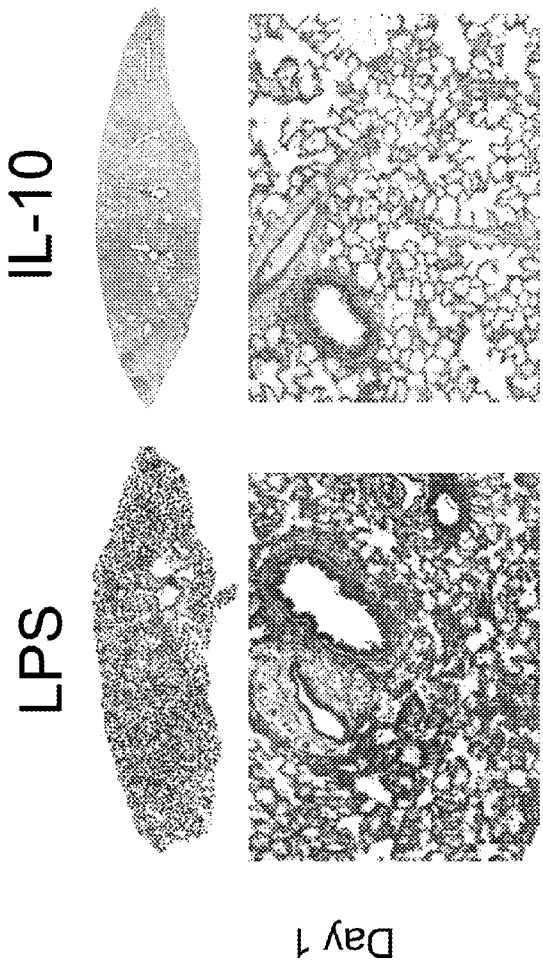

FIG. 26A

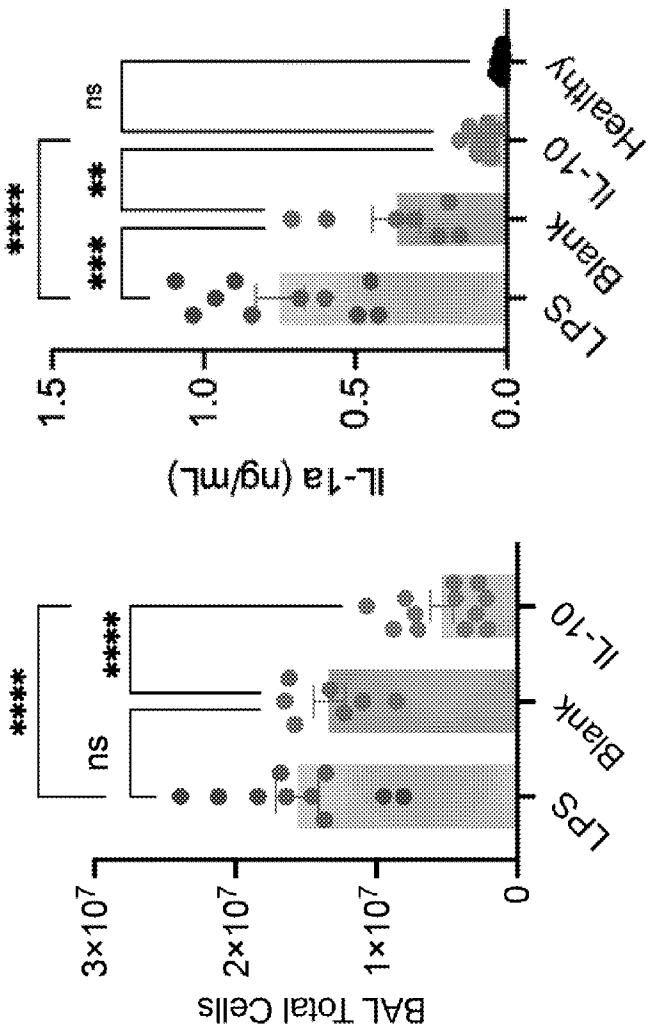
IL-1a (ng/mL)
1.5
1.0
0.5
0.0
LPS
Blank
IL-10
Healthy
****
***
**
ns
BAL Total Cells
3×10^7
2×10^7
1×10^7
0
LPS
Blank
IL-10
****
ns
****

FIG. 26B

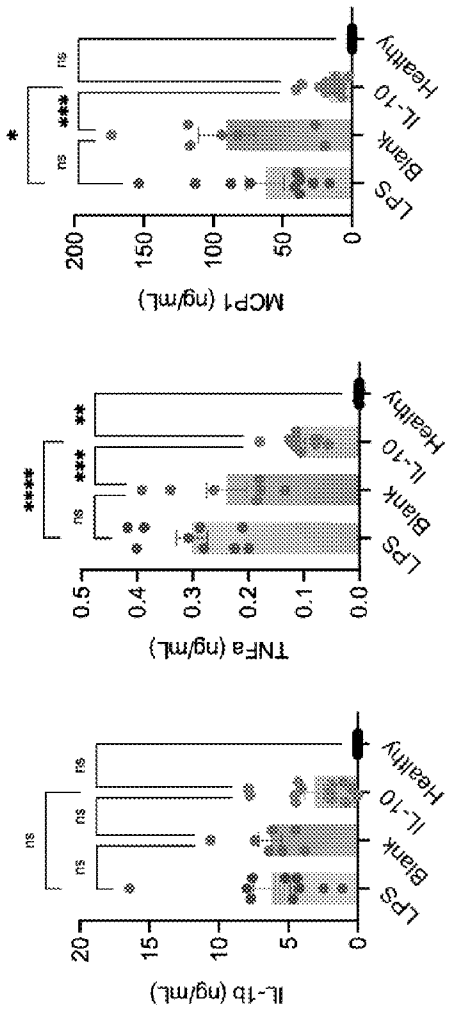
MCP1 (ng/mL)
0
50
100
150
200
LPS
Blank
IL-10
Healthy
*
***
ns
ns
TNFa (ng/mL)
0.0
0.1
0.2
0.3
0.4
0.5
LPS
Blank
IL-10
Healthy
****
***
**
ns
ns
IL-1b (ng/mL)
0
5
10
15
20
LPS
Blank
IL-10
Healthy
ns
ns
ns
ns

FIG. 26C

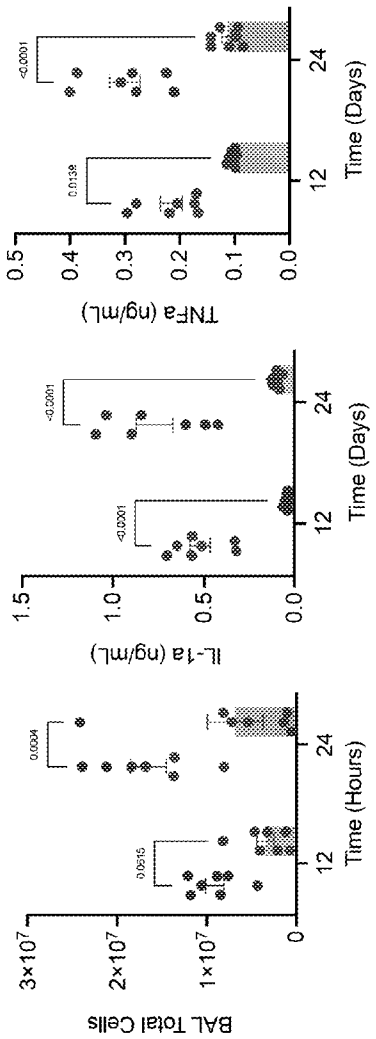
BAL Total Cells
3×10^7
2×10^7
1×10^7
0
0.0515
0.0004
12
24
Time (Hours)
IL-1a (ng/mL)
1.5
1.0
0.5
0.0
<0.0001
<0.0001
12
24
Time (Days)
TNFa (ng/mL)
0.5
0.4
0.3
0.2
0.1
0.0
0.0138
<0.0001
12
24
Time (Days)

FIG. 27A

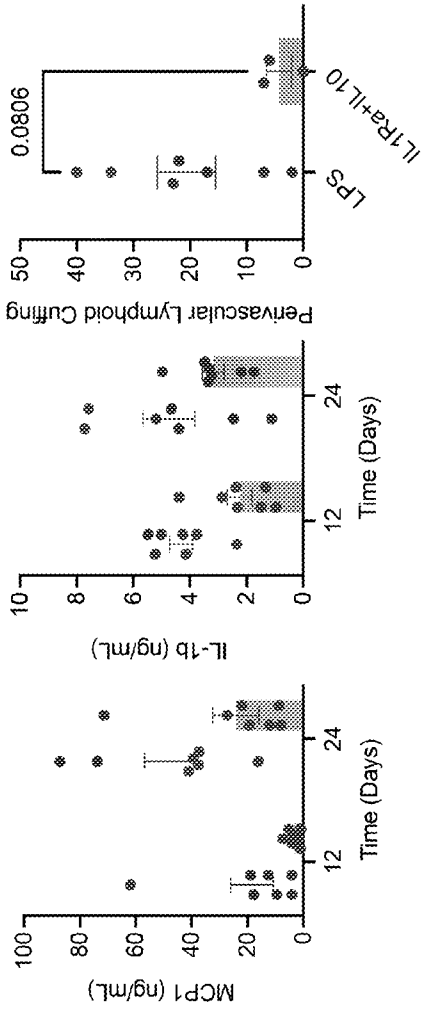
MCP1 (ng/mL)
IL-1b (ng/mL)
Perivascular Lymphoid Cuffing
Time (Days)
Time (Days)
0.0806
LPS
IL17Ra+IL10

FIG. 27B

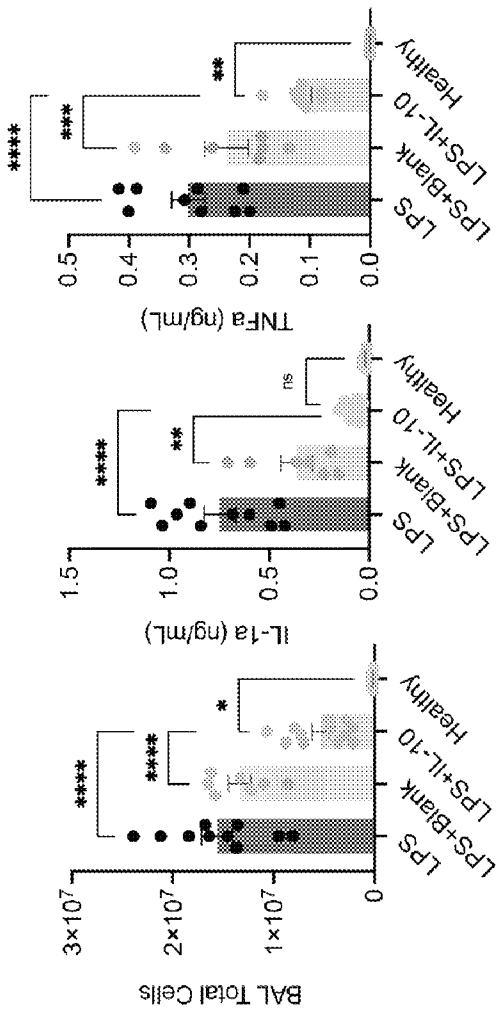
BAL Total Cells
3×10^7
2×10^7
1×10^7
0
****
****
*
IL-1a (ng/mL)
1.5
1.0
0.5
0.0
****
**
ns
TNFa (ng/mL)
0.5
0.4
0.3
0.2
0.1
0.0
****
***
**
LPS
LPS+Blank
LPS+IL-10
Healthy

FIG. 28A

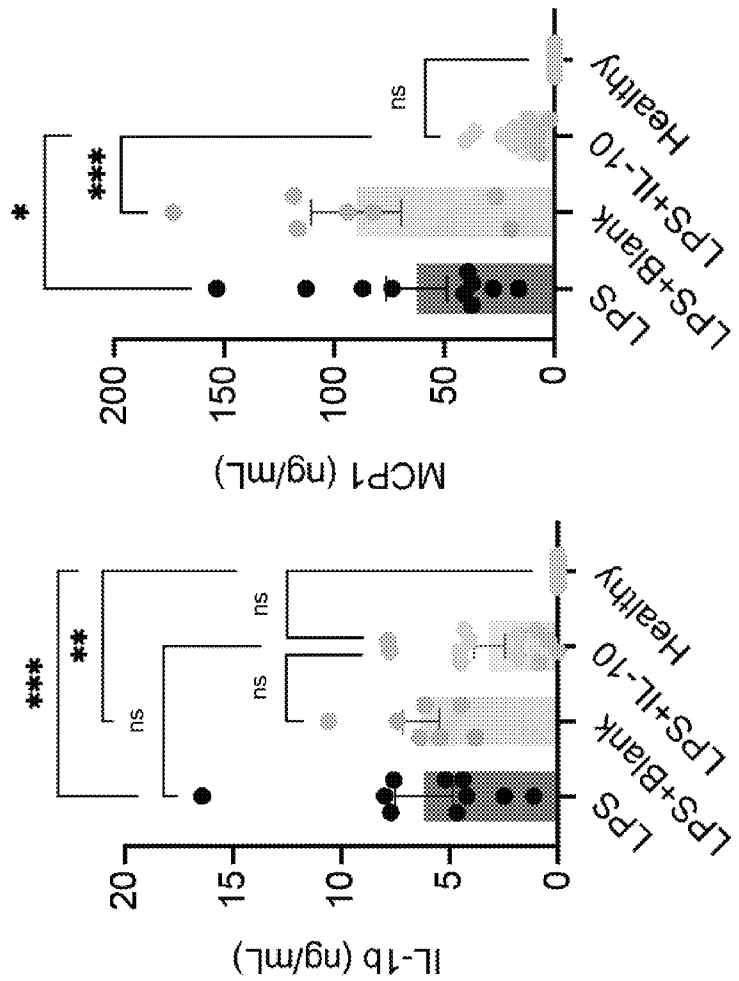
MCP1 (ng/mL)
0
50
100
150
200
LPS
LPS+Blank
LPS+IL-10
Healthy
*
***
ns
IL-1b (ng/mL)
0
5
10
15
20
LPS
LPS+Blank
LPS+IL-10
Healthy
***
**
ns
ns

FIG. 28B

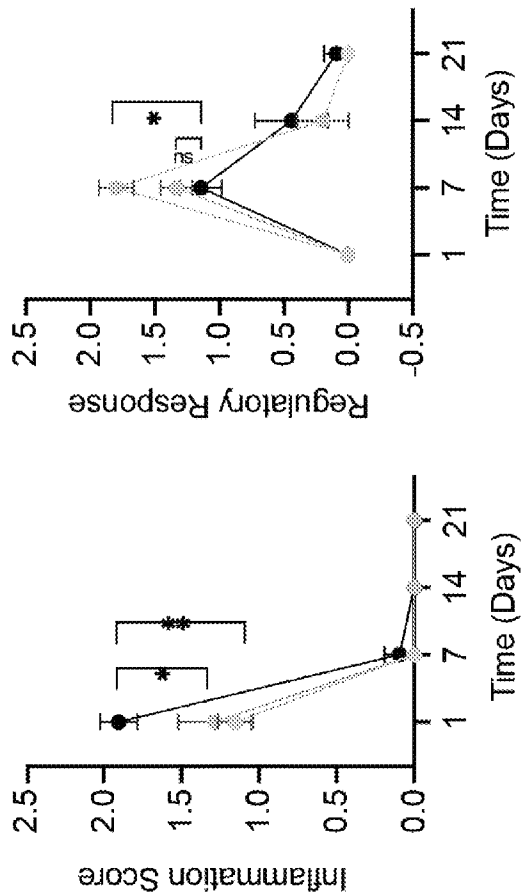
Inflammation Score
2.5
2.0
1.5
1.0
0.5
0.0
1
7
14
21
Time (Days)
*
**
Regulatory Response
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1
7
14
21
Time (Days)
ns
*

FIG. 28C

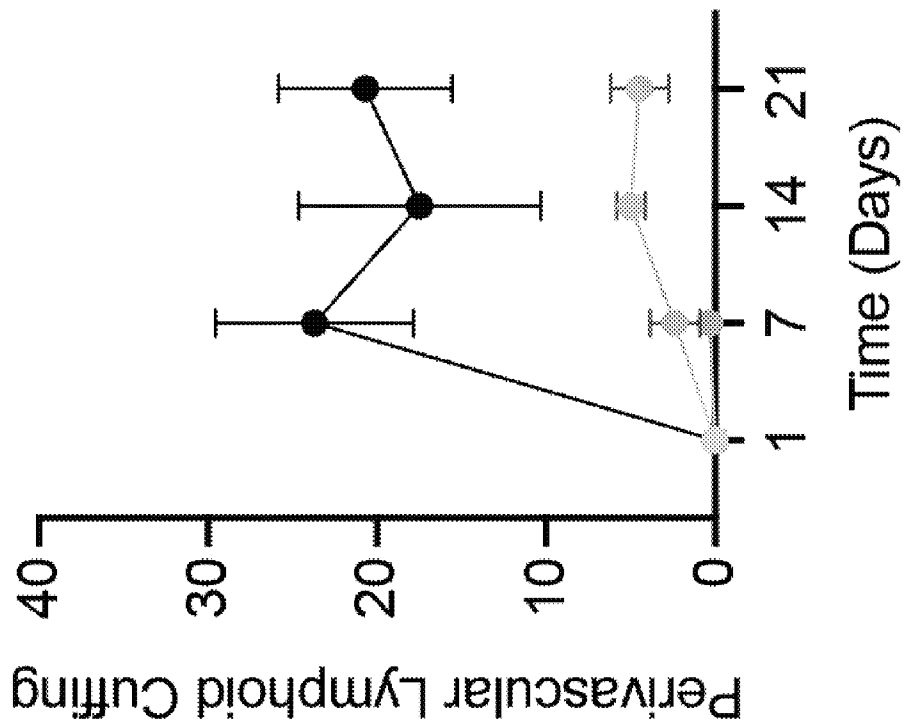
Perivascular Lymphoid Cuffing
40
30
20
10
0
1
7
14
21
Time (Days)

FIG. 28D

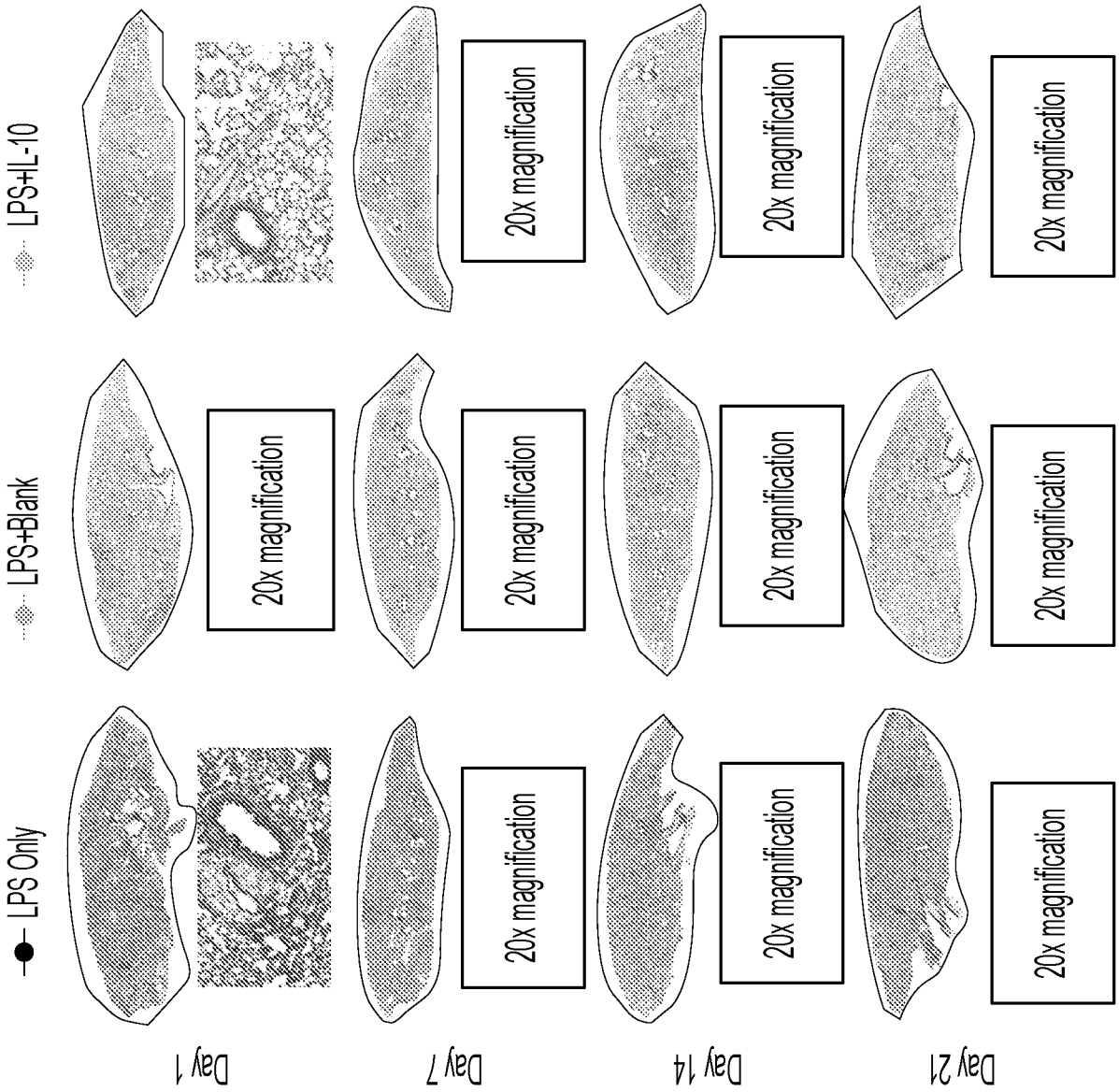
LPS Only
LPS+Blank
LPS+IL-10
Day 1
Day 7
Day 14
Day 21
20x magnification

FIG. 28E

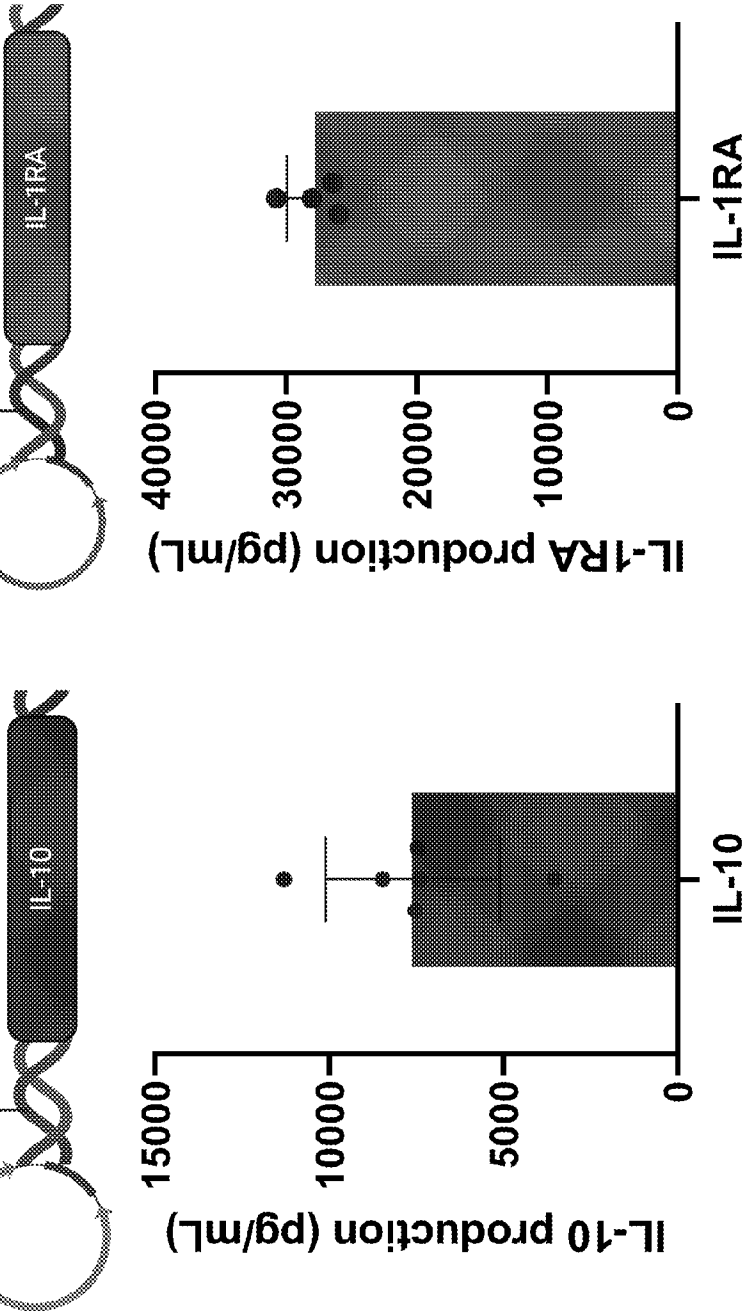

FIG. 29C

FIG. 29B

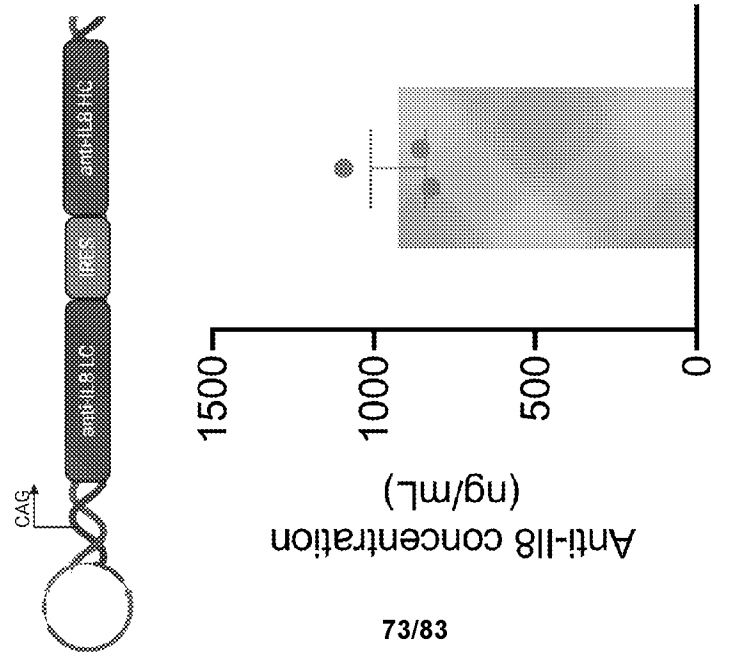

FIG. 29A

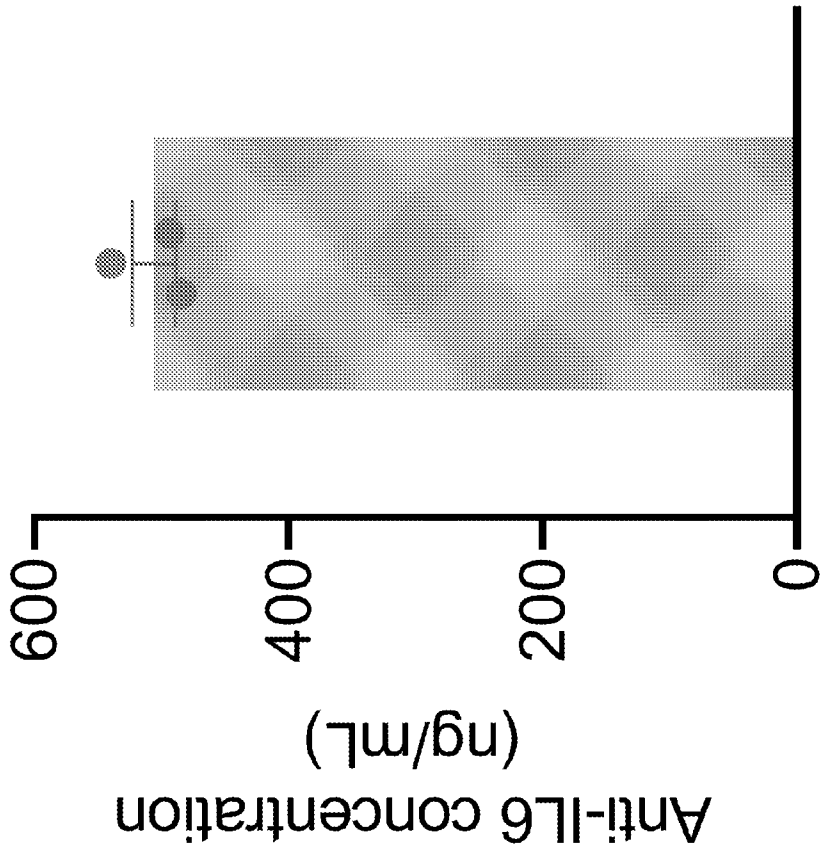

FIG. 30

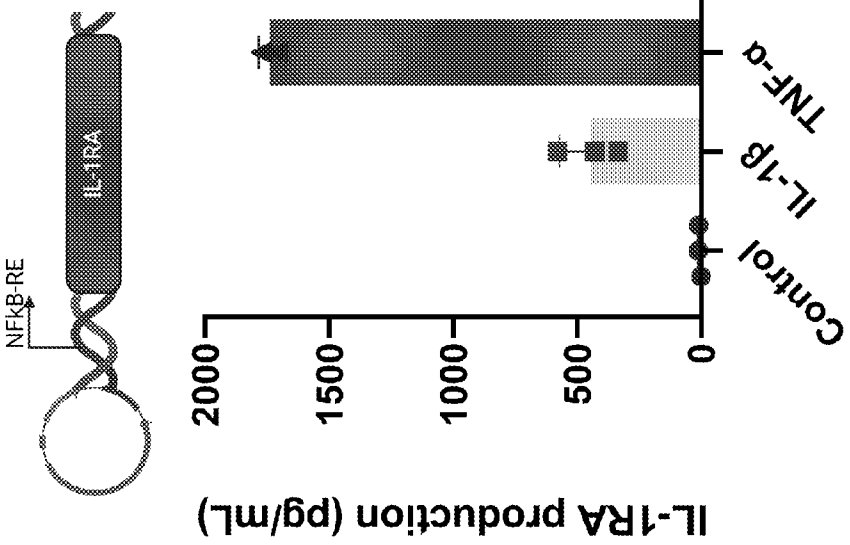

FIG. 31C

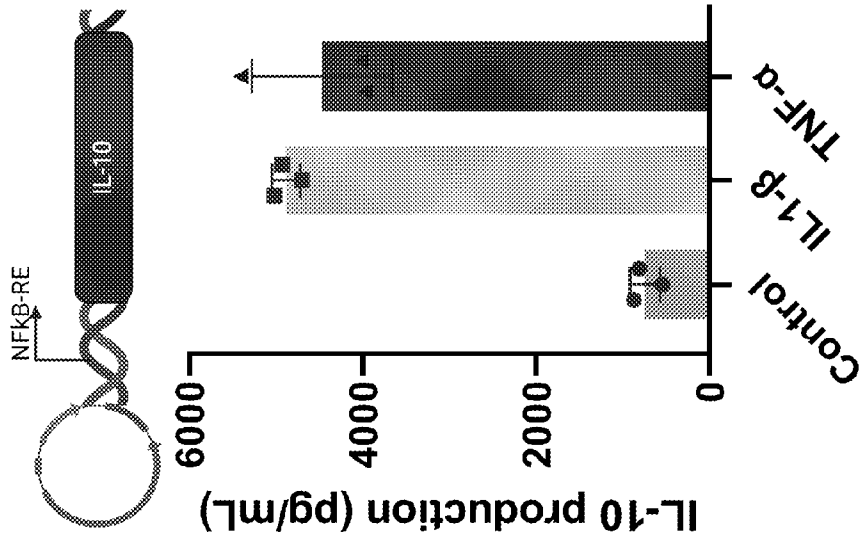

FIG. 31B

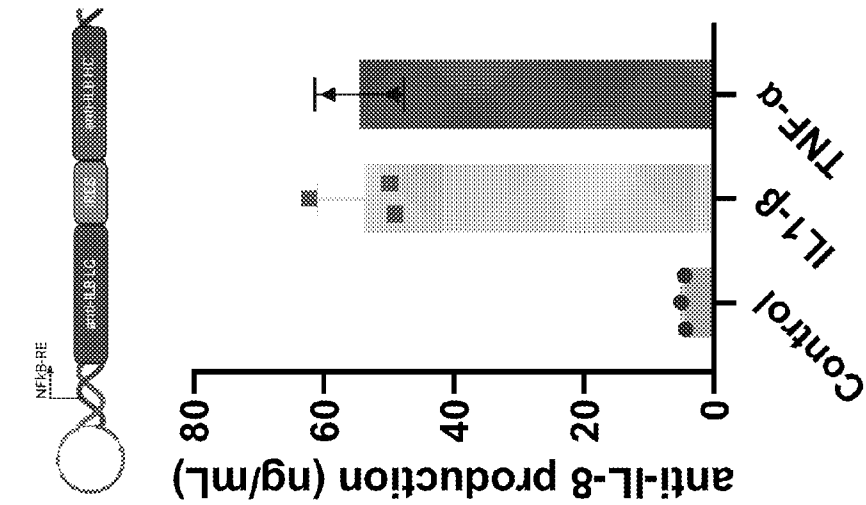

FIG. 31A

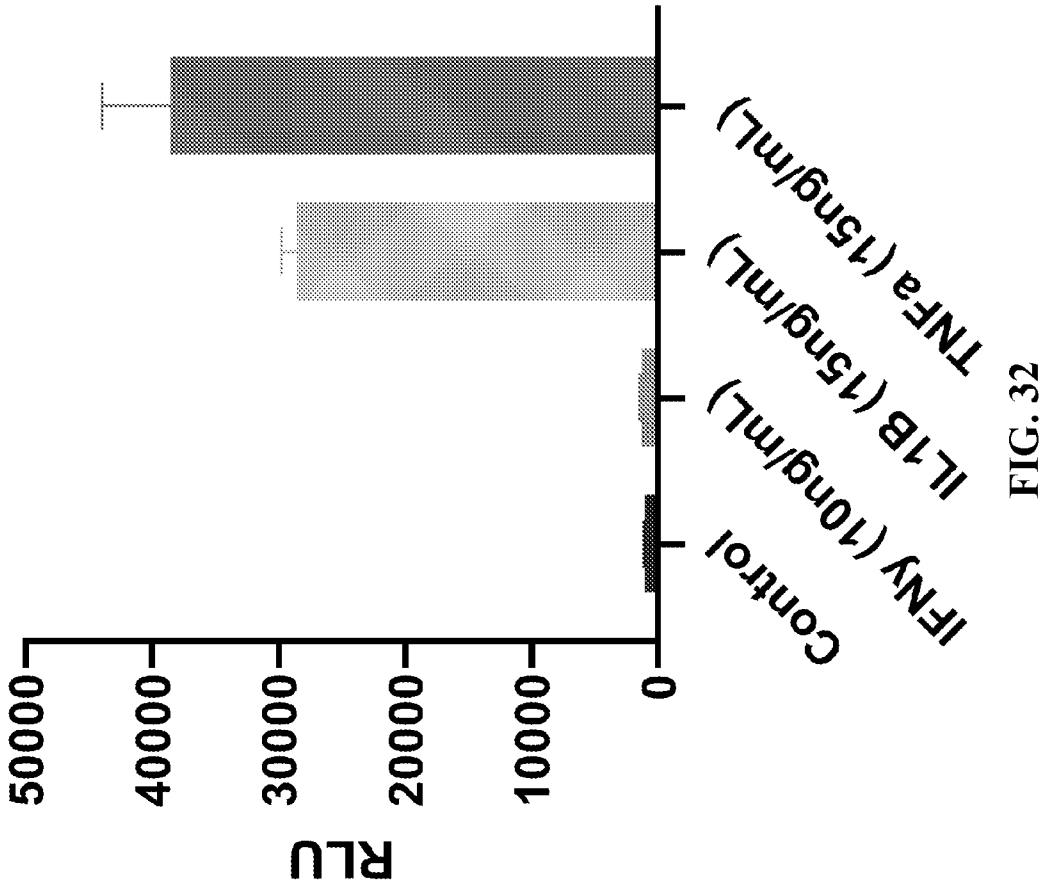
RLU
50000
40000
30000
20000
10000
0
Control
IFNγ (10ng/mL)
IL-1β (15ng/mL)
TNFa (15ng/mL)

FIG. 32

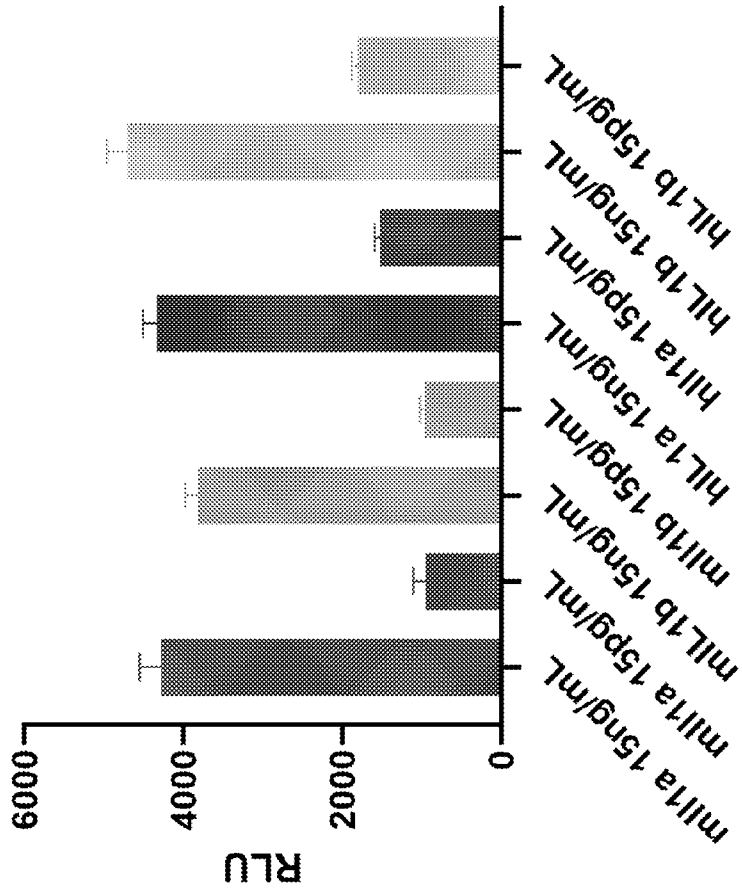
RLU
6000
4000
2000
0
mIl1a 15ng/mL
mIl1a 15pg/mL
mIL-1b 15ng/mL
mIL-1b 15pg/mL
hIl1a 15ng/mL
hIl1a 15pg/mL
hIL-1b 15ng/mL
hIL-1b 15pg/mL

FIG. 33

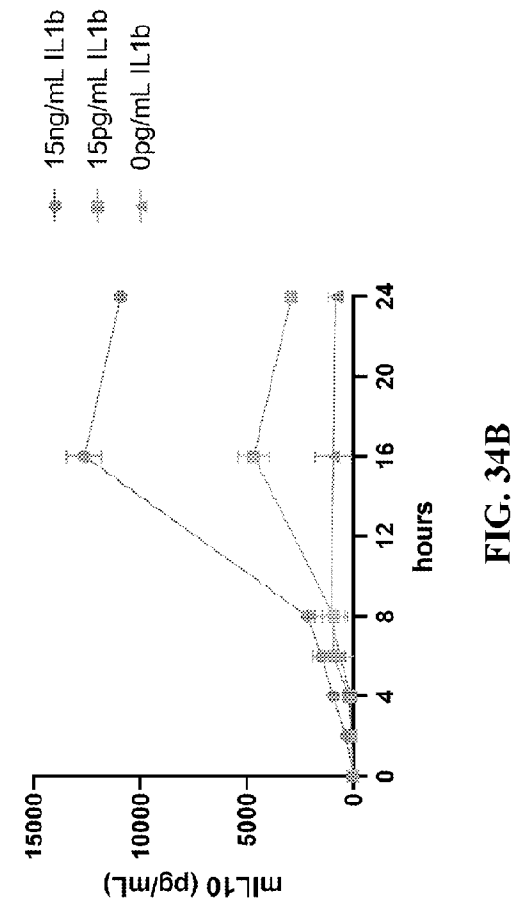

FIG. 34B

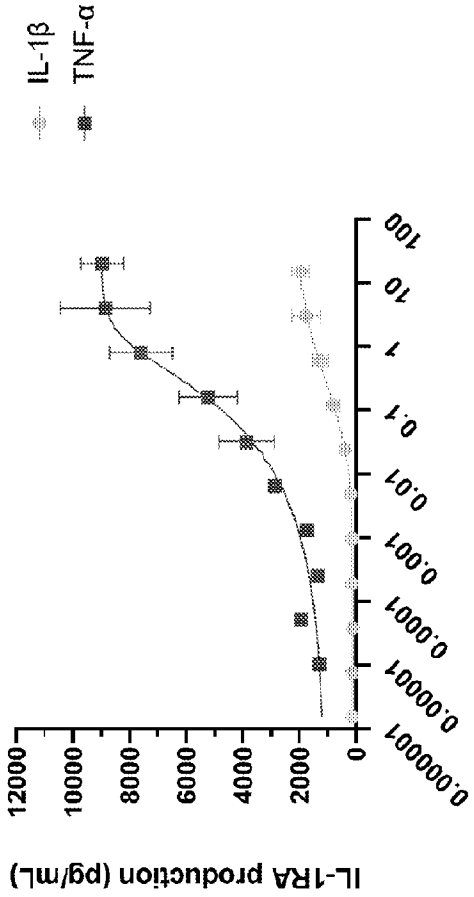

FIG. 34A

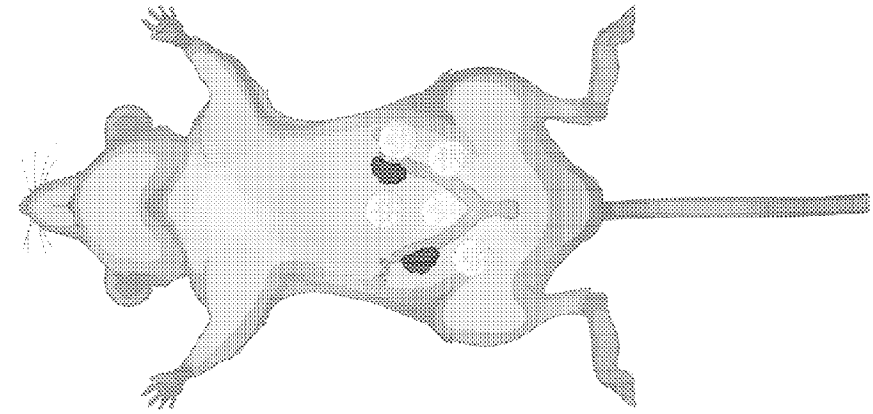

FIG. 35C

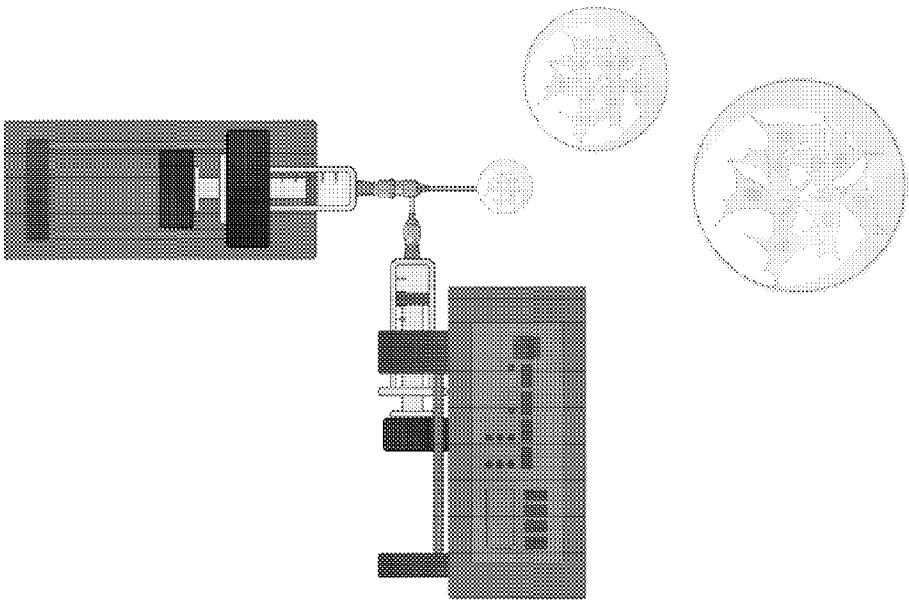

FIG. 35B

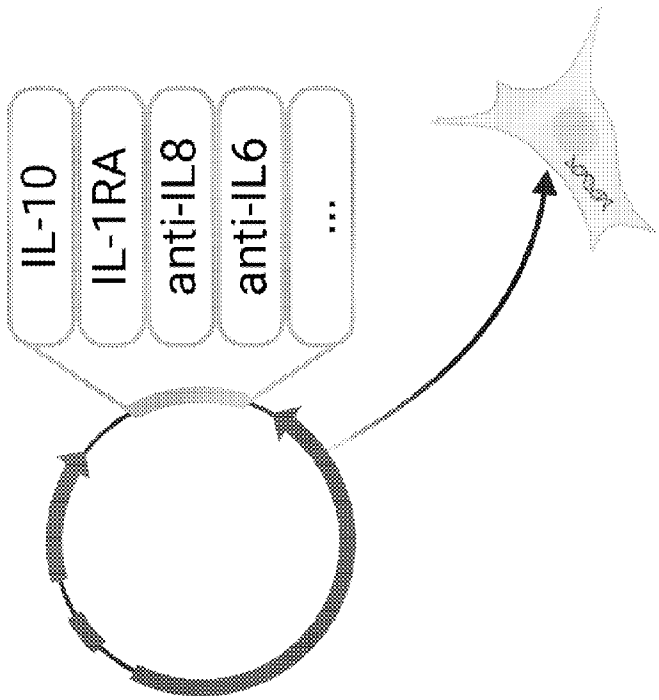

FIG. 35A

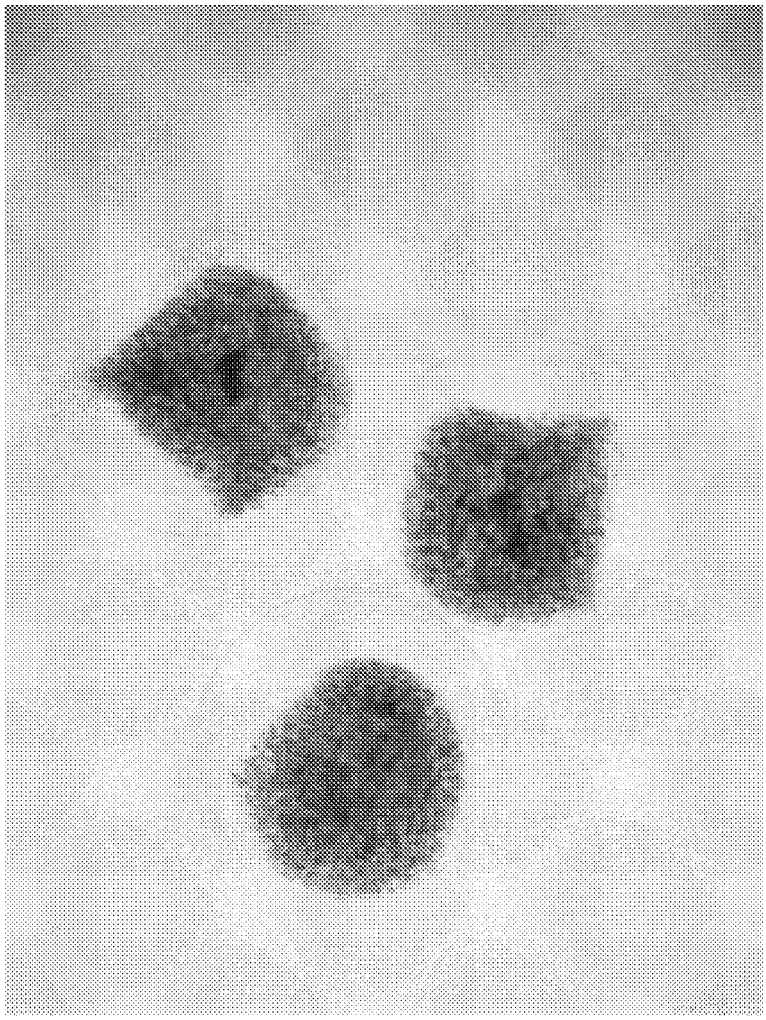

FIG. 36A

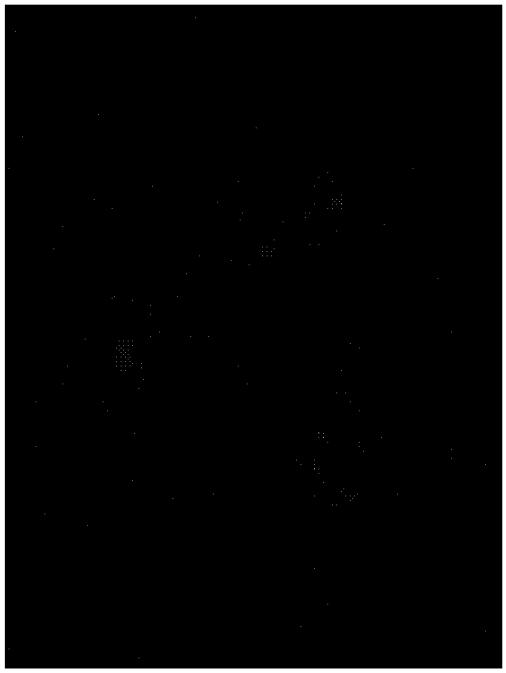

FIG. 36C

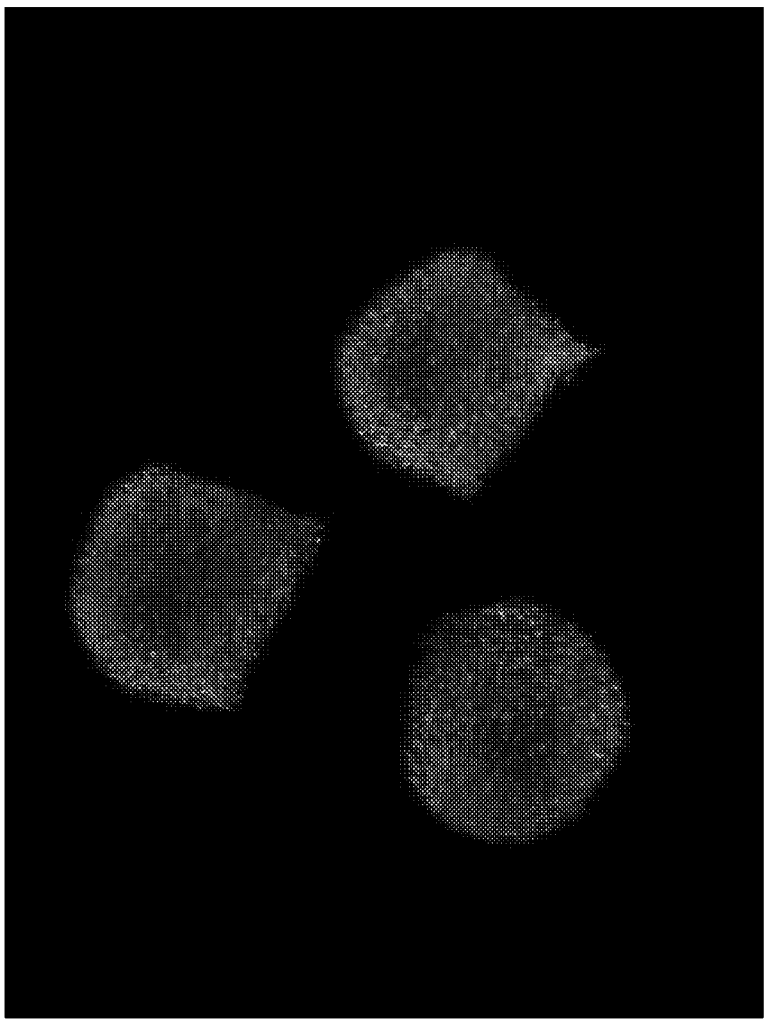

FIG. 36B

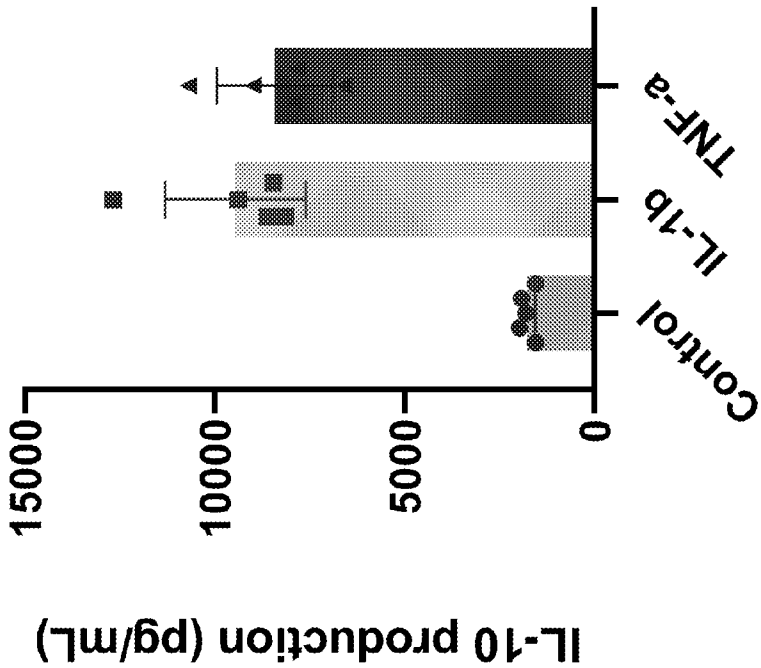

FIG. 36D

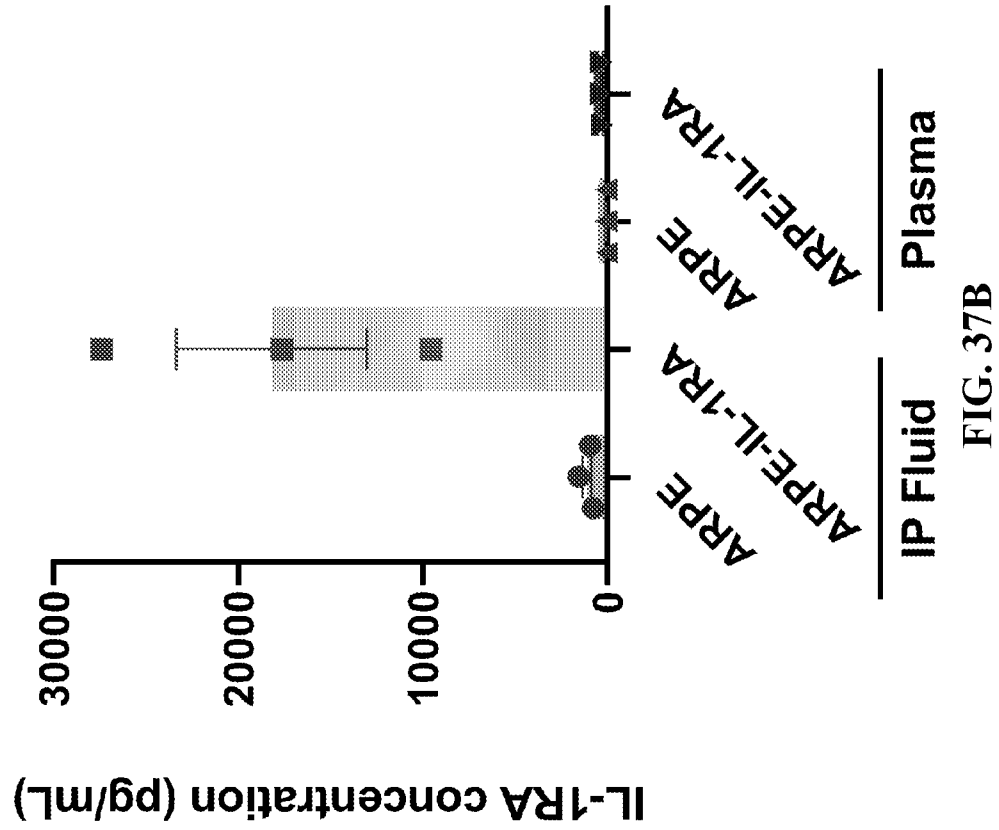

FIG. 37B

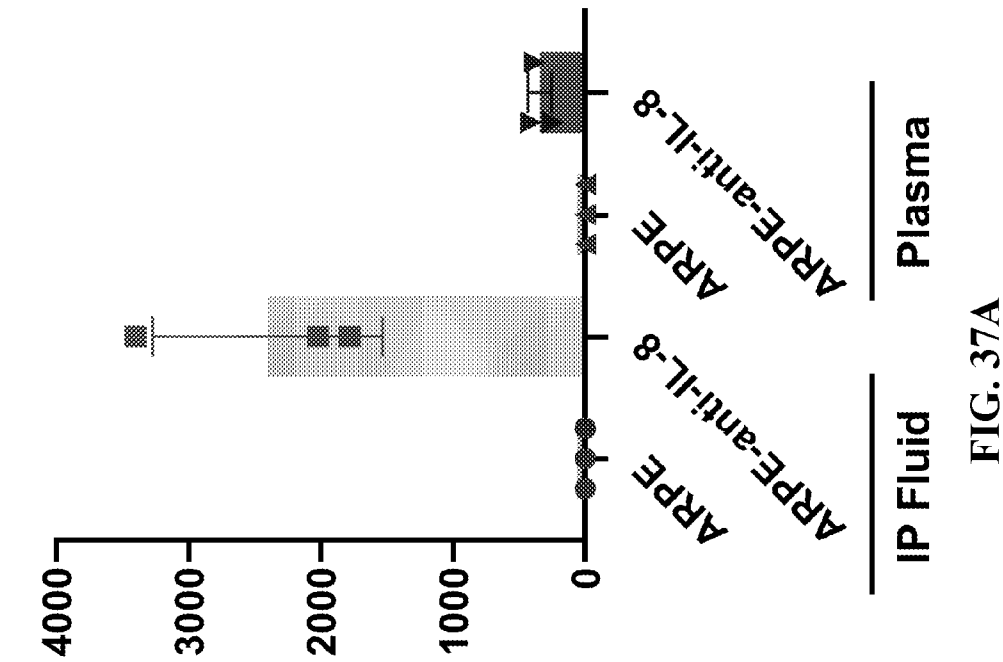

FIG. 37A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/010263

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 35/12*** (2025.01); ***A61K 9/00*** (2025.01); ***A61K 9/50*** (2025.01); ***A61K 38/20*** (2025.01); ***A61P 11/00*** (2025.01); ***C12N 5/00*** (2025.01); ***C12N 5/071*** (2025.01); *A61K 39/00* (2025.01); *A61K 47/36* (2025.01); *C07K 14/545* (2025.01)

CPC: ***A61K35/12; A61K9/0078; A61K9/5036; A61K38/2006; A61P11/00; C12N5/0012; C12N5/0682;*** *A61K47/36; A61K2035/128; A61K2039/5156; C07K14/545; C12N2510/00; C12N2533/74*

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)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 2023/070000 A1 (WILLIAM MARSH RICE UNIVERSITY) 27 April 2023 (27.04.2023)	
X	Abstract; page 3, lines 4-6, 9-12; page 7, lines 7-10; page 15, lines 9-10, 24-25, 27-28, 32-33; page 16, lines 1-17; page 17, lines 4-5, 20-22; page 18, lines 5-10; page 21, lines 33-34; page 27, lines 23-24, 32-33; page 28, lines 18-21; page 30, lines 15-16; page 41, lines 16-19; page 51, lines 31-32; page 53, lines 31-33; page 54, lines 27-29; page 58, line 14; page 76, lines 25-26 and page 77, lines 6-7	1-10, 12-14, 20, 22-27, 31-39, 41-43 and 45-47
Y		21 and 44
	US 8,377,442 B2 (MASAT ET AL.) 19 February 2013 (19.02.2013)	
X	column 35, lines 44-46; column 42, lines 18-23, 29-34; column 45, lines 61-65; column 46, lines 1-8, 16-21 and column 48, lines 48-52	1-3, 11, 14-15, 18, 27, 30, and 40
	US 2010/0063005 A1 (FIALA) 11 March 2010 (11.03.2010)	
Y	Abstract; paragraphs [0007], [0018], [0020], [0030], [0034], [0038] and [0045]; claim 7	21 ad 44

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"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
19 April 2025 (19.04.2025)	**01 May 2025 (01.05.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**SHANE THOMAS**
Facsimile No. **571-273-8300**	Telephone No. **PCT Helpdesk: (571) 272-4300**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	GHANTA. "Cell Based Immunomodulation to Suppress Lung Inflammation and Promote Repair" 1-7. NIH. Https://reporter.nih.gov/project-details/10940864. 16 August 2024	
P,X	Entire Document	1-10, 23-27, 30-39, 42, 46-47
P,A		11-15, 18, 20-22 and 40-41, 43-45
	AGHLARA FOTOVAT. "Poster W19 - Alginate Encapsulated Cells for Local Delivery ofImmunomodulatory Cytokines in Acute Respiratory Distress Syndrome" 1-3. BMES. Https://2024bmesannual.eventscribe.net/fsPopup.asp?PresentationID=1501367&mode=presInfo. 24 October 2024	
P,X	Entire Document	1-10, 23-27, 30-39, 42, 46-47
P,A		11-15, 18, 20-22 and 40-41, 43-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/010263

Box No. III 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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-47, and interleukin-1 (cytokine), Chinese hamster ovary cell (engineered cells), respiratory infection (disease or disorder in the lung or deriving from the lung), and acute bronchitis (respiratory infection) are directed towards a method of treating a disease or disorder in the lung or deriving from the lung.

The method of claims 1-3, 4 (in part), 5-10, 11 (in part), 12, 13 (in part), 14, 15 (in part), 18 (in part), 20-27, 30 (in part), 31-32, 33 (in part), 34-39, 40 (in part), 41, 42 (in part) and 43-47 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass interleukin-1 (cytokine), Chinese hamster ovary cell (engineered cells), respiratory infection (disease or disorder in the lung or deriving from the lung), and acute bronchitis (respiratory infection). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 4,11,13, 15,18, 30, 33, 40 and 42 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional cytokine, engineered cells, disease or disorder in the lung or deriving from the lung, and respiratory infection to be searched. Additional cytokine, engineered cells, disease or disorder in the lung or deriving from the lung, and respiratory infection will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected cytokine, engineered cells, disease or disorder in the lung or deriving from the lung, and respiratory infection. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Exemplary elections would be interleukin-1 alpha (cytokine), retinal pigment epithelial cell (engineered cells), autoimmune disease (disease or disorder in the lung or deriving from the lung), and anti-glomerular basement membrane disease (autoimmune disease).

Groups I+ share the technical features including: a method of treating a disease or disorder in the lung or deriving from the lung, the method comprising: providing a capsule comprising a plurality of encapsulated engineered cells; wherein one or more of the encapsulated engineered cells in the plurality produces a therapeutic agent, e.g., a protein, e.g., a cytokine; and administering the capsule to the subject, thereby treating the disease or disorder in the lung or deriving from the lung in the subject. These shared technical features are previously disclosed by the publication entitled "Microencapsulation of Lefty-secreting engineered cells for pulmonary fibrosis therapy in mice" by Ma et al. (hereinafter "Ma").

Ma discloses a method of treating a disease or disorder in the lung or deriving from the lung (engineered HEK293 cells with Lefty A can attenuate pulmonary fibrosis "lung disease" in vivo, thus providing a novel method to treat human pulmonary fibrotic disease; Abstract), the method comprising: providing a capsule comprising a plurality of encapsulated engineered cells (engineered HEK293 cells were encapsulated in alginate microcapsules; Abstract); wherein one or more of the encapsulated engineered cells in the plurality produces a therapeutic agent, e.g., a protein, e.g., a cytokine (Lefty A "a protein", a potent inhibitor of transforming growth factor-beta signaling for treating organ fibrosis, produced and released from the microencapsulated cells; Abstract; page L742, 1st column, 2nd paragraph; page L744, 1st column, 1st paragraph); and administering the capsule to the subject (The engineered HEK293 cells were encapsulated in alginate microcapsules and then subcutaneously implanted in ICR mice that had 1 wk earlier been intratracheally administered BLM to induce pulmonary fibrosis; Abstract), thereby treating the disease or disorder in the lung or deriving from the lung in the subject (The engineered HEK293 cells with Lefty A attenuate pulmonary fibrosis in vivo in the mice, thus providing a novel method to treat human pulmonary fibrotic disease; Abstract; L746, 1st column, 3rd paragraph).

Since none of the special technical features of Groups I+ inventions are found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Ma reference, unity of invention is lacking.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2025/010263

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

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.: **Claims 1-3, 4 (in part), 5-10, 11 (in part), 12, 13 (in part), 14, 15 (in part), 18 (in part), 20-27, 30 (in part), 31-32, 33 (in part), 34-39, 40 (in part), 41, 42 (in part) and 43-47, interleukin-1 (cytokine), Chinese hamster ovary cell (engineered cells), respiratory infection (disease or disorder in the lung or deriving from the lung), and acute bronchitis (respiratory infection).**

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.