magnetic-bead based products lack the ability to release bound cells readily from capture molecules in a manner that does not alter the viability and phenotype of the isolated cells.

Given the significant interest in, and rapid expansion of, T cell engineering-based cancer therapies, there is a significant need for improved T cell expansion and harvesting methods that overcome the above limitations of existing approaches, particularly for downstream clinical applications. Specifically, there is a substantial need for technologies to enable ex vivo cell expansion protocols to meet clinical specifications, to expand T and other immune cells consistently and reproducibly, to preserve cell viability and function, and to be applicable to different cell sources and expansion agents.

SUMMARY OF THE INVENTION

In one aspect, the present disclosure refers to a method of generating a population of expanded immune cells, the method comprising contacting a starting population of immune cells with a plurality of particles comprising a complex comprising a hydrogel and a binding moiety, wherein: (a) the hydrogel comprises an alginic acid-polyethylene glycol (PEG) copolymer; and (b) the binding moiety is configured to bind a cell surface component of an immune cell;; wherein the contact is operative to induce a metabolic change in the starting population of immune cells, thereby generating a population of expanded immune cells.

The present disclosure features a biocompatible hydrogel complex capable of binding to, activating, and expanding immune cell, e.g., T cells. In certain examples, the hydrogel complex can be dissolved, e.g., simply by reducing cation concentration, e.g., by introducing a chelating agent, enabling efficient production of large numbers of T cells for adoptive transfer systems and other uses of immune cells. Also provided herein are methods for producing hydrogel complexes and methods of generating expanded and/or activated immune cell, e.g., T cell, populations using the hydrogel complexes of the disclosure.

In one example, the disclosure features a particle including a complex including a hydrogel and a binding moiety, wherein the hydrogel includes a polymer; and the binding moiety is configured to bind a cell surface component of an immune cell.

In some examples, the polymer includes a natural polymer. Exemplary natural polymers are alginate, agarose, carrageenan, chitosan, dextran, carboxymethylcellulose, heparin, hyaluronic acid, polyamino acids, collagen, gelatin, fibrin, fibrous protein-based biopolymers, and any combination thereof. In other examples, the polymer includes a synthetic polymer. Exemplary synthetic polymers are alginic acid-polyethylene glycol copolymer, poly(ethylene glycol) (PEG), poly(2-methyl-2-oxazoline) (PMOXA), poly(ethylene oxide), poly(vinyl alcohol), and poly(acrylamide), poly(n-butyl acrylate), poly-(α-esters), poly(glycolic acid), poly(lactic-co-glycolic acid), poly(L-lactic acid), poly(N-isopropylacrylamide), butyryl-trihexyl-citrate, di(2-ehtylhexyl)phthalate, di-iso-nonyl-1,2-

cyclohexanedicarboxylate, polytetrafluoroethylene (e.g., expanded), ethylene vinyl alcohol copolymer, poly(hexamethylene diisocyanate), poly(ethylene) (e.g., high density, low density, or ultrahigh molecular weight), highly crosslinked poly(ethylene), poly(isophorone diisocyanate), poly(amide), poly(acrylonitrile), poly(carbonate), poly(caprolactone diol), poly(D-lactic acid), poly(dimethylsiloxane), poly(dioxanone), polyether ether ketone, polyester polymer alloy, polyether sulfone, poly(ethylene terephthalate), poly(hydroxyethyl methacrylate), poly(methyl methacrylate), poly(methylpentene), poly(propylene), polysulfone, poly(vinyl chloride), poly(vinylidene fluoride), poly(vinylpyrrolidone), poly(styrene-b-isobutylene-b-styrene), and any combination thereof.

The polymer may also be a copolymer, e.g., a copolymer of a natural polymer, e.g., alginate, with a synthetic polymer, e.g., PEG or PMOXA. Alternatively, the polymer is not a copolymer of alginate and PEG. Other examples include a copolymer of hyaluronic acid with PEG or dextran with PEG. In other examples, the polymer is not a copolymer of alginate with a fluoropolymer or a silicone.

In some examples, the cell surface component is CD2, CD3, CD19, CD24, CD27, CD28, CD31, CD34, CD45, CD46, CD80, CD86, CD133, CD134, CD135, CD137, CD160, CD335, CD337, CD40L, ICOS, GITR, HVEM, Galtectin 9, TIM-1, LFA-1, PD-L1, PD-L2, B7-H3, B7-H4, ILT3, ILT4, CDTL-4, PD-1, BTLA, MHC-I, MHC-II, Delta-like ligand (e.g., DLL-Fc, DLL-1, or DLL-4), WNT3, stem cell factor, or thrombopoietin. In some examples, the cell surface component is CD2, CD3, CD27, CD28, CD46, CD80, CD86, CD134, CD137, CD160, CD40L, ICOS, GITR, HVEM, Galtectin 9, TIM-1, LFA-1, PD-L1, PD-L2, B7-H3, B7-H4, ILT3, ILT4, CDTL-4, PD-1, BTLA, MHC-I, or MHC-II. Alternatively, the cell surface component is not CD2, CD3, CD27, CD28, CD46, CD80, CD86, CD134, CD137, CD160, CD40L, ICOS, GITR, HVEM, Galtectin 9, TIM-1, LFA-1, PD-L1, PD-L2, B7-H3, B7-H4, ILT3, ILT4, CDTL-4, PD-1, BTLA, MHC-I, or MHC-II, in particular when the polymer is a copolymer of alginate, e.g., a copolymer of alginate and PEG. In other examples, the cell surface component is CD24, CD31, CD34, or CD45. Alternatively, the cell surface component is not CD24, CD31, CD34, or CD45, in particular when the polymer is a copolymer of alginate, e.g., a copolymer of alginate and PEG. In other examples, the cell surface component is CD19, CD34, CD45, CD133, CD135, CD335, CD337, DLL-Fc, DLL-1, or DLL-4, WNT3, stem cell factor, or thrombopoietin, e.g., CD19, CD133, CD135, CD335, CD337, Delta-like ligand (e.g., DLL-Fc, DLL-1, or DLL-4), WNT3, stem cell factor, or thrombopoietin.

In some examples, the surface of the particle includes at least one binding moiety per square μm (e.g., at least 1 binding moiety per square μm, at least 2 binding moieties per square μm, at least 3 binding moieties per square μm, at least 4 binding moieties per square μm, at least 5 binding moieties per square μm, at least 10 binding moieties per square μm, at least 20 binding moieties per square μm, at least 30 binding moieties per square μm, at least 40 binding moieties per square μm, or at least 50 binding moieties per square μm). In some instances, one or more of the binding moieties is an antibody or antigen-binding fragment thereof.

The particle may include one, two, three, or more distinct binding moieties.

A binding moiety can be an antibody or antigen-binding fragment thereof. For example, a binding moiety is a monoclonal antibody or antigen-binding fragment thereof, a Fab, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, a monovalent antibody or antigen-binding fragment thereof, a chimeric antibody or antigen-binding fragment thereof, a single-chain Fv molecule, a bispecific single chain Fv ((scFv') 2) molecule, a domain antibody, a diabody, a triabody, an affibody, a domain antibody, a SMIP, a nanobody, a Fv fragment, a Fab fragment, a F(ab') 2 molecule, or a tandem scFv (taFv) fragment. The antibody or antigen binding fragment thereof is, for example, anti-CD2, anti-CD3, anti-CD19, anti-CD24, anti-CD27, anti-CD28, anti-CD31, anti-CD34, anti-CD45, anti-CD46, anti-CD80, anti-CD86, anti-CD133, anti-CD134, anti-CD135, anti-CD137, anti-CD160, anti-CD335, anti-CD337, anti-CD40L, anti-ICOS, anti-GITR, anti-HVEM, anti-Galtectin 9, anti-TIM-1, anti-LFA-1, anti-PD-L1, anti-PD-L2, anti-B7-H3, anti-B7-H4, anti-ILT3, anti-ILT4, anti-CDTL-4, anti-PD-1, anti-BTLA, anti-MHC-I, anti-MHC-II, anti-Delta-like ligand (e.g., anti-DLL-Fc, anti-DLL-1, or anti-DLL-4), anti-WNT3, anti-stem cell factor, or anti-thrombopoietin. In some examples, the antibody or antigen-binding fragment thereof is anti-CD2, anti-CD3, anti-CD27, anti-CD28, anti-CD46, anti-CD80, anti-CD86, anti-CD134, anti-CD137, anti-CD160, anti-CD40L, anti-ICOS, anti-GITR, anti-HVEM, anti-Galtectin 9, anti-TIM-1, anti-LFA-1, anti-PD-L1, anti-PD-L2, anti-B7-H3, anti-B7-H4, anti-ILT3, anti-ILT4, anti-CDTL-4, anti-PD-1, anti-BTLA, anti-MHC-I, or anti- MHC-II. Alternatively, the antibody or antigen-binding fragment thereof is not anti-CD2, anti-CD3, anti-CD27, anti-CD28, anti-CD46, anti-CD80, anti-CD86, anti-CD134, anti-CD137, anti-CD160, anti-CD40L, anti-ICOS, anti-GITR, anti-HVEM, anti-Galtectin 9, anti-TIM-1, anti-LFA-1, anti-PD-L1, anti-PD-L2, anti-B7-H3, anti-B7-H4, anti-ILT3, anti-ILT4, anti-CDTL-4, anti-PD-1, anti-BTLA, anti-MHC-I, or anti- MHC-II, in particular when the polymer is a copolymer of alginate, e.g., a copolymer of alginate and PEG. In some examples, the antibody or antigen-binding fragment thereof is anti-CD24, anti-CD31, anti-CD34, or anti-CD45. Alternatively, the antibody or antigen-binding fragment thereof is not anti-CD24, anti-CD31, anti-CD34, or anti-CD45, in particular when the polymer is a copolymer of alginate, e.g., a copolymer of alginate and PEG. In other examples, the antibody or antigen-binding fragment thereof is anti-CD19, anti-CD34, anti-CD45, anti-CD133, anti-CD335, anti-CD337, anti-DLL-Fc, anti-DLL-1, anti-DLL-4 anti-WNT3, anti-stem cell factor, or anti-thrombopoietin, e.g., anti-CD19, anti-CD133, anti-CD335, anti-CD337, anti-Delta-like ligand (e.g., anti-DLL-Fc, anti-DLL-1, or anti-DLL-4), anti-WNT3, anti-stem cell factor, or anti-thrombopoietin.

The binding moiety may be a signal 1 stimulus (e.g., anti-CD3) or a signal 2 stimulus (e.g., anti-CD28). In complexes having both a signal 1 stimulus (e.g., anti-CD3) and a signal 2 stimulus (e.g., anti-CD28), the molar ratio of the signal 1 stimulus and the signal 2 stimulus can be between about 1:100 and about 100:1 (e.g., from 1:80 to 80:1, from 1:60 to 60:1, from 1:50 to 50:1, from 1:40 to 40:1, from 1:30 to 30:1, from 1:20 to 20:1, from 1:10 to 10:1, from 1:5 to 5:1, from 1:2 to 2:1, or about 1:1). In some examples, the signal 1 stimulus is antigen-specific.

Alternatively, a complex may include three or more types of binding moieties. For example, in some examples, the complex includes a signal 1 stimulus (e.g., anti-CD3), a signal 2 stimulus (e.g., anti-CD28), and an additional stimulus, such as an activating stimulus, a suppressive stimulus, or a polarizing stimulus, such as a cytokine (e.g., a surface-bound cytokine, e.g., a trans-presented interleukin).

In certain examples, the binding moiety is a cytokine. For example, the cytokine is IL-1, IL-2, IL-3, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, TNF-α, or IFN-γ. In other examples, the binding moiety is chemokine (C-X-C motif) ligand 12 or low-density lipoprotein.

The immune cell is, for example, naïve and memory T cells, T helper cell, regulatory T cell, NK cell, NK T cell, CIK cell, TIL cell, HS cell (undifferentiated and differentiated), MS cell (undifferentiated and differentiated), iPS cell (undifferentiated and differentiated), B cell, macrophage, dendritic cell, neutrophil, stromal cell, and ES cell (undifferentiated and differentiated). In certain examples, the immune cell is an NK cell, CIK cell, TIL cell, HS cell (undifferentiated and differentiated), MS cell (undifferentiated and differentiated), iPS cell (undifferentiated and differentiated), or ES cell (undifferentiated and differentiated). In other examples, the immune cell is a T cell, e.g., a naïve or memory T cell, T helper cell, regulatory T cell, Natural Killer T cell, or Cytotoxic T Lymphocyte, or a B cell, macrophage, dendritic cell, neutrophil, or stromal cell.

In some examples, the polymer can change from a solid matrix into a solution or suspension, e.g., in response to a sufficient decrease of cationic concentration in the environment of the polymer, change in temperature, change in pH, due to hydrolysis, oxidation, enzymatic degradation, physical degradation, or other mechanism. For example, the decrease in the cationic concentration in the environment of the polymer can be caused by the presence of EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyridyl sulfonate, dioxane, DME, diglyme, or triglyme. In some examples, the cation is Li^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, or Al^{3+}. In some examples, the particle completely liquefies, e.g., in response to a sufficient decrease of cationic concentration in the environment of the particle, e.g., the particle does not further include a separation unit, such as a magnetic bead substrate or magnetic particles.

In some examples, the hydrogel has an elastic modulus of less than 1 gigapascal (GPa), e.g., 0.8 GPa, 0.6 GPa, 0.4 GPa, 0.2 GPa, 0.1 GPa, 0.08 GPa, 0.06 GPa, 0.04 GPa, 0.02 GPa, 0.01 GPa, 0.008 GPa, 0.006 GPa, 0.004 GPa, 0.002 GPa, 0.001 GPa, 0.0008 GPa, 0.0006 GPa, 0.0004 GPa, 0.0002 GPa, or 0.0001 GPa. In some examples, the hydrogel has an elastic modulus of less than 100,000 pascals (Pa).

In certain examples, the particle has at least one cross-sectional dimension of between about 50 nm and about 100 µm (e.g., from 1 µm to 50 µm). For example, the complex is substantially spherical and has a diameter of between about 1 µm and 100 µm (e.g., from 2 µm to 80 µm, from 3 µm to 50

μm, from 4 μm to 25 μm, from 5 μm to 15 μm, from 8 μm to 12 μm, or about 10 μm). In some examples, the average diameter of a plurality of complexes is between about 1 μm and 100 μm (e.g., from 2 μm to 80 μm, from 3 μm to 50 μm, from 4 μm to 25 μm, from 5 μm to 15 μm, from 8 μm to 12 μm, or about 10 μm).

The binding moiety of the complex may be covalently or non-covalently attached to the hydrogel. In some examples, the binding moiety is attached through a linker, such as an avidin-biotin linker (e.g., a streptavidin-biotin linker). For example, the hydrogel is covalently conjugated with streptavidin, followed by non-covalent conjugation to a biotinylated binding moiety.

In certain examples, the polymer is a copolymer of alginate, e.g., with PEG or PMOXA. In such examples, the immune cell is, for example, an NK cell, CIK cell, TIL cell, HS cell (undifferentiated and differentiated), MS cell (undifferentiated and differentiated), iPS cell (undifferentiated and differentiated), or ES cell (undifferentiated and differentiated). In other such examples, the binding moiety is a cytokine, e.g., IL-1, IL-2, IL-3, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, TNF-α, or IFN-γ, or the binding moiety is chemokine (C-X-C motif) ligand 12 or low-density lipoprotein. In further such examples, the cell surface component is CD19, CD133, CD134, CD335, CD337, Delta-like ligand (e.g., DLL-Fc, DLL-1, or DLL-4), WNT3, stem cell factor, or thrombopoietin. For example, the binding moiety is anti-CD19, anti-CD133, anti-CD134, anti-CD335, anti-CD337, anti-Delta-like ligand (e.g., anti-DLL-Fc, anti-DLL-1, or anti-DLL-4), anti-WNT3, anti-stem cell factor, or anti-thrombopoietin.

In a further example, the disclosure features a method of generating a population of expanded immune cells by contacting a starting population of immune cells with a plurality of particles of the disclosure, wherein the contact is operative to induce a metabolic change in the starting population of immune cells, thereby generating a population of expanded immune cells.

In certain examples, the particles change from a solid matrix into a solution or suspension, e.g., in response to a sufficient decrease of cationic concentration in the environment of the polymer. In other examples, the particles are administered to a culture comprising the population of immune cells at a particle-to-cell ratio from 1:20 to 20:1. For example, the particle-to-cell ratio is a particle-to-immune cell ratio, e.g., about 5:1. Alternatively, the particle-to-cell ratio is a complex-to-peripheral blood mononuclear cell (PBMC) ratio, e.g., about 10:1. In certain examples, the population of expanded immune cells includes 100-fold the number of immune cells relative to the starting population. In other examples, the population of expanded immune cells includes activated immune cells.

In one example, the disclosure features a complex including a hydrogel and a binding moiety, wherein the hydrogel comprises an alginic acid-polyethylene glycol (PEG) copolymer; and the binding moiety is configured to bind a cell surface component of a T cell. The alginic acid-PEG copolymer can change from a solid matrix into a solution or suspension in response to a sufficient decrease of cationic concentration in the environment of the polymer. For example, the decrease in the cationic

concentration in the environment of the polymer can be caused by the presence of EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyridyl sulfonate, dioxane, DME, diglyme, or triglyme. In some examples, the cation is Li^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, or Al^{3+}.

In some examples, the complex completely liquefies in response to a sufficient decrease of cationic concentration in the environment of the complex, e.g., the complex does not further include a separation unit, such as a magnetic bead substrate or magnetic particles.

In some examples, the elastic modulus of the hydrogel is sufficient both to induce expansion and skew the phenotype of an expanding population. For example, the hydrogel may have an elastic modulus of less than 100,000 pascals (Pa). Such properties can be imparted by the molecular structure of the copolymer. For example, the alginic acid-PEG copolymer can include a multi-arm PEG molecule (e.g., a four-arm PEG molecule).

The geometry of the hydrogel complex influences its contact with cells and therefore may impact expansion. In some examples, the complex has at least one cross-sectional dimension of between about 50 nm and about 100 µm (e.g., from 1 µm to 50 µm). For example, the complex may be substantially spherical and have a diameter of between about 1 µm and 100 µm (e.g., from 2 µm to 80 µm, from 3 µm to 50 µm, from 4 µm to 25 µm, from 5 µm to 15 µm, from 8 µm to 12 µm, or about 10 µm). In some examples, the average diameter of a plurality of complexes is between about 1 µm and 100 µm (e.g., from 2 µm to 80 µm, from 3 µm to 50 µm, from 4 µm to 25 µm, from 5 µm to 15 µm, from 8 µm to 12 µm, or about 10 µm).

The binding moiety of the complex may be covalently or non-covalently attached to the hydrogel (e.g., the hydrogel particle, e.g., covalently attached to an alginic acid domain of the alginic acid-PEG copolymer). In some examples, the binding moiety can be can be attached through a linker, such as an avidin-biotin linker (e.g., a streptavidin-biotin linker). For example, the hydrogel can be covalently conjugated with streptavidin, followed by non-covalent conjugation to a biotinylated binding moiety.

In some examples, the surface of the hydrogel complex includes at least one binding moiety per square µm (e.g., at least 1 binding moiety per square µm, at least 2 binding moieties per square µm, at least 3 binding moieties per square µm, at least 4 binding moieties per square µm, at least 5 binding moieties per square µm, at least 10 binding moieties per square µm, at least 20 binding moieties per square µm, at least 30 binding moieties per square µm, at least 40 binding moieties per square µm, or at least 50 binding moieties per square µm). In some instances, one or more of the binding moieties is an antibody or antigen-binding fragment thereof.

The binding moiety may be a signal 1 stimulus (e.g., anti-CD3) or a signal 2 stimulus (e.g., anti-CD28). In complexes having both a signal 1 stimulus (e.g., anti-CD3) and a signal 2 stimulus (e.g.,

anti-CD28), the molar ratio of the signal 1 stimulus and the signal 2 stimulus can be between about 1:100 and about 100:1 (e.g., from 1:80 to 80:1, from 1:60 to 60:1, from 1:50 to 50:1, from 1:40 to 40:1, from 1:30 to 30:1, from 1:20 to 20:1, from 1:10 to 10:1, from 1:5 to 5:1, from 1:2 to 2:1, or about 1:1). In some examples, the signal 1 stimulus is antigen-specific.

Alternatively, a complex may include three or more types of binding moieties. For example, in some examples, the complex includes a signal 1 stimulus (e.g., anti-CD3), a signal 2 stimulus (e.g., anti-CD28), and an additional stimulus, such as an activating stimulus, a suppressive stimulus, or a polarizing stimulus, such as a cytokine (e.g., a surface-bound cytokine, e.g., a trans-presented interleukin).

A binding moiety can be an antibody or antigen-binding fragment thereof. For example, a binding moiety can be a monoclonal antibody or antigen-binding fragment thereof, a Fab, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, a monovalent antibody or antigen-binding fragment thereof, a chimeric antibody or antigen-binding fragment thereof, a single-chain Fv molecule, a bispecific single chain Fv ((scFv') 2) molecule, a domain antibody, a diabody, a triabody, an affibody, a domain antibody, a SMIP, a nanobody, a Fv fragment, a Fab fragment, a F(ab') 2 molecule, or a tandem scFv (taFv) fragment. In some examples, the antibody or antigen-binding fragment thereof is anti-CD2, anti-CD3, anti-CD27, anti-CD28, anti-CD46, or anti-CD137.

In one example, the disclosure features a complex including a hydrogel particle and at least two binding moieties, wherein the hydrogel particle includes an alginic acid-PEG copolymer and Ca^{2+}, and the binding moieties include anti-CD3 and anti-CD28, wherein the alginic acid-PEG copolymer changes from a solid matrix into a solution or suspension in response to a sufficient decrease of Ca^{2+} concentration in the environment of the copolymer.

In some examples, the complex completely liquefies in response to a sufficient decrease of cationic concentration in the environment of the complex, e.g., the complex does not further include a separation unit, such as a magnetic bead substrate or magnetic particles.

In another example, the disclosure features a complex of the disclosure produced by atomization of an alginic acid-PEG copolymer. For example, an alginic acid-PEG copolymer solution can be passed through an atomizer to produce an atomized spray. The spray can be directed into a receiving solution having a cationic concentration sufficient to result in crosslinking of the alginic acid-PEG copolymer, thereby generating an alginic acid-PEG particle (e.g., a microparticle or nanoparticle). To produce the complex, the particle is then conjugated with a binding moiety.

In some examples, the alginic acid-PEG copolymer solution flows through the atomizer at a volumetric percentage from 30% to 90%. The droplets may be produced in the atomizer by an injection of gas

(e.g., pressurized gas, e.g., pressurized air or nitrogen), e.g., from 1 to 200 pounds per square inch (psi). In some examples, the alginic acid-PEG copolymer solution may flow through the atomizer at a rate from 0.1 to 100 mL per minute. In other examples, the rate of gas flow through the atomizer is independent from the rate of alginic acid-PEG copolymer solution, such as in the case of an external mix atomizer. In further examples, the atomizer produces a round spray pattern, e.g., at a spray angle from 10° to 30°.

In some examples, the complex produced by atomization completely liquefies in response to a sufficient decrease of cationic concentration in the environment of the complex, e.g., the complex does not further include a separation unit, such as a magnetic bead substrate or magnetic particles.

In another example, the disclosure provides a method of producing an alginic acid-PEG particle by passing an alginic acid-PEG copolymer solution through an atomizer to produce an atomized solution. The atomized solution is contacted with a receiving solution having a cation, which generates an alginic acid particle. In some examples, a binding moiety is further conjugated to the alginic acid-PEG particle to produce a hydrogel complex. The hydrogel complex can have an elastic modulus of less than 100,000 Pa. In some examples, the binding moiety binds to a cell surface component of a T cell.

In some examples, the alginic acid-PEG copolymer solution flows through the atomizer at a volumetric percentage from 30% to 90%. The droplets may be produced in the atomizer by an injection of gas (e.g., pressurized gas, e.g., pressurized air or nitrogen), e.g., from 1 to 200 pounds per square inch (psi). In some examples, the alginic acid-PEG copolymer solution may flow through the atomizer at a rate from 0.1 to 100 mL per minute. In other examples, the rate of gas flow through the atomizer is independent from the rate of alginic acid-PEG copolymer solution, such as in the case of an external mix atomizer. In further examples, the atomizer produces a round spray pattern, e.g., at a spray angle from 10° to 30°.

In another example, the disclosure features a method of generating a population of expanded T cells, wherein the method involves contacting a starting population of T cells with a plurality of complexes of any of the preceding examples, wherein the contact induces a metabolic change in the starting population of T cells, thereby generating a population of expanded T cells. In some examples, the method further includes liquefying some of all of the complexes by exposing a cation chelator to the complexes and the population of expanded T cells. In some examples, the complex completely liquefies in response to a sufficient decrease of cationic concentration in the environment of the complex, e.g., the complex does not further include a separation unit, such as a magnetic bead substrate or magnetic particles.

The complexes can be administered to a culture having the population of T cells at a complex-to-cell ratio from 1:1 to 20:1, or from 1:20 to 20:1 (e.g., a ratio of complexes to any phenotype of cells, including T cells and other cell types, for example, B cells, macrophages, dendritic cells, neutrophils,

or stromal cells). Additionally or alternatively, the complexes can be administered to a culture comprising the population of T cells at a complex-to-T cell ratio from about 1:1 to 20:1 (e.g., about 5:1). Additionally or alternatively, the complexes can be administered to a culture comprising the population of T cells at a complex-to-peripheral blood mononuclear cell (PBMC) ratio from about 1:1 to about 20:1 (e.g., about 10:1).

Methods of the disclosure enable expansion of a T cell population such that the expanded T cells differ in phenotype compared to the starting population. For example, an expanded population may have a greater number of activated T cells compared to the starting population. Additionally or alternatively an expanded population may include a greater number or percentage of $CD8^+$ T cells than the starting population. Conversely, the population of expanded T cells may include a lower number or percentage of $CD4^+$ T cells than the starting population. Additionally or alternatively, the population of expanded T cells may include a greater CD8-to-CD4 T cell ratio than the starting population. In general, the population of expanded T cells will include a greater overall number of T cells relative to the starting population (e.g., 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or over 100-fold the number of T cells). All or a portion of the expanded T cells may have an activated phenotype. In some examples, the complex is completely liquefied in response to a sufficient decrease of cationic concentration in the environment of the complex.

In some examples, the resulting population of cells has at least 2% naïve T cells (e.g., at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, or more, e.g., about 5%, about 10%, about 15%, or more). In some examples, the resulting population of cells has a greater number or percentage of naïve T cells relative to a reference population (e.g., cells expanded by control beads, e.g., 10% more, 20% more, 50% more, 100% more, or greater, e.g., 2-fold more, 3-fold more, 4-fold more, 5-fold more, 10-fold more, or greater). Additionally or alternatively, the resulting population of cells may have a greater number or percentage of central memory T cells than the reference population (e.g., 10% more, 20% more, 30%, more, 40% more, 50% more, 75% more, 100% more, 150% more, 200% more, 300% more, 400% more, 500% more, 1,000% more, 5,000% more, 10,000% more, or greater). In some examples, the naïve T cells are $CD45RA^+$ cells, $CD45RA^+CD62L^+$ cells, or $CD45RA^+CCR7^+$ cells. In other examples, the naïve T cells secrete lower quantities of IL-4 and/or IFN-γ than a reference population of cells (e.g., wherein the reference population is the starting population, a central memory cell population, an effector memory cell population, or an activated population).

In one example, the population of T cells is isolated from a subject. In a different example, the population of T cells is derived from a cell line. In one example, the starting population of T cells comprises a genetic modification, such as that resulting from a chimeric antigen receptor (CAR) modification.

In one example, the metabolic change induced in the T cells by contacting them with the complexes includes a biochemical or a morphological change. This change may be a greater frequency of cell division, a change in cytokine secretion profile (e.g., of IL-4 and/or IFN-γ), an increase in median cell diameter, a change surface molecule expression profile, or a change in cellular motility.

As used herein, the term "average" refers broadly to any representative value of a set of values or characteristic of a population of discrete objects. For example, an average diameter of a particle (e.g., nanoparticle or microparticle) or complex may refer to a mean, median, mode, or any weighted variant thereof, including average values derived from excluding outlying data. Unless otherwise specified, the "size" of a particle or complex is its diameter.

As used herein, the term "droplet" refers to a liquid product of an atomizer, whereas a "particle" refers herein to a solid (e.g., gel or hydrogel) spherical or substantially spherical construct (e.g., a nanoparticle or microparticle). A droplet becomes a particle upon solidification (e.g., gelling, e.g., upon exposure to a cation, resulting in alginate crosslinking).

As used herein, the term "complex" refers to a hydrogel construct (e.g., a particle, disk, rod, or other shape) that is associated with (e.g., conjugated to) one or more binding moieties.

As used herein, "volumetric percentage of liquid passed through an atomizer," and grammatical variants thereof, refer to the volume of liquid passing through an atomizer relative to the total volumetric flow through the atomizer, including a gas. For example, the volumetric percentage V_l of liquid flowing through an atomizer over a given period of time is given as:

$$V_l = \frac{l}{l+g} \times 100$$

where l is the volume of liquid passed through the atomizer over the given period of time, and g is the volume of gas passed through the atomizer over the given period of time.

As used herein, a "reference population" refers to any suitable control population of cells. For example, a characteristic of a population of cells can be compared to the starting population from which it has expanded. Alternatively, the reference population can be an untreated control or a control that has been treated (e.g., expanded) by an alternative means. Alternative means of T cell expansion include any conventional methods, such as use of soluble, plate-bound, and/or bead- or particle-bound antibodies or cytokines (e.g., T cell activating antibodies, such as anti-CD3 and/or anti-CD28). For example, a reference population of an expanded population of T cells can be generated using custom-made or commercially available control beads (e.g., DYNABEADS®).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: A schematic drawing showing a cross-sectional view of an exemplary spray apparatus.

FIGS. 2A-2D: Photomicrographs showing hydrogel particles of the invention. FIGS. 3A and 3B show the hydrogel particles prior to binding moiety conjugation at high density (FIG. 2A; 1.16×10^8 particles/mL) and at low density (FIG. 2B; 1.16×10^7 particles/mL). FIGS. 2C and 2D show the

CLAIMS

What is claimed is:

1. A method of generating a population of expanded immune cells, the method comprising contacting a starting population of immune cells with a plurality of particles comprising a complex comprising a hydrogel and a binding moiety, wherein:
(a) the hydrogel comprises an alginic acid-polyethylene glycol (PEG) copolymer; and
(b) the binding moiety is configured to bind a cell surface component of an immune cell;;
wherein the contact is operative to induce a metabolic change in the starting population of immune cells, thereby generating a population of expanded immune cells.

2. The method of claim 1, wherein the particles change from a solid matrix into a solution or suspension in response to a sufficient decrease of cationic concentration in the environment of the polymer, wherein the cation is Li^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, or Al^{3+}.

3. The method of claim 1 or 2, wherein the particles are administered to a culture comprising the population of immune cells at a particle-to-cell ratio from 1:20 to 20:1.

4. The method of any one of claims 1-3, wherein the population of expanded immune cells comprises more than 100-fold the number of immune cells relative to the starting population.

5. The method of any one of claims 1-4, wherein the population of expanded immune cells comprises activated immune cells.

6. The method of any one of claims 1-5 wherein the cell surface component is CD2, CD3, CD19, CD24, CD27, CD28, CD31, CD34, CD45, CD46, CD80, CD86, CD133, CD134, CD135, CD137, CD160, CD335, CD337, CD40L, ICOS, GITR, HVEM, Galtectin 9, TIM-1, LFA-1, PD-L1, PD-L2, B7-H3, B7-H4, ILT3, ILT4, CDTL-4, PD-1, BTLA, MHC-I, MHC-II, DLL-Fc, DLL-1, or DLL-4.

7. The method of any one of claims 1-6, wherein the binding moiety is a cytokine, or an antibody or antigen binding fragment thereof.

8. The method of claim 7, wherein the binding moiety is a cytokine selected from IL-1, IL-2, IL-3, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, TNF-α, and IFN-γ.

9. The method of claim 7, wherein the binding moiety is an antibody or antigen binding fragment thereof, selected from a monoclonal antibody or antigen-binding fragment thereof, a Fab, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, a monovalent antibody or antigen-binding fragment thereof, a chimeric antibody or antigen-binding fragment thereof, a single-chain Fv molecule, a bispecific single chain Fv ((scFv') 2)

molecule, a domain antibody, a diabody, a triabody, an affibody, a domain antibody, a SMIP, a nanobody, a Fv fragment, a Fab fragment, a F(ab') 2 molecule, or a tandem scFv (taFv) fragment.

10. The method of claim 7, wherein the binding moiety is an antibody or antigen binding fragment thereof, selected from anti-CD2, anti-CD3, anti-CD19, anti-CD24, anti-CD27, anti-CD28, anti-CD31, anti-CD34, anti-CD45, anti-CD46, anti-CD80, anti-CD86, anti-CD133, anti-CD134, anti-CD135, anti-CD137, anti-CD160, anti-CD335, anti-CD337, anti-CD40L, anti-ICOS, anti-GITR, anti-HVEM, anti-Galtectin 9, anti-TIM-1, anti-LFA-1, anti-PD-L1, anti-PD-L2, anti-B7-H3, anti-B7-H4, anti-ILT3, anti-ILT4, anti-CDTL-4, anti-PD-1, anti-BTLA, anti-MHC-I, anti-MHC-II, anti-DLL-Fc, anti-DLL-1, and anti-DLL-4.

11. The method of any one of claims 1-10, wherein the binding moiety is chemokine (C-X-C motif) ligand 12 or low-density lipoprotein.

12. The method of any one of claims 1-11, wherein the immune cell is selected from the group consisting of regulatory T cell, NK cell, NK T cell, CIK cell, TIL cell, HS cell (undifferentiated and differentiated), MS cell (undifferentiated and differentiated), iPS cell (undifferentiated and differentiated), and ES cell (undifferentiated and differentiated).

13. The method of any one of claims 2-12, wherein the decrease in the cationic concentration in the environment of the polymer is caused by the presence of EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyridyl sulfonate, dioxane, DME, diglyme, or triglyme.

14. The method of any one of claims 1-13, wherein the binding moiety is covalently attached to the hydrogel.

15. The method of any one of claims 1-14, wherein the binding moiety comprises a signal 1 stimulus.

16. The method of claim 15, wherein the binding moiety further comprises a signal 2 stimulus.

17. The method of any one of claims 1-16, wherein the alginic acid-PEG copolymer comprises a four-arm PEG molecule.

18. The method of any one of claims 1-17, wherein the immune cell is a T-cell, and the method generates a population of expanded T-cells.

19. The method of claim 18, wherein the population of expanded T cells comprises a greater number or percentage of $CD8^+$ T cells than the starting population, and/or wherein the population of expanded T cells comprises a lower number or percentage of $CD4^+$ T cells than the starting population, and/or wherein the population of expanded T cells comprises a greater CD8-to-CD4 T cell ratio than the starting population.

20. The method of claim 18 or 19, wherein the population of expanded T cells comprises activated T cells.