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, wherein the immune cells are selected from T-cells and NK 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 selected from a T-cell and an NK 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-

CLAIMS

What is claimed is:

1. A method of generating a population of expanded immune cells, wherein the immune cells are selected from T-cells and NK 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 selected from a T-cell and an NK 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 2-11, 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.

13. The method of any one of claims 1-12, wherein the binding moiety is covalently attached to the hydrogel.

14. The method of any one of claims 1-13, wherein the binding moiety comprises an anti-CD3 antibody or antigen-binding fragment thereof.

15. The method of claim 14, wherein the binding moiety further comprises an anti-CD28 antibody or antigen-binding fragment thereof.

16. The method of any one of claims 1-15, wherein the alginic acid-PEG copolymer comprises a four-arm PEG molecule.

17. The method of any one of claims 1-16, wherein the immune cell is a T-cell, and the method generates a population of expanded T-cells.

18. The method of claim 17, 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.

19. The method of claim 17 or 18, wherein the population of expanded T cells comprises activated T cells.