ARTIFICIAL ANTIGEN PRESENTING CELL SYSTEM AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No. 63/022,289, filed May 8, 2020, the content of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

Direct modulation of T cells and their function via antigen presenting cells (APCs), including naturally occurring APCs or artificial APCs (aAPCs), is an attractive therapeutic strategy against diseases ranging from cancer, viral infection, and autoimmune diseases. Constantino et al., Transl Res 2016, 168, 74-95; and Palucka et al., Nat Rev Cancer 2012, 12 (4), 265-77. Recent clinical interest and advances in T cell-based adoptive cell therapies have led to further adoption of APCs for *ex vivo* T cell modulation, which allows for expansions of neoantigen-specific T cells, tumor infiltrating lymphocytes, virus-targeted T cells, as well as general T cell expansion for subsequent genetic modification. Constantino et al., 2016; and Fucikova et al., Front Immunol 2019, 10, 2393.

Development of artificial APCs was of interest due to maintenance, storage, and aseptic requirement considerations. Sunshine et al., Nanomedicine (Lond) 2013, 8 (7), 1173-89; Turtle et al., Cancer J 2010, 16 (4), 374-81; Durai et alCancer Immunol Immunother 2009, 58 (2), 209-20; Latouche et al., Nat Biotechnol 2000, 18 (4), 405-9; and Sasawatari et al., Immunol Cell Biol 2006, 84 (6), 512-21. However, it is still a challenge to construct aAPCs that can mimic naturally-occurring APCs such as live dendritic cells (DCs) to a great extent.

SUMMARY OF THE DISCLOSURE

The present disclosure is based, at least in part, on the development of an efficient artificial antigen presenting cell (aAPC) system that comprises (a) gelated human dendritic cells capable of presenting antigen peptides to T cells and (b) a controlled release system capable of secreting one or more cytokines. The human dendritic cells may be genetically modified. Alternatively, the human dendritic cells may be naturally-occurring. The aAPC system disclosed herein is capable of fully activate immune cells such as T cells via triggering three signaling pathways, including the primary signaling pathway triggered by the engagement of MHC/peptide complex with T cell receptors, the co-stimulatory signaling pathway triggered by interaction between a co-stimulatory receptor on T cells and a ligand thereof, and the signaling pathway triggered by binding of stimulatory cytokines to their receptors on the immune cells.

Accordingly, one aspect of the present disclosure provides an artificial antigen

presenting cell complex comprising: (a) one or more gelated human dendritic cells; and (b) a controlled release system associated with one or more cytokines; whereinthe one or more gelated human dendritic cells are attached to the controlled release system to form the artificial antigen presenting cell complex. In some examples, the artificial antigen presenting cell complex may further comprise an antigenic peptide displayed on the surface of the one or more gelated human dendritic cells.

In some examples, the controlled release system comprises a microparticle, which may encapsulate the one or more cytokines. Exemplary cytokines include, but are not limited to, IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-12, IL-15, IL21, IL-23, IL-10, TNF-α, IFN-α, IFN-β, IFN-γ, IFNλ, and/or granulocyte-macrophage colony-stimulating factor. In some examples, the microparticle may comprise one or more degradable polymers, for example, poly(lactic-co-glycolic acid), polylactic acid, polycaprolactone, polyurethane, polyacrylate, or a combination thereof.

In some examples, the antigenic peptide can be displayed by a major histocompatibility complex class I molecule on the gelated human dendritic cell. In some examples, the gelated human cells may comprise intracellular cross-linked polyethylene glycol diacrylate (PEG-DA).

In another aspect, the present disclosure features a method for producing a gelated human dendritic cell, the method comprising: (a) providing a population of human dendritic cells; (b) permeating a gelation buffer into the human dendritic cells, wherein the gelation buffer comprises a photo initiator, a polymer or a polymerizable monomer, and dimethyl sulfoxide; and (c) exposing the human dendritic cells permeated with the gelation buffer to a light to cause crosslinking the polymer, thereby forming the gelated human dendritic cells. In some instances, the method may further comprise (d) coupling the gelated human dendritic cells to a controlled release system, which is associated with one or more cytokines.

In some examples, the polymer in the gelation buffer can be an acrylic polymer, for example, poly(ethylene glycol) diacrylate (PEG-DA). In some examples, the acrylic polymer is PEG-DA, which may have an average molecular weight of about 250 to about 2,000 dalton. In other examples, the PEG-DA may have an average molecular weight of about 600 to about 800 dalton. In some examples, the gelation buffer may comprise a polymerizable monomer, for example, wh 2-hydroxyethyl methacrylate, (hydroxyethyl)methacrylate, acrylic acid, sodium acrylate, ethylene glycol dimethacrylate, or a combination thereof.

In some examples, the photo initiator is 2-hydroxy-4′-(2-hydroxyethoxy) -2-methylpropiophenone.

In yet another aspect, the present disclosure provides the artificial antigen presenting human dendritic cells produced by any of the methods disclosed herein.

In yet another aspect, the present disclosure provides a method of activating an immune cell, comprising: (i) providing any of the artificial antigen presenting cell complexes disclosed herein; (ii) priming the artificial antigen presenting cell complex with an antigenic peptide to display the peptide on the surface of the gelated human dendritic cell in the artificial antigen presenting cell complex; and (iii) contacting the artificial antigen presenting cell complex produced in step (ii) with a cell population comprising immune cells to activate the immune cells. Optionally, the method may further comprise administering the activated immune cells produced in step (iii) to a subject in need thereof. In yet another aspect, the present disclosure provides a use of the activated immune cells as described herein in the manufacture of a medicament for treating a subject in need thereof.

In some examples, the immune cells comprise T cells. In some examples, the peptide is derived from a viral antigen, a bacterial antigen, a fungal antigen, a parasite antigen, a cancer antigen, or a self antigen associated with an autoimmune disease.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1D are diagrams showing preparation and characterization of gelated dendritic cells (G-DCs) via DMSO-mediated monomer and photoinitiator permeation. **FIG. 1A** is a schematic illustration showing preparation of gelated dendritic cells following direct permeation of hydrogel monomers and photoinitiators into the intracellular domain of cells in the presence of DMSO. A UV crosslinker is used for photo-activated crosslinking. **FIG. 1B:** PEG quantification of cells following incubation in 10 wt% PEG-DA gelation buffer shows that intracellular PEG-DA monomer concentration approaches saturation after 5 min of incubation. **FIG. 1C:** Fluorescence and bright-field microscopic observations of gelated dendritic cells prepared with fluorescein-DA show that gelated dendritic cells retain structural integrity upon suspension in PBS and in water. **FIG. 1D**: The stability of G-DCs is assessed following 21 days of suspension in PBS.

FIGs. 2A-2F are diagrams showing that G-DC antigens presentation can be modulated by peptide pulsing post intracellular hydrogelation. **FIG. 2A:** A schematic illustration showing that peptide antigens can be added to JAWSII cells after intracellular hydrogelation. **FIGs. 2B-**

2C: JAWSII cells were treated with LPS and then gelated by a photoactivated hydrogel system. In the non-activated G-DC group, control JAWSII cells without peptide and LPS activation were prepared for gelation. In the activated G-DC group, SIINFEKL peptides were added during the

What Is Claimed Is:

1. An artificial antigen presenting cell complex comprising:

(a) one or more gelated human dendritic cells; and

(b) a controlled release system associated with one or more cytokines;

wherein the one or more gelated human dendritic cells are attached to the controlled release system.

2. The artificial antigen presenting cell complex of claim 1, further comprising an antigenic peptide displayed on the surface of the one or more gelated human dendritic cells.

3. The artificial antigen presenting cell complex of claim 1 or claim 2, wherein the controlled release system comprises a microparticle, which encapsulates the one or more cytokines.

4. The artificial antigen presenting cell of any one of claims 1-3, wherein the one or more cytokines comprise IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-12, IL-15, IL21, IL-23, IL-10, TNF-α, IFN-α, IFN-β, IFN-γ, IFNλ, granulocyte-macrophage colony-stimulating factor, or a combination thereof.

5. The artificial antigen presenting cell complex of any one of claims 1-4, wherein the microparticle comprises one or more degradable polymers.

6. The artificial antigen presenting cell complex of claim 5, wherein the one or more degradable polymers comprise poly(lactic-co-glycolic acid), polylactic acid, polycaprolactone, polyurethane, polyacrylate, or a combination thereof.

7. The artificial antigen presenting cell complex of any one of claims 2-6, wherein the antigenic peptide is displayed by a major histocompatibility complex class I molecule on the gelated human dendritic cell.

8. The artificial antigen presenting cell complex of any one of claims 1-7, wherein the gelated human cells comprise intracellular cross-linked polyethylene glycol diacrylate (PEG-DA).

9. A method for producing a gelated human dendritic cell, the method comprising:

(a) providing a population of human dendritic cells;

(b) permeating a gelation buffer into the human dendritic cells, wherein the gelation buffer comprises a photo initiator, a polymer or a polymerizable monomer, and dimethyl sulfoxide; and

(c) exposing the human dendritic cells permeated with the gelation buffer to a light to cause crosslinking the polymer, thereby forming the gelated human dendritic cells.

10. The method of claim 9, further comprising:

(d) coupling the gelated human dendritic cells to a controlled release system, which is associated with one or more cytokines.

11. The method of claims 9 or 10, wherein the gelation buffer comprises the polymer, which is an acrylic polymer.

12. The method of claim 11, wherein the acrylic polymer comprises poly(ethylene glycol) diacrylate (PEG-DA).

13. The method of claim 12, wherein the acrylic polymer is PEG-DA having an average molecular weight of 250 to 2,000 dalton.

14. The method of claim 13, wherein the PEG-DA has an average molecular weight of 600 to 800 dalton.

15. The method of claim 9 or claim 10, wherein the gelation buffer comprises the polymerizable monomer.

16. The method of claim 15, wherein the polymerizable monomer is 2-hydroxyethyl methacrylate, (hydroxyethyl)methacrylate, acrylic acid, sodium acrylate, ethylene glycol dimethacrylate, or a combination thereof.

17. The method of any one of claims 9-16, wherein the photo initiator is 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone.

18. An artificial antigen presenting human dendritic cell, which is produced by a method of any one of claims 9-17.

19. A method of activating an immune cell, comprising:

(i) providing an artificial antigen presenting cell complex of any one of claims 1-8 and 14;

(ii) priming the artificial antigen presenting cell complex with an antigenic peptide to display the peptide on the surface of the gelated human dendritic cell in the artificial antigen presenting cell complex; and

(iii) contacting the artificial antigen presenting cell complex produced in step (ii) with a cell population comprising immune cells to activate the immune cells.

20. The method of claim 19, wherein the immune cells comprise T cells.

21. The method of claims 17 or 18, wherein the peptide is derived from a viral antigen, a bacterial antigen, a fungal antigen, a parasite antigen, a cancer antigen, or a self antigen associated with an autoimmune disease.

22. Use of the activated immune cells produced in step (iii) of any one of claims 19-21 in the manufacture of a medicament for treating a subject in need thereof.