

SUMMARY

We have developed an efficient affinity chromatography cell sorting system that can purify multiple cell types with high purity and yield with minimal functional impact on purified cell populations. The disclosure adapts a unique biocompatibly dissolvable hydrogel for use in an affinity chromatography system, enabling positive selection without residual affinity tags (e.g., immunofluorescent or magnetic tags).

In one aspect, the present disclosure refers to a kit comprising:

a) a substrate attached to a binding unit configured to bind a surface of a target cell, wherein all or a portion of the substrate liquefies upon decreased availability of a crosslinking agent wherein the substrate comprises hydrogel microbeads, and the hydrogel comprises a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 μm , wherein the PEG is a branched PEG; and

b) a strainer through which cells can pass but the substrate cannot, optionally wherein the binding unit:

(i) comprises an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein, optionally wherein the antibody or antigen binding fragment thereof 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; or

(ii) binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).

In another aspect, the present disclosure refers to a composition comprising a plurality of hydrogel microbeads, each attached to a binding unit configured to bind a surface of a target cell, wherein the hydrogel microbeads have a diameter from about 1-200 μm and wherein all of the hydrogel microbeads liquefy upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the PEG is a branched PEG, optionally wherein:

(a) the cation of the crosslinking agent is Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} ; or

(b) the hydrogel microbeads:

(i) have a median viscosity of between about 0.01 and 400 centipoise (cP);

(ii) have a median viscosity of about 80 cP;

(iii) have a polydispersity index of 1-2; or

(iv) have a diameter from 3-200 μm .

In another aspect, the present disclosure refers to a device comprising a container comprising an inlet and an outlet, the container containing a porous substrate comprising hydrogel microbeads and being configured to permit passage of cells from the inlet through the outlet while retaining the porous substrate within the container, wherein a binding unit configured to bind a surface of a target cell is attached to the porous substrate, and wherein all or a portion of the porous substrate liquefies upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 μm , wherein the PEG is a branched PEG, optionally wherein:

(a) the container:

(i) comprises a column or trough; or

(ii) comprises a strainer that permits passage of cells, optionally wherein the strainer comprises a mesh or sieve; or

(b) the porous substrate:

(i) is contained within a packed bed.

In another aspect, the present disclosure refers to a method for separating a target cell, the method comprising:

a) providing a suspension of cells comprising the target cell and non-target cells;

b) contacting the suspension with a porous substrate comprising hydrogel microbeads to allow target cells to bind to the substrate, wherein a binding unit configured to bind a surface of the target cell is attached to the substrate, and wherein all or a portion of the substrate liquefies upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 μm , wherein the PEG is a branched PEG; and

c) removing unbound cells from the target cell bound to the porous substrate, thereby separating the target cell.

In one example, the disclosure features a kit including a substrate, e.g., a porous substrate, attached to a binding unit configured to bind a surface of a target cell, wherein all or a portion of the substrate liquefies upon decreased availability of a crosslinking agent; and a strainer, e.g., housing in a container, through which cells can pass but the substrate cannot. In certain examples, the kit further includes a chelator for a cation that crosslinks the substrate, such as EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyrindyl sulfonate, dioxane, DME, diglyme, or triglyme. The substrate may include microbeads, e.g., having a diameter from 1-1000 μm , such as 2-500 μm , 3-200 μm , 4-100 μm , or 5-20 μm . In certain examples, the microbeads have a polydispersity index of 1-2. The binding unit is, for example, attached to the surface of the

microbeads. The strainer may include a mesh or sieve and have a pore size of 1-1000 μm , e.g., 1-30 μm , 10-40 μm , 20-30 μm , 5-50 μm , 20-80 μm , 1-100 μm , 200-500 μm , or 500-1000 μm .

In certain examples, the substrate includes a cross-linkable polymer, such as a hydrogel. In other examples, the crosslinking agent is an ion, e.g., a cation, such as Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} , in particular Ca^{2+} . In further examples, the hydrogel includes alginic acid, e.g., in the form of an alginic acid-polyethylene glycol (PEG) copolymer. The hydrogel has, for example, a median viscosity of between about 0.01 and 400 centipoise (cP), e.g., about 80 cP.

The binding unit may include an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein. Examples of antibodies or antigen binding fragments thereof include 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 certain examples, the binding unit binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).

In another example, the disclosure features a composition including a plurality of hydrogel microbeads, each attached to a binding unit configured to bind to a surface of a target cell, wherein the hydrogel microbeads have a diameter from about 1-1000 μm and wherein all of the hydrogel microbeads liquefy upon decreased availability of a crosslinking agent. The hydrogel microbeads may include alginic acid, e.g., in

the form of an alginic acid-polyethylene glycol (PEG) copolymer. The hydrogel has, for example, a median viscosity of between about 0.01 and 400 centipoise (cP), e.g., about 80 cP. In certain examples, microbeads have a polydispersity index of 1-2. The binding unit is, for example, attached to the surface of the microbeads. In certain examples, the crosslinking agent is an ion, e.g., a cation, such as Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} , in particular Ca^{2+} . The binding unit may include an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein. Examples of antibodies or antigen binding fragments thereof include 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 certain examples, the binding unit binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR). The decreased availability may be caused by the presence of a chelating agent, such as EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyrindyl sulfonate, dioxane, DME, diglyme, or triglyme.

In a further example, the disclosure features a device including a container, e.g., including a column or trough, having an inlet and an outlet, the container containing a porous substrate and being configured to permit passage of cells from the inlet through the outlet while retaining the porous substrate within the container, wherein a binding unit configured to bind to a surface of a target cell is attached to the porous substrate, and wherein all or a portion of the porous substrate liquefies upon decreased availability of a crosslinking agent. In certain example, the container includes a strainer, e.g., including a mesh or sieve, that permits passage of cells. The strainer may include a mesh or sieve and have a pore size of 1-1000 μm , e.g., 1-30 μm , 10-40 μm , 20-30 μm , 5-50 μm , 20-80 μm , 1-100 μm , 200-500 μm , or 500-1000 μm .

The porous substrate may be contained within a packed bed. In certain examples, the porous substrate includes a cross-linkable polymer, such as a hydrogel. In other examples, the crosslinking agent is an ion, e.g., a cation, such as Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} , in particular Ca^{2+} . In further example, the hydrogel includes alginic acid, e.g., in the form of an alginic acid-polyethylene glycol (PEG) copolymer. The hydrogel has, for example, a median viscosity of between about 0.01 and 400 centipoise (cP), e.g., about 80 cP. The porous substrate may include microbeads, e.g., having a diameter from 1-1000 μm , such as 2-500 μm , 3-200 μm , 4-100 μm , or 5-20 μm . In certain examples, the microbeads have a polydispersity index of 1-2. The binding unit is, for example, attached to the surface of the microbeads.

The decreased availability may be caused by the presence of a chelating agent, such as EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyridyl sulfonate, dioxane, DME, diglyme, or triglyme.

5 The binding unit may include an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein. Examples of antibodies or antigen binding fragments thereof include 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
10 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 certain examples, the binding unit binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c,
15 CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).

The device may further include one or more additional containers operatively connected to the outlet, wherein the one or more additional containers each includes a porous substrate having the same or different binding unit. The additional devices may be connected by one or more three-way valves.

20 In a related example, the disclosure features a method for separating a target cell by providing a suspension of cells including the target cell and non-target cells; contacting the suspension with a substrate to allow target cells to bind to the substrate, wherein a binding unit configured to bind to a surface of the target cell is attached to the substrate, and wherein all or a portion of the substrate liquefies
25 upon decreased availability of a crosslinking agent; and removing unbound cells from the target cell bound to the substrate, e.g., in a container, thereby separating the target cell. In certain examples, the suspension and substrate are contacted prior to introduction into the container. Alternatively, the suspension and substrate are contacted in the container. The method may further include decreasing the availability of the crosslinking agent to liquefy the substrate, e.g., to release cells bound to the substrate.

30 In certain examples, the method produces a subpopulation including the target cell from the suspension. In some examples, the one or more target populations of cells accounts for at least 80% (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the isolated population of cells. In some examples, the one or more target populations of
35 cells accounts for 50% or less (e.g., 50%, 45%, 40%, 35%, 30%, 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%, or less) of the general population of cells.

40 For any example of the disclosure, an exemplary target cell is a stem cell, circulating tumor cell, a cancer stem cell, or a white blood cell. For example, the target cell is a T cell, a T regulatory cell, a B cell, an NK cell, or a genetically engineered T cell, e.g., a chimeric antigen receptor T cell (CAR-T cell). Exemplary

stem cells include undifferentiated or differentiated stem cells. A stem cell may be selected from the group consisting of a mesenchymal stem cell, a hematopoietic stem cell, an embryonic stem cell, and an induced pluripotent stem cell. In certain examples of any example of the disclosure, the target cells are bound to a second binding unit to which the binding unit of the substrate, e.g., porous substrate, or microbead binds.

The disclosure features a device including a column containing a porous substrate and a binding unit attached to the porous substrate. The binding unit is configured to bind to a surface of a target cell. All or a portion of (e.g., a surface layer of) the porous substrate liquefies upon decreased availability of a crosslinking agent. In some examples, the column further includes an inlet and an outlet, wherein the porous substrate is configured to permit passage of cells from the inlet to the outlet.

In some examples, the porous substrate includes a polymer (e.g., a cross-linkable polymer). In some examples, the polymer includes a hydrogel. In some examples, the hydrogel includes alginic acid or an alginic acid-polyethylene glycol (PEG) copolymer. In some examples, the hydrogel has a median viscosity from about 0.01 and about 400 cP when liquid. In some examples, the porous substrate consists of a plurality of microbeads. The microbeads can have a diameter from 1-1000 μm (e.g., 2-500 μm , 3-200 μm , 4-100 μm , or 5-20 μm) and/or a polydispersity index of 1-2 (e.g., as measured by gel permeation chromatography).

In some examples, the crosslinking agent is an ion (e.g., a cation, e.g., Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+}). In some examples, the decreased availability is caused by the presence of a chelating agent. In some examples, the decreased availability is 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 binding unit is attached to the porous substrate (e.g., the surface of the microbeads). In some examples, the binding unit includes an antibody or an antigen-binding fragment thereof (e.g., 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 device is configured to produce an isolated population of cells from a general population of cells, wherein the isolated population of cells is enriched for one or more target populations of cells. In some examples, the general population of cells contains stem cells, cancer stem cells, or white blood cells, which can be separated from the general population. In some examples, the one or more target populations of cells are each characterized by the expression of one or more cell surface molecules (e.g., one or more of T cell receptor, CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15,

CD16, CD19, CD25, CD33, CD34, CD44, CD45RO, CD56, CD69, major histocompatibility complex (MHC), or a chimeric antigen receptor).

In some examples, the one or more target populations of cells accounts for at least 80% (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the isolated population of cells.

In some examples, the one or more target populations of cells accounts for 50% or less (e.g., 50%, 45%, 40%, 35%, 30%, 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%, or less) of the general population of cells.

In some examples, the device further includes one or more additional columns operatively connected to the outlet, wherein the one or more additional columns each including a substrate having a different binding unit. In some examples, the device further includes one or more three-way valves, e.g., for separating an isolated population of cells from a remainder of cells (e.g., of the general population).

In another example, the disclosure features a method for producing an isolated population of cells enriched for the target population of cells using a device of the disclosure.

In another example, the disclosure features a method for isolating a target population of cells by providing a suspension of cells including the target population of cells; contacting the suspension with a porous substrate to produce a population of bound cells (including the target cells) and a population of unbound cells; separating the population of unbound cells from the column; and liquefying all or a portion of the porous substrate to release the population of bound cells to isolate a target population of cells.

In some examples, the target cell is a T cell, a T regulatory cell, a B cell, an NK cell, a genetically engineered T cell, or a stem cell. In some examples, the genetically engineered T cell is a chimeric antigen receptor T cell (CAR-T cell). In some examples, the stem cell is an undifferentiated stem cell. In some examples, the stem cell is a differentiated stem cell. In some examples, the stem cell is a mesenchymal stem cell, a hematopoietic stem cell, an embryonic stem cell, or an induced pluripotent stem cell.

In certain examples of any example of the disclosure, all of the substrate liquefies upon decreased availability of a crosslinking agent. A column may be employed as a container for any purpose herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A schematic workflow for the isolation of target cells by a device of the invention.

Claims

1. A kit comprising:
 - a) a substrate attached to a binding unit configured to bind a surface of a target cell, wherein all or a portion of the substrate liquefies upon decreased availability of a crosslinking agent wherein the substrate comprises hydrogel microbeads, and the hydrogel comprises a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 μm , wherein the PEG is a branched PEG; and
 - b) a strainer through which cells can pass but the substrate cannot, optionally wherein the binding unit:
 - (i) comprises an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein, optionally wherein the antibody or antigen binding fragment thereof 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; or
 - (ii) binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).
2. The kit of claim 1, wherein the substrate:
 - (a) is porous;
 - (b) optionally wherein the hydrogel:
 - (i) has a median viscosity of between about 0.01 and 400 centipoise (cP); or
 - (ii) has a median viscosity of about 80 cP; or
 - (c) wherein the microbeads:
 - (i) have a diameter from 3-200 μm ;
 - (ii) have a diameter from about 5-20 μm ; or
 - (iii) have a polydispersity index of 1-2,

optionally wherein the binding unit is attached to the surface of the microbeads.
3. The kit of claim 1 or 2, wherein:
 - (a) the strainer:

- (i) is housed within a container;
- (ii) comprises a mesh or sieve; or
- (iii) has a pore size of 1-1000 μm ; or

(b) wherein the cation of the crosslinking agent is Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} .

4. A composition comprising a plurality of hydrogel microbeads, each attached to a binding unit configured to bind a surface of a target cell, wherein the hydrogel microbeads have a diameter from about 1-200 μm and wherein all of the hydrogel microbeads liquefy upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the PEG is a branched PEG, optionally wherein:

- (a) the cation of the crosslinking agent is Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} ; or
- (b) the hydrogel microbeads:
 - (i) have a median viscosity of between about 0.01 and 400 centipoise (cP);
 - (ii) have a median viscosity of about 80 cP;
 - (iii) have a polydispersity index of 1-2; or
 - (iv) have a diameter from 3-200 μm .

5. The composition of claim 4, wherein the binding unit:

- (a) is attached to the surface of the microbeads;
- (b) comprises an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein, optionally wherein the antibody or antigen binding fragment thereof 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; or
- (c) binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).

6. A device comprising a container comprising an inlet and an outlet, the container containing a porous substrate comprising hydrogel microbeads and being configured to permit

passage of cells from the inlet through the outlet while retaining the porous substrate within the container, wherein a binding unit configured to bind a surface of a target cell is attached to the porous substrate, and wherein all or a portion of the porous substrate liquefies upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 μm , wherein the PEG is a branched PEG,

optionally wherein:

(a) the container:

(i) comprises a column or trough; or

(ii) comprises a strainer that permits passage of cells, optionally wherein the strainer comprises a mesh or sieve; or

(b) the porous substrate:

(i) is contained within a packed bed.

7. The device of claim 6, wherein:

(a) the cation of the crosslinking agent is Li^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , Cu^{2+} , or Al^{3+} ; or

(b) the decreased availability is caused by the presence of a chelating agent, optionally wherein the chelating agent is selected from the group consisting of EDTA, EGTA, sodium citrate, BAPTA, crown ether, cryptand, phenanthroline sulfonate, dipyrldyl sulfonate, dioxane, DME, diglyme, and triglyme.

8. The device of claim 6 or 7, wherein:

(a) the hydrogel:

(a) has a median viscosity of between about 0.01 and 400 centipoise (cP);

(b) has a median viscosity of about 80 cP; or

(b) the microbeads:

(i) have a diameter from 3-200 μm ;

(ii) have a diameter from about 5-20 μm ; or

(iii) have a polydispersity index of 1-2.

9. The device of any one of claims 6-8, wherein the binding unit:

(a) is attached to the surface of the microbeads;

(b) comprises an antibody or antigen-binding fragment thereof, biotin, or a biotin binding protein, optionally wherein the antibody or antigen binding fragment thereof 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, or

(c) binds one or more cell surface molecules selected from the group consisting of T cell receptor (TCR), CD3, CD4, CD8, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD19, CD25, CD33, CD34, CD45RO, CD56, major histocompatibility complex (MHC), and chimeric antigen receptor (CAR).

10. The device of claim any one of claims 6-9, further comprising one or more additional containers operatively connected to the outlet, wherein the one or more additional containers each comprises the same or different binding unit, optionally further comprising one or more three-way valves.

11. A method for separating a target cell, the method comprising:
- a) providing a suspension of cells comprising the target cell and non-target cells;
 - b) contacting the suspension with a porous substrate comprising hydrogel microbeads to allow target cells to bind to the substrate, wherein a binding unit configured to bind a surface of the target cell is attached to the substrate, and wherein all or a portion of the substrate liquefies upon decreased availability of a crosslinking agent, wherein the hydrogel microbeads comprise a cross-linkable polymer, wherein the cross-linkable polymer is a hydrogel comprising an alginic acid-polyethylene glycol (PEG) copolymer, wherein the crosslinking agent is a cation, wherein the microbeads have a diameter of 1-200 µm, wherein the PEG is a branched PEG; and
 - c) removing unbound cells from the target cell bound to the porous substrate, thereby separating the target cell.

12. The method of claim 11, wherein step (c):
- (a) occurs in a container, optionally wherein the suspension and substrate are contacted prior to introduction into the container or are contacted in the container; or
 - (b) produces a subpopulation comprising the target cell from the suspension, optionally wherein:
 - (a) at least 80% of the subpopulation is target cells;
 - (b) at least 95% of the subpopulation is target cells;
 - (c) 50% or less of the suspension is target cells; or
 - (d) 10% or less of the suspension is target cells.

13. The method of claim 11 or 12, further comprising decreasing the availability of the crosslinking agent to liquefy the substrate.
14. The method of any one of claims 11-13, wherein the target cell:
- (a) is a stem cell, circulating tumor cell, a cancer stem cell, or a white blood cell,
optionally wherein the stem cell is an undifferentiated stem cell or a differentiated stem cell;
 - (b) is a T cell, a T regulatory cell, a B cell, an NK cell, or a genetically engineered T cell, optionally wherein the genetically engineered T cell is a chimeric antigen receptor T cell (CAR-T cell); or
 - (c) is bound to a second binding unit to which the binding unit of the substrate binds.
15. The method of claim 14, wherein the stem cell is selected from the group consisting of a mesenchymal stem cell, a hematopoietic stem cell, an embryonic stem cell, and an induced pluripotent stem cell.
16. The method of any one of claims 11-15, wherein the microbeads have a diameter from 3-200 μm .