NANOPARTICLE FORMULATIONS

PRIORITY

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/429,466, filed December 2, 2016, the content of which is incorporated by reference in its entirety.

BACKGROUND

[0002] The quaternary structure of polypeptides can greatly influence their physicochemical and biological character. Protein aggregation, or non-native aggregation, refers to the process by which protein molecules assemble into stable complexes composed of two or more proteins, with the individual proteins denoted as the monomer. Aggregates are often held together by strong non-covalent contacts, and require some degree of conformational distortion (unfolding or misfolding) in order to present key stretches of amino acids that form the strong contacts between monomers. While aggregation tends to increase the stability of protein, it often does so at the cost of biological activity of the protein, decreased uniformity of the composition, and can, in some cases, increase the immunogenicity of the protein. These properties adversely affect the ability to use such proteins as a biologic for treatment.

SUMMARY

[0003] The present technology relates to the controlled assembly of MYC-containing polypeptides into populations of biologically active particles of defined size range. Methods are provided herein for the production of stable preparations of MYC-containing nanoparticles that retain biologic activity. Also provided are methods using the biologically active particles for treatment, including *in vitro* and *in vivo* methods of treating cells.

[0003a] In one aspect, the present disclosure refers to a composition comprising a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides wherein: a) the average diameter of the biologically active nanoparticles is between about 80 nm and about 150 nm; b) the pH of the formulation is at least about pH 6.0, and no greater than about pH 8; and c) contacting an anti-CD3 or anti-CD28 activated T-cell with the MYC-containing polypeptide

nanoparticle composition under conditions suitable for T-cell proliferation, augments one or more of the activation, survival, or proliferation of the T-cell compared with an anti-CD3 or anti-CD28 activated T-cell that is not contacted with the MYC polypeptide-containing composition; and wherein the MYC-containing polypeptide comprises a MYC fusion protein, comprising a protein transduction domain linked to a MYC polypeptide.

[0004] Disclosed herein are compositions comprising MYC-containing polypeptides formulated as biologically active, stable nanoparticles. In some examples, the MYC-containing polypeptides comprise a fusion peptide, wherein the fusion peptide comprises: (i) a protein transduction domain; (ii) a MYC polypeptide sequence, and wherein the nanoparticles exhibit the biological activity of MYC. In some examples, the fusion peptide comprises SEQ ID NO: 1. In some examples, the fusion peptide comprises SEQ ID NO: 10.

[0005] Provided herein, in certain examples, are compositions comprising a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides. In some examples, the number average diameter of the biologically active nanoparticles is between about 80 nm and about 150 nm. In some examples, pH of the formulation is at least about pH 6.0, but is no greater than about pH 8. In some examples, contacting an anti-CD3 or anti-CD28 activated T-cell with the MYC-containing polypeptide nanoparticle composition under conditions suitable for T-cell proliferation, augments one or more of the activation, survival, or proliferation of the T-cell compared with an anti-CD3 or anti-CD28 activated T-cell that is not contacted with the MYC polypeptide-containing composition. In some examples, the MYC polypeptide is acetylated. In some examples, the MYC-containing polypeptide comprises a MYC fusion peptide, comprising a protein transduction domain linked to a MYC polypeptide. In some examples, the MYC fusion peptide further comprises one or more molecules that link the protein transduction domain and the MYC polypeptide. In some examples, the MYC-containing polypeptide comprises a MYC fusion peptide with the following general structure: protein transduction domain-X-MYC sequence, wherein -X- is molecule that links the protein transduction domain and the MYC sequence. In some examples, the protein transduction domain sequence is a TAT protein transduction domain sequence. In some examples, the TAT protein transduction domain sequence is

selected from the group consisting of TAT[48-57] and TAT[57-48]. In some examples, the MYC polypeptide is a MYC fusion peptide comprising SEQ ID NO: 1. In some examples, the MYC polypeptide is a MYC fusion peptide comprising SEQ ID NO: 10. In some examples, the nanoparticles have a number average diameter of between about 80 nm and about 150 nm. In some examples, the nanoparticles have a number average diameter of between about 100 nm and about 110 nm. In some examples, the composition further comprises, a pharmaceutically acceptable excipient. In some examples, the composition is formulated for topical administration, oral administration, parenteral administration, intranasal administration, buccal administration, rectal administration, or transdermal administration.

[0006] Also provided herein, in certain examples, are methods of increasing one or more of activation, survival, or proliferation of one or more immune cells or increasing an immune response in a subject in need thereof by administering a therapeutically effective amount of a composition provided herein comprising a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides. In some examples, the one or more immune cells comprise one or more anergic immune cells. In some examples, the one or more immune cells are T cells. In some examples, the T cells are selected from the group consisting of naïve T cells, CD4+ T cells, CD8+ T cells, memory T cells, activated T cells, anergic T cells, tolerant T cells, chimeric B cells, and antigen-specific T cells. In some examples, the one or more immune cells are B cells. In some examples, the B cells are selected from the group consisting of naïve B cells, plasma B cells, activated B cells, memory B cells, anergic B cells, tolerant B cells, chimeric B cells, and antigen-specific B cells.

Also provided herein, in certain examples, are methods of priming hematopoietic stem cells to enhance engraftment, following hematopoietic stem cell transplantation (HSCT) comprising, contacting one or more hematopoietic stem cells, *in vitro*, with the composition provided herein comprising a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides prior to transplantation of the hematopoietic stem cells.

[0007] Also provided herein, in certain examples, are methods for the preparation of a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides, the method comprising: (a) solubilizing MYC-containing

polypeptides in a solubilization solution comprising a concentration of a denaturing agent to provide solubilized MYC-containing polypeptides; (b) performing a first refolding step on the solubilized MYC-containing polypeptides with a first refold buffer comprising about 0.35 to about 0.65 the concentration of the denaturing agent of step (a) and about 100mM to about 1M alkali metal salt and/or alkaline metal salt for at least about 30 to 180 minutes to provide a first polypeptide mixture; (c) performing a second refolding step on the first polypeptide mixture with a second refold buffer comprising about 0.10 to about 0.30 the concentration of the denaturing agent of step (b) and about 100mM to 1M alkali metal salt and/or alkaline metal salt at least about 30 to 180 minutes to provide a second polypeptide mixture; (e) performing a third refolding step on the second polypeptide mixture with a third refold buffer comprising about 100mM to 1M alkali metal salt and/or alkaline metal salt for at least about 30 to 180 minutes; and (f) maintaining the MYC-containing polypeptides in the third refold buffer for a period of time sufficient to produce biologically active nanoparticles having a number average diameter of between about 80 nm and about 150 nm, wherein contacting an anti-CD3 or anti-CD28 activated T-cell with the biologically active nanoparticles under conditions suitable for T-cell proliferation, augments one or more of the activation, survival, or proliferation of the T-cell compared with an anti-CD3 or anti-CD28 activated T-cell that is not contacted with the biologically active nanoparticles. In some examples, the first refolding step, second refolding step, and/or third refolding step comprise performing the step by buffer exchange. In some examples, buffer exchange is performed using tangential flow filtration. In some examples, the alkali metal salt comprises one more of a sodium salt, a lithium salt, and a potassium salt. In some examples, the alkali metal salt comprises one or more of sodium chloride (NaCl), sodium bromide, sodium bisulfate, sodium sulfate, sodium bicarbonate, sodium carbonate, lithium chloride, lithium bromide, lithium bisulfate, lithium sulfate, lithium bicarbonate, lithium carbonate, potassium chloride, potassium bromide, potassium bisulfate, potassium sulfate, potassium bicarbonate, and potassium carbonate. In some examples, the alkaline salt comprises one more of a magnesium salt and a calcium salt. In some examples, the alkaline metal salt comprises one or more of magnesium chloride, magnesium bromide, magnesium bisulfate, magnesium sulfate, magnesium bicarbonate, magnesium carbonate, calcium chloride, calcium bromide, calcium bisulfate, calcium sulfate, calcium bicarbonate, and calcium carbonate. In some

examples, the alkali metal salt comprises sodium chloride (NaCl). In some examples, the first, second, and/or third refold buffers comprise about 500mM NaCl. In some examples, the concentration of denaturing agent in step (a) is from about 1 M to about 10 M. In some examples, the denaturing agent comprises one or more of guanidine , guanidine hydrochloride, guanidine chloride, guanidine thiocyanate, urea, thiourea, lithium perchlorate, magnesium chloride, phenol, betain, sarcosine, carbamoyl sarcosine, taurine, dimethylsulfoxide (DMSO); alcohols such as propanol, butanol and ethanol; detergents, such as sodium dodecyl sulfate (SDS), N-lauroyl sarcosine, Zwittergents, non-detergent sulfobetains (NDSB), TRITON X-100, NONIDET™ P-40, the TWEEN™ series and BRIJ™ series; hydroxides such as sodium and potassium hydroxide. In some examples, the first refold buffer, the second refold buffer, and/or third refold buffer each independently comprise a buffering agent. In some examples, the buffering agent comprises one or more of TRIS (Tris[hydroxymethyl]aminomethane), HEPPS (N-[2-Hydroxyethyl]piperazine-N'-[3-propane-sulfonic acid]), CAP SO (3-[Cyclohexylamino]-2-hydroxy-l-propanesulfonic acid), AMP (2-Amino-2-methyl-1-propanol), CAPS (3-[Cyclohexylamino]-1-propanesulfonic acid), CHES (2-[N-Cyclohexylamino]ethanesulfonic acid), arginine, lysine, and sodium borate. In some examples, the buffering agent is independently present at a concentration from about 1 mM to about 1M. In some examples, the first refold buffer, second refold buffer, and/or third refold buffer each independently comprise an oxidizing agent and a reducing agent, wherein a mole ratio of oxidizing reagent to reducing agent is from about 2:1 to about 20:1. In some examples, the oxidizing agent comprises cysteine, glutathione disulfide (“oxidized glutathione”), or both. In some examples, the oxidizing agent is included in a concentration from about 0.1 mM to about 10 mM. In some examples, the reducing agent comprises one or more of beta-mercaptoethanol (BME), dithiothreitol (DTT), dithioerythritol (DTE), tris(2-carboxyethyl)phosphine, (TCEP), cystine, cysteamine, thioglycolate, glutathione, and sodium borohydride. In some examples, the reducing agent is included in a concentration from about 0.02 mM to about 2 mM. In some examples, the denaturing agent comprises urea. In some examples, the denaturing agent comprises 6-8M urea. In some examples, the first, second, and/or third refold buffers comprise glutathione and/or oxidized glutathione. In some examples, the first, second, and/or third refold buffers comprise 5mM glutathione and/or 1mM oxidized glutathione. In some examples, the first, second,

and/or third refold buffers comprise glycerol. In some examples, the step (f) is performed for at least 5 hours. In some examples, the step (f) is performed for at least 10 hours. In some examples, the step (f) is performed for 10 -12 hours. In some examples, the step (f) further comprises stirring the MYC-containing polypeptides in the third refold buffer at less than 1000 rpm.

[0008] In some examples, the methods provided herein further comprise isolating a recombinant MYC-containing polypeptide from a microbial host cell. In some examples, the microbial host cell is *E. coli.* In some examples, isolating a recombinant MYC-containing polypeptide from a microbial host cell comprises expressing the MYC-containing polypeptide from an inducible promoter. In some examples, isolating a recombinant MYC-containing polypeptide from a microbial host cell comprises purifying the MYC-containing polypeptide using affinity chromatography and/or anion exchange chromatography. In some examples, the MYC-containing polypeptide is acetylated.

In some examples, the MYC-containing polypeptides of the nanoparticle compositions and methods for the production thereof provided herein are recombinant polypeptides. In some examples, the MYC-containing polypeptide of the nanoparticle compositions provided herein comprises a MYC fusion peptide, comprising a protein transduction domain linked to a MYC polypeptide. In some examples, the MYC fusion peptide further comprises one or more molecules that link the protein transduction domain and the MYC polypeptide. In some examples, the MYC-containing polypeptide comprises a MYC fusion peptide with the following general structure: protein transduction domain-X-MYC sequence, wherein -X- is molecule that links the protein transduction domain and the MYC sequence. In some examples, protein transduction domain sequence is a TAT protein transduction domain sequence. In some examples, TAT protein transduction domain sequence is selected from the group consisting of TAT[48-57] and TAT[57-48]. In some examples, the MYC-containing polypeptide is a MYC fusion peptide comprising SEQ ID NO: 1. In some examples, the MYC-containing polypeptide is a MYC fusion peptide comprising SEQ ID NO: 10. In some examples, the nanoparticles have a number average diameter from about 80 nm and about 150 nm. In some examples, the nanoparticles have a number average diameter from about 100 nm and about 110 nm.

[0009] In an exemplary example, provided herein is a method for the preparation of a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides, the method comprising: (a) denaturing MYC-containing polypeptides in a buffered solubilization solution comprising 6-8M Urea to provide denatured MYC-containing polypeptides; (b) performing a first refolding step on the denatured MYC-containing polypeptides with a first refold buffer comprising about 3M Urea and about 500mM NaCl for at least about 120 minutes to provide a first polypeptide mixture; (c) performing a second refolding step on the first polypeptide mixture by buffer exchange with a second refold buffer comprising about 1.5M Urea and about 500mM NaCl at least about 120 minutes to provide a second polypeptide mixture; (d) performing a third refolding step on the second polypeptide mixture by buffer exchange with a third refold buffer comprising about 500mM NaCl for at least about 120 minutes; and (f) maintaining the MYC-containing polypeptides in the third refold buffer for a period of time sufficient to produce biologically active nanoparticles having a number average diameter of between about 80 nm and about 150 nm, wherein contacting an anti-CD3 or anti-CD28 activated T-cell with the biologically active nanoparticles under conditions suitable for T-cell proliferation, augments one or more of the activation, survival, or proliferation of the T-cell compared with an anti-CD3 or anti-CD28 activated T-cell that is not contacted with the biologically active nanoparticles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **FIG. 1A** and **FIG. 1B** show the effect of MYC-containing nanoparticle formulation C2A on proliferation of anti-CD3 and anti-CD28 activated T-cells using flow cytometry techniques. Figure 1A shows the proliferation of T-cells in the buffer control sample. Figure 1B shows flow the proliferation of T-cells incubated for 24 h with MYC-containing nanoparticle formulation C2A.

[0011] **FIG. 2A** and **FIG. 2B** show chromatograms of select MYC-containing nanoparticle formulations analyzed by Asymmetrical Flow Field-flow Fractionation (AF4) and Multiangle Laser Light Scattering (MALLS). FIG. 2A and 2B show the chromatograms obtained from the analysis of Formulation F01 and Formulation F02, respectively. Trace t1 and t2 were obtained from samples stored at 25°C. Trace t2*

and t4* were obtained from samples stored at 5°C. Time (t) was measured in weeks and is shown in the numerical value that follows the t (i.e., t1= one week, t2=two weeks etc.).

[0012] **FIG. 3** shows a comparison of chromatograms derived by fractionating biologically active and inactive MYC-containing nanoparticle formulations by size exclusion chromatography (SEC) using Sepax SRT-C 2000 and 300 columns in tandem. Traces showing fractionation of biologically active nanoparticle formulations C12 and C13 and the biologically inactive formulation R147 are indicated.

[0013] **FIG. 4A** and **FIG. 4B** show analysis of MYC-containing nanoparticle formulation C2A using Size Exclusion Chromatography with Multi-Angle Light Scattering analysis

WHAT IS CLAIMED IS:

1. A composition comprising a population of biologically active nanoparticles comprising one or more MYC-containing polypeptides wherein:

a) the average diameter of the biologically active nanoparticles is between about 80 nm and about 150 nm;

b) the pH of the formulation is at least about pH 6.0, and no greater than about pH 8; and

c) contacting an anti-CD3 or anti-CD28 activated T-cell with the MYC-containing polypeptide nanoparticle composition under conditions suitable for T-cell proliferation, augments one or more of the activation, survival, or proliferation of the T-cell compared with an anti-CD3 or anti-CD28 activated T-cell that is not contacted with the MYC polypeptide-containing composition; and

wherein the MYC-containing polypeptide comprises a MYC fusion protein, comprising a protein transduction domain linked to a MYC polypeptide.

2. The composition of claim 1, wherein the nanoparticles have an average diameter of about 90-140 nm.

3. The composition of claim 1, wherein the nanoparticles have an average diameter of between about 100 nm and about 110 nm.

4. The composition of claim 1, wherein the nanoparticles have a molecular mass of about 10^4 - 10^6 daltons.

5. The composition of claim 1, wherein the nanoparticles comprise about 200 molecules of the MYC-containing polypeptide.

6. The composition of claim 1, wherein the MYC polypeptide is acetylated.

7. The composition of claim 1, wherein the MYC fusion peptide further comprises one or more linkers that link the protein transduction domain and the MYC polypeptide.

8. The composition of claim 1, wherein the MYC-containing polypeptide comprises a MYC fusion peptide with the following general structure:

protein transduction domain-X-MYC sequence,

wherein -X- is a linker that links the protein transduction domain and the MYC sequence.

9. The composition of claim 1, wherein the protein transduction domain sequence is a TAT protein transduction domain sequence.

10. The composition of claim 9, wherein the TAT protein transduction domain sequence is selected from the group consisting of TAT[48-57] and TAT[57-48].

11. The composition of claim 1, wherein the MYC polypeptide is a MYC fusion peptide comprising SEQ ID NO: 1 or 10.

12. The composition of claim 1, further comprising a pharmaceutically acceptable excipient.

13. The composition of claim 1, formulated for topical administration, oral administration, parenteral administration, intranasal administration, buccal administration, rectal administration, or transdermal administration.