There is a need to provide a microcarrier system or a polymeric microsphere that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary

There is provided a polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group.

In an aspect, there is provided a polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine

Advantageously, the polymeric microsphere may be suitable for the scalable expansion of stems cells or stromal cells (such as human mesenchymal stem/stromal cells) under serum- and xeno-free conditions, and may allow efficient non-invasive harvesting. This may be achieved by (i) generating an entirely thermoresponsive polymeric microsphere containing a thermally responsive monomer crosslinked with a functional group monomer throughout the matrix, that is capable to change in volume and surface area when the temperature of the microsphere is lowered to a reduced temperature (such as at room temperature or 20 ºC). Additionally, the entire thermoresponsive microcarrier may be coated with a polymer coating.

There is provided a method of preparing a polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group, wherein the method comprises the steps of:

i. providing a microemulsion mixture of the thermally responsive monomer, the functional group monomer and the cross-linker stirring at a temperature for a period of time; and

ii. polymerizing said microemulsion mixture with an initiator, stirring at a temperature for a period of time to thereby synthesize said microsphere.

In another aspect, there is provided a method of preparing a polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the method comprises the steps of:

i. providing a microemulsion mixture of the thermally responsive monomer, the functional group monomer and the cross-linker stirring at a temperature for a period of time;
ii. polymerizing said microemulsion mixture with an initiator stirring at the temperature for a period of time to thereby synthesize said polymeric microsphere
iii. coating the polymeric microsphere with a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

There is provided use of a polymeric microsphere for cultivating stem cells or stromal cells, wherein the polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group.

In another aspect, there is provided the use of a polymeric microsphere for cultivating stem cells or stromal cells, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

There is a method of culturing stem cells or stromal cells comprising the step of cultivating the stem cells on a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group.

In another aspect, there is provided a method of culturing stem cells or stromal cells comprising the step of cultivating the stem cells or stromal cells on a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

There is a method of harvesting stem cells or stromal cells comprising the step of detaching the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group.

In another aspect, there is provided a method of harvesting stem cells or stromal cells comprising the step of detaching the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

There is a method of expanding and

harvesting stem cells or stromal cell comprising the steps of growing and detaching the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group or an amine functional group.

In another aspect, there is provided a method of expanding and harvesting stem cells or stromal cell comprising the steps of growing and detaching the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

The following words and terms used herein shall have the meaning indicated:

The term “serum-free condition” as used herein refers to cultivation of a cell in a condition which essentially is free of serum of any type. Non-limiting example of a serum which is not used in the present application is fetal bovine serum.

The term “xeno-free condition” as used herein refers to cultivation of a cell in a condition which does not have any human or animal component. It is possible that the term “xeno-free condition” may include trace amounts of human or animal component depending on the type of cell to be cultured but it is expected that such components, if present, would be less than 1% and were not intentionally added, and were also not regular part of any of the reagents or media components that were used.

The term “room temperature” as used herein and commonly known are temperatures between 20 °C to 25 °C or 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, or 25 °C.

The term “initiator” as used herein refers to a compound or a source of any chemical species that reacts with a monomer (single molecule that can form chemical bonds) to form an intermediate compound capable of linking successively with a large number of other monomers into a polymeric compound. Such compounds are capable of initiating or catalyzing chemical reactions.

The term “thermally responsive” when referring to an article, refers to the ability of the article to change at least one of its physical properties when subjected to a change in temperature. This physical property can be an increase or a decrease in the volume, surface area or at least one dimension (height, length, breadth, etc) of the article when the temperature that the article is subjected to increases or decreases. The change in the physical property may be proportional to the change in the temperature (for example, as the temperature increases, the magnitude of the physical property increases, or decreases as the temperature decreases), or may be inversely proportional (for example, as the

temperature increases, the magnitude of the physical property decreases, or increases as the temperature decreases). In addition to a change in physical properties, there can be a change in chemical properties. When referring to the thermoresponsive microcarriers, the surface and volume changes, and in addition, the surface hydrophilicity changes as well.

The term “fold” as used herein refers to the equation of [expanded size of the polymeric microsphere (new size)]/[original size of the polymeric microsphere].

Claims

1. A polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

2. The polymeric microsphere of claim 1, wherein the thermally responsive monomer is selected from the group consisting of N-isopropylacrylamide, N,N-diethylacrylamide, 2-(dimethylamino)ethyl methacrylate, N,N-dimethylacrylamide, acrylamide, 2-(diethylamino)ethyl acrylate, 2-(acryloyloxyethyl) trimethylammonium chloride, vinylcaprolactam, methyl vinyl ether, hydroxyethylmethacrylate, 4-hydroxybutyl acrylate, 2-hydroxyethyl methacrylate, 3-hydroxypropyl methacrylate, 2-carboxyethyl acrylate, 2-carboxyethyl acrylate oligomers, and poly(ethylene glycol) methacrylate.

3. The polymeric microsphere of claim 1, wherein the carboxylic acid functional group monomer is selected from the group consisting of methacrylic acid, acrylic acid and 2-carboxyethyl acrylate.

4. The polymeric microsphere of claim 1, wherein the amine functional group monomer is selected from the group consisting of primary amine, secondary amine and tertiary amine.

5. The polymeric microsphere of claim 1, wherein the amine functional group monomer is selected from the group consisting of 2-aminoethyl methacrylate hydrochloride, N-[3-(dimethylamino)propyl]methacrylamide, (3-acrylamidopropyl)trimethylammonium chloride, 3-aminopropyl methacrylate hydrochloride, N-(3-aminopropyl)methacrylamide hydrochloride, (2-boc-amino)ethyl methacrylate, 2-(tert-butylamino)ethyl methacrylate, 2-(dimethylamino)ethyl methacrylate, 2-(diethylamino)ethyl acrylate, 3-(dimethylamino)propyl acrylate, 2-(diethylamino)ethyl methacrylate, 2-(diisopropylamino)ethyl methacrylate, 2-(dimethylamino)ethyl acrylate, [3-(methacryloylamino)propyl]-trimethylammonium chloride, [2-(acryloyloxy)ethyl]trimethylammonium chloride and [2-(methacryloyloxy)ethyl]trimethylammonium chloride.

6. The polymeric microsphere of claim 1, wherein the thermally responsive monomer is crosslinked with the functional group monomer by a crosslinker comprising N,N'-methylenebis(acrylamide).

7. The polymeric microsphere of any one of the preceding claims, wherein the microsphere has a particle size in the range of 100 to 200 µm.

8. The polymeric microsphere of claim 7, wherein the particle size is in the range of 125 to 177 µm.

9. The polymeric microsphere of any one of the preceding claims, wherein the particle size of the polymeric microsphere increases in the range of more than 1-fold to 300-fold of the original polymeric microsphere when the polymeric microsphere is subjected to a reduced temperature.

10. The polymeric microsphere of any one of the preceding claims, wherein the microsphere has a hydrophilic surface when the microsphere is subjected to a reduced temperature.

11. A method of preparing a polymeric microsphere comprising a thermally responsive monomer crosslinked with a functional group monomer, wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the method comprises the steps of:

i. providing a microemulsion mixture of the thermally responsive monomer, the functional group monomer and the cross-linker stirring at a temperature for a period of time;
ii. polymerizing said microemulsion mixture with an initiator stirring at the temperature for a period of time to thereby synthesize said polymeric microsphere
iii. coating the polymeric microsphere with a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

12. The method of claim 11, wherein the temperature is room temperature.

13. The method of claims 11 and 12, wherein the initiator is ammonium persulfate and/or N,N,N',N'-tetramethylethylenediamine.

14. The method of claim 11, wherein the temperature is in the range of 50 °C to 70 °C.

15. The method of claims 11 and 14, wherein the initiator is 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride.

16. Use of a polymeric microsphere for cultivating stem cells or stromal cells, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

17. A method of culturing stem cells or stromal cells comprising the step of cultivating the stem cells or stromal cells on a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

18. The method of claim 17, wherein the culturing step is performed for a duration of up to 7 days, up to 21 days or up to 24 days, preferably up to 21 days or up to 24 days.19. A method of harvesting stem cells or stromal cells comprising the step of detaching

the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

20. A method of expanding and harvesting stem cells or stromal cell comprising the steps of growing and detaching the stem cells or stromal cells from a surface coated on a polymeric microsphere, wherein said polymeric microsphere comprises a thermally responsive monomer crosslinked with a functional group monomer, and wherein the functional group monomer comprises at least one of a carboxylic acid functional group, an amine functional group or a quaternary ammonium functional group, wherein the microsphere further comprises a polymer coating selected from the group consisting of polymerized catecholamines, dopamine, L-3,4-dihydroxyphenylalanine, epinephrine and norepinephrine.

21. The method of any one of claims 17 to 20, wherein the method is undertaken in a serum-free condition.

22. The method of any one of claims 17 to 21, wherein the method is undertaken in a xeno-free condition.

23. The method of any one of claims 16 to 22, wherein the stem cells or stromal cells are human mesenchymal stem/stromal cells.