US20100322908A1 describes biodegradable microspheres, with a large size distribution, ranging over an order of magnitude (i.e. from 1 to 10 times a given size). WO2009014441A2 describes the fabrication of polycaprolactone (PCL) microspheres by fluidics; however, the size control of their method is unrefined and does not allow for low coefficient of variation (CV) values, as described in the present disclosure. Cultured cells seeded at a uniform density reach confluence at different times on such microcarriers.

J Mater Sci: Mater Med (2008) 19:1703–1711; DOI 10.1007/s10856-007-3253-9, Biomaterials, Volume 22, Number 20, 15 October 2001 , pp. 2785-2794(10), and Journal of Controlled Release 161 (2012) 927–932; doi:10.1016/j.jconrel.2012.05.003 describe fabrication of PCL microspheres for encapsulating and releasing bioactive molecules. US20080020049A1 describes microspheres with a high porosity, interconnected, and their positioning within a scaffold. These documents do not provide for size uniformity of the microspheres.

Similarly, US20110245456A1 does not similarly provide for achieving a uniform size distribution with low CV, of the fabricated biodegradable microspheres. The spray method described does not allow for a high degree of size uniformity of the fabricated microspheres.

US20080095822A1 describes embedding microspheres within a hydrogel, which may be cross-linked. It discloses uniform dispersion of microspheres within the hydrogel, although it does not provide for or describe fabrication of microspheres with uniform size.

Summary of the Invention

In one aspect, the present invention refers to a plurality of PCL microcarriers, having a mean diameter of about 50 to about 400 μm, and a coefficient of variation (CV) of the diameter of less than 10%. In another aspect, the present invention refers to PCL microcarrier, or plurality thereof, having a density of 1.01 to 1.09 g/cm3. In still another aspect, the present invention refers to a method of culturing cells, optionally stem cells, the method comprising: (i) attaching cells to a PCL microcarrier, or to a plurality of PCL microcarriers, according to to the present invention to form microcarrier-cell complexes; and (ii) culturing the microcarrier-cell complexes. In one aspect, the present invention refers to a method of differentiating stem cells, the method comprising: (i) attaching stem cells to a PCL microcarrier, or to a plurality of PCL microcarriers, according to the present invention to form microcarrier-stem cell complexes; and (ii) inducing differentiation of the stem cells. In another aspect, the

present invention refers to a sealed packaging containing at least 0.1 gram of PCL microcarriers according to the present invention. Also described herein are microcarriers and a plurality of microcarriers comprising a bioresorbable, biodegradable polymer. The use of a biodegradable, bioresorbable, polymer that is FDA approved for implants allows cells cultured on the microcarriers to be implanted in-vivo, as an integral part of the tissue engineering process. This may be used in the context of bone, cartilage, or even neural regeneration.

In some examples, the bioresorbable, biodegradable polymer is polycaprolactone (PCL).

In some examples the microcarriers comprise a second biodegradable polymer, blended into PCL. In some examples the second biodegradable polymer is included as a biphasic emulsion, within the PCL. In some examples, the second biodegradable polymer is more rapidly biodegradable than PCL or can be dissolved in a solvent different from PCL (for

example, aqueous or ethanol medium) as a porogen, capable of forming a porous structure within the PCL. In some examples the pores are isolated pores or interconnected pores. In some examples the pores are macropores.

In some examples, the microcarriers have a hydrophilic surface. In some examples, the microcarriers are wettable in water. In some examples the microcarriers have less than 0.01% surfactant by weight.

In some examples polyelectrolyte is included within the PCL. This may be blended into the PCL or in the form of a diblock copolymer, with one segment compatible with PCL and a second segment being the polyelectrolyte. One example of this is polystyrene-polyacrylic acid (PS-PAAc) diblock copolymer, which has been successfully used to confer negative charge to polystyrene microspheres, formed from dichloromethane (DCM).

Another advantage offered by PCL is its ability to release molecules, including bioactive molecules. The release of these molecules in direct contact with cultured cells can efficiently direct their evolution, be it to maintain pluripotency or to induce differentiation into a specific lineage. The range of molecular weight spans from small (a few hundred daltons) to proteins or polysaccharides, with molecular weights in the tens of kilodaltons.

Accordingly, in some examples, the microcarriers comprise a bioactive material, such as hydroxyapatite for bone formation, or other bioactive molecules, within the PCL. This can be achieved by direct blending of PCL with these materials or by encapsulation to incorporate them within the PCL.

In some examples the microcarriers comprise a compatible polymer with density less than 1 g/cm^3, e.g. at 25°C). Advantageously, this can be used to adjust the density of microcarriers that can be used within an agitated bioreactor.

The present disclosure provides microcarriers having a coating. In some examples the coating comprises poly-L-lysine, an adhesion promoting protein or bovine serum albumin. In some examples, the microcarriers are substantially or completely coated with a coating comprising one or more adhesion promoting molecules. Adhesion promoting molecules include polypeptides, glycopolypeptides, polysaccharides and cationic polypeptides capable of binding a mammalian surface cell polypeptide, glycopolypeptide, cationic polyelectrolyte or polysaccharide. In some examples, the cationic polyelectrolyte is polybrene. In some

examples, the coating comprises one or more matrix components. Matrix components include components of biological matrices. In some examples, the matrix component is an extracellular matrix component.

Also described herein is a PCL microcarrier or a plurality of PCL microcarriers coated with an adhesion promoting molecule. In some examples, the coating is a multilayer coating, and the multilayer coating comprises (i) a first layer, comprising an adhesion promoting molecule, and; (ii) another layer, comprising an adhesion promoting molecule. In some examples, the adhesion promoting molecule of the first layer is different from the adhesion promoting molecule of the second layer. In some examples, the multilayer coating comprises more than two layers. In some examples, the multilayer coating comprises 3, 4, 5, 6, 7, 8, 9 or 10 layers.

In some examples the adhesion promoting molecule is a matrix component. In some examples, the matrix component is selected from polylysine (or poly-L-lysine), laminin, gelatin, collagen, keratin, fibronectin, vitronectin, elastin, heparan sulphate, dextran, dextran sulphate, chondroitin sulphate or a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1. In some examples, the matrix component is an extracellular matrix component. In some examples the coating comprises one or more extracellular matrix proteins crosslinked with a dialdehyde, for example glutaraldehyde

In some examples, the microcarriers are coated with combinations of adhesion promoting molecules, including combinations of matrix components. In some examples the microcarriers are coated with combinations of fibronectin, and/or poly-L-lysine, and/or vitronectin. In some examples the combinations of attachment proteins are provided in layers, on the same microcarrier. In some examples the microcarrier may be a PCL microcarrier coated with layers of fibronectin, then poly-L-lysine, then fibronectin. In some examples the microcarrier may be a PCL microcarrier coated with fibronectin and/or poly-L-lysine, and/or vitronectin, or fibronectin, then poly-L-lysine, then fibronectin.

Advantageously, combinations of attachment substrates may give better adhesion of cells to and/or proliferation of cells on the microcarriers. For example, use of fibronectin, and/or poly-L-lysine, and/or vitronectin, may allow mesenchymal stem cell proliferation in static or agitated culture, where such attachment substrates allow cell adhesion and proliferation during agitation. In some examples, the coating may be a fibronectin-PLL-fibronectin (FN-PLL-FN) coating, or a fibronectin-PLL-vitronectin (FN-PLL-VN) coating.

In some examples, the PCL microcarrier or plurality of PCL microcarriers is further coated with cells, and/or has cells attached thereto.

In the present disclosure cells may be of any kind, e.g. somatic cells. In some preferred examples the cells are stem cells, which may be pluripotent (e.g. embryonic stem cells of induced pluripotent stem cells) or multipotent (e.g. adult stem cells). The cells may be mesenchymal stem cells (MSCs), embryonic stem cells (ESCs) or induced pluripotent stem cells (iPS). Cells may be non-human, e.g. rabbit, guinea pig, rat, mouse or other rodent (including cells from any animal in the order Rodentia), cat, dog, pig, sheep, goat, cattle, horse, non-human primate or other non-human vertebrate organism; and/or non-human mammalian cells; and/or human cells.

While human embryonic stem cells typically grow in aggregates, mesenchymal stem cells are generally cultured in a single, or quasi-single layer. The latter presents a significant challenge, as the cells' evolution is altered after reaching confluence (i.e. when the cells cover the entire surface and begin to laterally exert pressure on each other). For this application, uniform microcarrier size enables the cultured cells to reach confluence at approximately the same time. Were the surface area of the microcarriers to differ greatly, cultured cells would reach confluence at different times, resulting in an inhomogeneous cell population. Hence the advantage of a low CV of their diameter (coefficient of variation, representing the ratio of the standard deviation to the mean diameter), less than 10% and preferably less than 5%.

Accordingly, the present disclosure provides a PCL microcarrier or plurality of PCL microcarriers having a coefficient of variation (CV) of their diameter of less than 10%. In some examples, the CV of their diameter is one of less than 9%, less than 8%, less than 7% or less than 6%. In some examples, the CV of the diameter is less than 5%. In some examples, the CV of their diameter is one of less than 4%, less than 3%, less than 2% or less than 1%. Advantageously, cultured cells, seeded on such microcarriers at uniform density reach confluence at approximately the same time.

The present disclosure also provides a PCL microcarrier or a plurality thereof having a mean diameter of about 50 to about 400 microns. In some examples the mean diameter is about 75 to about 300 microns, about 100 to 250 microns, about 150 to 200 microns.

Microcarrier density is preferably suitable to allow suspension of the microcarriers in suspension cell cultures, e.g. in agitated bioreactors.

The present disclosure provides a PCL microcarrier or a plurality thereof having a density of 1.01 to 1.09 g/cm^3. This may be one of:
1.01 to 1.09 g/cm^3, 1.01 to 1.085 g/cm^3, 1.01 to 1.08 g/cm^3, 1.01 to 1.07 g/cm^3, 1.01 to 1.06 g/cm^3, 1.01 to 1.05 g/cm^3, 1.01 to 1.04 g/cm^3, 1.01 to 1.03 g/cm^3, 1.01 to 1.02 g/cm^3;

1.02 to 1.09 g/cm^3, 1.02 to 1.085 g/cm^3, 1.02 to 1.08 g/cm^3, 1.02 to 1.07 g/cm^3, 1.02 to 1.06 g/cm^3, 1.02 to 1.05 g/cm^3, 1.02 to 1.04 g/cm^3, 1.02 to 1.03 g/cm^3;

1.03 to 1.09 g/cm^3, 1.03 to 1.085 g/cm^3, 1.03 to 1.08 g/cm^3, 1.03 to 1.07 g/cm^3, 1.03 to 1.06 g/cm^3, 1.03 to 1.05 g/cm^3, 1.03 to 1.04 g/cm^3;

1.04 to 1.09 g/cm^3, 1.04 to 1.085 g/cm^3, 1.04 to 1.08 g/cm^3, 1.04 to 1.07 g/cm^3, 1.04 to 1.06 g/cm^3, 1.04 to 1.05 g/cm^3;

1.05 to 1.09 g/cm^3, 1.05 to 1.085 g/cm^3, 1.05 to 1.08 g/cm^3, 1.05 to 1.07 g/cm^3, 1.05 to 1.06 g/cm^3.

A preferred density is in the range about 1.02 to about 1.05 g/cm^3. Suitable densities include one of about 1.02, 1.025, 1.03, 1.035, 1.04, 1.045, 1.05 g/cm^3.

In some examples the density is about 1.03 to 1.1 g/ml.

In some examples the microcarriers are substantially spherical in shape.

PCL microcarriers may be provided in dried form. They may be substantially purified and isolated from contaminants. They may be provided in sterile or non-sterile form.

Also described herein is sealed packaging containing at least 0.1 gram of PCL microcarriers as described herein. The PCL microcarriers may be provided in dried form.

Packaging may comprise a container, e.g. bag, box, vial, bottle, or jar. Packaging may be made from a plastics material and may be waterproof so as to keep the contents dry. The microcarriers may be non-sterile or sterile. Packaging may contain one of at least 0.2 g, 0.3 g, 0.4 g. 0.5 g, 0.6 g, 0.7 g, 0.8 g, 0.9 g, 1.0 g, 2.0 g, 3.0 g, 4.0 g, 5.0g, 10 g, 20g, 30g, 40 g,

50 g, 100 g, 200 g, 300 g, 400 g, 500 g, 600 g, 700 g, 800 g, 900 g, 1 kg or more of PCL microcarriers. The weight is preferably dry weight.

The present disclosure provides a process, e.g. method of manufacture, for making microspheres comprising (i) injecting a first liquid through an outlet in an injector into a second liquid, said outlet being immersed in the second liquid which flows past said outlet during said injecting, wherein the first liquid comprises a polymer and wherein the first and second liquids are sufficiently immiscible that droplets of the first liquid form in the second liquid; and (ii) forming the microspheres from the droplets. In some examples the microspheres are biodegradable and/or bioresorbable. In some examples the polymer is caprolactone or a copolymer thereof.

Also described herein is a process for producing a PCL microcarrier, comprising (i) injecting a first liquid through an outlet in an injector into a second liquid, the outlet being immersed in the second liquid which flows past the outlet during the injecting, wherein the first liquid comprises caprolactone or a copolymer thereof and wherein the first and second liquids are sufficiently immiscible that droplets of the first liquid form in the second liquid, and (ii) forming microcarriers from the droplets.

In some examples the rate of flow of the first liquid is the same or slower than the rate of flow of the second liquid. In some examples the ratio of the flow rate of the first liquid to second liquid is from about 1:1 to about 1:10, optionally one of about 1:2 to about 1:5, or about 1:2 to about 1:3. It may be one of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10.

In some examples, the outlet diameter has a diameter of about 0.05 mm to about 0.3 mm, and the ratio of the flow rate of the first liquid and the flow rate of the second liquid is about 1:1 to about 5:1.

Advantageously, the resulting microcarriers obtained after step (ii) may have a mean diameter of about 50 to 400 microns, and/or a coefficient of variation of the diameter of less than 10%, preferably less than 5%.

In some examples the method is a solvent-free method, whereby PCL is heated and extruded by microfluidics into a cold water bath.

In some examples, the first liquid additionally comprises any one or more of gelatin, heparin, chitosan, hydroxyapatite, chondroitin sulfate, collagen, a polypeptide, a polysaccharide, or an aqueous solution such as a hydrogel, optionally comprising an artificial molecule, such as polyethylene oxide, polyoxazoline, or polyacrylamide. In some examples the polymer has an average molecular weight of about 2000 to about 100,000, or about 60,000 to 100,000 or about 50,000 to about 100,000 as measured by GPC. In some examples the first liquid additionally comprises a solvent for the polymer, whereby the first liquid is a solution of the polymer in the solvent. In some examples the first liquid has a specific gravity greater than that of the second liquid.

In some examples, the solvent is a chlorinated or brominated solvent. In some examples the solvent is a chlorinated hydrocarbon, optionally dichloromethane (DCM).

In some examples the polymer has a concentration in the first liquid of about 0.1 to about 10% w/v, optionally one of about 0.1 to about 50%, about 0.5 to about 10%, or about 0.5 to about 4% w/v. In some examples the first liquid is solvent free, whereby the first liquid comprises a melt of the polymer.

In some examples the second liquid is an aqueous liquid. In some examples the second liquid comprises a polymeric stabiliser in solution.

In some examples neither the first nor the second liquids contains a surfactant other than the polymeric stabiliser. In some examples the polymeric stabiliser is polyvinylalcohol or a copolymer thereof. In some examples the polyvinylalcohol has a degree of hydrolysis of at least about 75%. In some examples the polymeric stabiliser has a concentration in the second liquid of about 0.2 to about 5% w/v, optionally about 0.5 to about 4% w/v.

In some examples of the process described herein the polymer comprises ionic monomer units, whereby said polymer is a polyelectrolyte copolymer.

In some examples the first liquid additionally comprises a second polymer. In some examples the second polymer is a polyelectrolyte.

In some examples the first liquid comprises a porogen, optionally a polymeric porogen.

In some examples of the process described herein, injecting is such that the first liquid and the second liquid flow in the same direction. In some examples, the ratio between

the flow rate of the first liquid and the flow rate of the second liquid is about 1:1 to about 5:1, optionally about 2:1 to about 3:1, optionally about 2:1.

In some examples of the process described herein, the outlet has an inner diameter of about 0.05 to about 0.3 mm, optionally one of about 0.05 to about 0.2 mm, 0.05 to about 0.1 mm, 0.1 to about 0.3 mm 0.1 to about 0.2 mm 0.1 to about 0.3 mm, or 0.2 to about 0.3 mm. Other injector/needle sizes and tubing diameters may also be used. Different injector/needle diameters, tubing diameters, and flow rates of the continuous and dispersed phases regulates the diameter range of the microspheres.

In some examples the injector is in the form of an inner tube and the second liquid flows inside an outer tube which surrounds the inner tube. In some examples the first liquid flows at a rate such that droplets of the first liquid form within the second liquid. In some examples the flow rate of the second liquid is such that the second liquid is in a laminar flow regime. In some examples the flow rate of the first liquid and the flow rate of the second liquid are, independently, between about 1 and about 10,000 microliters per minute. In some examples the flow rate of the first liquid is between about 10 and about 500 microliters per minute, optionally between about 10 and 400 microliters per minute. In some examples the flow rate of the second liquid is between about 10 and about 500 microliters per minute, optionally between about 10 and 400 microliters per minute.

In some examples of the process described herein, the injector comprises a syringe needle and the second liquid flows through a tube, whereby an injecting portion of the syringe needle is substantially concentric with the tube.

In some examples of the process described herein, step (i) results in formation of gel microcarriers and step (ii) comprises allowing the gel microcarriers to partially separate from the second liquid, removing the majority of the second liquid from the gel microcarriers and allowing the gel microcarriers to form solid polymer microcarriers.

In preferred examples the microcarrier droplets are separated from the liquid phase. In some examples the microcarrier droplets are dried.

In some examples of the process described herein, the first liquid comprises a bioactive substance such as hydroxyapatite, and the microcarriers comprise the bioactive substance.

In some examples, the process described herein additionally comprises the step of treating the microcarriers with an aqueous hydroxide solution. In some examples the treatment is for about 1 to about 6 hours, and increases the wettability of the microcarriers. In some examples, treatment confers a negative surface charge to the microcarriers. In some examples the treatment is for about 1 to about 10 days, and results in porous microcarriers. In some examples the hydroxide is sodium hydroxide.

In some examples, the process described herein additionally comprises the step of crosslinking the polymer in the microcarriers. In some examples crosslinking comprises heating the microcarriers in the presence of a peroxide such as benzoyl peroxide.

In some examples, the process additionally comprises coating the microcarriers with a coating comprising one or more adhesion promoting molecules. Adhesion promoting molecules include polypeptides, glycopolypeptides, polysaccharides and cationic polypeptides capable of binding a mammalian surface cell polypeptide, glycopolypeptide, cationic polyelectrolyte or polysaccharide. In some examples, the cationic polyelectrolyte is polybrene. In some examples the coating comprises poly-L-lysine, an adhesion promoting protein or bovine serum albumin. In some examples, the coating comprises one or more matrix components. Matrix components include components of biological matrices. In some examples, the matrix component is an extracellular matrix component.

In some examples, the process comprises coating the microcarriers with a multilayer coating, wherein a multilayer coating comprises (i) a first layer, comprising an adhesion promoting molecule, and (ii) another layer, comprising an adhesion promoting molecule. In some examples the adhesion promoting molecule of the first layer is different from the adhesion promoting molecule of the second layer.

In some examples, the microcarriers are sequentially coated with layers of adhesion promoting molecules. In some examples, the process comprises first

coating the microcarrier with a layer comprising an adhesion promoting molecule, and subsequently coating the microcarriers with another layer comprising an adhesion promoting molecule. In some examples the adhesion promoting molecule of the first layer is different from the adhesion promoting molecule of the second layer. In some examples, the multilayer coating comprises more than two layers. In some examples, the process comprises sequentially coating the microcarrier to yield a microcarrier having a multilayer coating comprising 3, 4, 5, 6, 7, 8, 9 or 10 layers.

In some examples the adhesion promoting molecule is a matrix component. In some examples, the matrix component is selected from polylysine (or poly-L-lysine), laminin, gelatin, collagen, keratin, fibronectin, vitronectin, elastin, heparan sulphate, dextran, dextran sulphate, chondroitin sulphate or a mixture of laminin, collagen I, heparan sulfate proteoglycans, and entactin 1. In some examples, the matrix component is an extracellular matrix component. In some examples process additionally comprises treating the microcarriers with a dialdehyde such as glutaraldehyde.

In some examples microcarriers are coated with a polypeptide is capable of binding a mammalian cell surface protein. In some examples the polypeptide is a polyaminoacid comprising lysine or a lysine derivative. In some examples, the polypeptide is an extracellular matrix protein. In some examples the extracellular matrix protein is vitronectin, fibronectin, laminin, osteopontin or collagen, or a derivative thereof.

In some examples, the process comprises coating the microcarrier with combinations of fibronectin, and/or poly-L-lysine, and/or vitronectin. In some examples the process comprises sequentially coating the microcarrier with layers of coating on the same microcarrier. As such, a microcarrier may be coated in a layer of polylysine and/or fibronectin and/or vitronectin, in any order. In some examples the process comprises first coating the microcarrier with a layer comprising fibronectin, then a layer comprising poly-L-lysine, then another layer comprising fibronectin. In some examples the process comprises first coating the microcarrier with a layer comprising fibronectin then a layer comprising poly-L-lysine, then another layer comprising vitronectin.

In some examples, the PCL microcarrier or plurality of PCL microcarriers is further coated with cells, and/or has stem cells attached thereto. In some examples the stem cells are mesenchymal stem cells (MSCs) or embryonic stem cells (ESCs). In some examples, the MSCs or HSCs are human MSCs or human HSCs, respectively.

Accordingly, in some examples, the process additionally comprises exposing the microcarriers to embryonic stem cells or mesenchymal stem cells, to attach the stem cells to the microcarriers. In some examples, the process described herein comprises (i) exposing the microcarriers to one or more polypeptides capable of binding a mammalian cell surface protein, and (ii) exposing the microcarriers to embryonic stem cells or mesenchymal stem cells, so as to attach said stem cells to the microcarriers.

Also described herein is a PCL microcarrier or plurality thereof obtained by or obtainable by using the process of the invention.

Also described herein is a scaffold comprising, containing or supporting a microcarrier or microcarriers as described herein.

Also described herein is an implantable device comprising (i) a scaffold comprising a plurality of strands; and (ii) a plurality of PCL microcarriers as described herein disposed in and/or on the scaffold.

In some examples, the implantable device comprises a radiopaque marker. In some examples, the radiopaque marker is barium sulfate or gold nanoparticles.

In some examples the strands of the implantable device have a diameter of about 200 to about 350 microns, optionally from about 230 to about 250 microns. In some examples, the strands are overlaid in a lattice. In some examples the lattice has a 60° angle change between adjacent layers. In some examples, spacing between adjacent strands is about 400 microns.

In some examples of the implantable device, the scaffold is made from a biodegradable or bioresorbable material.

The microcarrier or plurality of microcarriers described herein may be used (i) in static or stirred spinner or bioreactor culture of cells; (ii) in tissue engineering; (iii) in generation of inflammatory/ectopic effects; (iv) for expansion of human mesenchymal stem cells and/or embryonic stem cells; (v) in differentiation of human mesenchymal stem cells; (vi) in treatment of, or implantation into, a patient wherein differentiated or undifferentiated mesenchymal stem cells cultured on said microcarriers are implanted into said patient; (vii) in treatment of, or implantation into, a patient wherein differentiated or undifferentiated mesenchymal stem cells are cultured on said microcarriers which are then located in and/or on a scaffold prior to said treatment or implantation into said patient; and (viii) as a vehicle for a radiopaque dye.

Also described herein is a method of culturing stem cells, the method comprising (i) attaching cells, optionally stem cells, to a PCL microcarrier, or to a plurality of PCL microcarriers described herein and (ii) culturing the microcarrier-cell complexes.

Optionally, the surface of the microcarriers is coated in a matrix or in two or more layers of matrix.

In some examples, the culture is a static or stirred spinner or bioreactor culture. In some examples the method comprises introducing the microcarrier or plurality of microcarriers described herein into a liquid compatible with said microcarrier or plurality of microcarriers.

In some examples, the number of stem cells after step (ii) is expanded. In some examples, culturing the microcarrier-cell complexes comprises suspension culture under static conditions and/or suspension culture with agitation. In some examples, the microcarriers support expansion of various mesenchymal stem cells (bone marrow, fetal, adipose, etc.) in static or stirred spinner or bioreactor cultures.

In some examples, culturing the microcarrier-stem cell complexes comprises culture in serum free media.

In some examples the cells are stem cells and the method may comprise (iii) passaging the cultured cells from (ii); and repeating steps (i)-(iii) through at least 3 passages, wherein the cells preferably retain their pluripotent or multipotent status after passaging. In some examples the method may further comprise the step of inducing differentiation of the stem cells obtained.

In some examples the method comprises placing microcarrier-stem cell complexes under conditions which induce the differentiation of the stem cells. Such methods may comprise attaching stem cells obtained after step (iv) to a plurality of second microcarriers to form microcarrier-stem cell complexes, wherein the surface of the second microcarriers is coated in a matrix or is uncoated; and culturing the microcarrier-stem cell complexes in suspension culture under conditions that induce the differentiation of the stem cells.

In some examples the method may comprise the step of separating stem cells from the microcarriers and culturing the separated stem cells in non-microcarrier culture under conditions which induce differentiation of the stem cells.

Also described herein is a method of differentiating stem cells, the method comprising (i) attaching stem cells to a PCL

microcarrier or a plurality of PCL microcarriers described herein and (ii) inducing differentiation of the stem cells. In some examples, the microcarriers support *ex vivo* differentiation of various MSCs (bone marrow, fetal, adipose, etc.) into bone or cartilage in static or stirred spinner or bioreactor

cultures. In some examples, the microcarriers support *ex vivo* differentiation of various pluripotent cells (for example human embryonic stem cells, induced pluripotent stem cells, etc.) into differentiated cells from the three germ layers (ectoderm, mesoderm and endoderm) in static or stirred spinner or bioreactor cultures.

Also described herein are PCL microcarriers or a plurality of PCL microcarriers as disclosed herein for use in a method of tissue engineering. Also described herein is the use of PCL microcarriers or a plurality of PCL microcarriers as disclosed herein in the manufacture of a medicament or pharmaceutical composition is provided for use in a method of tissue engineering. In some examples, the methods comprise introducing the microcarriers described herein into a living organism, optionally by injection. In some examples, the methods comprise *in vivo* implantation of PCL microcarriers covered with MSC (derived from bone marrow, fetal, adipose etc.), into the body for tissue engineering or generation of anti-inflammatory/ectopic effects. Cells can be used in their non-differentiated form or differentiated into bone or cartilage. In some examples, the methods comprise *in vivo* implantation of PCL microcarriers covered with various pluripotent cells (for example human embryonic stem cells, induced pluripotent stem cells, etc.) differentiated to cells from the three germ layers (ectoderm, mesoderm and endoderm) into the body for tissue engineering purposes.

The benefit of not dissociating the cells from the support on which they are cultured is an improved viability and, potentially, a more rapid adaptation to their role in the tissue engineering application.

Advantageously, in some examples the microcarriers at least partially resorb or degrade within the organism without production of products which are toxic or otherwise harmful to the organism.

Also described herein are PCL microcarriers or a plurality of PCL microcarriers as disclosed herein for use in methods of medical treatment. Further described herein is the use of PCL microcarriers or a plurality of PCL microcarriers disclosed herein in the manufacture of a medicament or pharmaceutical composition is provided for use in a method of medical treatment.

Also described herein is a method comprising implanting PCL microcarriers into the human or animal body, the PCL microcarriers preferably being attached to cells intended to provide a therapeutic effect at or near the site of implantation.

Claims:

1. A plurality of PCL microcarriers, having a mean diameter of about 50 to about 400 µm, and a coefficient of variation (CV) of the diameter of less than 10%.

2. The plurality of PCL microcarriers according to claim 1, wherein the CV of the diameter is less than 5%.

3. The plurality of PCL microcarriers according to claim 1 or 2, having a mean diameter of about 150 to about 200 µm.

4. The plurality of PCL microcarriers according to any one of claims 1 to 3, having a density of 1.01 to 1.09 g/cm^3.

5. A PCL microcarrier, or plurality thereof, having a density of 1.01 to 1.09 g/cm^3.

6. The PCL microcarrier, or plurality thereof, according to claim 5, having a density of 1.02 to 1.05 g/cm^3.

7. The PCL microcarrier, or plurality thereof, according to claim 5, having a density of 1.04 to 1.07 g/cm^3.

8. The PCL microcarrier, or plurality thereof, according to claim 5, having a density of 1.03 to 1.07 g/cm^3.

9. A method of culturing cells, optionally stem cells, the method comprising:

(i) attaching cells to a PCL microcarrier, or to a plurality of PCL microcarriers, according to any one of claims 1 to 6 to form microcarrier-cell complexes; and

(ii) culturing the microcarrier-cell complexes.

10. The method of claim 9, wherein the number of cells after step (ii) is expanded.

11. The method of claim 9 or claim 10 wherein culturing the microcarrier-cell complexes comprises suspension culture under static conditions and/or suspension culture with agitation.

12. The method of any one of claims 9 to 11, wherein culturing the microcarrier-cell complexes comprises culture in serum free media.

13. A method of differentiating stem cells, the method comprising:

(i) attaching stem cells to a PCL microcarrier, or to a plurality of PCL microcarriers, according to any one of claims 1 to 8 to form microcarrier-stem cell complexes; and

(ii) inducing differentiation of the stem cells.

14. The PCL microcarrier or plurality thereof according to any one of claims 1 to 8, for use in a method of tissue engineering.

15. The microcarrier or plurality thereof according to any one of claims 1 to 8, for use in a method of medical treatment.

16. Sealed packaging containing at least 0.1 gram of PCL microcarriers according to any one of claims 1 to 8.