SYNTHETIC SURFACES FOR CULTURING STEM CELL DERIVED CARDIOMYOCYTES

PRIORITY

[0001] This application claims priority to US provisional Appl. No. 61/062,921, filed January 30, 2008, and is incorporated by reference in its entirety.

FIELD

[0002] The present disclosure relates to cell culture articles and methods of use thereof, and more particularly to articles suitable for supporting the culture of stem cell derived cardiomyocytes.

BACKGROUND

[0003] Pluripotent stem cells such as human embryonic stem cells (hESCs) have the ability to differentiate into any of the three germ layers, giving rise to any adult cell type in the human body. This unique property provides a potential for developing new treatments for a number of serious cell degenerative diseases, such as diabetes, spinal cord injury, heart diseases and the like. For example, unlike organs such as the skin or liver, the heart is not capable of regenerate sufficient cardiomyocytes to undergo extensive repair. Therefore cardiac repair may benefit from cardiomyocytes, which can be differentiated from hESCs or other pluripotent stem cells, being transplanted into the heart.

[0004] However there remain obstacles in the development of such hESC-based treatments. Such obstacles include obtaining and maintaining adequate numbers of undifferentiated hESCs in tissue culture and controlling their differentiation in order to produce specific cell types. Stem cell cultures, such as hES cell cultures are typically seeded with a small number of cells from a cell bank or stock and then amplified in the undifferentiated state until differentiation is desired for a given therapeutic application. To accomplish this, the hESC or their differentiated cells are currently cultured in the presence of surfaces or media containing animal-derived components, such as feeder layers, fetal bovine serum,

or MATRIGEL. These animal-derived additions to the culture environment expose the cells to potentially harmful viruses or other infectious agents which could be transferred to patients or compromise general culture and maintenance of the hESCs. In addition, such biological products are vulnerable to batch variation, immune response and limited shelf-life.

[0005] Some steps have been taken to culture hESCs either in media or on surfaces that are free of animal-derived components. However, the response of hESCs or their differentiated derivatives is difficult to predict as components of the surface or culture medium change. Yet some advances have been made. For example, hESC-derived cardiomyocytes have been cultured in defined serum-free medium. While such culture systems are not completely xeno-free culture systems when the matrices employed contain animal-derived components, such as gelatin and MATRIGEL, they do provide a step toward the eventual clinical application of hESC-derived cardiomyocytes. By way of further example, some synthetic surfaces have been identified that can support differentiation of human epithelial stem cells into epithelial cells. However, the systems employed relied on serum medium for the cell culture, which still potentially causes problem as described before for all biological animal derived components. To date, a completely animal free system employing a chemically defined medium and a synthetic surface has not yet been identified for culturing stem cells or cells derived from stem cells.

BRIEF SUMMARY

[0006] The present disclosure describes, *inter alia*, synthetic surfaces useful in the culture of stem cell-derived cardiomyocytes in chemically defined media.

[0007] In an embodiment, a method for culturing a stem cell-derived cardiomyocytes is provided. The method includes depositing a suspension containing the stem cell-derived cardiomyocyte on a polymer material and culturing the deposited stem cell-derived cardiomyocyte in a cell culture medium. The polymer material comprises a homopolymer or copolymer of selected one or more acrylate monomers.

[0008] In an embodiment, a culture of a stem cell-derived cardiomyocyte is provided. The culture includes an article having a polymeric material disposed on a surface. The culture

further includes the stem cell-derived cardiomyocyte disposed on the polymer material and a culture medium in which the stem cell-derived cardiomyocyte is cultured. The polymer material comprises a homopolymer or copolymer of selected one or more acrylate monomers.

[0009] In an embodiment, a cell culture article for culturing stem cell-derived cardiomyocytes in a chemically defined medium is provided. The article includes a substrate having a surface and a polymer material disposed on the surface. The polymer material comprises a homopolymer or copolymer of selected one or more acrylate monomers.

[0010] One or more of the various embodiments presented herein provide one or more advantages over prior surfaces for culturing stem cell-derived cardiomyocytes. For example, the synthetic surfaces reduce potential contamination issues associated with surfaces having components obtained from or derived from animal sources. Such surfaces may also provide for improved shelf life compared to those surfaces with biological components. The ability to culture stem cell-derived cardiomyocytes in chemically-defined media further reduces potential contamination issues. In addition, there will likely be less batch to batch variation in the ability of the synthetic surfaces or chemically defined media, resulting in improved reproducibility of culture results and expectations. These and other advantages will be readily understood from the following detailed descriptions when read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIG. 1A-B** are schematic diagrams of side views of synthetic polymer layer coated articles.

[0012] **FIG. 2A-C** are schematic diagrams of cross sections of a multi-well cell culture plate. The plate is uncoated in **FIG. 2A** and coated in **FIGs. 2B-C.**

[0013] **FIG. 3** is a fluorescent image of stem cell derived cardiomyocytes cultured in chemically defined medium and on a surface of formulation 1 (A), formulation 18 (B), and MATRIGEL (C) as described in Example 1. Green: Nkx 2.5. Red: Alpha-actinin.

[0014] The drawings are not necessarily to scale. Like numbers used in the figures refer to like components, steps and the like. However, it will be understood that the use of a number to refer to a component in a given figure is not intended to limit the component in another figure labeled with the same number. In addition, the use of different numbers to refer to components is not intended to indicate that the different numbered components cannot be the same or similar.

DETAILED DESCRIPTION

[0015] In the following detailed description, reference is made to the accompanying drawings that form a part hereof, and in which are shown by way of illustration several specific embodiments of devices, systems and methods. It is to be understood that other embodiments are contemplated and may be made without departing from the scope or spirit of the present disclosure. The following detailed description, therefore, is not to be taken in a limiting sense.

[0016] All scientific and technical terms used herein have meanings commonly used in the art unless otherwise specified. The definitions provided herein are to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0017] As used in this specification and the appended claims, the singular forms "a", "an", and "the" encompass embodiments having plural referents, unless the content clearly dictates otherwise. As used in this specification and the appended claims, the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

[0018] Unless stated otherwise, ratios of compounds in a composition, such as a solution, are stated on a by volume basis.

[0019] As used herein, "have", "having", "include", "including", "comprise", "comprising" or the like are used in their open ended sense, and generally mean "including, but not limited to".

[0020] As used herein the term "acrylate" includes compounds containing an acrylate moiety or a methacrylate moiety. An acrylate moiety is moiety of the following formula: $CH_2CHC(O)O$-. A methacrylate moiety is a moiety of the following formula: $CH_2C(CH_3)C(O)O$-. For the purposes of this disclosure, the term "acrylate" includes specific compounds disclosed in Table 1. "Acrylate" and "methacrylate" are used herein interchangeable, except when content clearly dictates otherwise; e.g. when a specific compound or group of compounds are named.

[0021] The present disclosure describes, *inter alia*, articles having synthetic surfaces for culturing stem cell-derived cardiomyocytes and methods for culturing stem cell-derived cardiomyocytes on such surfaces. In some embodiments, the synthetic surfaces are used in combination with a chemically defined medium to culture stem cell-derived cardiomyocytes. The surfaces may be useful in differentiating stem cells, such as hESCs, into cardiomyocytes.

[0022] 1. Cell Culture Article

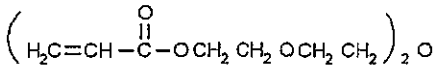
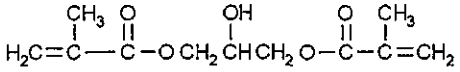
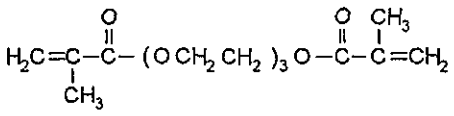
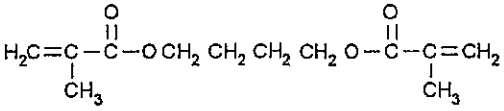
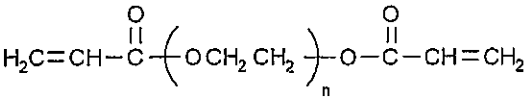
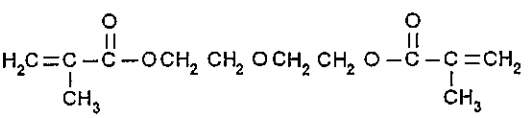
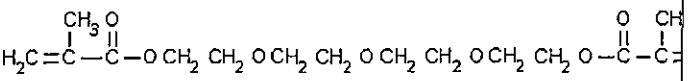
[0023] Referring to **FIG. 1**, a schematic diagram of article **100** for culturing cells is shown. The article **100** includes a base material substrate **10** having a surface **15**. A synthetic polymer coating layer **20** is disposed on the surface **15** of the base material **10**. While not shown, it will be understood that synthetic polymer coating **20** may be disposed on a portion of base material **10**. The base material **10** may be any material suitable for culturing cells, including a ceramic substance, a glass, a plastic, a polymer or co-polymer, any combinations thereof, or a coating of one material on another. Such base materials **10** include glass materials such as soda-lime glass, pyrex glass, vycor glass, quartz glass; silicon; plastics or polymers, including dendritic polymers, such as poly(vinyl chloride), poly(vinyl alcohol), poly(methyl methacrylate), poly(vinyl acetate-maleic anhydride), poly(dimethylsiloxane) monomethacrylate, cyclic olefin polymers, fluorocarbon polymers, polystyrenes, polypropylene, polyethyleneimine; copolymers such as poly(vinyl acetate-co-maleic anhydride), poly(styrene-co-maleic anhydride), poly(ethylene-co-acrylic acid) or derivatives of these or the like.

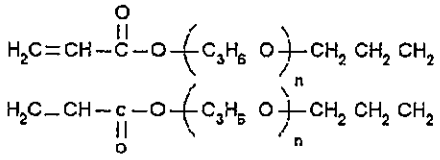
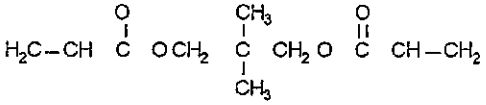
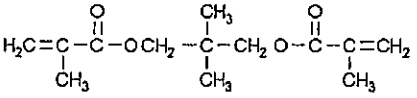
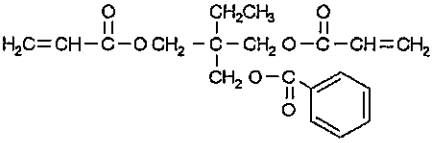
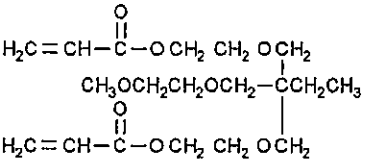
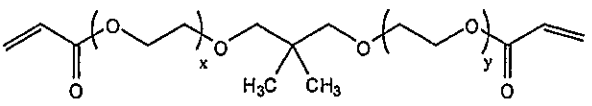
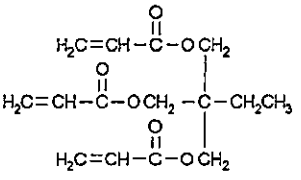
[0024] Examples of articles **100** suitable for cell culture include single and multi-well plates, such as 6, 12, 96, 384, and 1536 well plates, jars, petri dishes, flasks, beakers, plates,

roller bottles, slides, such as chambered and multichambered culture slides, tubes, cover slips, cups, spinner bottles, perfusion chambers, bioreactors, and fermenters.

[0025] Synthetic polymer coating **20** provides a surface **25** on which cells may be cultured. The synthetic polymer surface **20** includes polymerized (meth)acrylate monomers, selected from the group of monomers provided in **Table 1** below. Other materials (not shown), such as peptides, may be incorporated into or conjugated to synthetic polymer surface to produce a biomimetic surface.

Table 1: List of acrylate and methacrylate monomers

Monomer name	Monomer structure
Tetra(ethylene glycol) diacrylate	
Glycerol dimethacrylate	
Triethylene glycol dimethacrylate	
1,4-Butanediol dimethacrylate	
Poly(ethylene glycol) diacrylate (average M_n ~258)	
Di(ethylene glycol) dimethacrylate	
Tetra(ethylene glycol)	

Monomer name	Monomer structure
dimethacrylate	
1,6-Hexanediol propoxylate diacrylate	
Neopentyl glycol diacrylate	
Neopentyl glycol dimethacrylate	
Trimethylolpropane benzoate diacrylate	
Trimethylolpropane ethoxylate (1 EO/OH) methyl	
Tricyclo[5.2.1.$0^{2,6}$]decanedimethanol diacrylate	
Neopentyl glycol ethoxylate diacrylate	
Trimethylolpropane triacrylate	

[0026] The acrylates listed in **Table 1** may be synthesized as known in the art or obtained from a commercial vendor, such as Polysciences, Inc., Sigma Aldrich, Inc., and Sartomer, Inc.

[0027] As shown in **FIG. 1B**, an intermediate layer **30** may be disposed between surface **15** of base material **10** and the synthetic polymer coating **20**. Intermediate layer **30** may be configured to improve binding of coating **20** to substrate **10**, to facilitate monomer spreading, to render portions of the surface **10** that are uncoated cytophobic to encourage cell growth on coated areas, to provide a substrate compatible with a monomer or solvent where the monomer or solvent is incompatible with the base material **10**, to provide topographical features if desired through, for example, patterned printing, or the like. For example, if substrate **10** is a glass substrate, it may be desirable to treat a surface of the glass substrate with an epoxy coating or a silane coating. For various polymer base materials **10** it may be desirable to provide an intermediate layer **30** of polyamide, polyimide, polypropylene, polyethtylene, or polyacrylate. While not shown, it will be understood that synthetic polymer coating **20** may be disposed on a portion of intermediate layer **30**. It will be further understood that intermediate layer **30** may be disposed on a portion of base material **10**.

[0028] In various embodiments, surface **15** of base material **10** is treated, either physically or chemically, to impart a desirable property or characteristic to the surface **15**. For example, and as discussed below, surface **15** may be corona treated or plasma treated. Examples of vacuum or atmospheric pressure plasma include radio frequency (RF) and microwave plasmas both primary and secondary, dielectric barrier discharge, and corona discharge generated in molecular or mixed gases including air, oxygen, nitrogen, argon, carbon dioxide, nitrous oxide, or water vapor.

[0029] Synthetic polymer coating layer **20**, whether disposed on an intermediate layer **30** or base material **10**, preferably uniformly coats the underlying substrate. By "uniformly coated", it is meant that the layer **20** in a given area, for example a surface of a well of a culture plate, completely coats the area at a thickness of about 5 nm or greater. While the thickness of a uniformly coated surface may vary across the surface, there are no areas of the uniformly coated surfaces through which the underlying layer (either intermediate layer **30** or base material **10**) is exposed. Cell responses across non-uniform surfaces tend to be more variable than cell responses across uniform surfaces.

[0030] Synthetic polymer coating layer **20** may have any desirable thickness. However, it has been found that thicker coatings, e.g. coatings of greater than about 10 micrometers, tend to have unevenness around the periphery of the coating due to surface tension. In various embodiments, the thickness of the coating layer **20** is less than about 10 micrometers. For example, the thickness may be less than about 5 micrometers, less than about 2 micrometers, less than about 1 micrometers, less than about 0.5 micrometers or less than about 0.1 micrometers.

[0031] The polymer material forming synthetic polymer layer **20** may be cross-linked to any suitable degree. Higher degrees of cross-linking may result in reduced waste product and reduced cell toxicity.

[0032] Article **100**, in numerous embodiments, is cell culture ware having a well, such as a petri dish, a multi-well plate, a flask, a beaker or other container having a well. Referring now to **FIG. 2**, article **100** formed from base material **10** may include one or more wells **50**. Well **50** includes a sidewall **55** and a surface **15**. Referring to **FIG. 2B-C**, a synthetic polymer coating **20** may be disposed on surface **15** or sidewalls **55** (or, as discussed above with regard to **FIG. 1** one or more intermediate layer **30** may be disposed between surface **15** or sidewall **55** and synthetic polymer coating **20**) or a portion thereof.

[0033] In various embodiments, article **100** includes a uniformly coated layer **20** having a surface **25** with an area greater than about 5 mm^2. When the area of the surface **15** is too small, reliable cell responses may not be readily observable because some cells, such as human embryonic stem cells, are seeded as colonies or clusters of cells (e.g., having a diameter of about 0.5 mm) and adequate surface is desirable to ensure attachment of sufficient numbers of colonies to produce a quantitative cell response. In numerous embodiments, an article **100** has a well **50** having a uniformly coated surface **15**, where the surface **15** has an area greater than about 0.1 cm^2, greater than about 0.3 cm^2, greater than about 0.9 cm^2, or greater than about 1cm^2.

[0034] 2. Coating of Synthetic Polymer Layer

[0035] A synthetic polymer layer may be disposed on a surface of a cell culture article via any known or future developed process. Preferably, the synthetic polymer layer provides a uniform layer that does not delaminate during typical cell culture conditions. The synthetic polymer surface may be associated with the base material substrate via covalent

or non-covalent interactions. Examples of non-covalent interactions that may associate the synthetic polymer surface with the substrate include chemical adsorption, hydrogen bonding, surface interpenetration, ionic bonding, van der Waals forces, hydrophobic interactions, dipole-dipole interactions, mechanical interlocking, and combinations thereof.

[0036] In various embodiments, the base material substrate surface is coated according to the teachings of co-pending application serial no. _____, filed on even date herewith, naming Gehman et al. as inventors, having attorney docket number 20726, and entitled STEM CELL CULTURE ARTICLE AND SCREENING, which is hereby incorporated herein by reference in its entirety for all purposes to the extent that it does not conflict with the disclosure presented herein.

[0037] In numerous embodiments, monomers are deposited on a surface of a cell culture article and polymerized in situ. In such embodiments, the base material will be referred to herein as the "substrate" on which the synthetic polymer material is deposited. Polymerization may be done in solution phase or in bulk phase.

[0038] As many of the monomers identified in **Table 1** above are viscous, it may be desirable to dilute the monomers in a suitable solvent to reduce viscosity prior to being dispensed on the surface. Reducing viscosity may allow for thinner and more uniform layers of the synthetic polymer material to be formed. One of skill in the art will be able to readily select a suitable solvent. Preferably the solvent is compatible with the material forming the cell culture article and the monomers. It may be desirable to select a monomer that is non-toxic to the cells to be cultured and that does not interfere with the polymerization reaction. Alternatively, or in addition, selection of a solvent that can be substantially completely removed or removed to an extent that it is non-toxic or no longer interferes with polymerization may be desirable. In such circumstances, it may be desirable that the solvent be readily removable without harsh conditions, such as vacuum or extreme heat. Volatile solvents are examples of such readily removable solvents.

[0039] Some solvents that may be suitable in various situations for coating articles as described herein include ethanol, isopropanol, acetyl acetate, dimethylformamide (DMF), and dimethylsulfoxide (DMSO). As described in co-pending application serial no.

_____, ethanol may be a particularly suitable solvent when it is desired to remove solvent prior to polymerization.

[0040] The monomers may be diluted with solvent by any suitable amount to achieve the desired viscosity and monomer concentration. Generally the monomer compositions used according to the teachings presented herein contain between about 1% to about 99% monomer. By way of example, the monomer may be diluted with an ethanol solvent to provide a composition having between about 1% and about 50% monomer, or from about 1% to about 10% monomer by volume. The monomers may be diluted with solvent so that the polymer layer **20** achieves a desired thickness. As discussed above, if the deposited monomers are too thick, a non-uniform surface may result. As described in further details in the Examples, non-uniform surfaces may be observed when the monomer-solvent composition is deposited on a surface **15** of a well **50** at a volume of greater than about 8 microliters per square centimeter of the surface **15**. In various embodiments, the monomer-solvent compositions are deposited on a surface **15** of a well **50** in a volume of about 7 microliters or less per square centimeter of the surface **15**. For example, the monomer-solvent compositions may be deposited on a surface **15** of a well **50** in a volume of about 5 microliters or less per square centimeter of the surface **15**, or about 2 microliters or less per square centimeter of the surface **15**.

[0041] In various embodiments, synthetic polymer surface is deposited on a surface of an intermediate layer that is associated with the base material via covalent or non-covalent interactions, either directly or via one or more additional intermediate layers (not shown). In such embodiments, the intermediate layer will be referred to herein as the "substrate" onto which the synthetic polymer surface is deposited.

[0042] In various embodiments, the surface of the base material is treated. The surface may be treated to improve binding of the synthetic polymer surface to the base material surface, to facilitate monomer spreading on the base material surface, or the like. Of course, the base material may be treated for similar purposes with regard to an intermediate layer. In various embodiments, the surface is corona treated or vacuum plasma treated. High surface energy obtainable from such treatments may facilitate monomer spreading and uniform coating. Examples of vacuum plasma treatment that may be employed include microwave vacuum plasma treatments and radio frequency vacuum plasma treatments.

The vacuum plasma treatments may be performed in the presence of reactive gases, such as oxygen, nitrogen, ammonia or nitric oxide.

[0043] To form the synthetic polymer surface, one or more monomers presented in **Table 1** above are polymerized. If one monomer is used, the polymer will be referred to as a homopolymer of the monomer. If two or more different monomers are used, the polymer will be referred to as a copolymer of the monomers. The monomers employed may be monofunctional, difunctional, or higher-functional. When two or more monomers are used, the ratio of the monomers may be varied. In various embodiments, two monomers are used and the ratio, by volume of the first monomer to the second monomer ranges from between about 5:95 to about 95:5. For example, the ratio of the first monomer to the second monomer ranges from between about 10:90 to about 90:10, about 20:80 to about 80:20, from about 30:70 to about 70:30. In some embodiments, the ratio of the first monomer to the second monomer is about 50:50, 30:70, or 10:90. It will be understood that the molecular weight of the polymer may be controlled by varying the concentration of monomers or the ratios of difunctional or higher-functional monomers to monofunctional monomers. Increased concentrations of difunctional or higher-functional monomers will increase the degree of cross-linking in the chains.

[0044] In addition to the monomers that form the polymer layer, a composition forming the layer may include one or more additional compounds such as surfactants, wetting agents, photoinitiators, thermal initiators, catalysts, activators, and cross-linking agents.

[0045] Any suitable polymerization initiator may be employed. One of skill in the art will readily be able to select a suitable initiator, e.g. a radical initiator or a cationic initiator, suitable for use with the monomers listed in **Table 1**. In various embodiments, UV light is used to generate free radical monomers to initiate chain polymerization.

[0046] Any suitable initiator may be used. Examples of polymerization initiators include organic peroxides, azo compounds, quinones, nitroso compounds, acyl halides, hydrazones, mercapto compounds, pyrylium compounds, imidazoles, chlorotriazines, benzoin, benzoin alkyl ethers, diketones, phenones, or mixtures thereof. Examples of suitable commercially available, ultraviolet-activated and visible light-activated photoinitiators have tradenames such as IRGACURE 651, IRGACURE 184, IRGACURE 369, IRGACURE 819, DAROCUR 4265 and DAROCUR 1173 commercially available from

Ciba Specialty Chemicals, Tarrytown, N.Y. and LUCIRIN TPO and LUCIRIN TPO-L commercially available from BASF (Charlotte, N.C.)

[0047] A photosensitizer may also be included in a suitable initiator system. Representative photosensitizers have carbonyl groups or tertiary amino groups or mixtures thereof. Photosensitizers having a carbonyl groups include benzophenone, acetophenone, benzil, benzaldehyde, o-chlorobenzaldehyde, xanthone, thioxanthone, 9,10-anthraquinone, and other aromatic ketones. Photosensitizers having tertiary amines include methyldiethanolamine, ethyldiethanolamine, triethanolamine, phenylmethyl-ethanolamine, and dimethylaminoethylbenzoate. Commercially available photosensitizers include QUANTICURE ITX, QUANTICURE QTX, QUANTICURE PTX, QUANTICURE EPD from Biddle Sawyer Corp.

[0048] In general, the amount of photosensitizer or photoinitiator system may vary from about 0.01 to 10% by weight.

[0049] Examples of cationic initiators include salts of onium cations, such as arylsulfonium salts, as well as organometallic salts such as ion arene systems.

[0050] In various embodiments where the monomers are diluted in solvent before being deposited on the substrate surface, the solvent is removed prior to polymerizing. The solvent may be removed by any suitable mechanism or process. As described in copending application serial no. _____, it has been found that removal of substantially all of the solvent prior to curing, allows for better control of curing kinetics and the amount of monomer converted. When conversion rates of the monomers are increased, waste generation and cytotoxicity are reduced.

[0051] Whether polymerized in bulk phase (substantially solvent free) or solvent phase, the monomers are polymerized via an appropriate initiation mechanism. Many of such mechanisms are well known in the art. For example, temperature may be increased to activate a thermal initiator, photoinitiators may be activated by exposure to appropriate wavelength of light, or the like. According to numerous embodiments, the monomer or monomer mixture is cured using UV light. The curing preferably occurs under inert gas protection, such as nitrogen protection, to prevent oxygen inhibition. Suitable UV light combined with gas protection may increase polymer conversion, insure coating integrity and reduce cytotoxicity.

[0052] The cured synthetic polymer layer may be washed with solvent one or more times to remove impurities such as unreacted monomers or low molecular weight polymer species. In various embodiments, the layer is washed with an ethanol solvent, e.g. greater than about 70% ethanol, greater than about 90% ethanol, greater than about 95% ethanol or greater than about 99% ethanol. Washing with an ethanol solvent may not only serve to remove impurities, which may be cytotoxic, but also can serve to sterilize the surface prior to incubation with cells.

[0053] 3. Incubating cells on synthetic polymer layer

[0054] Stem cell-derived cardiomyocytes may be cultured on a synthetic polymer layer, as described above, according to any suitable protocol. As used herein, "stem cell derived cardiomyocyte" means a cardiomyocyte obtained from differentiation of a stem cell. In some embodiments, the stem cells are multipotent, totipotent, or pluripotent stem cells. In some embodiments the cells may be present in an organ or tissue of a subject. In numerous embodiments, the stem cells are embryonic stem cells, such as human embryonic stem cells.

[0055] Because human embryonic stem cells (hES) have the ability to grown continually in culture in an undifferentiated state, the hES cells for use in this invention may be obtained from an established cell line. Examples of human embryonic stem cell lines that have been established include, but are not limited to, H1, H7, H9, H13 or H14 (available from WiCell established by the University of Wisconsin) (Thompson (1998) *Science* 282:1145); hESBGN-01, hESBGN-02, hESBGN-03 (BresaGen, Inc., Athens, GA); HES-1, HES-2, HES-3, HES-4, HES-5, HES-6 (from ES Cell International, Inc., Singapore); HSF-1, HSF-6 (from University of California at San Francisco); I 3, I 3.2, I 3.3, I 4, I 6, I 6.2, J 3, J 3.2 (derived at the Technion-Israel Institute of Technology, Haifa, Israel); UCSF-1 and UCSF-2 (Genbacev et al., Fertil. Steril. 83(5):1517-29, 2005); lines HUES 1-17 (Cowan et al., NEJM 350(13):1353-56, 2004); and line ACT-14 (Klimanskaya et al., Lancet, 365(9471):1636-41, 2005). Embryonic stem cells used in the invention may also be obtained directly from primary embryonic tissue. Typically this is done using frozen in vitro fertilized eggs at the blastocyst stage, which would otherwise be discarded.

[0056] Cardiomyocytes according to the invention may also be differentiated from induced primate pluripotent stem (iPS) cells. iPS cells refer to cells, obtained from a juvenile or

adult mammal such as a human, that are genetically modified, e.g., by transfection with one or more appropriate vectors, such that they are reprogrammed to attain the phenotype of a pluripotent stem cell such as an hES cell. Phenotypic traits attained by these reprogrammed cells include morphology resembling stem cells isolated from a blastocyst as well as surface antigen expression, gene expression and telomerase activity resembling blastocyst derived embryonic stem cells. The iPS cells typically have the ability to differentiate into at least one cell type from each of the primary germ layers: ectoderm, endoderm and mesoderm and thus are suitable for differentiation into cardiomyocytes. The iPS cells, like hES cells, also form teratomas when injected into immuno-deficient mice, e.g., SCID mice. (Takahashi et al., (2007) Cell 131(5):861; Yu et al., (2007) Science318:5858).

[0057]

[0058] Stem cell derived cardiomyocytes may be obtained by any suitable methods. One way to obtain such cells is described in Laflamme et al., "Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infracted rats, *Nature Biotechnology*, 25: 1015 - 1024 (2007). Briefly, undifferentiated human embryonic stem cells, such as those derived from the female H7 human embryonic stem cell line, may be seeded on MATRIGEL-coated plates at a density of about 100,000 cells/cm^2 and refed daily with hES cell growth medium (KO DMEM + 20% Serum replacement, 1 mM l-glutamine, 1% NEAA, 0.1 mM 2-ME plus hbFGF at 80 ng/ml and TGFb1 at 0.5 ng/ml) To induce differentiation, growth media may be replaced with RPMI-B27 medium (available from Invitrogen) supplemented with about 100 ng/ml human recombinant activin A (available from R&D Systems) for about 24 hours, followed by 10 ng/ml human recombinant BMP4 (available from R&D Systems) for four days. Of course, any other suitable method may be employed (see, e.g., US Patent No. 7,425,448).

[0059] Prior to seeding cells, the cells may be harvested and suspended in a suitable medium, such as a growth medium in which the cells are to be cultured once seeded onto the surface. For example, the cells may be suspended in and cultured in serum-containing medium, a conditioned medium, or a chemically-defined medium. As used herein, "chemically-defined medium" means cell culture media that contains no components of unknown composition. Chemically defined media may, in various embodiments, contain no proteins, hydrosylates, or peptides of unknown origin. In some embodiments,

conditioned media contains polypeptides or proteins of known composition, such as recombinant growth hormones. Because all components of chemically-defined media have a known chemical structure and composition, variability in culture conditions can be reduced and thus cell response may be more reproducible. In addition, the possibility of contamination is reduced. Further, the ability to scale up is made easier due, at least in part, to the factors discussed above. Chemically defined cell culture media are commercially available from Invitrogen (Invitrogen Corporation, 1600 Faraday Avenue, PO Box 6482, Carlsbad, California 92008) as StemPro® a fully defined, serum- and feeder-free medium (SFM) specially formulated for the growth and expansion of human embryonic stem cells (hESCs) and StemCell Technologies, Inc as mTeSR™1 maintenance media for human embryonic stem cells.

[0060] The cells may be seeded at any suitable concentration. Typically, the cells are seeded at about 10,000 cells/cm^2 of substrate to about 500,000 cells/cm^2. For example, cells may be seeded at about 50,000 cells/cm^2 of substrate to about 150,000 cells/cm^2. However, higher and lower concentrations may readily be used. The incubation time and conditions, such as temperature CO_2 and O_2 levels, growth medium, and the like, will depend on the nature of the cells being cultured and can be readily modified. The amount of time that the cells are incubated on the surface may vary depending on the cell response being studied or the cell response desired.

[0061] Any suitable method may be used, if desired, to confirm that the stem cell derived cardiomyocytes are indeed cardiomyocytes or that the stem cells employed have successfully differentiated into cardiomyocytes. For example, the presence of certain cardiomyocyte-selective markers may be investigated. Such markers include Nkx2.5 and α-actinin, cardiac troponin I. Antibodies to such markers may be used in standard immunocytochemical or flow cytometry techniques. In addition or alternatively, cellular morphology or functionality, by observing beating cardiomyocytes in culture or by performing various electrophysiological analyses to determine whether the cells have characteristics of cardiomyocytes.

[0062] The cultured stem cell derived cardiomyocytes may be used for any suitable purpose, including investigational studies in culture, in animals, for developing therapeutic uses, for drug discovery and toxicology or for therapeutic purposes. One potential therapeutic or investigational purpose is repairing cardiac damage due to an infarct, e.g., as described

in Laflamme et al., *Nature Biotechnology*, 25: 1015 - 1024 (2007). The cells may also be used to create cDNA libraries according to known methods. The cDNA libraries may be used to study gene expression in the differentiated cardiomyocytes. For example the library obtained from the differentiated cardiomyocytes may be compared to a cDNA library from the undifferentiated stem cells from which the cardiomyocytes were derived, thus allowing for the identification and isolation of genes related to the differentiation and development of cardiomyocytes.

[0063] In the following, non-limiting examples are presented, which describe various embodiments of the articles and methods discussed above.

EXAMPLES

[0064] EXAMPLE 1: Identification of Acrylic Coating Surfaces Suitable for Culturing Stem Cell Derived Cardiomyocytes in a Chemically Defined Medium

[0065] 1. Coating Preparation

[0066] Acrylic coating surfaces were prepared from homomonomers or copolymers of various acrylate and methacrylate monomers. For copolymers two different acrylate or methacrylate monomers were used. Briefly, the monomers were mixed with 1% w/w of photoinitiator Irgacure 819 (Ciba Specialty Chemiscals, Inc.) and used along or blended with other monomer formulation (monomer with 1% w/w of photoinitiator) according to volume ratio of 70:30. Then formulation was placed in a well of a vacuum plasma treated cyclic olefin copolymer plate (provided by Corning Life Science Development group) at a volume of 2.5 μL. The plate was allowed to lay horizontally flat for 30 min for the formulation to spread out. The coatings were cured with 13mW/cm^2 pulsed (100 Hz) UV light (Xenon RC-700) for 1 min in N_2 purged box (with fused silica window). All the plates were sterilized by 25-35 kGy Gamma radiation prior to cell culture.

[0067] 2. Cell preparation and assays

[0068] Prior to the experiments, H7 hES cells were maintained in the undifferentiated state on MATRIGEL-coated plates in SR medium (KO-DMEM, 20 % KO-serum replacement, 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% non- essential amino acids, 80 ng/ml

hbFGF and 0.5 ng/ml TGFb1). hESC-derived cardiomyocytes were generated using direct differentiation protocol. Briefly, undifferentiated H7 cells were harvested by 200 U/ml collagenase IV and seeded on MATRIGEL-coated plates at the density of 100,000 cells/cm^2 in SR medium. Cells were cultured for 6 days with daily medium exchange. Cardiac differentiation was initiated by replacing SR medium with RPMI-B27 medium, supplemented with 100 ng/ml human recombinant activin A for 24 h, followed by 10 ng/ml human recombinant BMP4 for 4 days. At this point, cells were detached by Accutase treatment, re-suspended in RPMI-B27 medium w/o growth factors, and seeded at the density of100,000 cells/cm^2 onto Corning CB/TOPAS 96-well plates coated with different binary mixtures of acrylates or MATRIGEL as positive control. Cells were cultured for another 2-3 weeks with the same medium exchange every 2-3 days. The wells were microscopically examined, and spontaneous beating activity was recorded.

[0069] At the end of the differentiation protocol, cells were fixed with 4% paraformaldehyde immunostained for cardiomyocyte (CM) specific markers, Nkx2.5 and α-actinin, and counterstained with 4'-6-Diamidino-2-phenylindole (DAPI) to stain the nucleus. After scanning each plate with ArrayScan, the following quantitative analyses were performed for each surface: 1) TNC: total number of cells based on DAPI positive cell number, 2) TNC: total number of CM, based on Nkx2.5-positive cell number, 3) CM yield = TNC/TNC.

[0070] 3. Results

[0071] It was found that only a small portion of the tested surfaces supported the cell growth while maintaining some key characteristics and functions. Examples of the coating surfaces which supported growth of differentiated human embryonic cardiomyocytes in chemically defined medium are listed in **Table 2**, where the volume ratio of monomer (1) to monomer (2) is 70:30. These surfaces were characterized and given a "grade" based on qualitative assessments of the morphology of the cells and their adhesion to the surfaces. Acrylate surface rating was performed based on consideration of the following criteria: 1) total cell number, 2) total CM number, 3) CM yield, 4) presence of beating areas, 5) similarity to Matrigel derived CM morphology. Notes on the qualities of the differentiated embryonic myocytes that were observed are shown in Table 2, as well as the grade that was assessed. For example, "Sim MA" is a note that indicates that cells growing on that surface were similar to cells growing on Matrigel™. "Sim TOP" is a

note that indicates that the cells growing on that surface were similar to cells growing on plasma-treated cyclic olefin copolymer surface, sold as TOPAS® surface by TOPAS, Florence, Kentucky. The notes also indicate that some of the surfaces supported differentiated embryonic myocytes that began to beat in culture.

Table 2. Example compositions of acrylic polymers which support the culture of hES cell derived cardiomyocytes in chemically defined medium.

Formuation ID	Monomer (1)	Monomer (2)
95-1	Tetra(ethylene glycol) diacrylate 100%	
95-2	Glycerol dimethacrylate100%	
90-2	Triethylene glycol dimethacrylate 100%	
90-4	1,4-Butanediol dimethacrylate 100%	
95-3	Poly(ethylene glycol) diacrylate 100%	
122-1	Tetra(ethylene glycol) diacrylate 70%	Glycerol dimethacrylate 30%
122-3	Tetra(ethylene glycol) diacrylate 70%	1,4-Butanediol dimethacrylate 30%
27-2	Tetra(ethylene glycol) diacrylate 70%	Trimethylolpropane triacrylate 30%
123-1	Glycerol dimethacrylate 70%	Tetra(ethylene glycol) diacrylate 30%
123-2	Glycerol dimethacrylate 70%	Tri(ethylene glycol) dimethacrylate 30%
123-3	Glycerol dimethacrylate70%	1,4-Butanediol dimethacrylate 30%
123-4	Glycerol dimethacrylate 70%	Poly(ethylene glycol) diacrylate 30%
123-6	Triethylene glycol dimethacrylate 70%	Glycerol dimethacrylate 30%
123-7	Triethylene glycol dimethacrylate 70%	1,4-Butanediol dimethacrylate 30%
124-2	1,4-Butanediol dimethacrylate 70%	Glycerol dimethacrylate 30%
124-3	1,4-Butanediol dimethacrylate 70%	Triethylene glycol dimethacrylate 30%
124-5	Poly(ethylene glycol) diacrylate 70%	Tetra(ethylene glycol) diacrylate 30%
133-1	Di(ethylene glycol) dimethacrylate 70%	Tetraethylene glycol dimethacrylate 30%

134-1	Tetraethylene glycol dimethacrylate 70%	Di(ethylene glycol) dimethacrylate 30%
140-1	Trimethylolpropane triacrylate 70%	Neopentyl glycol ethoxylate diacrylate 30%

TABLE 3. Cell count, cell surface Marker, and functions of h7 differentiated cardiomyocyted cultured on acrylate polymer surfaces.

ID	Rating	Beating	Average Cell Count (per well)	Average % Nkx2.5	Cardio yield (based on count*Nkx2.5)	Note
95-2	A	y	19970	7	1397.9	Sim MA
90-2	C-		5420	14	758.8	Sim MA, peeled lumpy
90-4	B-	y	6907	24	1657.68	Sim TOP
95-3	C	y	3788	24	909.12	
122-3	B		6061	3	181.83	Sim as MA, not beating
123-1	B+	y	5250	5	262.5	Sim MA, beating
123-2	A		6327	7	442.89	Good attachment, no beating
123-3	A		6223	8	497.84	Good attachment, no beating
123-4	B-		4954	4	198.16	Many attached, dying
123-7	C		4246	15	636.9	peeled
124-2	A	y	3798	21	797.58	Sim TOP beating
124-3	B	y	2267	8	497.84	Sim TOP beating
124-5	C	y	940	27	253.8	Beating but poor attachment
140-1	B-	y	2886	33	952.38	Sim TOP

[0072] For other tested homopolymers and copolymer combinations, very low or no cardiomyocytes yield was observed at the end of the differentiation procedure, due to either low total cell number or low Nkx2.5-positive cells present on those surfaces

[0073] **FIG. 3** shows fluorescent images of differentiated cardiomyocytes cultured on surfaces of formulation 95-2 (**A**), formulation 27-2 (**B**), and MATRIGEL (**C**). Cell morphology, Nkx 2.5 marker expression (green) and alpha-actinin marker expression (red) are similar to MATRIGEL. In addition, the cells depicted in **FIG. 3** exhibited beating prior to fixing with paraformaldehyde.

[0074] Thus, embodiments of SYNTHETIC SURFACES FOR CULTURING STEM CELL DERIVED CARDIOMYOCYTES are disclosed. One skilled in the art will appreciate that the arrays, compositions, kits and methods described herein can be practiced with embodiments other than those disclosed. The disclosed embodiments are presented for purposes of illustration and not limitation.

CLAIMS

What is claimed is:

1. A culture of a pluripotent stem cell-derived cardiomyocyte, comprising:

an article comprising a polymeric material disposed on a surface; the pluripotent stem cell-derived cardiomyocyte disposed on the polymer material; and

a culture medium in which the pluripotent stem cell-derived cardiomyocyte is cultured,

wherein the polymer material comprises

(i) tetra(ethylene glycol) diacrylate, glycerol dimethacrylate, triethylene glycol dimethacrylate, 1,4-butanediol dimethacrylate, or poly(ethylene glycol) diacrylate, or

(ii) a copolymer formed from tetra(ethylene glycol) diacrylate and 1,4-butanediol dimethacrylate, glycerol dimethacrylate and tetra(ethylene glycol) diacrylate, glycerol dimethacrylate and tri(ethylene glycol) dimethacrylate, glycerol dimethacrylate and 1,4-butanediol dimethacrylate, glycerol dimethacrylate and poly(ethylene glycol) diacrylate, triethylene glycol dimethacrylate and 1,4-butanediol dimethacrylate, poly (ethylene glycol) diacrylate and tetra(ethylene glycol) diacrylate, di(ethylene glycol) dimethacrylate and tetraethylene glycol dimethacrylate, tetra(ethylene glycol) diacrylate and trimethylolpropane triacrylate, trimethylolpropane triacrylate and neopentyl glycol ethoxylate diacrylate, or tetra(ethylene glycol) dimethacrylate and di(ethylene glycol) dimethacrylate.

2. The culture of claim **1**, wherein the polymer material comprises a homopolymer formed from tetra(ethylene glycol) diacrylate or glycerol dimethacrylate or a copolymer formed from (i) glycerol dimethacrylate and tri(ethylene glycol) dimethacrylate, (ii) glycerol dimethacrylate and 1,4-butanediol dimethacrylate, or (iii) tetra(ethylene glycol) diacrylate and trimethylolpropane triacrylate.

3. The culture of claim **1**, wherein the polymer material consists essentially of a homopolymer formed from tetra (ethylene glycol) diacrylate or glycerol dimethacrylate or a copolymer formed from (i) glycerol dimethacrylate and tri (ethylene glycol) dimethacrylate, (ii) glycerol dimethacrylate and 1,4-butanediol dimethacrylate, or (iii) tetra(ethylene glycol) diacrylate and trimethylolpropane triacrylate.

4. The culture of claim 1, wherein culture medium is a chemically defined medium.

5. The culture of claim 1, wherein the stem cell-derived cardiomyocyte is a human embryonic stem cell-derived cardiomyocyte.