METHOD OF FABRICATING CELL ARRAYS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. application Serial No. 62/070,508, filed on August 28, 2014, the disclosure of which is incorporated by reference herein.

BACKGROUND

Induced pluripotent stem cells iPSCs are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three germ layers.

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. In the original protocol, viruses were used to introduce the reprogramming factors into adult cells. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers have now developed non-viral delivery strategies that are believed to have less chance of causing cancers.

This breakthrough discovery of iPSCs has created a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system. The iPSCs strategy creates pluripotent stem cells that, together

with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

However, creating viable large arrays of iPSCs for high throughput drug screening is problematic due to their sensitivity to environmental factors, available nutrients, fabrication techniques, handling, contamination, and dehydration, as well as the cells immediate three dimensional macro structure environment and the precision equipment necessary to dispense nano liter quantities of molecules of interest to specific array locations.

SUMMARY

The present disclosure provides a fabrication process that results in creating large arrays of living cells, e.g., stem cells, which are subsequently exposed to nanoliter quantities of compounds to test the efficacy on cellular metabolism. In one example, induced pluripotent stem cells iPS are mixed with a ultra violet curable hydrogel matrix, coated onto a support or substrate, then mated to an embossing master tool with a pre defined micro geometric pattern in a N x N matrix array where N can range from 1 to 10,000, exposed to UV radiation to harden the gel, the embossing tool removed, and the patterned gel, cell, support structure then transferred to an incubation chamber to enhance cellular growth and viability. The N x N matrix of living cells is subsequently exposed to a drug compound delivery system that can address any N in the N x N matrix and dispense nano liter quantities of drug compounds to the site of interest.

In one embodiment, the present disclosure provides for the use of a micro embossing fabrication technology in combination with a nano liter dispensing system that can address individual locations within a cellular array, e.g., an iPSCs matrix array, to deliver drug candidates of interest, thereby providing large matrix arrays for high throughput drug screening.

In one embodiment, this disclosure provides a method of fabricating arrays by the use of micro embossing an ultraviolet curing gel that contains dispersed cells such as stem cells or iPSCs.

In one embodiment, this disclosure provides a method of fabricating cell s arrays by the use of micro embossing where distinct three dimensional micro structures are created that contain dispersed cells.

In one embodiment, this disclosure provides a method of fabricating cell arrays by the use of micro embossing where an array matrix is formed in an N x N pattern where N can range from 1 to 10,000.

In one embodiment, this disclosure provides for exposing arrays, such as those formed either on a film or microwell plate, as to a high precision nano liter dispensing system. In one embodiment, this disclosure provides for exposing arrays to a high precision nano liter dispensing system that can address individual location sites on a N x N iPSCs array.

BRIEF DESCRIPTION OF THE DRAWING

Figure1 shows a cross sectional view of one embodiment of the present invention that shows iPSCs dispersed into a hardened gel linear matrix.

DETAILED DESCRIPTION

In one embodiment, Figure 1 shows a cross sectional view of one example of the present invention that uses iPSC cardiomyocyte cells 30 that have been dispersed and cured into an ultraviolet sensitive hydrogel 20, that was deposited onto a support 10. The support 10 can be comprised of a number of materials such as metal, glass, ceramic, polymer sheet or film and optionally may be a rigid support. The thickness of the support can range from about 12 microns to about 10 millimeters and in one embodiment, about 0.2 to about 1 millimeters. In most cases, the substrate is transparent to allow visual inspection of the contents of the fabricated array on the surface or to allow UV radiation to pass through the material in order to cure the hydrogel- cell mixture onto the surface. In one embodiment, a polymer substrate such as heat stabilized polyester film, polycarbonate, or 1 mm glass is employed. In addition, it may be desirable to have the substrate be porous in order to allow water and nutrients to diffuse into the bottom of the micro embossed 20 hydrogel array. Some porous materials that may be employed are Vycor glass made by Corning, open cell foam film and sheet, or micro porous polymer films that are readily available from multiple suppliers.

In one embodiment, a hydrogel 20, such as one having methacrylate, e.g., gelatin methacrylate, that has iPSCs dispersed into the volume of the gel is coated onto the support 10 and then the gel is cured into the desired geometric

shape. In the example of Figure 1, that shape is a linear array with dimensions of approximately 1.5 mm x 1.5 mm x 0.25 mm. The linear array shape can be fabricated by mask less direct write photolithography or by using a micro embossing template into which the inverse pattern has been created by diamond engraving, laser ablation, or photolithography, that is pressed into the gel and the gel cured with UV light through a transparent micro embossing template such as silicone or glass or a transparent polymer or glass support 10. A N x N array can be formed onto the support where the number of discrete geometric shapes can range from 2 to over millions. N can have an integer value from 1 to 10,000 but for practical purposes an N x N where N= 50 is sufficient and practical. In the present example a 1 X 16 linear array was fabricated.

In addition to the aforementioned method one skilled in the art can also take a pre-embossed film that has microstructures fabricated as in Figure 1 then optionally depositing onto the surface an adhesion promoter such as fibronectin, gelatin, hyaluronic acid, carrageen, cellulose, polylysine, polyvinylprylidone, collagen, polyvinylalcohol, or polyethylene oxide or combinations of the aforementioned molecules including polymers thereof. Differentiated iPSCs can then be deposited onto the pre-embossed film and laminated to a N x N where N can range from 1 to 10,000, microplate to form an array.

After the iPSCs array is fabricated, the device is then placed in an incubation chamber at 37°C with a 5% carbon dioxide gas atmosphere. A number of additives, such as RB+ and Rock inhibitor, that are well known in the art is/are added to a nutrient solution to nourish and prevent the cells from premature death. The cells may be incubated and nourished for up to 3 days or more prior to exposing them to a drug dosing procedure.

In one example, a Labcyte Echo Model 555 nano liter sonic dispensing system was used to deliver 2 heart drugs, Satolol an Antiarrhythmic drug and Isoproteronol a nitric oxide generating compound, to the fabricated array. The drugs were diluted to 2.25 uM in cell media nutrient solution to prevent dehydration. The Echo 555 liquid handler revolutionizes liquid handling with acoustic energy. Sound waves eject precisely sized droplets from a source onto a microplate, slide or other surface suspended above the source. This product does not use tips, pin tools or nozzles completely eliminating contact between the instrument and the liquid. Fluids are transferred in nanoliter increments. Larger

volumes are transferred at the rate of hundreds of droplets per second. By using this non contact method it minimizes any contact that could damage or kill the cells as found in other dispensing methods. It also allows for the use of very small quantities thus allowing users to screen molecules that are scarce or difficult to manufacture thus reducing cost. In addition, the X and Y position of the dispensing head can be precisely located over the N x N array location of interest thereby allowing different locations to have a drug of interest administered.

The invention will be described by the following non-limiting example.

Example 1

Induced pluripotent stem cells: beating H9 embryonic stem cell derived cardiomyocytes were used on day 25 of differentiation. After disassociation a vial with 1 mL of suspension was produced. The concentration of the suspension was 1.38 x 10^6 mL total cells. Within those it was determined that 8.38 x 10^5 live cells were present with tryptan blue. The food solution provided was a 50 mL aliquot of RB+ medium (RPMI supplemented with B27 with insulin) for the cells after gel encapsulation. A single drop of Rock inhibitor was added to the nutrient solution. This drug is used to avoid cell death until the cells sense that they are attached to a substrate. The vial was used at a 10 mM concentration. The cells were centrifuged and mixed with GelMa a UV curable matrix and kept at 37°C. The GelMa cell mixture was them placed on a glass support and the area defined by hydrophobic tape. Then a TI DLP mirror chip was used in combination with UV light with a peak frequency of 370 nm to direct write a 1 x 16 linear array that was approximately 1.5 mm x 1.5 mm by 0.25 mm high which takes about 10 seconds. The uncured GelMa was washed away. The support and array was placed in a petri dish and filled with nutrient solution and placed in an incubator at 37°C in a 5% CO_2 atmosphere. Each day the nutrient solution is replaced with fresh media for up to 3 days. After 3 days the arrays were demonstrating a strong beating action and were ready for drug dosing. The array was placed in a Labcyte 555 sonic dispensing machine and the drug molecules precisely dispensed onto the beating cells. During this process the cells were observed with a Lumenera Camera with infinity capture software to visualize the beating cells during the drug dosing.

During the dosing with 10 nM of Satolol an increase in the heart cells beating by 30-40% was observed. During the dosing with Isoproteronol it was observed that the drug removed the refectory period (pause in beating) of some irregularly beating heart cells.

The invention herein is described by example and one particular way of practicing the invention has been described. However the invention as claimed herein is not limited to that specific embodiment are not limited to use therewith and may be used separately or in conjunction with other embodiments disclosed herein. For example, instead of using visual cameras to observe drug effects one can use electronic, magnetic, fluorescence, or fiber optic methods to examine cellular response to drug dosing. Equivalence to the description as hereinafter claimed is considered to be in the scope of protection of this patent. While particular embodiments of the method for fabricating cell micro arrays with subsequent drug dosing has been described it will be appreciated by those skilled in the art that changes and modifications may be made thereto without departing from the invention in its broader aspects as set forth in the following claims.

CLAIMS

1. A method of screening compounds on a fabricated array having cells, comprising:

providing a mixture of cells and a UV curable hydrogel matrix, wherein the cells are embryonic stem cells, induced pluripotent stem cells (iPSC) or cardiomyoytes;

applying a volume of the mixture in a N x N matrix array to a support in a specific geometry;

exposing the support to UV light to cure each volume in the array and incubating the array under conditions that provide for cell viability;

contacting each volume of cells in the array with a nanoliter volume of the one or more compounds using ultrasonic deposition;

detecting whether the one or more compounds affect the cells.

2. The method of claim 1 wherein the cells are human iPSCs.

3. The method of claim 1 wherein the cells are human cardiomyocytes.

4. The method of claim 1 wherein the support is a rigid support.

5. The method of claim 1 wherein the support is a flexible support.

6. The method of claim 1 wherein the support comprises glass, ceramic or a polymer sheet or film.

7. The method of claim 1 wherein the array is coated with an adhesion polymer.

8. The method of claim 1 wherein the specific geometry is provided by a mold or by microstructures in the array.

9. The method of claim 1 wherein the support is transparent.

10. The method of claim 1 wherein the support is a polyester film, comprises polycarbonate or comprises glass.

11. The method of claim 1 wherein the support is porous.

12. The method of claim 1 wherein the supportcomprises a pre-embossed film substrate having microstructures that provide for the specific geometry, wherein the microstructures are coated with one or more cellular adhesion polymers; and

wherein the support is laminated to a microplate.

13. The method of claim 1 wherein the hydrogel comprises gelatin.

14. The method of claim 13 wherein the hydrogel comprises methacrylated gelatin (GelMA).

15. The method of claim 1 wherein the cells are incubated in the presence of RPMI supplemented with B27 with insulin (RB+) or Rho-associated protein kinase (Rock) inhibitor.

16. The method of claim 1 wherein the volume of cells is applied to the support and then mated to an embossing master tool with a pre-defined micro geometric pattern in the N x N matrix array.