Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays
(Microfludic_Platform)

Project Design Specifications
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Function:

Cellular microarrays contain populations of living cells that are spatially separated from one another. Because of the numerous discrete populations, these devices are beneficial in high-throughput screening applications. Last semester, we adapted a microfluidic device, originally created by Jeon et al., to be capable of establishing a concentration gradient over the intended cellular microarray portion of the platform. This semester, we aspire to integrate the microfluidic and microarray components in a way that enables them to be compatible with a standard microscope stage. Along with fitting in the stage, the platforms must be able to generate concentration gradients across the field of flow, form a watertight seal, and be reusable in order for the devices to be effective. By accomplishing this, our client will be able to perform live-cell imaging and high-throughput analysis to determine how various culture conditions effect stem cell differentiation.

Client Requirements:

- A prototype microfluidic platform that can:
  - Generate concentration gradients across the field of flow
  - Form a water-tight seal with a microscope slide
  - Be reusable for multiple cellular microarrays
  - Fit on top of a microscope stage and be used for live-cell imaging
  - Allow for diffusion dominated flow or convection dominated flow

1. Physical and Operational Characteristics

A. ***Performance Requirements:*** The device must be able to house cellular microarrays and enable a concentration gradient of fluids to continuously flow, without leakage, while the arrays are imaged with a confocal microscope.
B. ***Safety:*** The apparatus cannot be harmful to the cells that it will contain or the researchers who will be working with it.
C. ***Accuracy and Reliability:*** An accurate and reliable concentration gradient must be able to be established and maintained across the field of flow. The developed concentrations must be within one percent of their expected values. This will ensure that the data obtained from experiments utilizing the microfluidic platform will be repeatable and that the results are truly representative of how certain conditions effect stem cell differentiation.

D. ***Life in Service:*** The platform must be able to be continuously used for the duration of various types of stem cell experiments, which typically range from 1 to 10 days in length. The molds to create the platforms should be capable of being reused for 10 PDMS devices.
E. ***Shelf Life:*** When not in use, the device mold will be stored on a laboratory shelf at 20 °C and standard pressure. It must be capable of retaining its full functionality at these conditions for up to two years.
F. ***Operating Environment:*** While experiments are running, the temperature of the apparatus will be 20-37 °C. During imaging, the laser used may increase the temperature to slightly above 37 °C; however, this change is not expected to be significant and therefore should not affect the efficacy of the device. As testing will be performed in a standard laboratory, humidity and pressure will be within the typical ranges for this type of environment. In order to sterilize the device, it will be autoclaved, temporarily exposing it to high pressure saturated steam at 121 °C, or washed with sterilizing chemicals, which may potentially be corrosive.
G. ***Ergonomics:*** The platform should be easy to use by trained researchers and should not impose any physical strain on their part to assemble or disassemble for experiments. The microarray insert and microfluidic device must have alignment markers that are easy to align using a microscope and must be accurate within 5 microns.
H. ***Size:*** The maximum dimensions for the portion of the microfluidic device that will fit within the microscope stage for imaging are 158 x 105 mm. Gradient generation will need to be accomplished within 67.5 mm before the fluid reaches the culture channels. The insert containing the microarray has dimensions of 50 x 60 mm while the microarray has dimensions of 36 x 46 mm. Each pixel within the microarray has a diameter of 300 microns. The microarray will be microprinted on a 0.16 mm thick glass slide, allowing for maximal magnification if needed. The device should be under 2.5 cm in height to fit within the stage area.
I. ***Weight:*** The device must weigh less than 0.5 kg, the maximum recommended load for the piezoelectric microscope stage that it will be mounted on for imaging.
J. ***Materials:*** The materials used must be biocompatible, nontoxic, and able to withstand sterilization techniques such as autoclaving and the use of sterilizing chemicals. Materials that have a history of use in microfluidic devices and entail simple fabrication and design processes are ideal.
K. ***Aesthetics, Appearance, and Finish:*** The portion of the apparatus that will contain the cellular arrays must be transparent so that the cells can be properly imaged and analyzed by researchers.

2. Production Characteristics

A. ***Quantity:*** One mold that can be used to create PDMS devices for use in multiple experiments is required.
B. ***Target Product Cost:*** If the device does not require temperature control for effective usage, then the target total manufacturing costs should be less than $5,000. If temperature control is required, the costs will likely need to be between $10,000 and $20,000.

3. Miscellaneous

A. ***Standards and Specifications:*** There are no federal regulations that need to be met for this device; however, as the apparatus will be used with cultured cells, it must adhere to standard cell culture protocols.
B. ***Customer:*** Intended customers for this device will desire a microfluidic platform that can be easily applied, removed, and reused on cellular microarrays. Other devices in the competitive market are not removable and thus limit the potential for expansion of cell lines after experimentation.
C. ***Patient-related Concerns:*** Induced pluripotent and embryonic stem cells will be seeded in this apparatus. As a result, it must be able to be sterilized between uses. There are no concerns regarding data storage or confidentiality involved with this project, as the subjects are not patients.
D. ***Competition:*** Several research efforts have used PDMS microfluidic devices to deliver soluble factors to cells and establish concentration gradients (pioneered by Whitesides *et al*., 2000). One notable competing device developed for a similar research goal was patented by David J. Beebe *et al.* in 2007. This apparatus is titled *Microfluidic platform and method of generating a gradient therein*, and implements a single microfluidic channel with porous membranes and source/sink action to generate a gradient of particles. A comparable device using a source and sink gradient bridge titled *Microfluidic gradient devices* was developed and patented by Noo Li Jeon *et al.* in 2011.