ABSTRACT

Titanium is a viable material for prosthetic implants due to its biocompatible surface which facilitates cell adhesion. In this study, titanium discs were used as a substrate for bone marrow stromal cells from a rodent. The stromal cells have been genetically altered with Green Fluorescent Protein (GFP) in order to utilize fluorescent microscopy for cell growth analysis.

Previous research by a graduate student focused on automatically recording periodic images of live cell growth using image acquisition software coupled with a fluorescent microscope camera (Jensen, 2013). During the duration of these trials, the cells were enclosed in a micro-incubator system which replicated the environment of the control incubator. The micro-incubator was set to maintain an internal temperature of 37.0°C and a 5% CO₂ / 95% air atmosphere. This research focused on investigating different ways to optimize the environment of the micro-incubator, since it was concluded from Jensen’s work that there was a huge disparity between the cells’ life cycle, adhesion, and expansion in the control incubator environment versus that of the micro-incubator environment.

RESULTS AND DISCUSSION

Temperature Experiment

For the temperature experiments, the micro-incubator was filled with 12 ml of α-MEM Growth Media + 10% by volume FBS. It was placed on the fluorescence stage and prepared as though a live cell imaging experiment was to be performed, as detailed in Jensen. The changes in this procedure included greasing the thermo-resistor with Dow Corning 705 silicon diffusion pump oil and inserting it into the side of the micro-incubator to measure the micro-incubator’s external temperature. The thermocouple, used to measure the micro-incubator’s internal temperature, was then placed inside the Tygon tubing lead until it hit the bottom of the Teflon well inside the micro-incubator. The set temperature on the controller was then set at 37.0°C, the optimal temperature for rat cell growth. Once the external temperature read by the thermo-resistor reached 37.0°C, the timer was started and the temperatures were recorded for a total of 30 minutes.

Poison Experiment

The three “poisons” encountered by the cell suspension in the micro-incubator that were not present in the control incubator were 1) 316 surgical steel, 2) Dow Corning 734 silicon sealant, and 3) the steel used to make the top of the micro-incubator. The locations of these poisons are highlighted in Figure 1. The cells were thawed, fed, and passaged, following standard cellular biology techniques. After passaging, the appropriate amount of cell suspension needed for the desired viable inoculation density and its balance of growth media was placed on the titanium discs that were within silicon wells. These wells were placed in six wells plates. For each poison trial, two control wells and two poisoned wells were used. The six well plates were placed in the control incubator and the time was recorded. Fluorescence pictures were then taken every 24 hours for 7 days using the Pro Plus digital and video capture software in conjunction with an Olympus BX41 microscope.

REFERENCES


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