Osteoblastic Proliferation on Surgical Implant Materials

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Abstract

According to the United States Bone and Joint Initiative in 2013 nearly 270,000 spinal surgeries involving a spinal-fusion device were performed. Fusion implant materials have been engineered and used for implantation for many years; among these materials include: Titanium and PEEK (Polyether Ether Ketone). A medical device company based out of Salt Lake City specializes in the manufacturing of silicon nitride (Si3N4), a new material for implantation. This company reached out to InnovaBio® to assess their material. It is important to understand which materials will lessen recovery times and save on medical costs. Recovery times can be tested in part based on how bone cells attach and proliferate on the fusion-device material.

Fig. 1
Example of silicon nitride disc

For the assessment, the company's material silicon nitride and the lead implant material PEEK are being compared through standard and growth curves. Using an osteoblastic cell line, MG-63, cell proliferation was determined by staining with crystal violet which was detected through spectrophotometry. Part of the research data presented consists of current conditions of the project and the complications in which occur within a cell culture laboratory. Future experiments will consist of perfecting and analyzing current experiment procedures and laboratory technique.

Purpose

The purpose of this project is to test the viability of PEEK and Si3N4 for potential use as an orthopedic implant by performing in vitro growth assays on disc samples of various properties with the human osteoblast cell line, MG-63.

Background

There are several types of spinal implants. They fall into two categories: Fusion and non-fusion devices. Fusion devices are used in hundreds of thousands of surgeries a year – nearly 270,000 according to The United States Bone and Joint Initiative. The devices are used in treating arthritis, scoliosis deformities, broken or misaligned vertebrae, and herniated disks. They help stabilize the spine and take pressure off the nerves. There are various materials these devices are made from; such as Titanium and PEEK – each with their own advantages. There is a trade-off between the strength and flexibility of these materials, but the success of these devices greatly lies in how they help the bone grow or fuse together. A local medical device company specializes in a new spinal fusion material – silicon nitride. Innovabio® has been tasked with testing the attachment and growth of cells on this material. To do this an osteoblastic model was used – a cancer strain know as MG-63. The growth of these cells are compared to both the new silicon nitride material and PEEK.

Fig. 2
Example of PEEK disc

In order to properly assess each material, consistent growth in our cell line was first maintained. The first task was to verify that the storage methods were not damaging our cells. This was done by cryopreservation along with subsequent thaws and monitoring the growth/health of these cultures. Next, the doubling times at specific seeding densities were quantified and selected the best, most consistent numbers to use in the passaging methods. In performing this task, seeding and counting methods were perfected before testing with implant materials. To determine the accurate range of our equipment and the proper well sizes and volumes that would give readings in this range, a period of trial and error experiments were performed. Scientific papers were used to guide in the attempts which provided a baseline and proper calculations to use.

Background Cont.

There have been several set-backs, the largest of which is the production of standard curves. Each experiment needs a standard curve performed first so there is an accurate model to calculate growth from. Each of these seem to have a fair degree of variance and error depending on which points are used. This leads to unreliable growth data later on. The range has been scaled back (namely on the high end) of these curves which has given better results. This however means scaled back seedings and growth on the material as to stay within accurate range. One additional problem that has faced this project is culture contamination. What appears to be fungus has plagued the cultures for the last few months creating variability in cell proliferation.

Methods

Phase 1

Propagation and cryopreservation of MG-63 cells

Create Standard and Growth curve by adding various densities of cells into 24-well plates.

Stain cells with crystal violet* and measure absorbance to count amount of cells.

Graph and analyze data to determine optimal density and doubling time of cells.

Phase 2

In 24-well plates add MG-63 cells at the optimal density found in phase 1 on Si3N4, Peek, positive and negative controls along with a standard curve.

Fix cells to wells and stain cells with crystal violet*. Measure the amount of cells through absorbance.

Graph and analyze data by comparing cell amounts of growth mediums and control groups.

*Crystal violet is a stain that adheres to the DNA in a cell. The stain is allowed to absorb into the cell and excess is removed. Once the cells have dried the crystal violet is resuspended in acetic acid and the absorbance is read at 570 nm.

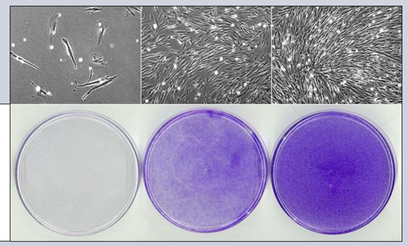
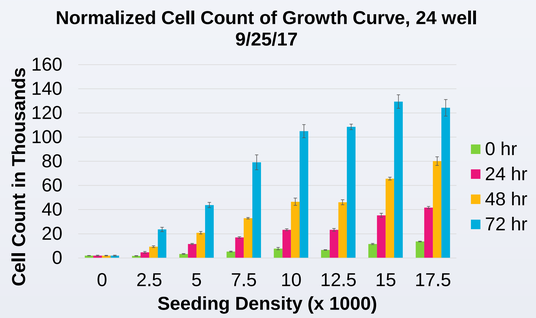


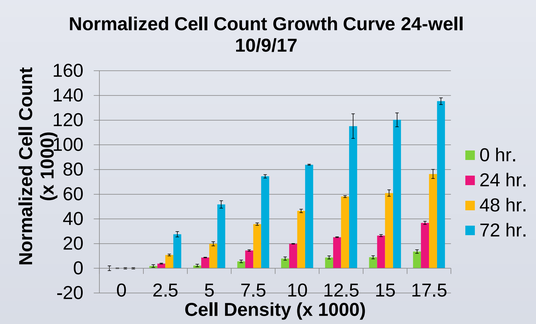
Fig. 3 Example of crystal violet staining. Cells used were human lung cells. cellpro.csc.mrc.ac.uk/genetic-screenings.html

Results

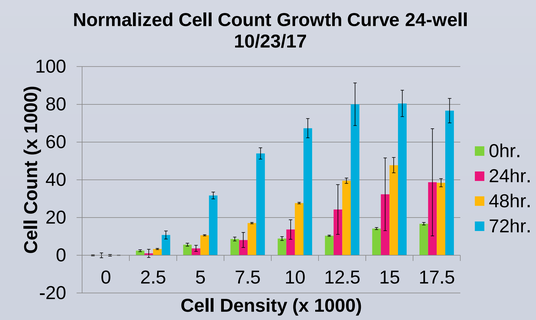
The following graphs are growth curves of cell counts that were calculated using the standard curve. The standard curve was made from the same cell stock and performed at the same time as the growth curve. The error bars indicate the difference between cell counts in each category over 3 wells.



Graph 1
Cell count growth curve from experiment performed the week of September 25, 2017.



Graph 2
Growth Curve with cell count calculated from experiment done the week of October 9, 2017.



Graph 3
Cell count growth curve from experiment that was performed the week of October 23, 2017.

Conclusions/Future Work

Cell proliferation has decreased over the span of the three growth curve experiments indicated in the result portion of this poster. After completion of the three experiments, cultures were showing a presence of what looked like fungus hyphae followed by an immediate pH change in cell culture media. The addition of an antifungal reagent showed an increase in cell growth indicated in the images below:

Figure A is untreated cells. Figure B are cells treated with Fungin. Figure C is cells treated with amphotericin B

Further growth of cells without treatment were compared to treated cells since hyphae is no longer visible in current cultures. A new vial of MG-63 cells was purchased and cultured to compare against the old batches of cells to determine if cell metabolism has decreased in the older cell batches. Observations showed the new cell's metabolism was healthier in the first passage, but then slowed down to the old cells growth rate in the second passage of culturing. It was determined that this was the new cell proliferation rate and doubling times need to be calculated. Current experimentation is determining the doubling times by standard and growth curve in 24- well tissue cultured plates diagramed below.

	1	2	3	4	5	6
A	0	0	0	10	10	10
B	2.5	2.5	2.5	12.5	12.5	12.5
C	5	5	5	15	15	15
D	7.5	7.5	7.5	17.5	17.5	17.5

Fig. 4
This figure shows the setup for the growth curve. The amounts are in a thousand. The standard curve will have cell densities from 0 – 80,000.

When doubling times are calculated, material testing will be the next stage of the project.

Sources

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