

Designing a Scaffold for High Density Mammalian Cell Growth

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Abstract

The purpose of this research project is to design a multi-layered scaffold using rapid prototyping to maximize the growth of 3T3 fibroblast cells within a culture plastic ware. 3T3 fibroblast cells grow as a monolayer which puts a limitation as to the number of cells that can be grown in particular area. Maximizing the number of the cells grown without adding surface area is a cost effective way of creating a solution to a problem. Specifically, the selective laser sintering (SLS) method of rapid prototyping plays an integral part in this research. The SLS used DuraForm Polyamide (PA) to make four scaffolds that provided the appropriate mechanism for cells to cultivate and thrive. By using different parameters such as coating one set of scaffolding with collagen or changing the porosity of the structures within a culture well plate provided information that would be applicable to other cultures as well. The four lattice structures had the following porosity size 45% dense, 29% dense, 16% dense, and 6% dense. A density of 100% is equivalent to solid DuraForm PA. Experimental evidence showed a strong correlation between a high surface area and population growth. Designing a scaffold led to higher cell growth as compared to the monolayer. The cell growth increased well over 200% in 6 out of 8 scaffolds tested. A multilayer scaffold will open up the possibility of growing cells in a way that is not only cost effective but also provides data that would be very beneficial to the scientific community and biotechnology industries.

Keywords: 3T3 Fibroblast, Scaffold, DuraForm Polyamide

1. Introduction:

1.1. background:

Technology is constantly advancing in the medical field in order to keep up with the increasing demands, and one of those demands requires figuring out a way to design a scaffold in order to grow tissues for organ transplants. Last year only 23,683 people were fortunate enough to receive a transplant, while another 80,000 people waited⁸. Since the demands for this resource cannot meet the needs of the patients, it is up to the scientist, engineers, and doctors alike to develop a model that would no longer allow the accessibility of not having a transplant to become an issue. Researching cell growth allows the researcher to figure out how cells are grown onto a scaffold, determine the complexities and challenges of growing vital organs outside the body, and pave the way for more complex organs to be embedded onto the scaffold .

3T3 fibroblast cells are the core foundation to this research experiment and originally come from a cell line of embryonic Swiss mice. The unequivocal growth rate of fibroblast 3T3 cells make this organ an excellent model to study. Mice skin cells are used as opposed to human skin cells because of the extreme precautions that would have to be addressed if human cells were studied instead. Since the genetic makeup of mice to humans is so different the spreading of harmful diseases would be minimized.

The hypothesis for this research is that by creating a multilayer scaffold this will maximize the cell population without having to add surface area. Also coating the scaffold with collagen would cause the cell population to

increase even greater. In the body 3T3 fibroblast cells are covered in collagen, and replicating this environment would aid in the reproduction process. Lastly, of the four lattice structures, lattice structure (LS4) would provide the highest population growth.

2. Methodology:

2.1. cell culture:

The process of designing a scaffold for high density cell mammalian growth is one that involves many systematic techniques. The first procedure requires the culturing of 3T3 fibroblast cells. Culturing is the process where cells are cultivated in a laboratory under conditions that are controlled in *ex vivo* and the specific idea to maintain cells separate from their original environment. The media is composed of four hundred and fifty milliliters (ml) of Dulbecco's modified eagles medium (DEME), five ml of penicillin/streptomycin, five ml of l-glutamine, and fifteen ml of fetal calf serum (FCS). The other materials involved in this experiment include trypsin and phosphate buffer saline, which is used for extracting the cells off the cell walls of the flask.

Cell culturing is a continuous process that entails growing cells under aseptic conditions. Once all safety measures have been completed, removing cells from the incubator is the first step. The incubator maintains the proper conditions for cells by controlling temperature, humidity, and creating an environment that is conducive to cell growth. Removing 3T3 fibroblast cells from the surface of the flask is the next procedure. By adding ten ml of phosphate buffer saline, this removes any medium remaining. Trypsin is the enzyme used in facilitating the detachment of cells at a faster pace. One ml of this enzyme is the proper amount followed by placing the fibroblast into the incubator for another seven minutes. Diluting the enzyme with an additional ten ml of PBS is essential before centrifuging the cells. Cells are spun down in the form of a pellet using a centrifuge and followed by removal of trypsin and resuspension in media. The next procedure requires preparing two test tubes with the following amount: one test tube is filled with 9 ml of medium with 1 ml of cells, while the other test tube is filled with 9.5 ml of medium and .5 ml of the cells. Preparing two test tubes at all times eliminates any room for error. The process of culturing cells should be carried out every three to four days to eliminate the competition of resources and promote cell growth.

2.2. autoclaving:

Sterilization is the chief component that allows for the killing of bacteria, germs, fungi or any other harmful diseases that would contaminate the fibroblast 3T3 cells. Specifically, autoclaving is the methodological process by which any traces of these diseases would be destroyed. Scaffolds were sterilized in an autoclave at 121°C and a pressure of 1.1 bars.

2.2.1. materials:

Choosing a material that is compatible with the rapid prototyping and also autoclaveable is a requirement when designing a multilayered scaffold. During this experiment a test was completed in order to ensure both of those requirements were met. In the Rapid Prototyping Center at the Milwaukee School of Engineering (MSOE) there were eight materials that could be examined for experimentation. The materials included: DuraForm GF (glass filled), DuraForm Flex, DuraForm PA (polyamide), Accura25, Polycarbonate, Acrylonitrile-Butadiene Styrene (ABS), Polyphenyl Sulfone (PPSF), and ZP-131.

2.3. collagen coating:

Research has shown that creating environments that mimic the body maximizes cell population of fibroblast 3T3 cells. The experiment, *A Co-Cultured Skin Model Based on Cell Support Membranes*¹, showed a 55% increase in the cell population when using a collagen coating to carry out the experiment. Thus, collagen coating of the scaffold was used to determine whether or not this would have an effect on population growth. Type one collagen was used to coat the surface of the scaffold. Specifically, this type is known to facilitate the reproduction rate of cells.

The procedures for coating the collagen include first reconstituting the concentration of .5mg/ml in .25% of acetic acid, occasionally swirling for several hours. Once that task has been completed, dissolving the solution for several hours at a temperature of 2 to 8°C is the following procedure. The next step involves coating the scaffold and

allowing the solution to sit overnight. The final procedure involves sterilizing the scaffold by rinsing with seventy percent ethanol.

2.4. cell seeding:

Cell seeding has a profound effect as to how the cell is able to proliferate onto the scaffold. In literature, methods have been reported with the purpose of figuring out how to efficiently seed the cells onto a scaffold, ranging from simple techniques such as static seeding all the way to more advanced seeding methods similar to pulsatile perfusion seeding. Depending on the function of the scaffold, this will determine which method would be applicable for cell seeding. In this experiment, centrifugal force was the method that was replicated. Centrifugal force seeding allows high efficiency seeding at low cell concentrations using fewer disposal items. Also being able to yield a fairly even distribution of cells throughout the entire construct made this an appropriate technique to replicate. This procedure is a step that determines how well the fibroblast cells are able to attach and proliferate throughout the scaffold. According to the Biomaterials Journal, a common issue with cell seeding is the inability to deliver cells deep inside the scaffold with a uniform distribution². By using a technique such as centrifugal force, that utilizes different physical principles and allows high levels of vascularization, this would meet the common challenges expressed in previous research.

2.5. cell counting:

The method utilized to calculate cell population involved using a hemacytometer. Once the cells have been counted, an equation can be used to determine the total number of cells in each scaffold. The equation used is as follows:

$$\frac{\text{Total cells counted}}{\text{Number of squares}} \times \text{dilution factor} \times 10,000 \quad (1)$$

2.6. rapid prototyping:

Rapid prototyping is a unique process that allows a scaffold to mimic the function of a natural extra cellular matrix in the body. The capability to increase the bioactivity in the scaffold makes this technology an advancement in designing a scaffold because of the increased cell proliferation. The goal of the scaffold is to create a design in such a way that the cells would be in close proximity but at the same time allow the growth of differing skin to be separated in layers mimicking *in vivo*. On a broad spectrum, rapid prototyping takes virtual designs from computer aided design of CAD software then transforms them into thin, virtual, horizontal cross-sections creating cross sections in physical space. This process is replicated one after the other until the model comes to completion⁶. There are several machines by which this process can be completed. Specifically the selective laser sintering method was applicable to the scaffold design because the SLS machine is constructed in such a way that uses a high power laser to selectively fuse small particles that would later be represented into a three dimensional object. This preferred fabrication process is ideal for producing an intricate structure that require precision and accuracy. Figure 1 shows the four lattice structures along with varying porosities. Overall, this technology provides a greater understanding in the cell's physiology, and cell growth pattern that can be applied to other cultures as well.

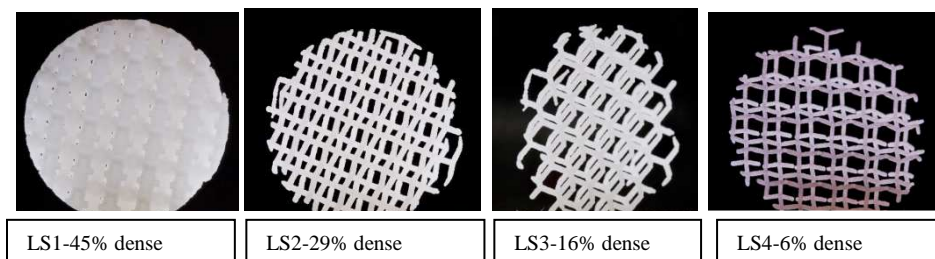


Figure 1. The four lattice structures used to test cell growth.

3. Experimentation:

The set up for this experimentation involved using two six well plates. Figure 2 shows the experimental set up of the first well plate. The first well plate contained one side that was used for collagen coating, while the other half had no collagen coating. On each side of the well plate there were three different lattice structures with varying porosities, together holding six scaffolds in the first well plate.

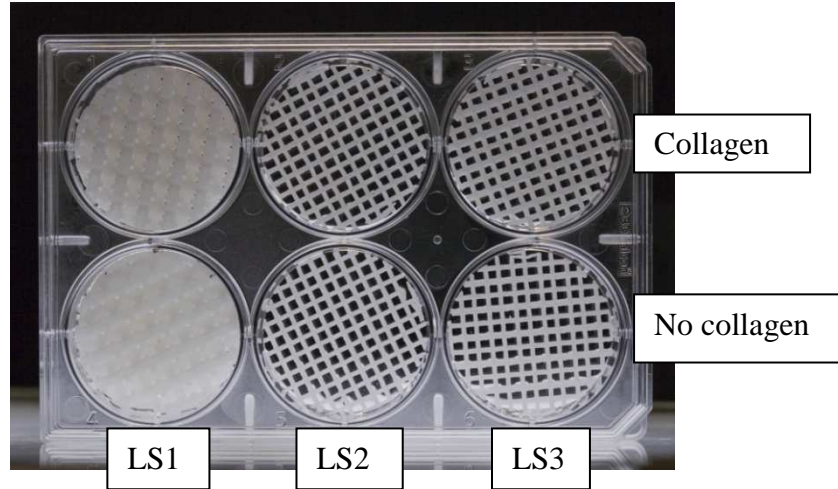


Figure 2. Experimental set up showing scaffolds inserted in a six well plate.

In the second well plate, lattice structure four and the control monolayer were the materials used in this well plate.

4. Results:

4.1. rapid prototyping materials:

Out of the eight materials, five could not withstand the adverse conditions required for autoclaving. Those materials included: DuraForm Flex, Accura25, Acrylonitrile Butadiene Styrene (ABS), Polyphenyl Sulfone (PPSF), and ZP-131. Warping, bending, cracks in the structure, and a change of the color and texture of the materials were all indications that these materials could not be used to design a multilayered structure. Of the three remaining materials that could be autoclaved only DuraForm PA was used to carry out the experiment.

Table 1. Autoclaving ability of rapid prototyping material

Material	DuraForm P.A	Accura25	ABS	DuraForm Flex	PSF	ZP-131	DuraForm GF	PC
Passed	X						X	X
Failed		X	X	X	X	X		

4.2. cell growth:

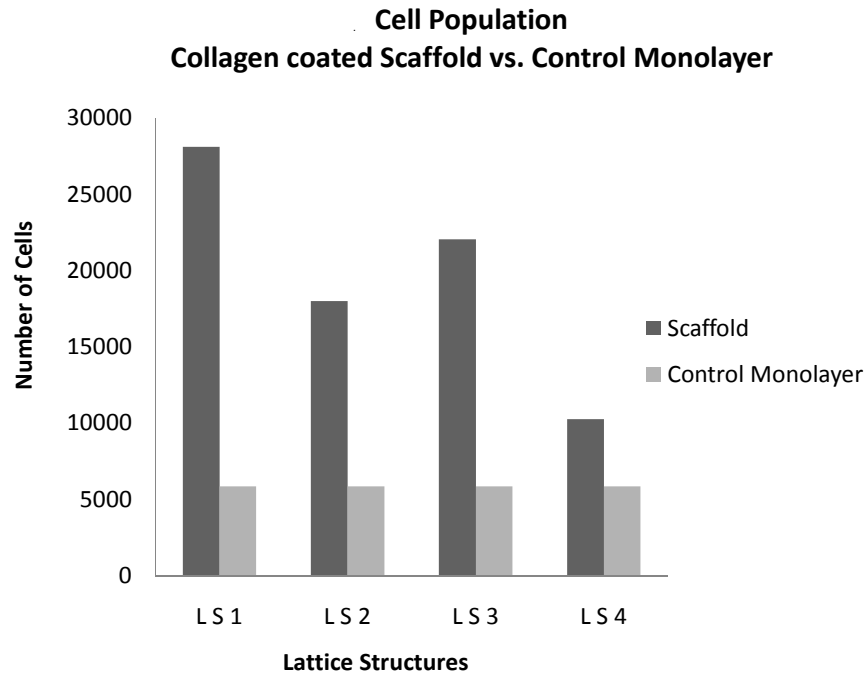


Figure 3. Cell population for the collagen coated scaffold vs. control monolayer.

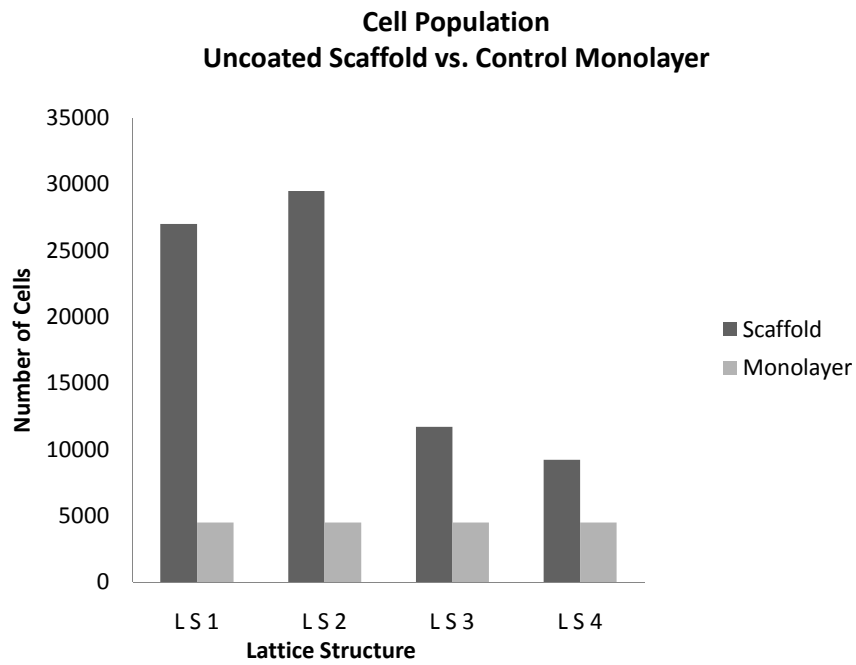


Figure 4. Cell population for uncoated scaffold versus the control monolayer.

Figure 3 shows the cell population for the collagen coated scaffold versus the control monolayer. The control monolayer produced a cell population of 5,850 cells. LS1 generated a cell population of 28,130 a 380% increase as compared to the control monolayer. LS2 two obtained a cell population of 18,000 which is a 208% increase. In LS3 the cell population was 22,052, a 276% increase. Lastly, in LS4 the population was 10,250, an increase of 75% as compared to the monolayer.

Figure 4 shows the cell population for the uncoated scaffold versus the control monolayer. In Figure 3, the monolayer yielded a population of 4,500. LS1 had a cell population of 27,000 a 500% increase. For LS2 the population growth was 29,500 a 556% increase. For LS3, 11,700 was the population growth, and the percentage increase was 160%. Lastly, LS4 harvested 9,225 cells with a 105% increase.

5. Discussion:

This research showed a strong correlation between surface area and cell growth. The larger the surface area, the higher the cell growth tended to be. LS1 and LS2 demonstrated this idea as both of these lattice structures had a high surface area yielding a higher cell growth.

Cell seeding is the primary explanation as to why in one graph one lattice structure produced the highest cell growth while in another graph a different lattice structure had the highest cell growth. When a higher density of cells is seeded onto a scaffold this causes an increase in the overall cell growth for that particular lattice structure. Although the same number of cells was placed inside each scaffold, cell proliferation was dependent on centrifugal force seeding.

Lastly, time was a factor in this experiment as to why the uncoated scaffolds produced a higher cell growth versus the collagen coated scaffold. Based on previous research, it is hypothesized that the longer the cells are kept on the scaffold the more mice keratinocytes would be produced, allowing a greater cell proliferation onto the scaffold². Also having a collagen coating would produce an even higher proliferation rate. In this experiment the cell count was taken only after a week. If the cell count would have been taken after three or four weeks, as opposed to a week, the results should be dramatically different.

6. Conclusions:

In conclusion, the hypothesis for this research stated that by creating a multilayer scaffold, this will maximize the cell growth without having to add surface area. The experiment showed that by building a multilayer scaffold this increased cell growth in every one of the lattice structures and well over 200% in 6 out of 8 scaffolds tested, as compared to monolayer. The second assumption for this experiment states that by coating the scaffold with collagen this would cause the cell population to increase even greater because fibroblasts are covered in collagen in the body, creating a condition that is favorable for the cells. The finding showed that collagen did not cause a greater increase in the cell growth. In fact the uncoated scaffolds showed a dramatically higher cell growth. Lastly, it was theorized that LS4 would provide the highest population growth because the large pore size would make it easier for nutrients to flow in and out of the scaffold. The finding LS4 produced the lowest cell growth. The pore size was too large for the cells, causing the cell attachment and proliferation rate to be low.

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