UNIVERSITY OF WISCONSIN – MADISON
DEPARTMENT OF BIOMEDICAL ENGINEERING
BME 301 – DESIGN

Cancer Cell Scaffold

Mid-Semester Report

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Abstract

A great deal of research is ongoing into methods to better identify cancerous tissues, stage the progression of cancer, and assess the progress of cancer treatment. One current approach is to characterize the metabolism of the tissue being studied using MRI Hyperpolarization of ^{13}C-labeled pyruvate. Cancer cells demonstrate unusually high metabolic rate and can thus be differentiated from surrounding tissue. Before these methods can be applied *in vivo*, the detection methods must be optimized *in vitro* by observing cancerous cells in a consistent culture. A cell scaffold and bioreactor system is required to maintain the viability of these cells and promote growth to a high density in order to clearly measure the ^{13}C signal. Thus, the objective of this project is to develop a scaffold that is compatible with a bioreactor, can grow cells to a high density, and maintain cell viability throughout the duration of the MRI hyperpolarization experiment. At this point, the team has chosen to pursue amicrocarrier cell scaffold and will proceed with testing in the upcoming weeks.

Background

MRI ^{13}C Hyperpolarization

Hyperpolarization of ^{13}C-labeled compounds,such as pyruvate, provide high contrast in magnetic resonance imaging (MRI) and can be used to track metabolic pathways [1]. Hyperpolarization involves inducing nuclear polarization of a material beyond the thermal equilibrium conditions [1]. This is accomplished by cooling a small amount of ^{13}C-labeled pyruvate to 1.4K. At this low temperature, the spins of the electrons are aligned with the magnetic field and microwave irradiation will transfer the spin to the nuclei, polarizing the protons [1]. During imaging, it is the decay of the hyperpolarized protons that enables the ^{13}C-labeled compounds to be tracked. ^{13}C-labeled pyruvate is ideal for such studies because ^{13}C has a relatively long decay time (approximately one minute) compared to other compounds, is inexpensive, and degrades via known metabolic pathways [1].

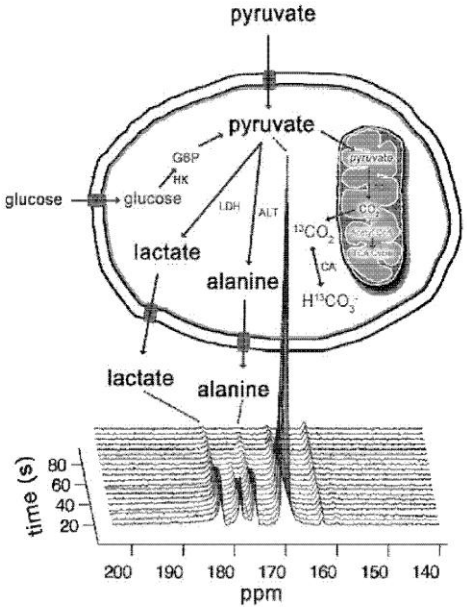

Figure 1: Schematic demonstrating the metabolic breakdown of pyruvate and the resulting signals [1].

This method can be applied to stage cancer and assess progress of treatment by injecting ^{13}C-labeled pyruvate into a cell culture and monitoring its metabolism [2]. Metabolic activity, especially glycolysis is up-regulated in cancerous tissues. Glycolysis is the conversion of glucose to pyruvate to smaller metabolites such as lactate and alanine. Thus, using MRI, the amount of pyruvate breakdown products can be measured to evaluate the celluar metabolism and assess the agressiveness of cancer cells [2]. Figure 1 shows a diagram depicting the breakdown of pyruvate and the chemical shifts observed from these compounds.

The client for this project would like to apply this method to study various cancer cell lines. To accomplish this a bioreactor and cell scaffold is needed to sustain high cell density and viability in order to obtain clear signals in the MRI machine.

Bioreactors

A bioreactor is a system used to grow and sustain cell cultures by delivering nutrients to cells and removing cellular waste. There are many types of bioreactors available commercially. However, all bioreactors are mixing patterns of two basic types of reactors: the stirred tank reactor and the tubular reactor. The stirred tank bioreactor contains propellers that stir the contents in the bioreactor tank such that the fluids and gases perfused are homogeneous. The tubular reactor uses plug-flow and nutrient concentration decreases from the inlet to the outlet [4]. A bioreactor system of this type is needed for the MRI hyperpolarization experiment described previously to culture cancer cells high density. Figure 2 shows an example bioreactor containing a cell scaffold.

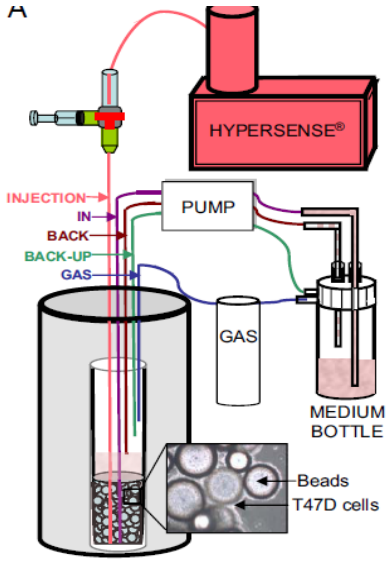

Figure 2: Diagram of bioreactor system including injection and output lines for cell media and gases. A scaffold made of many microscopic beads is conatined within this bioreactor [3].

Cell Scaffolds

The cell scaffold provides a site for cell attachment and is encased within the bioreactor [4]. There are various methods and materials that can be used to efficiently culture the cells in the bioreactor without being substantially affected by the perfusion system. 2D culture environments would not create a high enough density to observe a clear signal during MRI imaging. Additionally, 3D cultures better mimic the physiological environment as compared to 2D structures that may change certain properties of the cell including phenotype [5]. For this reason, the team will focus on scaffolds with 3D structures. Such scaffolds offer a large surface area for cell attachment which is desirable to grow cell cultures to high densities. Scaffolds can be coated with extracellular matrix (ECM) components that promote the adhesion and proliferation of cells. The 3D structure also allows better diffusion of nutrients for growing cells. Depending on the micro- or macrostructures of the scaffolds, cells may even be protected from hydrodynamic damage in the bioreactor [4].

Cancer Cells

The focus of this project is to promote cancer cell adhesion and proliferation on a cell scaffold. To accomplish this it is important to understand the biology of cancer cells and the conditions in which they thrive. In general, cancer cells exhibit unregulated growth due to increased or unregulated production of growth factors and ECM components [6]. Included in excessive growth are up-regulated metabolic processes such as lipid synthesis and degradation, bioenergetics, and glycolysis [3]. Cancer cells also demonstrate unregulated cell division leading to higher proliferation rates [6]. This property will be

advantageous to this project because cancer cells will naturally proliferate and can thrive in many environments.

Several cells lines are of particular interest to the client and will be studied in this project. These cell lines are all adherent cancer cells and thus need a scaffold to grow on. The cell lines of interest include:

- Breast cancer: T47D
- Prostate cancer: PC-3, LNCaP, DU145
- Brain glioma: U87, U251

Project Motivation

In order to observe the metabolism of cells, MRI imaging is used to track the decay of ^{13}C-labeled pyruvate. The samples of cells used during the MRI imaging process have to be of high density and viability for images to be clear. The main type of cells that the client is concerned with is cancer cells, which are self-proliferating and tend to overproduce ECM components. This makes cancer cells relatively easy to cultivate to obtain high cell density and viability. However, different cell lines have different requirements in terms of culture media and attachment surface. Hence, it is necessary to find the optimal scaffold for all cell lines to achieve the maximum cell density and viability.

Design Criteria

There are seven key design criteria the cell scaffold needs to fulfill. The first and most important is that the scaffold has a large surface area to volume ratio. This is extremely important so that the cells can grow to a high cell density in a small space. The density that the cells need to reach in order to successfully measure their metabolism is $5x10^7$cells/mL. Another important condition is to maintain cell viability for the duration of the seeding, growth, and imaging of the cells, which should take about four or five days. Maintaining cell viability means that the cells remain alive and remain nurished. The scaffold should also allow the cells to be perfused with both oxygen and media to continuously nourish the cells as well as remove all cell waste. An additional requirement the scaffold needs to satisfy is to ensure proper inoculation of the cells. The scaffold should allow the cells to be easily seeded and readily allow them to grow and proliferate. Finally, the scaffold should not contain any ferrous material that would interfere with the MRI machine's magnet and must fit in the $3,000 budget for the entire bioreactor and cell scaffold project.

Design Alternatives

Encapsulation

The first design alternative involves the formation of beads that contain the cancer cells. The most common type of scaffold is a calcium alginate bead as shown in Figure 3. These beads can be created in one of two ways, which are similar but also have key differences. The first way to create the beads is to start by creating a solution of 2% sodium alginate solution mixed with the cells in a one to one ratio. This solution is then drawn into a 1-cc syringe fitted with a 24-gauge angiocatheter that has a

23-gauge needle pierced at its hub to act as the positive electrode in the electrostatic casting process. The syringe is then placed in a syringe pump system set up so droplets fall orthogonal to the calcium chloride solution. This solution has a concentration of 125mM and contains a grounded electrode. This allows an electrostatic potential to be created across the angiocatheter tip and calcium chloride solution using a high voltage DC source. Using this method the bead size is controlled by adjusting the applied potential [7]. The other way to create calcium alginate beads does not use an electrostatic potential to control bead size. The protocol is the same as the first procedure except the beads are made entirely by dropping the sodium alginate cell solution into the calcium chloride solution with a syringe and syringe pump [8]. The latter method is older and the sizes of the beads vary greatly, meaning the cell densities inside vary as well. Therefore the method of using an electrostatic potential is a better method although it is more costly because a high voltage DC source is needed.

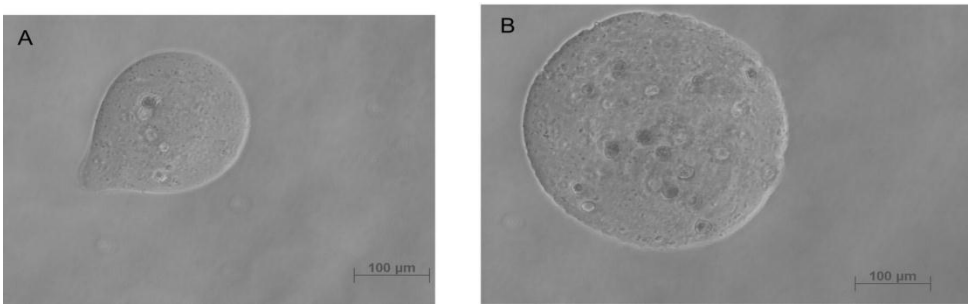

Figure 3: Photo of cells encapsulated in calcium alginate beads showing range of sizes that result from fabrication [7].

This method of encapsulation has a strong presence in hyperpolarized ^{13}C spectroscopy research and NMR-compatible bioreactor systems to assess cellular metabolism. The hyperpolarized ^{13}C is able to penetrate the beads and be taken up by the cells. This method has been shown to grow the cells to a density between $5x10^7$ cells/mL and $1.2x10^8$ cells/mL which is consistent with the density that the client is looking for [3]. This technique has also been used to assess cancer cells in past experiments but not with the target cell types. This technique is very cost efficient costing under $10 per experiment. The main disadvantage of this type of scaffold is time consuming construction, because it would take over a day to fabricate enough beads to run a single experiment.

Microcarriers

Microcarriers are spherical beads that usually range from 60-300 microns in diameter (Figure 4). They are made of a variety of materials such as dextran (a polysaccharide), glass, polystyrene, acrylamide, and collagen [9]. The microcarriers can have different surface modifications which promote cell adhesion. They may be coated with collagen, FACT which is a modified collagen, or ProNectinF which is a polymer [9]. Positive or negatively charged molecules may also be introduced to the bead's surface. The beads may be nonporous or have micro or macropores which enhance the surface area of the bead and allow for a greater cell density. Microcarriers are often used with bioreactors because of their versatile composition and because the

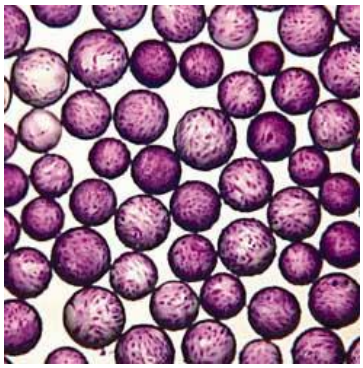

Figure 4: Photograph of microcarriers seeded with cells [2]

spacing in between the beads allows for adequate perfusion of fluids. For the design alternatives, three different microcarriers were considered: BiosilonNunclon Delta Microcarriers, Cytodex 3, and Sigma-Solohill Microcarrier Beads.

Biosilon Nunclon Delta Microcarriers

The Biosilon microcarrier is a nonporous polystyrene bead with surface treatment. They are radiation-sterilized and come ready to use, but are not autoclavable. The beads range from 160-300 microns in diameter [10]. The client provided a paper in which these beads were used in studies of metabolism in T47D breast cancer cells [2]. The cell density of 5×10^7 cells/mL which the client desires was achieved using 0.5 g of these beads, so the surface area of these beads is sufficient for the needs of this project. Metabolism of the cells was monitored using NMR perfusion in a bioreactor [2]. These beads were sold by Krackeler Scientific, Inc. and when enquiring about the price, it was discovered that the product was discontinued. This was not known when this design was first considered. However, these beads were expensive due to the specialized surface modification. Other distributors have not been found, but it is possible that with further research one could be found.

Cytodex 3

Cytodex 3 is a microporous microcarrier sold by GE Healthcare. It is made of cross-linked dextran and has a coating of acid-denatured porcine collagen. The beads come in powder and must be swelled in PBS prior to use. The beads range from 60-87 microns in diameter [11]. This diameter is smaller than that of the Biosilon beads, thus the surface area is smaller as well. A greater amount of beads would have to be used to achieve the same cell density as with the Biosilon beads. 10 g costs $141.00, and assuming 0.5 g is needed for an experiment, the estimated cost is $7.05 per experiment [12]. This type of microcarrier was used to investigate the differences between metabolite levels through enzymes regulating phospholipid and mitochondrial metabolism in normal mammary epithelial cells and in the breast cancer cell lines 21PT and 21NT. The NMR perfusion experiments for this study were done using a bioreactor [12]. Thus, these beads have been used with cancer cells and with a bioreactor, but have not been used with any of the specific cell lines for this project.

Sigma-Solohill Microcarriers

The Sigma-Solohill microcarrier is a nonporous polystyrene bead with a porcine collagen coating. The beads range from 125-212 microns in diameter [13]. This diameter is smaller than the Biosilon bead, so greater amount of beads would have to be used to achieve the same cell density. 20 g cost $160.70, thus the estimated cost per experiment is $4.02 assuming 0.5 g of beads is used [13]. Before use, the beads must be suspended in deionized water and then autoclaved [14]. This microcarrier has been used with studies using a mouse mammary tumor cell line EMT6, even though this is not one of the project specific cell lines. Mancuso *et al.* (2004) used this microcarrier in a bioreactor with EMT6 to develop a method "for obtaining high signal-to-noise ^{13}C NMR spectra of intracellular compounds in metabolically active cultured cells" [15]. The specific cell lines for this project should adhere to the collagen coating because Wozniak and Keely (2005) used 3D collagen gels with T47D breast cancer cells [16].

Hollow Fibers

The third category of design alternatives is the hollow fiber scaffold as seen in Figure 5. This type of scaffold is commercially sold pre-incorporated into a perfusion bioreactor system consisting of a plastic outer casing containing a cartridge of several thousand hollow tubule membranes in a parallel array [17].These thousands of fibers provide a large surface area ideal for cell attachment. Cells are most commonly grown in the extra-capillary space (ECS) because of the larger area for growth and proliferation, but cells can also be grown in the lumen of the tubules [17]. When cells are grown in the ECS, they adhere and proliferate on the outside of the tubule membranes. Nutrients (medium and oxygen) and experimental agents (^{13}C-labeled pyruvate or drugs) are perfused into the input ports and through the hollow fibers. The nutrients are exchanged for metabolic waste products across the permeable tubule membrane and the waste products are carried out via the output ports [17]. Figure 6 shows a diagram of the setup described.

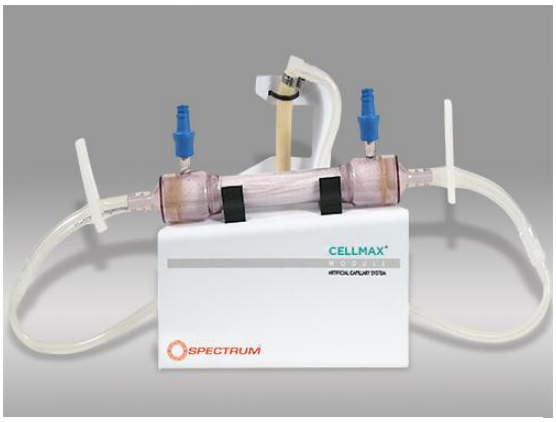

Figure 5: Photo of Spectrum Labs CellMax hollow fiber bioreactor [18].

FiberCell Sytems Inc. and Specturm Labs are two companies that sell these hollow fiber bioreactor systems. Common materials for the hollow membrane include polysulfone, polypropylene, regenerated cellulose, and polyethylene [18, 19]. The bioreactors also are available in a variety of sizes. For this MRI hyperpolarization experiment, a smaller bioreactor is required to fit within the bore of the MRI magnet. The suppliers also recommended the polypropylene membranes because these fibers have established protocols for being coated with extracellular matrix (ECM) proteins to further promote cell adhesion and growth. Furthermore, the molecular weight cutoff of the membranes can also be chosen based upon the size of the molecules needed to diffuse across the membrane. The specific option that was investigated for this project was a polypropylene fiber bioreactor with a surface area of 100 cm^{2}and a cost of $420 [18].

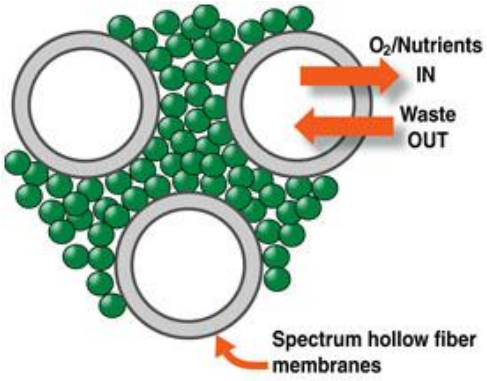

Figure 6:Diagram of cells growing in the extra-capillary space and nutrient/waste diffusion through hollow fiber scaffold [18].

There are several advantages to this system with the most important being larger surface area leading to increased cell density [20]. Furthermore, the thousands of tubules provide consistent and physiologic perfusion allowing the bioreactor to sustain cell cultures for up to six months [1]. Because the conditions within the bioreactor mimic the physiologic environment, studying the cellular metabolism in the hollow fiber bioreactor may provide results closer to what is seen *in vivo*. Another advantage of the hollow fiber scaffold is that the cells are protected from high shear forces of perfusing

medium because the membrane acts as a barrier [20]. This will minimize the amount of cells that detach from the scaffold during use. Additionally, hollow fiber bioreactors have been used with cancer cell lines in the past including brain glioma U87 and several breast cancer cell types [21]. However, there are also several shortcomings with this design. For instance, the membranes may cause noise in the MRI and would require preliminary testing to determine if the bioreactor cartridge would be usable for the MRI hyperpolarization experiment [2]. Also, the ECM proteins used to coat the fibers can be very expensive. Lastly, the bioreactor is difficult to autoclave, requiring a specific protocol with a slow ramp up time to the maximum temperature [2]. Even then, leaks in the membranes can occur which would lead to loss of cells due to perfusion. For the purposes of this project, if the scaffold is expensive it must be reusable and the hollow fiber scaffold may not be able to accomplish this.

3D Scaffold Structures

Porous Structures

Porous structures such as alginated (Figure 7) and fibrin scaffold types have macroporous structures that allow for easy cell seeding and nutrient delivery [22]. Both alginated and fibrin scaffolds provide a defined substrate commonly used for cancer cell lines to study disease states and drug models [23].

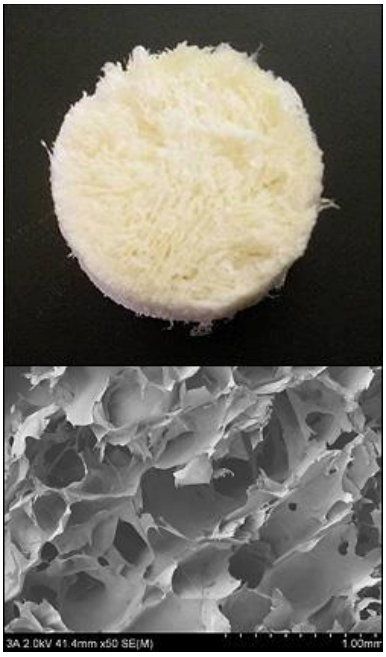

Figure 7: Dry alginated scaffold (top). Microstructure of dry alginated scaffold (bottom) [26].

Generally a solution of proposed substrates, for example 4% chitosan-alginate, 2% acetic acid, 4% alginate for an alginated scaffold, are added to a solution, mixed with a blender, and then cast in welled plate and frozen at -20^{o}C for eight hours [5]. Samples are then lyophilized, sectioned and cross-linked in a 0.2 M $CaCl_2$ solution for 10 minutes in vacuum conditions. Samples are washed for several minutes to remove salts and are sterilized in 70% ethanol for an hour. Scaffolds are stored in PBS in a shaker to remove ethanol. Scaffolds are then ready for use and seeded with cells.

The complex methods of fabrication for alginated and fibrin scaffolds require very specific resources and time that it seems the graduates students working on this project do not find attractive [5, 24]. Additionally, upon looking for commercial products of this type, it seems that they come pre-arranged in various plate formats that would not be compatible with the final bioreactor container. There has been a previous study that utilized alginated hollow fibers [25], but the disadvantages of hollow fiber usage, as discussed in the hollow fiber design alternative section, prevent this from being a viable option. Additionally, the use of this scaffold is dependent on the size and shape of the bioreactor cartridge which is presently unknown.

ECM Protein Gel Structures

ECM proteins provide structure and anchorage to mammalian cells *in vivo*. In labs, ECM proteins, commonly collagen, are used to provide cell cultures with anchorage, provide extracellular cues, and better mimic the *in vivo* environment. ECM proteins are typically used in 2D coating

applications. A surface is coated with said protein to encourage attachment of cells to a plate or surface with complex geometry.

ECM proteins are usually expensive. Depending on the type of protein, source, and purity, these proteins can range from $50-500/ml. When performing a literature review, no bioreactor used a scaffold of pure ECM protein. This product is often used as a coating in cases such as hollow fibers or microcarrier beads [2, 16]. An ECM coating would provide the scaffold with an environment that strongly encourages cell attachment. For the cell lines of interest, any ECM protein would encourage attachment since cancer cells are known to overproduce many ECM proteins to create their own niche.

Design Matrix

Table 1 shows the design matrix used to assess the four design alternatives. The four design options are listed in the left-most column. The microcarriers and 3D scaffold structures categories were broken down into three subgroups each because there is a wide variety available on the market. Each subgroup was scored as a separate design. The criteria used to rate the design alternatives are listed in the top row with their respective weights. These weights total to 100; thus, each design was scored out of 100 possible points.

Of the seven criteria, surface area was weighted the highest with 25 points because it is proportional to the maximum density that cells in culture can attain and high cell density was the most important requirement given by the client. Attaining a high cell density is required to visualize the ^{13}C-labeled pyruvate.

The design alternatives were also weighed based on their presence in bioreactor research and use with the target cell lines. The scores in these categories were based on a literature search conducted by the team. The highest scores were given to designs that have been used in previous studies with the target cell lines. The next highest scores were given to designs having precedence with cancer lines not specific to this project as many cancer cells lines require the same conditions for adhesion and proliferation. These categories of cell specificity and presence in bioreactors were weighted highly (20 and 15, respectively) because it is extremely important to ensure that the chosen design will be compatible with the cells and bioreactor being used before the scaffold can be purchased.

Furthermore, cost and ease of fabrication were given a weight of 10 each because the client did not emphasize the importance of these categories; however, the selected scaffold will need to be fabricated at least 2-3 per week by the client. Thus, experiment preparation should be simple and inexpensive to replicate. A time consuming and expensive scaffold will impede the progress of the client's research. Therefore, a cost effective solution must be chosen for the final design.

The remaining two categories, maintaining cell viability and phenotype, refer to the condition of the cells. In certain cases, materials can cause changes in cell phenotype, which is undesirable for this project. The cells need to remain viable and in their standard proliferative phenotype throughout the duration of the experiment to properly observe the metabolism of pyruvate. Although together these categories hold significant weight, all of the scaffold design alternatives are capable of maintaining cell viability and phenotype based on the literature search conducted by the team. Thus, all design alternatives received the maximal score.

After researching the seven previously described criteria for all design alternatives, the scores seen in Table 1 were given. The 3D gel structures scored lowest of all alternatives. This was primarily due to the lack of use in bioreactors. Research into theses gel structures revealed that use in perfusion bioreactors can cause damage to the scaffold and can cause cells to fall off the structure. Additionally, the surface area for cell attachment in these 3D gel scaffolds is much smaller than that with the other design alternatives which is a major drawback for this project.

Although higher than the 3D gel structures, encapsulation also scored low on the design matrix. The largest disadvantage of calcium alginate encapsulation is that fabrication is complex and time consuming. This is not ideal for an experiment that is going to be repeated frequently. Furthermore, no journal articles or papers could be found that used encapsulation with the cell lines of interest. Thus, fewer points were given in the cell specificity category.

The hollow fiber scaffold scored very high compared to the 3D gel scaffolds and encapsulation; however, the shortcomings of this design prevented it from being the best alternative. The greatest disadvantage was the possibility of damage due to autoclaving. Considering the cost of the hollow fiber bioreactor cartridge, it must be used for several experiments necessitating that it must be sterilized (ideally via autoclave). Thus, potential damage to the fiber membranes from the autoclave will increase the cost because new scaffolds would need to be purchased frequently. Additionally, the ECM protein coatings are expensive and would further increase the cost and complexity of preparation for each experiment.

Microcarriers as a category are a more cost effective solution than hollow fibers and offer a large enough surface area for the cell densities desired for MRI hyperpolarization. In previous studies, microcarriers have been used successfully in bioreactors and with the cell lines of interest. Based on this precedence, microcarriers will likely be an excellent option to accomplish the objectives of this project. Of the three specific microcarriers considered, the polystyrene beads coated with collagen from Sigma-Solohill scored the highest. This high score was received because it has a lower cost than the BiosilonNunclon alternative and has been used with the specific cells lines of interest unlike the Cytodex 3 microcarriers. Therefore, the team chose to pursue the Sigma-Solohill microcarriers as the final design.

Table 1: Design matrix for the cancer cell scaffold showing the collagen coated polystyrene beads attained the highest score.

Type of Matrix	Description/ subcategory	Surface Area (Density) 25	Cell Specificity 20	Presence in Bioreactors 15	Cost 10	Maintains phenotype 5	Viability 15	Ease of Fabricat-ion 10	Total 100
Encapsulation	Calcium Alginate	21	14	12	9	5	15	1	77
Microcarriers	*Cytodex 3*	22	17	15	9	5	15	10	93
	Biosilon Nunclon microcarriers	23	19	15	8	5	15	10	95
	Collagen Coated Polystyrene microcarriers	23	19	15	9	5	15	10	96
Hollow Fibers		24	18	15	6	5	15	8	91
3D Scaffolds	*Algenated Bought/made*	15	14	0	1/8	5	15	8/1	58
	Fibrin Bought/made	15	14	0	1/8	5	15	8/1	58
	ECM Gels	15	14	0	2	5	15	8	59

Final Design

As shown in the design matrix (Table 1),the team chose the Sigma-Solohill microcarrier as the final design. While the diameter, and therefore surface area, of these beads is smaller than that of the Biosilon beads, it is a reasonable compromise to use these beads because they are cheaper and readily available. Since they are cheaper, it will not cost much more to use more beads to achieve the cell density required. This type of microcarrier has been used with the mouse mammary tumor cell line EMT6 [15]. This is not one of the specific cell lines, but most cancer cell lines should behave in a similar manner concerning cell adhesion. Furthermore, this type of microcarrier has a strong presence bioreactor research. The coating on the beads is collagen, and it has been shown that T47D breast cancer cells adhere to collagen gels [16]. Changes in cell phenotype will not occur because of the short duration of this experiment. Cell viability should also not be a problem because T47D cells have been cultured on collagen in previous studies.

Ergonomics

The primary concern is that the user is able to easily insert and remove the scaffold from the bioreactor. This should not be a problem when using microcarriers, as the beads will simply be placed

into the cartridge which will be compatible with the bioreactor. They will be easy to remove as well because they will not be tightly packed within the cartridge. Much of the ergonomics for this project cannot be controlled because the team will be purchasing the scaffold and almost no modifications can be made to it.

Ethical Considerations

This project does not involve stem cell lines so there are no ethical considerations of this nature. All experiments will be done *in vitro* meaning there will be no subjects, human or animal, although human cancer cells will be used. Also, there are currently no standards or guidelines for cell culturing techniques set by organizations or the government because the cells are not being used for any type of cell therapy. Therefore, the scaffold will be designed by the standards and guidelines of the client.

Future Work

Order Size

Before ordering this product, two pieces of information must be confirmed. First, the BME 301 team designing the bioreactor in which the cell scaffold will be placed must be consulted. Dependent on the bioreactor cartridge they decide to pursue, the volume of the container holding the scaffold will vary. The total volume of the cartridge will be between three different commercially available products. Secondly, the client must be consulted to understand the extent of the experiments that he will be running. The number of experiments the client will run, along with the size of the container holding the scaffold and cells, will determine the total amount of product needed for this project.Despite this, it might be advantageous to buy the smallest amount available. The team will be testing the product to ensure it will meet the density and proliferation standards of the project andif the tests do not give encouraging results, the client will not lose as much money.

Testing

Once the product has been delivered, testing will begin.The team would like to find an optimal amount of cells to use as a seed culture and would like to characterize the density of cells on the scaffold over time with various starting amounts. The optimal seeding amount will be the smallest amount of cells that can be used to reach the client's designated density in an ideal amount of time. The client's graduate students will choose this ideal time after testing has been completed. While the cells are attached and growing on the scaffold, density and proliferation rates of the cells will be quantified.

For these quantifications, the number of cells on a sample of each scaffold will be counted once a day over the course of two weeks. A standard cell culture method involving the use of trypsin and a hemocytometer will be used to accomplish this. A sample of each scaffold will be isolated and the volume measured. This sample will then be trypsinized, which causes the cells to detach and fall off of the scaffold. Then, the cells will be counted with a hemocytometerand this number will be used in Equation 1 to give cell density [27]. Once the testing is complete, a graph of density over time can be established with separate curves specifying initial seeding amount.Based on the graph, an optimum

seeding density can be established. After this initialtesting has been completed, the tests will be repeated with the optimal seeding density inside the bioreactor while using its perfusion system.

Equation 1: $$\frac{cells}{mL} = \frac{total\ cell\ count * dilution\ factor * 10^4}{number\ of\ squares\ counted}$$

Lab space for these experiments has not been confirmed yet. They will either be conducted in the lab space near the MRI room maintained by the client or in the laboratories of the client's collaborators HirakBasu and Manish Patankar who work in the cancer center at WIMR. The team will be hearing from the client or his graduate students about this in the near future.

Management Planning

The smallest amount available for sale of the Sigma-Solohill microcarrier is 20 g for$160.70. With shipping the total will be approximately $180.70. The client's collaborators will be providing common tissue culturing supplies.

After the product is ordered, the rest of the work will be in-lab testing that will take at most two weeks. During these two weeks, the team will need to assign at least one person to do the cell counting activities each day. It will take approximately 2-3 hours each day to collect a sample, measure volume, trypsinize cells, and count cells. It may take an additional one to two weeks to incorporate the scaffold testing with the bioreactor set up to ensure the components work properly with each other.

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Appendix A

Product Design Specifications – February 2, 2011

Project #60: Cell Scaffold

Team Members

Vivien Chen – BSAC

Sarah Czaplewski – BWIG

Vanessa Grosskopf – Communicator

Josh Kolz – Leader

Sarah Sandock – BSAC

Problem Statement

Assessing the progression and response to treatment of cancer may be possible by characterizing the metabolic state of cancer cells. Currently, our client uses MRI hyperpolarized carbon-13 labeled pyruvate to evaluate the metabolism of cancer. The objective of our project is to develop a cell scaffold for maintaining the cancer cells within a MR-compatible bioreactor. Cells must adhere and proliferate on this scaffold to sufficiently high densities and sustain viability for visualization of the pyruvate.

Client Requirements

- Grow specified cancer cells to a high density
- Maintain cell viability
- Use materials for scaffold that are compatible with the MRI machine and bioreactor
- Allow sufficient nutrients and experimental agents (i.e. ^{13}C-pyruvate) to reach the cells

Design Requirements

1. **Physical and Operational Characteristics**
    a. *Performance Requirements:* Cells must attach to scaffold and populate it. The scaffold should be coated with a substance that provides evenly distributed cell attachment

sites.Also, the chemical interaction between the cells and the scaffold must be minimized. Ideally, a universal scaffold will be used for all cell types tested including Lymphoma K562, Leukemia NKL, Prostate PC3, DU145, LNCaP, U251 and U87 Brain Glioma, and T47D human breast cancer cells.

b. *Safety:* No ferrous materials may be used in the construction of the scaffolds for the safety of the person conducting the study using the MRI machine.

c. *Accuracy and Reliability:* Scaffold should maintain cells at a density of approximately $50x10^6$ cells/mL and sustain cell viability for the duration of the experiment which is approximately 1 hour. Also, The scaffold must grow cells to approximately the same density in every use for easy comparison between experiments.

d. *Shelf Life:* The 3D structure of the scaffold should last 5 years and if a separate coating formulation is used, it should last at least one year.

e. *Life in Service:* The scaffold can either be disposable or reusable. If it is disposable, the scaffold will be used for one experiment (1 hour) plus the time it takes for the cells to adhere to the 3D structure. If the scaffold is reusable, it should last for at least one month.

f. *Operating Environment:* Scaffold will be perfused in a medium to the specific cell type being tested and housed in a MR-compatible bioreactor.

g. *Ergonomics:* User should be able to easily apply and remove scaffold from bioreactor.

h. *Size:* In previous studies, cell scaffolds were placed within an NMR tube inside a bioreactor. The bioreactor we are using is currently being developed by another team and the inner chamber size has not yet been specified. However, the bioreactor must be able to fit in the 3 inch diameter bore of the MRI machine magnet. Thus, the 3D structure of the scaffold will be smaller than 3 inches in diameter and the coating on the structure should be less than 1 mm thick.

i. *Weight:* The weight of the scaffold should not exceed 200 grams.

j. *Materials:* The scaffold materials should minimize chemical interaction with the cells and be biocompatible. Also, the material used should be MR-compatible and contain no ferrous metals. If the scaffold is designed to be reusable, the materials should be autoclave safe.

k. *Aesthetics:* The appearance of the scaffold is not pertinent to this project.

2. Production Characteristics

a. *Quantity:*The quantity depends on if the scaffold is designed to be reusable or disposable. However, the team should focus on making one scaffold which could be reproduced by the client as he sees fit. Also, one generic scaffold for all cell types is ideal but a scaffold tailored to each of the 8 cell types specified would suffice if needed.

b. *Product Cost:* The budget for the bioreactor and cell scaffold together is between $2000 and $3000. The scaffold should take up a smaller portion of this budget.

3. Miscellaneous

a. *StandardsandSpecifications*: There are no standards or specifications set by any organization that the project must follow. The scaffold will be designed with the standards and specifications set by our client.

b. *Competition*: Currently, there are a wide variety of scaffold structures and coating used in cell culture. Many types are commercially available such as microcarriers (small beads coated with ECM proteins), hollow fiber scaffolds, and 3D gel structures made of ECM proteins.Still, many other methods have been developed by research labs such as electrostatic calcium alginate encapsulation and the formation of cellular spheroids.