Automated Fluid Perfusion System for a Tissue Synthesis Bioreactor

ME493 Final Report - Year 2010

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**Project Sponsors**
- Dr. Sean Kohles
  - PSU Reparative Bioengineering Lab
Executive Summary

Millions of people suffer from some type of tissue loss, damage, or bone defect every year. Tissue engineering provides a medical solution to these problems by the development of substitutes that restore and maintain tissue functions. A bioreactor device is specifically designed to stimulate tissue growth using synthetic scaffolds seeded with cells and supplied with a nutritive fluid or gel. The nutritive fluid in the PSU bioreactor system needs to be replaced periodically in order to supply the cells with fresh nutrients and remove waste products.

The Bioreactor Capstone team mission was to design, prototype and install a device that transports a nutrient gel into small square test cells. The proposed device should fit inside an incubator with the bioreactor system and it is automatic, environmentally resistant, and easy to install.

The design selected to be produced was a dovetail system. This design utilizes a tool less mounting system. This was selected due to the design’s advantage in ease of manufacture, flexibility in sample type and shape, quick setup and repair, and ease of access to the side panel.

The designed system was manufactured in the PSU workshop. The implemented system was then assembled and tested for leakage, flow characteristics and overall performance to make sure it complies with the PDS requirements dictated by the customer.

A demonstration of the system in operation was attended by the customer for validation of the main requirements. The customer was satisfied with the performance of the fluid perfusion system developed by the Bioreactor capstone team.
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Introduction and Background

Millions of people suffer some type of tissue loss, damage, or bone defect every year. Medical treatments for such conditions include autografts (a tissue graft obtained from one part of the patient’s body for use on another part), allografts (a tissue graft from a donor genetically unrelated to the recipient), and metallic implants. These methods suffer from limited availability, reliance on a limited number of volunteer donors, and there are issues of potential immune system reaction from allografts and metallic implants resulting in rejection of the graft. For that reason, many patients are still suffering from tissue loss or bone defects.

However, the science of tissue engineering provides medical solutions to these problems by the development of substitutes that restore and maintain tissue functions. An in vitro (outside of the body) tissue-engineered bone for subsequent implantation in vivo (inside of the body) is being developed as one of these solutions. In particular, an in vitro engineered cartilage replacement is being pursued as a way to repair injuries and damage to cartilage.

Bioreactor devices are designed specifically to support such tissue engineering applications. A typical Bioreactor system will hold test samples consisting of synthetic scaffolds seeded with cells which form the base of the final engineered-tissue. These scaffolds are then supplied with a nutritive fluid or gel consisting of cells, biomaterials, and growth stimulants. Tissue growth is then encouraged by stimulating the cells. This can be done via several methods, including fluid proliferation through the scaffolds, and mechanical loading stimulation. The full device is placed in an incubator that maintains the temperature, gas percentages, and humidity at certain levels to simulate the environment inside of the human body.

The nutritive fluid in the bioreactor system needs to be replaced periodically in order to supply the cells with fresh nutrients, to remove waste products, and to allow effective scaffold proliferation. However, the current bioreactor system in development by the Portland State
University (PSU) bioengineering department research team does not have a perfusion system and the fluid replacement is done manually. The manual process involves opening the incubator and changing the fluid by hand. This disrupts the equilibrium within the incubator, which slows down tissue growth. Therefore, the capstone team was asked to design and fabricate a fluid perfusion system to be installed to the current bio reactor system to allow automated fluid replacement.

**Mission Statement**

The Bioreactor Capstone team will design, prototype and install a device that will transport a nutrient gel into small square test cells. The device will fit inside an incubator with bioreactor and not interfere with the other testing devices that monitor the cells. In addition the system will be automatic, pressure resistant, and easy to install. The project will be documented extensively with reports, visual aids, and presentations. A working prototype is to be installed by the end of June.

**Product Design Specifications**

Dr. Sean S. Kohles and the PSU Reparative Bioengineering lab are the main costumers for the bioreactor fluid perfusion system. The system is to be installed in the current bioreactor device to allow automated fluid replacement. After working with Dr. Kohles and the Bioengineering Lab team, the team developed the following major design specifications:

**A. The assembly must survive the incubator environment.**

The system must be able to withstand the inside temperature of the incubator which can reach up to 50 °C along with specific ranges of CO₂, O₂, and humidity for extended
periods of time (up to 7 days). CO$_2$ percentage ranges between 0.2 – 20 %, O$_2$ percentage ranges between 5 – 20 %, and the humidity percentage reaches up to 95 % RH.

B. **Time needed to replace any failed component.**

   During the replacement of any failed component, the open incubator time must not exceed 15 minutes.

C. **Unattended Runtime**

   The fluid system is required to run for up to 7 days without direct supervision.

D. **Time needed to disassemble, clean, and reassemble.**

   The process of taking apart the system for cleaning and reassembly must not take more than 180 minutes.

E. **Perfusion system physical specifications.**

   The system to be installed must interface with the current bioreactor system and at the same time fit inside the incubator. The inside dimensions (in mm) of the incubator are 520(W) x 426(L) x 690(H).

F. **Flow Rate.**

   The nutritive fluid must be supplied to the test samples at a minimum flow rate of 4 mL/min.

G. **Cost:**

   All purchased parts and materials, fabrication, prototyping and testing processes are funded with a 1000 USD budget provided by the customer.
Top Level Design Decisions

Cantilevered Sleeve:

One of the design decisions our team made was to extend the sleeves away from the tray in order to gain access to the left and right sides of its main body. This allowed us to drill holes into center chamber from opposite sides so fluid could be transported through the sleeve. The original designs had these faces blocked by the aluminum tray and were completely inaccessible. In addition the PDS specifications prevented us from altering the front and bottom faces. The team determined that moving the body of the sleeve horizontally provided the best geometry to create the simplest and controllable flow path inside the sleeve. The dovetail design also reduced the overall dimensions of the tray/sleeve system, minimizing material use and cost.

Dovetail Fixture:

The original bioreactor system uses sleeves with rectangular screw tapped flanges for fixing the sleeves to the tray using two screws. This system requires unscrewing and screwing back the screws whenever a sleeve is needed to be replaced which is time consuming. The proposed dovetail design utilizes a tool less mounting system to attach the sleeves to the outer ring of the

Figure 1 – Sample sleeves
sample tray. Five dovetail slots are cut into the carrying tray, and each individual sleeve has a dovetail shaped protrusion that allows the sleeve to slide into a slot with a tight fit. Highlights of this design include quick and simple installation and maintenance due to its tool less nature, simple machining requirements to produce the parts, and a reduction in material consumption due the reduced tray size. The Dovetail fixture also allows the applied load produced by the pistons to be distributed on the lateral surface of the dovetail which decreases stresses in the sleeves.

**Fluid Recirculation shape:**

The team initially looked cutting a square sample chamber into the carrier sleeve for the sake of simplicity. Examination of the design revealed a concerning level of fluid recirculation in the corners of the cubic sleeve. Due to the need to flush biological contaminants and waste products, this was considered unacceptable. The team utilized computational fluid dynamic models of the system to optimize the flow design. The selected design utilized rounded corners, and an indentation on the side of the chamber to create a uniform flow throughout the chamber, over the sample cube to minimize recirculation.
Materials:

Materials for the bioreactor device must withstand the incubator environment without corrosion or contamination of the nutrient fluid.

*Tray* – Aluminum; Aluminum is relatively light and easy to manufacture compared with other materials. The whole assembly must be placed inside the incubator at 50°C. At this temperature the properties of aluminum won’t be changed (Mangonon, 1999), so Aluminum is safe to use in the incubator with any adverse affect. 20~200N of loads are applied to each sleeve, but this loads are too low to result in deformation. Stainless steel can be used as an alternative material for the tray. Stainless steel has a strong resistant to corrosion and rust so it is suitable material. However, it is harder material than aluminum, so it is more difficult to manufacture it.

*Sleeves* – Acrylic; Acrylic is transparent material, so it does not disturb observation of tissue growth by ultrasound transducer and digital microscope that is part of customer’s requirements. The sleeves are inserted in the aluminum tray, so they also have to be available to be used at the incubator temperature, 50°C. It is safe to be used up to 200°F (93.3°C), so incubator temperature won’t affect the sleeves. (Mangonon, 1999) Loads are applied to the sleeves, but the analysis proves that acrylic can be used safely (Appendix A).
Final Design

Tray:

The tray is an aluminum disk that holds five sample sleeves. It is fixed to the bioreactor on a rotating base using three mounting screws. The tray has a U shaped slot cut into it to allow it to be fixed to the base without interfering with the center column that holds five load transducers. A circular array of five dovetail shaped slots is cut into the tray to attach the sleeves to the trays’ outer ring. Figure 5 shows the shape of the designed tray which allows the testing of five samples simultaneously. The disk shape is used so that the tray can be rotated without interfering with the back column of the bioreactor and to align the sleeves with the load transducers. The tray is rotated periodically for sample inspection. It is fabricated from a square (8x8x0.5) in$^3$ aluminum slab. The diameter of the tray is 5.8 in and its thickness is 0.5 in. The dovetail slots are not cut all the way through the part but are 0.38 in deep.

Sleeve:

The sleeve has a square main body connected to a dove shape cut into the back for mounting. The overall dimensions of this part are 30mmx20mmx12mm. In the center there is a hole drilled through it used to house the bioreactor.
cartilage samples. The curvature of this hole in the center was designed to reduce recirculation and help direct steady flow for a 5x5x5mm samples. There are two access ports that are used to connect the chamber to a reservoir and transport the fluid through the sleeve and out to waste receptacles. On the top is an extruded rectangular shape that will fit into the sleeve cap. The bottom will be glued sleeve base which with provide the sample holder and rest gently on the surface of the bioreactor platform. Lastly the dovetail backside fits snugly with the tray so that no hand tools are necessary to secure this component of the fluid perfusion system.

**Sleeve Base:**

Sleeve Base is made from acrylic, and its shape is square which dimension is 13 mm×14.5 mm. The thickness is 4 mm. This thickness corresponds to the tray thickness, so when the sleeve is inserted to the tray, the bottom of sleeve is aligned to the bottom of the tray.

There is a void, which is 5 mm×5 mm, on the base. This is for the scaffold. The scaffold is stuck against the wall and the material of sleeve is acrylic that is transparent, so a digital microscope and an ultrasound transducer can observe the growth of tissue from the side and the bottom of sleeve respectively.

**Sleeve Cap:**

Sleeve Cap is also made from acrylic. It is also square shape and its size is 16 mm ×16 mm. This sleeve cap fits over the top of the body sleeve. This cap is for preventing the nutrient fluid...
from spilling out of the inner chamber. A hole will be cut in this sleeve cap, and a force applicator goes through it and applies force to a scaffold. The cap is water sealed with and adhesive putty in order for the part to be removed during installation and disassembly.

Fluid Input: (Reservoir, Pump, Manifold)

Fluid input is handled by means of a peristaltic pump and a large beaker. A peristaltic pump was selected to maintain sterility of the solution. The pump draws the solution from the reservoir beaker, and pushes it to the manifold. The manifold then distributes the solution to each of the five sleeves. The pump is powered by an ATX 12 volt power supply.
**Fluid Output:** (Waste Containers)

For prototype demonstration purposes, the used fluid flows out of each sample sleeve through a medical tube and is directed into a graduated beaker to monitor the flow rate through each sleeve and to make sure all sleeves are equally supplied with fluid. The final product can maintain this setup, or allow for a single outflow tank that holds all used fluid flowing out of the five sleeves, depending on the needs of the current experiment.

**Microcontroller Pump Control:**

To meet the requirements for variable flow control, a custom digital pump controller was developed. This system uses an ATMega168 microprocessor with a DS1307+ real time clock. This allows runtime and interval to be set from zero to 1 year duration. The microprocessor uses pulse width modulation to control the speed of the pump, allowing adjustment from zero to 80 mL/min for each sleeve.

*Figure 11- Controller, first light*  
*Figure 112-Prototyping the controller*
**Evaluation and Verification**

Once the complete prototype was assembled, the various design requirements were measured to ensure compliance with the PDS (see Appendix D)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Target</th>
<th>Metric</th>
<th>Verification</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
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<td>Flow rate</td>
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<td>mL/min</td>
<td>Prototype</td>
<td>0 to 80 mL/min</td>
</tr>
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<td>Unattended Run Time</td>
<td>7</td>
<td>Days</td>
<td>Prototype</td>
<td>0-1 year</td>
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<tr>
<td>Adjustable Run Interval</td>
<td>0-72</td>
<td>Hours</td>
<td>Design</td>
<td>0-1 year</td>
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<td>°C</td>
<td>Design</td>
<td>80°C (tubing)</td>
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<tr>
<td>CO₂ Tolerance</td>
<td>0.2-20</td>
<td>%</td>
<td>Design</td>
<td>Materials Stable in these gasses Al, Brass, Acrylic</td>
</tr>
<tr>
<td>O₂ tolerance</td>
<td>5-20</td>
<td>%</td>
<td>Design</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>Prototype</td>
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<tr>
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<td>Prototype</td>
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<td>Physical Dimensions</td>
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<td>Design</td>
<td></td>
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<tr>
<td>Complete Maintenance Time</td>
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<td>1000</td>
<td>USD$</td>
<td>Prototype</td>
<td>&lt;$200</td>
</tr>
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</table>

[1]The bioreactor apparatus that the team’s project will interface with is not yet complete. As a result complete assembly/disassembly testing of the system cannot yet be performed. Estimates based on testing performed ex-situ show the targets should easily be met.


**Conclusion**

After our final design was completed, our team met with our customer and discussed the prototype. We demonstrated the fluid perfusion system and all the different capabilities of the design. He agreed with our results in the product verification/evaluation section and that the PDS requirements were met short of being able to install the device with the completed bioreactor. It was the intention of our customer to have the bioreactor up and running by the time our team completed the fusion perfusion system. Currently the lab bioreactor is unfinished and some components are either still in production or have yet to be ordered. This prevented us from being to verify some of the PDS requirements. However we are confident that when the bioreactor is finish our fluid perfusion system will satisfy the rest of the requirements. As for our final prototype design, all verifiable product specifications were met, delivered on time, and within the budget, therefore the project overall was a success.

*Figure 12-Final Prototype on Display*
Appendix A - Analysis
Appendix A-1: Volumetric Flow Analysis

Summary:

The objective of this analysis is to determine the proper size of the fluid reservoir for our fluid perfusion system to run uninterrupted for a period of 7 days. The incubator that the system has to fit into has a limited amount of space, and if a significant amount of gel is needed to operate over this time period then our team would need to changed the size of other components or alter the flow rate of the system. The figure to the right is a diagram of fluid flow in our system. This analysis will help us determine the limitations of our device.

Given:

5 sleeves with inner dimension of roughly 10mmx10mmx9.25mm
5 tubes with inner diameter .125in and 2ft long
1 inlet tube with inner diameter .25in and 1 ft long
Volume recycles every 4-6 hours
Target flow rate 4ml/min

Find:

Determine the minimum volume of nutrient gel to supply five sleeves for a period of 7 days.

Solution:

Flow Rate = Volume Sleeves / Recycle Time

\[ V_{\text{Sleeves}} := 5 \times H_{\text{Sleeve}} \times W_{\text{Sleeve}} \times L_{\text{Sleeve}} = 4.625\text{mL} \]

\[ \text{Cycle}_{\text{Sleeves}} := 4\text{hr} \]
Conclusion:

The reservoir in the incubator should be at a minimum able to hold 400 mL of nutrient gel. At the maximum flow rate of 4mL/min the reservoir would need to hold 40L in order to maintain the growth environment for a 7-day period, if a constant flow rate were used.

Utilization of the intermittent flow features will allow a longer run times with smaller fluid capacities.
Appendix A-2 Sleeve Pressure

Summary:
The objective of this analysis is to determine the maximum pressure in a sleeve. The sleeve needs to be designed in order to withstand the calculated pressure.

Given:
Maximum flow rate of the pump Q; 0.261 [gpm]
Approximate Atmospheric Pressure P; 14.7[psi]
Inner diameter of sleeve tube; 0.125 [inch]
Inner diameter of inlet tube; 0.25 [inch]
Tube length; 9 [in]
Assume change in elevation is negligible
Assume constant velocity
Assume nutrient gel properties are approximately the same as water at room temperature

\[
\gamma_{\text{water}} := 1.21 \times 10^{-5} \text{ ft}^2/\text{s}^2 \\
g = 9.807 \text{ m/s}^2 \\
\gamma_{\text{water}} := 9.8 \text{ kN/m}^3
\]

Find:
Maximum pressure in the sleeve

Solution:
The exit velocity of the pump

\[
D_{\text{Inlet}} := .125 \text{ in} \\
A_{\text{Inlet}} := \frac{\pi}{4} D_{\text{Inlet}}^2
\]

\[
V_{\text{Inlet}} := \frac{Q_{\text{Inlet}}}{A_{\text{Inlet}}} = 2.08 \text{ m/s}
\]

The maximum exit velocity of each sleeve

\[
D_{\text{Sleeve}} := .125 \text{ in} \\
A_{\text{Sleeve}} := \frac{\pi}{4} D_{\text{Sleeve}}^2
\]

\[
V_{\text{Sleeve}} := \frac{\frac{1}{5} Q_{\text{Inlet}}}{A_{\text{Sleeve}}} = 0.416 \text{ m/s}
\]
Calculating the Reynolds number for flow through sleeve tubes

\[ \text{Re}_{\text{Sleeve}} := \frac{V_{\text{Sleeve}} D_{\text{Sleeve}}}{v_{\text{water}}} = 1.175 \times 10^3 \quad \text{Laminar} \]

The major and minor losses

\[ A_{\text{Chamber}} := 101 \text{ mm}^2 \]

\[ f_{\text{tube}} := \frac{64}{\text{Re}_{\text{Sleeve}}} \]

\[ \text{L}_{\text{tube}} := 9 \text{ in} \]

\[ H_{\text{Lmajor}} := f_{\text{tube}} \frac{\text{L}_{\text{tube}}}{D_{\text{Sleeve}}} \left( \frac{V_{\text{Sleeve}}}{2 g} \right)^2 = 1.362 \text{ in} \]

\[ k_L := .48 \quad \text{(Munson Young Okiishi Figure 8.26)} \]

\[ H_{\text{Lminor}} := k_L \frac{V_{\text{Sleeve}}}{2 g}^2 = 0.16\gamma \text{in} \]

The pressure inside a sleeve is calculated using a modified Bernoulli equation assuming negligible changes in velocity and elevation.

\[ P_{\text{Inlet}} := P_{\text{atm}} + \gamma_{\text{water}} \left( H_{\text{Lmajor}} + H_{\text{Lminor}} \right) = 14.755 \text{ psi} \]

Calculating the difference in pressure gives

\[ P_{\Delta} := P_{\text{Inlet}} - P_{\text{atm}} = 0.055 \text{ psi} \]

**Conclusion:**
According to the calculation, the maximum pressure at each sleeve is 14.755 psi. This is when the pump is supplying maximum flow rate.
Appendix A-3 Computational Flow Dynamics Analysis

Summary:
The initial design used a simple square chamber to hold the tissue samples. This resulted in recirculation of the fluid within the chamber. Due to the biological nature of the samples, this is unacceptable. The shape of the chamber must be optimized to minimize such recirculation.

Find:
Optimize sleeve shape for the test chamber.

Solution:
The team had neither the time nor funds to create multiple prototypes and test them. The team utilized the computational fluid dynamic capabilities of SolidWorks to simulate the flow through several designs. The sleeve flow profile was refined from one run to the next, until the flow was sufficiently uniform over the sample scaffold.

Conclusion:
By simulating several prototype designs, the team was able to minimize recirculation. The final profile involved rounding the corners of the chamber, and adding a flow area reduction over the flat portion of the cube, to accelerate the fluid through the straight stretch.
Appendix A–4 Sleeve Stress Analysis

Summary:

The sample sleeves project from the sides of the carrier tray. When the bioreactor applies compressive loads to the scaffold structures, the sample sleeves can be subjected to load as though they were cantilevered beams. The factor of safety of the design must be determined to ensure it will take the applied loading without breaking.

Given:

The applied load will be less than 10N

The narrowest part of the dovetail shape is 4.6mm, with a height of 9.25 mm

Acrylic Yield Strength is 110 MPa on average, Shear Strength is 62 MPa

Load is applied approximately 4.2 mm from the clamped dovetail.

Find:

Factor of Safety

Solution:

An analysis of the design was performed using SolidWorks integrated finite element analysis capabilities. It was determined that the safety factor of the design was 46. This is high enough that neither moderately large errors in the software analysis nor statistical variances in the strength of the polymer compound would not result in failure.
Appendix B – Part Drawings
Appendix C – Bill of Materials

<table>
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<th>Source</th>
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**TOTAL**                                      |        |        |          | **$156.35**|

*Table 1 - Bill of materials for prototype fluid perfusion system*

**Sources:**

MMC - McMaster-Carr - http://www.mcmaster.com

MOU - Mouser Electronics - http://www.mouser.com

RS - Radio Shack - http://www.radioshack.com
# Appendix D – PDS Summary

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Customer</th>
<th>Metric</th>
<th>Target</th>
<th>Target Basis</th>
<th>Verification</th>
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<td>Flow Rate</td>
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<td>mL/min</td>
<td>&gt;4</td>
<td>Journal Article</td>
<td>Prototype</td>
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<td>Design</td>
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<td><strong>Environment</strong></td>
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<td>Able to withstand elevated temperatures</td>
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<td>Incubator Specifications</td>
<td>Design/Material Selection</td>
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<td>%</td>
<td>0.2-20</td>
<td>Incubator Specifications</td>
<td>Design/Material Selection</td>
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<td>Able to withstand wide range of O₂ Levels</td>
<td>Dr. Kohles</td>
<td>%</td>
<td>5-20</td>
<td>Incubator Specifications</td>
<td>Design/Material Selection</td>
</tr>
<tr>
<td>Able to withstand high humidity</td>
<td>Dr. Kohles</td>
<td>%RH</td>
<td>95</td>
<td>Incubator Specifications</td>
<td>Design/Material Selection</td>
</tr>
<tr>
<td><strong>Installation And Maintenance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of people needed to install</td>
<td>Bioengineering Research Team</td>
<td># of people</td>
<td>1</td>
<td>Research Team</td>
<td>Prototype</td>
</tr>
<tr>
<td>Open incubator time needed to replace any failed component</td>
<td>Dr. Kohles</td>
<td>Minutes</td>
<td>&lt;15</td>
<td>Sponsor</td>
<td>Prototype</td>
</tr>
<tr>
<td>Time needed to disassemble, clean, and reassemble</td>
<td>Bioengineering Research Team</td>
<td>Minutes</td>
<td>&lt;180</td>
<td>Sponsor</td>
<td>Prototype</td>
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<tr>
<td><strong>Manufacturing</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost</td>
<td>Dr. Kohles</td>
<td>USD$</td>
<td>&lt;1,000</td>
<td>Sponsor Budget</td>
<td>Design</td>
</tr>
<tr>
<td>Can be made in PSU machine shop</td>
<td>Bioengineering Research Team</td>
<td>--</td>
<td>No outside manufacturing</td>
<td>Sponsor</td>
<td>Design</td>
</tr>
<tr>
<td>Requirement</td>
<td>Customer</td>
<td>Metric</td>
<td>Target</td>
<td>Target Basis</td>
<td>Verification</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------------</td>
<td>--------</td>
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<td>----------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Physical Specifications</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Width</td>
<td>Dr. Kohles</td>
<td>mm</td>
<td>&lt;520</td>
<td>Incubator Specifications</td>
<td>Design</td>
</tr>
<tr>
<td>Depth</td>
<td>Dr. Kohles</td>
<td>mm</td>
<td>&lt;426</td>
<td>Incubator Specifications</td>
<td>Design</td>
</tr>
<tr>
<td>Height</td>
<td>Dr. Kohles</td>
<td>Mm</td>
<td>&lt;690</td>
<td>Incubator Specifications</td>
<td>Design</td>
</tr>
<tr>
<td>Weight of any component</td>
<td>Bioengineering Research Team</td>
<td>Lbf</td>
<td>&lt;30</td>
<td>Easy 1 person installation</td>
<td>Design</td>
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</tbody>
</table>
Appendix E – Concept Generation and Selection

The internal search portion of the product development process consisted of several steps. The team started by discussing ways in which the unique challenges presented by the design requirements could be overcome. The team then spent a week independently developing new ideas. During the following weekly meeting, the ideas were presented to the group, and discussed. A further brainstorming session took place during the meeting, and the next iteration debated. The team settled on six final contenders.

- Fluid tank – Rather than utilize individual sample sleeves, all samples are submerged in a large common pool of nutrient solution
- Dovetail – This system utilizes a tool less mounting system to attach the sleeves to the outer ring of the sample tray.
- Slot ports – Attaching machined rectangular tubes to the rear surface of the sleeves allowed this design to avoid some of the potential tangling issues, and may help minimize fluid recirculation
- Raised sleeve – By lifting the sample sleeves above the surface of the carrier tray, this design facilitates the needed access to the side panels of the sleeves.
- Raised tray – Similar to the raised design, this system drops sleeves below the tray by lifting the carrier with a horseshoe. This both allows access to the side panels, and gives an alternative hose routing option under the carrier tray.
- Channel routing – Machining the fluid flow channels into the surface of the tray, and then capping them with a secondary lid, minimized use of hoses in this design.

After a final panel of six candidates was selected, a scoring matrix was used to
objectively evaluate the ability of each design to handle the challenges presented. The team assigned a numeric value to each of the primary categories of criteria, the sum of which yielded an overall score for each design. The highest score determined the winner.

**Top-Level Final Design Evaluation and Selection**

The process of selecting the final design consisted of several steps. First, the team identified what criteria should be followed in the selection process. After meeting with the customer over a period of time, the team had a vivid idea of the main customer requirements, which were listed in our product design specification document. The customer made it clear that the main goal is to make a fluid perfusion system that is easy to install and maintain and that interfaces with the current bioreactor system. The team was also required to stick to the budget provided by the sponsor. Therefore, the main design selection criteria according to the PDS are:

1. **Maintenance:** All parts of the perfusion system have to be easily cleaned, accessible and have satisfactory service lifetimes.

2. **Installation:** The system has to interface with the existing device. The process of system disassembly and reassembly for maintenance purposes or for sample replacement has to be simple and quick.

3. **Cost:** All purchased parts and materials, fabrication, prototyping and testing processes are funded with a 1000 USD budget.

All design specifications described in the PDS document were revisited before the design process began to make sure that all the proposed designs comply with the main specifications as described by the customer. However, during the design process other criteria used in selecting
our final design were dictated by technical design parameters which the team found to be necessary for creating an efficient and reliable system. These criteria are fluid recirculation, tubing system and manufacturing and are explained below.

4. **Fluid recirculation**: Recirculation of the used fluid inside the sample wells has to be minimized to guarantee that the samples are always supplied with fresh nutritive fluid.

5. **Tubing system**: Fluid distribution system cannot interfere with other equipment fixed to the bioreactor. In case of using medical tubing, tangling of the tubes has to be avoided.

6. **Manufacturing**: The fabrication processes of implementing the prototypes and final product need to be simple and time efficient. After the first designs were finished, the team discussed the main pros and cons of each design and the results are shown on the next page.
<table>
<thead>
<tr>
<th>Raised Tray</th>
<th>Raised Sleeve</th>
<th>Dove-Tail</th>
<th>Slot Ports</th>
<th>Channel Routing</th>
<th>Fluid Tank</th>
</tr>
</thead>
</table>
| **Main Advantages** | • Hanging the sleeves provides more space for connecting the tubes.  
• Existing tray can be used.  
• Accessing the sleeves from beneath the tray avoids interference between the tubing and the actuators. | • Raising the sleeves provides more space for connecting the tubes.  
• Acrylic is cheap and easy to fabricate.  
• Sleeves can be of larger volume.  
• Tube connections can be easily checked. | • Cantilevering the sleeves provides more space for connecting the tubes.  
• Not too many restrictions on the sleeve size.  
• Samples are subjected to a direct cross flow.  
• Sleeves are easy to install and replace.  
• Tube connections can be easily checked. | • Not too many restrictions on the sleeve size.  
• Easy to install.  
• Tube connections can be easily checked.  
• Tangling is reduced by the use of slot ports and short hoses. | • No tangling of tubes.  
• Current tray can be modified to fit with the new system.  
• Can hold various sample sizes. |
| **Main Disadvantages** | • Assembling and disassembling the sleeves are time consuming.  
• Difficult to check connections hidden underneath the tray. | • Assembling and disassembling the sleeves are time consuming.  
• Tubes can be easily tangled together.  
• The tray and brackets assembly is too fragile.  
• Requires fabricating a new tray. | • Requires fabricating a new tray.  
• Difficult and expensive to fabricate.  
• Slot port connectors are too fragile.  
• Requires fabricating a new tray. | • Difficult to fabricate.  
• Difficult to detect leakage in tubes and disconnections hidden underneath the cover.  
• Requires fabricating a new tray.  
• Taking out the tubes to be cleaned requires unscrewing the cover which is time consuming. | • Difficult and expensive to fabricate.  
• Only used in the case of static fluid. |
Finally, a scoring matrix was used to objectively evaluate the ability of each design to handle the challenges presented. The team assigned a numeric value to each of the primary categories of criteria, the sum of which yielded an overall score for each design.

*Top Level Scoring Matrix*

<table>
<thead>
<tr>
<th>PDS Criteria</th>
<th>Raised Tray</th>
<th>Raised Sleeve</th>
<th>Dove-Tail</th>
<th>Slot Ports</th>
<th>Channel Routing</th>
<th>Fluid Tank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>2</td>
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<td>Installation</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cost</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fluid Recirculation</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>1</td>
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<tr>
<td>Tubing</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Manufacturing</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
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<td>23</td>
<td>20</td>
<td>17</td>
<td>9</td>
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</tbody>
</table>

The final design selected to move forward to the final refinement stage was the dovetail system. This was selected due to the design’s advantage in ease of manufacture, flexibility in sample type and shape, quick setup and repair, and ease of access to the side panel.
Appendix F - Bibliography


Overview of materials for Acrylic, Heat Resistant, Molded . (n.d.). Retrieved May 2010, from matweb.com:
http://www.matweb.com/search/DataSheet.aspx?MatGUID=6ba2ca129f0340f89ca6daab21fcb755&ckck=1


http://www.matweb.com/search/DataSheet.aspx?MatGUID=0cd1edf33ac145ee93a0aa6fc666c0e0