Identifying the Affects of Different Diffusion Limitations on Capillary Tube Formation from Endothelial Progenitor Cells in the Presence of Fibroblast

Abstract

A major limiting factor in the development of three-dimensional synthetic tissues is the development of a vascular network within the new tissue. Without blood supply to nourish the tissue, the size and scope of tissues able to be created is limited. Understanding capillary growth is necessary in developing vascular networks for three-dimensional synthetic tissues. This study investigates whether Endothelial Progenitor Cells isolated from human umbilical cord blood will form capillary tubes in a tissue matrix. In addition the study investigates the hypothesis that growth of capillary tubes is affected by the resistance of diffusion caused by an increase in the overall depth of the tissue matrix and an increase in the distance between the fibroblast and the beads.

Background

Recent advancement in the field of tissue engineering is extraordinary. There is an enormous amount of potential in being able to replace and restore functions to a variety of tissues. Most of this success has come in creating thin avascular tissues measuring in thickness of no more than 2 mm. To advance to create complex organs, such as lungs, heart and muscles, it will be necessary to create tissues in greater thickness than 2 mm, of approximately 1 cm in thickness. In a recent issue of *Nature* an article concerning the progress of engineered cardiac tissue stated that the development of thick tissues beyond the diffusion limits as one of the greatest challenges facing tissue engineering[1]. There has been great work done on the understanding of new blood vessel formation and also the collection of endothelial cells. Therefore science is ready for the development of vascularized thick implantable tissues.

There is a great clinical need for thick vascularized tissues. As previously stated there has been success in relatively thin tissues in which the supply of nutrients and oxygen is primarily thru diffusion. Some of the examples include the epidermis of skin which has received FDA approval, and cartilage such as the nasal saptae[2, 3]. More complex tissues such as cardiac muscle and liver have been tried but have been limited to very thin sections that are not useful. Some more homogenous tissues have had more success but again been limited in thickness to dimensions less than 2.5 mm. Bulkier tissues for reconstructive surgery have proved more difficult due to the need for immediate vascular supply to keep the tissue alive.

Tissues thicker than 2 mm have been a problem to generate because of lack of access to the nutrients needed to survive. Thin tissues have the advantage of receiving the nutrients needed through diffusion while thicker tissues require more. One of the major limiting factors in the development of three-dimensional synthetic tissues is the development of a vascular network within the new tissue. Without blood supply to nourish the tissue, the size and scope of created tissues is limited. A better understanding of capillary growth is necessary in developing these vascular networks for three-dimensional synthetic tissues.

It has been found that Endothelial Progenitor Cells are an important part of angiogenesis during wound healing[6]. Also Endothelial Progenitor Cells have been found in human umbilical cord blood[4, 5, 7]. We believe that for these two reasons Endothelial Progenitor Cells would be great

candidates for to grow capillary tubes in a tissue matrix. CD34+ has been used in other studies as a marker for Endothelial Progenitor Cells in different origins including umbilical cord blood[4, 5]. The isolation of Endothelial Progenitor Cells form umbilical cord blood to create a tissue matrix is a reasonable task.

Materials Method

To establish the diffusion limits the following procedures will be followed. The physical dimensions that we will be varying to establish the diffusion limitations are the distance separating the capillary network from the media (C), the distance separating the fibroblast from the media (F) and the distance between the fibroblasts and the capillary network (Δ). To do this we will move the fibroblast layer from the capillary network and the media. Endothelial Progenitor cells will be isolated from human umbilical cord blood using the Dynal CD34 Progenitor cell selection system. The cells will than be grown to confluence. The cells will than be seeded onto 150-µm diameter cytodex beads at a ratio of about 400 cells to one bead. The beads with the cells on them will than be allowed to culture overnight. After culturing the beads, they will be harvested from the flask and mixed with 2.5 mg/ml of fibrinogen, at a ratio of 200 beads/ml. Thrombin will than added to this mixture.

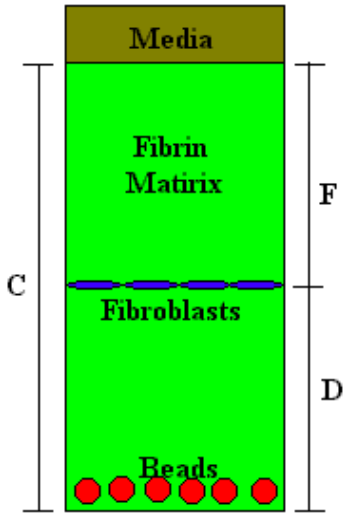

Figure 1. ***The Tissue Matrix is defined by different dimensions, which will be varied.*** The three critical dimensions are: 'C' the overall depth of tissue, 'D' the fibroblast distance from the beads and 'F' the fibroblast distance from media. A layer of fibrin will be placed to separate the beads, fibroblasts and media. In one case 'C' will be varied from 3.6 to 8.1 mm with 'D' held constant at 1.8 mm. In the second case 'D' will be varied from 1.8 to 4.5 mm with 'C' held constant at 5.4 mm.

Primary dermal fibroblasts will than be added to the matrix at a known distance from the beads. On top of the fibroblast will be another layer of fibrin followed by a layer of media (See Fig 1). These plates will be cultured at 37 °C for 7 days. The distance separating the capillary network from the media (C), the distance separating the fibroblast from the media (F) and the distance between the fibroblasts and the capillary network (D) will be altered. To do this C will have the depths of 3.6, 4.5, 5.4, 6.3, 7.2 and 8.1 mm. D will have the depths of 1.8, 2.7, 3.6, and 4.5 mm. When C is varied D will be held constant at 1.8 mm and when vice versa C was held constant at 5.4 mm. There will be 3 tissues and 5 beads for each combination of C and D, which will result in a total of 360 beads to quantify. The beads will be quantified using the following criteria: count the length of each continuous vessel sprout from bead, count the length of each vessel branching off vessel sprout, and count the total length of all vessels (See Fig. 2). Each researcher quantifying the beads will be blinded to the parameters step for each bead to avoid bias.

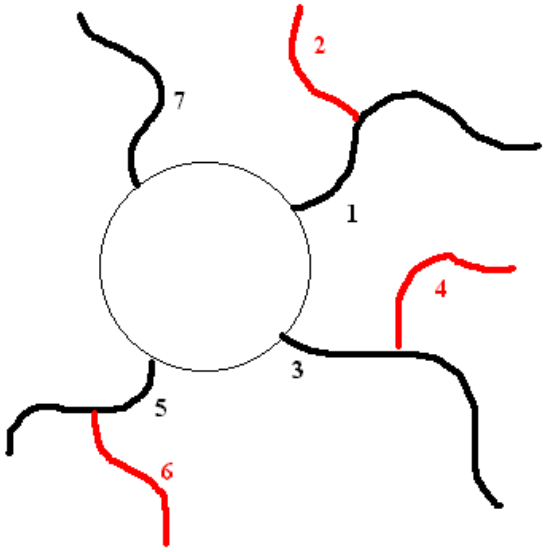

Figure 2. ***Quantification of capillary tubes forming off of cytodex bead.*** The following criteria will be used to quantify the total growth of capillaries: count the length of each continuous vessel sprout from bead (Black), count the length of each vessel (Red) branching off vessel sprout, and count the total length of all vessels. Each individual will be blind to the parameters of each bead that they quantified. This will eliminate bias based on what the expected results should be.

Student's Level of Experience

I have learned the techniques of tissue and cell culturing using fibroblast and also endothelial cells. Also I have constructed similar tissue matrixes using endothelial cells. My work in the lab thus far has allowed me to work with the many of the tools required to work on tissue cultures. I have also completed all the required courses to work in the lab provided by EH&S.

Student's Responsibilities

The student responsibilities will be to grow and passage the cells, do the tissue and cell culturing, isolate the Endothelial Progenitor Cells, and also construct the tissue matrix. The student will be running most of the protocol with the supervision of the Mentor and Graduate student.

Project Timeline

December – February: Isolation and growth of Endothelial Progenitor Cells. Construct tissue matrix and allow cells to form capillary tubes.

March-April: Quantification of the capillary tube sprouts and length.

References

1. Bryant SJ & Anseth KS. The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. *Biomaterials* **22**, 619-626 (2001).
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3. Masuda H & Asahara T. Post-natal endothelial progenitor cells for neovascularization in tissue regeneration. *Cardiovascular Research*. **58**, 390-398 (2003)
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6. Rosado C, Justesen J, Melsvik D, Ebbesen P, & Kassem M. The human umbilical cord blood: a potential source for osteoblast progenitor cells. *Calcif Tissue Int*. **72**, 135-142 (2003)
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Itemized Budget

Item	Manufacturer- cat #	Quantity	Size	Total Price
Fibroblast Growth Media	Cambrex- CC3131	1	500 ml	$60.00
Endothelial Growth Media-2	Cambrex- CC3156	1	500 ml	$55.00
EGM-2 Singlequots	Cambrex- CC4176	1	1 kit	$61.00
Reagent Pack	Cambrex- CC5034	1	300 ml	$45.00
1-100µl Pipet Tips	Fisher- 02-707-37	1	1000	$54.20
Thrombin (10 KU)	Sigma- T3399	4	1 KU	$107.04
Fibrinogen	Sigma- F4753	1	1g	$61.40
Glass pipet tips (5 3/4in)	Fisher- 13-678-6A	1	250	$17.88
T-75 Tissue Culture Flask	Fisher- S304661	1	5	$13.75
24 well Culture Plate	Fisher- 07-200-84	1	100	$224.48
Cytodex Microcarrier Bead (10g)	Sigma- C3275	1	10 g	$96.70
Normal Human Dermal Fibroblast	Cambrex- CC2611	1	T25 Flask	$455.00
Dynal CD34 Progenitor Cell Selection System	Dynal- 113.01	1	1 kit	$995.00
Dynal MPC-1 (Magnetic separation device)	Dynal- 120.01	1	1	$195.00
Total				$2,441.45