AD_____

Award Number: DAMD17-01-1-0152

TITLE: Lymphatic Regeneration Within Porous VEGF-C Hydrogels for Secondary Lymphedema

PRINCIPAL INVESTIGATOR: Mauricio A. Contreras, M.D.

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215

REPORT DATE: July 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2004	Annual (1 Jul 2003 - 30 Jun 2004)

4. TITLE AND SUBTITLE
Lymphatic Regeneration Within Porous VEGF-C Hydrogels for Secondary Lymphedema

5. FUNDING NUMBERS
DAMD17-01-1-0152

6. AUTHOR(S)
Mauricio A. Contreras, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215

E-Mail: mcontrer@BIDMC.harvard.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Introduction: Lymphedema is an abnormal swelling, in which lymph production exceeds drainage capabilities. This occurs as a result of lymphatic vessel destruction during the removal of lymph nodes or subsequent radiation therapy in breast cancer treatment. Management of lymphedema remains a clinical problem. In adult lymphangiogenesis, VEGF-C has been shown to be a specific mitogen for lymphatic endothelial cells (LEC) via the VEGF-3 receptor. Ang-2 has recently been shown to be required for proper lymphatic development via the Tie 2 receptor. Objective: In our model we incorporated into alginate gels, Ang-2 and VEGF-C to promote lymphoangiogenesis by stimulating LEC proliferation and migration. Methods: Sterile alginate gels with Ang-2 (2µg/ml) and VEGF-C (200ng/ml) were tested *in vivo* for LEC proliferation and migration. Results: Previous *in vitro* studies demonstrated that by adding Ang-2 to the VEGF-C alginate gels, LEC proliferation and migration increased, when compared to VEGF-C alginate gels alone. Our Preliminary animal (n=6) studies in a tail lymphedema animal model using these alginate gels (Ang-2/VEGF-C) suggests that lymphoangiogenesis does occur. Conclusions: Our preliminary *in vivo* results demonstrate that alginate gels are an effective delivery system of Ang-2 and VEGF-C, in which new tubular structures are formed resembling lymphatic vessels (Histology). Further animal studies (Lymphocintography) are required to evaluate these new tubular structures and their capability in restoring lymphatic function *in vivo*.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover..... 1

SF 298..... 2

Table of Contents..... 3

Introduction..... 4,5

Body..... 6-11

Key Research Accomplishments..... 12

Reportable Outcomes..... 12

Conclusions..... 12

References..... 13,14

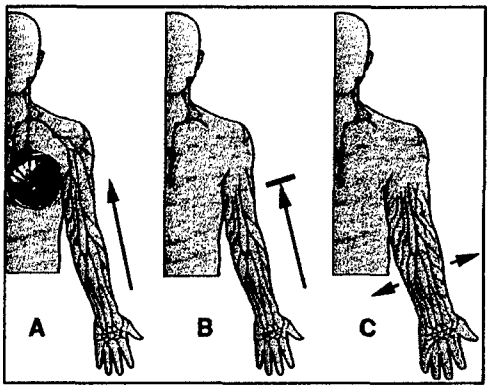
Appendices..... 14

Introduction:

Breast carcinoma continues to be the most frequently occurring carcinoma in women. Presently, approximately one in every eight women will develop the disease.[1] Great strides have been made in the treatment of breast carcinoma that have reduced the risk of recurrence and improved survival rates.

The removal of axillary lymph nodes has been an integral part of breast carcinoma treatment since the end of the last century.[2] Axillary lymph nodes are removed to provide accurate information for staging, to accomplish local control, and for prognosis in order to plan adjunctive, systemic therapy. The status of the axillary lymph nodes remains the single most important predictor of survival.[3,4] Therefore, axillary treatment surgically and/or with radiotherapy is still an essential component in the management of most patients with invasive breast carcinoma.[5,6]

Chronic edema of the arm, commonly called Lymphedema or post mastectomy edema (PME), was described first as a side effect of mastectomy operations by Halsted in 1921[7] *(Figure 1)*. Although there has been a trend toward more conservative surgery and greater use of radiotherapy, PME remains a common iatrogenic problem.

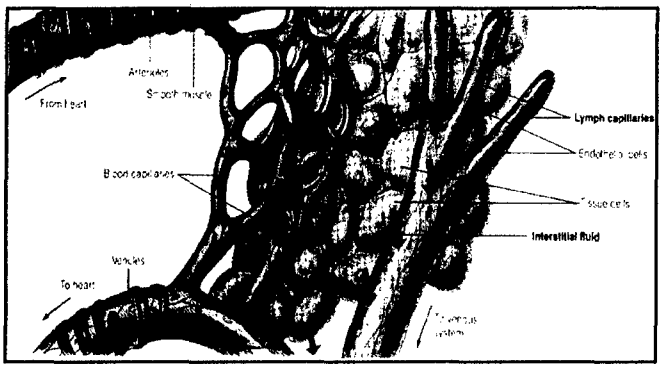


Edema represents an increase in interstitial fluid volume sufficient to manifest with swelling. Any edema, whatever the underlying cause, is due to an imbalance between capillary filtration and lymph drainage.[8] Most examples of limb edema are caused by an increase in capillary filtration, overwhelming lymph drainage capacity. Lymphedema, however, strictly occurs when swelling is due to a failure of lymph drainage in circumstances in which capillary filtration is not increased.

Figure 1: Schematic representation of Secondary Lymphedema. A) Normal lymphatic flow from the hand to the Thoracic duct (arrow). B) Removal of cancerous breast and axillary lymph nodes, lymphatic flow interrupted (arrow). C) Lymph (proteins) stagnation within the arm (arrows) and subsequent swelling.

The lymphatic system is a one-way drainage route designed to rid the "tissues" of unwanted material and excess fluid. It therefore represents a waste route and overflow pipe, with its essential function being to return to the blood vascular compartment protein, colloids, and particulate matter too large to reenter the blood compartment directly [9] *(Figure 2)*. Two types of lymphatic vessels exist: First, the smaller initial lymphatic, which includes the smallest lymphatic capillary and the larger precollector vessel, and second, the collecting lymphatic vessel into which the precollectors drain.[10]

The collecting lymphatics are the main limb lymphatic vessels that provide the afferent flow to the lymph nodes. They behave like a series smooth muscle "hearts" that are responsible mainly for the propulsion of lymph centripetally.[11] Intrinsic pumping of collecting vessels is the essential motor for lymph propulsion.
For initial lymphatics, however, flow of interstitial fluid and macromolecules is caused by intermittent changes in hydrostatic and oncotic pressures locally. [12]



Deformation or movement of the tissues by surface pressure or underlying muscle contractions and by other contractile structures, such as arterioles, causes expansion or compression of the of the initial lymphatics. The compression forces lymph along initial lymphatics. Valves in the initial and collecting lymphatics ensure that flow is unidirectional.

Figure 2. Schematic representation of Lymphatic Vessel, Arteriole and Venule.

Lymphedema arises when an intrinsic fault develops within the lymph-conducting pathways (primary lymphedema) or when damage occurs from one or more factors originating outside the lymphatic system, such as surgical removal of lymph nodes (secondary lymphedema). Lymphedema therefore, is a disease characterized by abnormal collections of fluid and proteins within the interstitial space. Treatment options for this debilitating condition have included drug therapy, physical therapy, and surgical approaches that have yielded limited success. Unfortunately, for lymphedema today, treatment options are all palliative, for there is **no effective treatment** that could offer a permanent cure to this disease.

Secondary Lymphedema resulting after iatrogenic, surgical disruption of the Lymphatic vessels in breast cancer surgery, has continued to be neglected in the U.S. in spite of the fact that it has now become an acceptable diagnosis (ICD9-457.0)[13]. Swelling of the upper limb, constitutes the most invalidating complication of breast carcinoma treatment.[14] Untreated, upper extremity lymphedema predisposes women to the development of severe acute or chronic infections with limitations on functioning and serious disturbances in a patient's quality of life. Psychological distress (disfigurement), depression and social inhibition.[15]

It is estimated that 15-20% of the 2 million breast carcinoma survivors, are living currently with post-treatment lymphedema .[16-17] The swollen arm, which can be as much as twice the normal size, is disfiguring and commonly causes functional impairment, psychosocial maladjustment and psychological morbidity.[18] Added to the physical symptoms that patients must cope with, is the pain caused unintentionally by clinicians that, interested in carcinoma recurrence, trivialize the no lethal nature of lymphedema.

Body:

Vascular Endothelium Growth Factor (VEGF) is produced by many different cell types both in tissue culture and in vivo. It binds to plasma membrane receptors on endothelial cells (EC) only with an extra cellular transmembrane glycoprotein linked to an intracellular tyrosine kinase domain, thus VEGF is an EC specific mitogen.

VEGF-C is a 38 kD homodimeric glycoprotein that is commercially available. This recombinant human VEGF-C is from a DNA sequence encoding the 165 amino acid residue variant of human VEGF expressed in Sf 21 insect cells using a baculovirus expression system. The product is lipolized from a sterile-filtered solution in 30% acotonitrile plus 0.1% TFA containing 50μg of cytokine. **VEGF-C** is of particular interest because it is a specific mitogen for **lymphatic endothelium** in adult tissues.

Angiopoietin-2 is a ligand for the endothelial Tie-2 receptor tyrosine kinase, it has a dual function in the processes of postnatal angiogenesis and vascular remodeling. Also **Ang-2** signals are required for the proper development and function of **lymphatic vessels.** Recent data also suggest that the roles of Ang-2 and VGEF-C in the lymphatic vessel development are analogous too the roles of Ang-1 and VEGF in blood vessel development. Thus, Ang-2 is the first endothelial cell-specific growth factor demonstrated to function in vessel formation or regression depending on the tissue context.[19] In combination with VEGF-C, Ang-2 potentiates its effect (*Figure 3).*

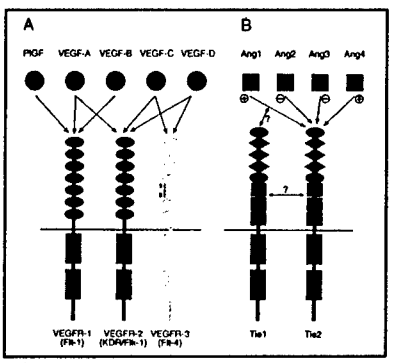


Figure 3.

Schematic representation of the different types of Vascular Endothelial Growth factor and the effect they have on specific receptors.

Tissue engineering is an interdisciplinary field that incorporates principles of engineering and polymer chemistry into the biological sciences, in efforts to develop biological substitutes for failed tissues and organs. It is a new and rapidly expanding field, in which the techniques are being developed for culturing or promoting regeneration of a variety of tissues, both in vitro and in vivo using polymer "scaffolds" to support tissue growth.[20]

Unlike the solid polymer systems currently used to create a cell-polymer construct, a liquid support matrix that polymerizes to a gel would have the potential for injectable delivery, which would be much less invasive than open implantation. **Alginate hydrogels** have been increasingly important in biotechnology applications, such as tissue engineering, artificial organs and as drug carriers.[21] Delivery rate can be predetermined and its local, rather than systemic administration be of great advantage (Alginates are approved by Food and Drug Administration for human use).

Alginate refers to a family of polyanionic copolymers derived from brown sea algae and comprising 1,4-linked β-D-manuronic (M) and α-L-glucoronic (G) acid residues in varying proportions. The fact that G and M are C5 epimers results in a switch-over of the monomer chair conformation, giving rise to all four possible glycosidic linkages and at the molecular level, large effects like cavity formations between G residues are observed. It has been shown that the occurrence of G and M within the alginate molecule is block-wise and not random.[22,23]

There is a direct dependence between the mechanical strength of an alginate gel and the porosity of the gel network. When gels are made from alginate rich in glucoronic (G) acid residues, higher moduli are obtained compared to gels made from alginates less enriched in G residues, and also higher diffusion rates. *(Figure 3)*. Gelling properties of alginate are a function of the M/G composition and the sequential structure of M and G along the alginate chain (*Figure 4*).

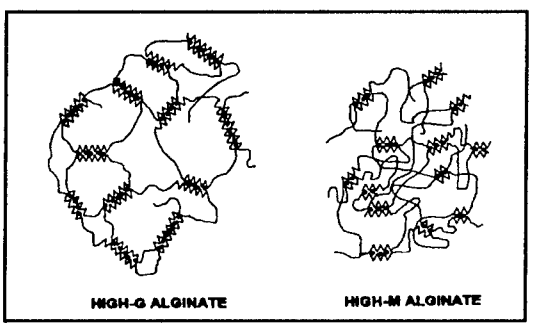


Figure 4

In general terms, an increase in G content (measured as $G_{n>1}$) as well as in molecular weight will give a stronger gel. In contrast to most gelling polysaccharides, alginate gels have the particular feature of being "cold setting"'. This implies that the setting of alginate gel is more-or-less independent of temperature.

Alginate gel incorporation: Essentially there are two main methods for the preparation of alginate gels: The dialysis/diffusion method and internal geleation method. In the dialysis/diffusion method (diffusion setting) gelling ions are allowed to diffuse into the alginate solution. In our study we will incorporated sterile G (50%) and M (50%) copolymers of alginate to a solution with Potassium Phosphate and Sodium Chloride (0.1 M K_2HPO_4 and 0.135 M NaCl, pH of 7.4), which was previously sterilized by autoclave. A 1.0% Sodium Alginate solution will then be mixed with 0.2 gm of $CaSO_4$ per milliliter, this solution will be placed in ice prior to extrusion. Because of all the different characteristics previously mentioned we decided to produce an alginate gel that would have the best properties of both, the G and M copolymers. Thus providing a gel that would be highly porous and relatively strong. Previously expanded EPC's which have been induce to differentiate into LEC by the growth factors, VEGF-C and Ang-2, will be incorporated into this gel for injection.

Preliminary in vitro studies were carried out to determine the ideal Ang-2 concentration to be incorporated into the alginate gels. *In vitro* culture with LEC and three different concentrations (as recommended by Regeneron): 0.22 μg/ml, 0.67 μg/ml and 2 μg/ml of Ang-2 reveled that the proliferative and migratory response of the LEC was greater at 2 μg/ml when compared to the other two concentrations.

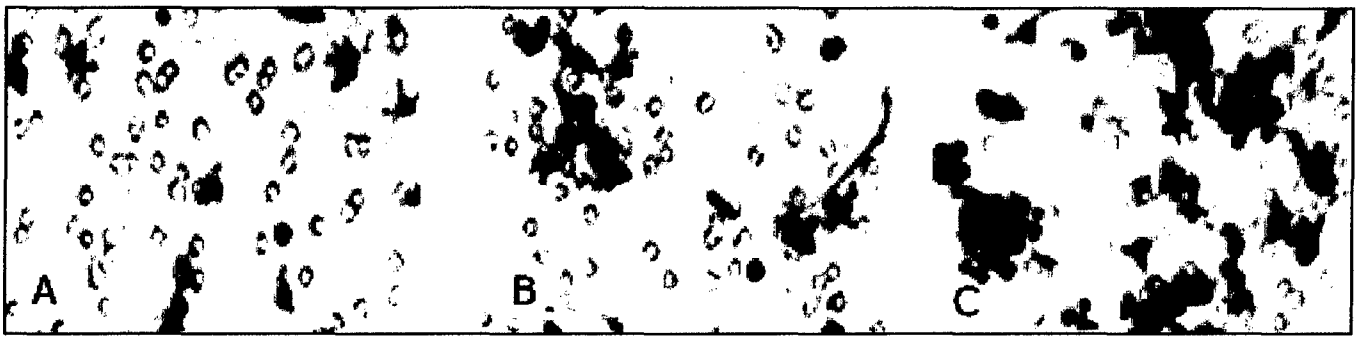


Figure 5. Photograph of in vitro Proliferation and migration Assays of LEC in a Bodin chamber at 7 days. A) Ang-2 concentration of 0.22 μg/ml, B) Ang-2 concentration of 0.67 μg/ml, C) Ang-2 concentration of and 2 μg/ml.

With these initial results we determined that the concentrations of Ang-2 that should be incorporated into alginate gels should be the same as the ones above, and determine if the release of the growth factor from the alginate gel would result in the same or comparable proliferation and migration of LEC.

LEC's were cultivated with Ham's modified F12K medium for EC with ECGS. Cells were serum starved (from 10% to 1%FBS) prior to plating on the petri-dish with the alginate gels. Once again the ideal concentration for Ang-2 was that of 2 μg/ml, where the proliferation and migration of LEC was more profound.

Alginate gels were then prepared to incorporate VEGF-C at a concentration of 200 ng/ml and Ang-2 at a concentration of 2 μg/ml. These gels will now be used *in vivo*, in the mouse tail animal model we have worked on.

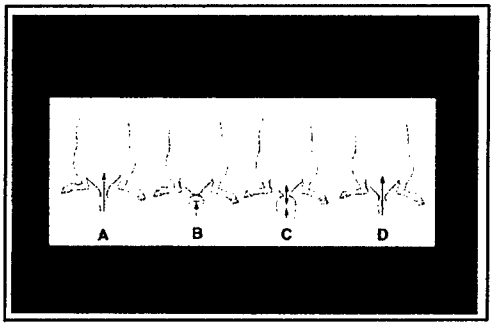
Lymphedema animal model: A lymphedema mouse model has recently been described in the literature.[24] This model provides a useful *in vivo* system, in which experimental treatments for lymphedema can be evaluated. We have started to operate on the tail of mice to cauterize the lymphatics and induce lymphedema (*Figure 6, 6a*).

In this model, hairless mice (SKH1/Charles River Laboratories, Pittsfield, NH) weighing between 20 are 30 g will be used. The hairless mice offer the advantage that lymphedema development and subsequent tail diameter measurements are easier to follow. Anesthesia and Analgesia: The animals are anesthetized by placing them inside an induction chamber with 1.5% isoflurane and 100% Oxigen (JA Webster Inc., Sterling, MA). Once the animals are anesthetized they are placed in a prone position and the head placed inside a conical mask to maintain anesthesia (0.8% Isoflurane and 100% Oxigen).

Analgesia is maintained post-op and for the next 7 consecutive days by daily subcutaneous (SC) injections of 3.3 mg/Kg butorphanol.

Prior to start the surgical procedure, 0.10 ml of 5% blue dextran 2M (Sigma, St. Louis, MO) in saline is injected SC into the tip of the tail in order to identify the lymphatic vessels. Then, the operative site at the base of the tail is cleansed with 70% ethanol and povidine/iodine, and a circumferential incision is made through the dermis to sever the superficial lymphatic network. The three deep lymphatic vessels located in the lateral and ventral aspects of the tail are isolated and destroyed by electrical cauterization. Finally, low cauterization is applied too the edges of the circumferential wound, which results in a 2-4 mm gap between the skin edges followed by secondary healing at the site (*Figure 6*).

Figure 6 .



Schematic of Lymphatic tail model.

A) Normal lymphatic flow.
B) Obliteration of tail lymphatics with subsequent lymphedema formation.
C) Lymphangiogenesis by transplantation (Injection) of Alginate gel with VEGF-C and Ang-2.
D) Restoration of normal lymphatic flow with subsequent lymphedema reabsorption.

Once the lymphedema of the tail has been developed (weeks), the transplantation (SC injection) of the alginate gel containing the growth factors, VEGF-C and Ang-2 will be performed in an attempt to induce lymphatic vessel proliferation and regeneration of the lymphatic network previously destroyed. Subsequent Lymphocintography studies will confirm if lymphatic flow has been restored.

Ang-2 & VEGF-C Alginate gels Transplantation Animal Model. All procedures are being performed in accordance with the Animal Care Act and Use Committee. Hairless mice (Charles River Laboratories) age 8-10 weeks and weighing 20-30 g are anesthetized with 160 mg/kg pentobarbital IP. for operative transplant (injection) of the lymphedematous tails.

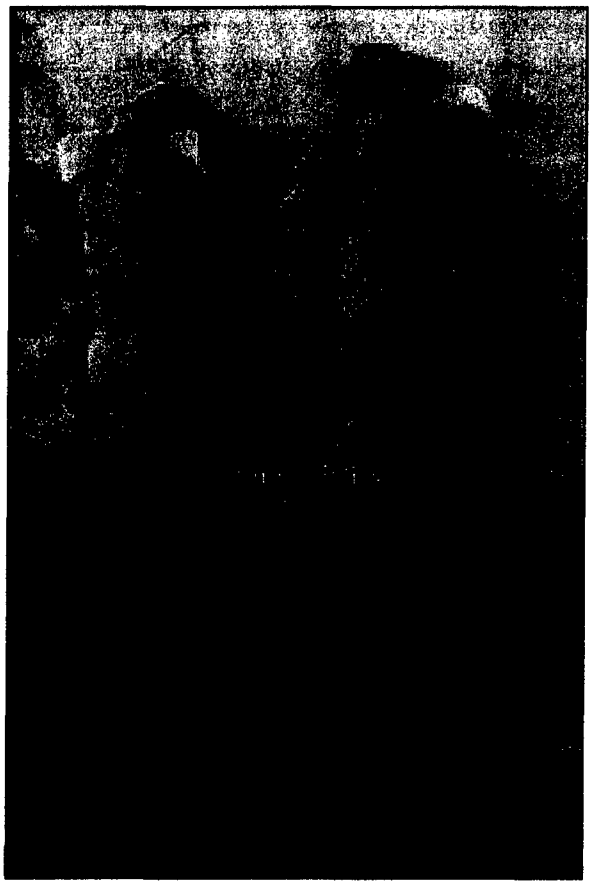


Figure 6a.

Photograph of Lymphedematous Tail in the mouse (A), at 2 weeks. The increase in diameter of the tail is more profound at a later time point 4 weeks.
When compared side-by-side to a normal (B) tail of a mouse, the Lymphedematos tail is also evident.
Alginate gels with Ang-2 & VEGF-C will be injected at different time intervals (3, 7, 14, and 28) to evaluate lymphatic regeneration and restoration of lymphatic flow.

Last Phase of Research: *In Vivo* model.

Histologic Assessment of Transplanted Animals. Tissue sections from the base of the Lymphedematous and healthy tails are being performed. Tails are being and will continue to be harvested on days 3, 7, 14, and 28 after transplantation. For conventional H&E Histology.

We have treated the mice lymphedematous tails with our Alginate gel (Ang-2 & VEGF-C) injections and have collected the tails 7 days thereafter for conventional histology. Our preliminary results show that tails harvested at 7 days after they have been treated with alginate gels alone (control) without any growth factors exhibit less lymphatics (*Figure 7*) when compared to those mouse tails that were treated with the alginate gels that contained both growth factors, VEGF-C and Ang-2 (*Figure 8*).

Even though these preliminary results are encouraging, we need to asses if these new lymphatics will be able to reconstruct the lymphatic network and re-establish lymphatic function by decreasing the volume on the lymphedematous tail. We will evaluate lymphatic function through lymphocintography studies prior to animal sacrifice and tail harvest for conventional histology and immunohistochemistry.

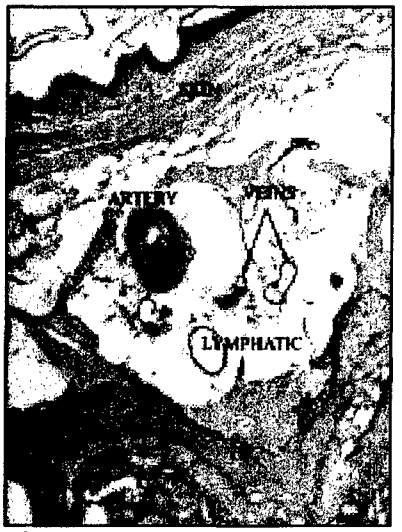


Figure 8

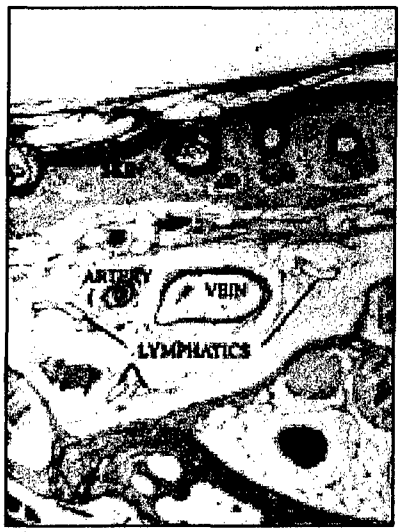


Figure 7

We requested for an extension at no additional cost to the DOD-BC for an additional year so that we may conclude our in vivo studies and our petition was granted. Pending are the 3,14 days time intervals for conventional histology.

On Going Studies:

Immunohistochemistry: Tissues will be embedded in OCT compound (Miles Scientific, Elkhardt, IN) and snap frozen in liquid nitrogen. Frozen sections of 6-μm thickness will be mounted on silane-coated glass slides, air-dried for 1 h, and counterstained with biotinylated antibodies to UEA-1, mouse and human CD31 (PECAM-1; Dako), and conjugated with FITC-streptavidin (Caltag), as previously described. Before direct immunofluorescent examination, slides will be covered with Vectashield mounting medium for fluorescence microscopy (Vector Laboratories). The extent of lymphangiogenesis will be assessed by measuring lymphatic density in light microscopic sections of tails retrieved from lymphedematous mousetails. Sections will be stained for LYVE-1 to identify LECs.. At each time point, 20 fields (×40 magnification) will be counted for each of 3 animals.

Physiological Assessment of Transplanted Animals. At 3,7,14 and 28 days after transplantation, Lymphocintography studies will be conducted to assess lymphatic regeneration. Immediately before sacrifice, mice will be injected with an overdose of pentobarbital.

Statistical Analysis. All data will be presented as mean ± SEM. Inter-group comparisons will be performed by paired Student's *t* test or ANOVA. The comparative incidence of lymphatic regeneration by decreased lymphedema will be evaluated. Probability values of $P < 0.05$ will be interpreted to denote statistical significance.

Key Research Accomplishments:

Our Specific aims for the third year of this award were successfully completed.

- We were able to incorporate into our previous porous, biodegradable alginate gel with VEGF-C a second growth factor, Ang-2 to serve as a receptor agonist, thus enhance the VEGF-C mitogenic effect in promoting Lymphatic Endothelial Cell proliferation and migration *in vitro and in vivo.*

- Next Phase (Last year of this award) we will continue to induce (surgically) lymphedema of the tail of mice to inject the VEGF-C and Ang-2 alginate gels to evaluate lymphoangiogenesis *in vivo* and evaluate if lymphatic function has occurred.

Reportable Outcomes:

- Rat Thoracic Duct Cell line maintained.

- We were invited by the New England Lymphedema Support Group at the Leahy Clinic in Woburn,MA to address their Lymphedema patients on the current research we are conducting.

- We were invited by Dr. Stanley G. Rokson, Chief editor of the Lymphatic Research and Biology Journal to submit a manuscript this fall. Manuscript in progress.

Conclusions:

A biodegradable Alginate gel, may be an effective delivery system for sustained slow release of VEGF-C and Ang-2 to promote lymphangiogenesis in those instances where the integrity of the VEGF-3 and Tie-2 receptor of the LEC are intact. We continue to test these alginate gels in a lymphedema animal model (mouse tail) to evaluate lymphoangiogenesis *in vivo* and determine if Lymphatic function can be reestablished.

References:

1) Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. *CA Cancer J Clin* 1997;47:5-27

2) Pressman PI. Surgical Treatment and Lymphedema. *Cancer* 1998;83(12):2782-87

3) Mustafa I, Bland K. Indications for axillary dissection in T1 breast cancer. *Ann Surg* 1998;5:4-8

4) Fisher B, Bauer M. Margolese R, Poisson R, Plich Y, Redmond C, et al. Five year results of a randomized clinical trial comparing total mastectomy and segmental mastectomy with or without radiation in the treatment of breast cancer. *N Engl J Med* 1985;312:665-73

5) Falk SJ. Radiotherapy and the management of the axilla in early breast cancer. *Br J Surg* 1994;82:1277-81

6) Recht A, Houlihan MJ. Axillary lymph nodes and breast cancer. *Cancer* 1995;76:1491-512

7) Halsted WS. The swelling of the arm after operations for cancer of the breast-elephantiasis chirurgica-its cause and prevention. *Bull John Hopkins Hosp* 1921;32:309-13

8) Levick JR. An introduction to cardiovascular physiology, 2nd ed. Oxford: *Butterworth- Heinemann*, 1995.

9) Yoffey JM, Courtice JM. Lymphatics, lymph and the lympho-myeloid complex. New York: *Academy press*, 1970

10) Kubik S, Manestar M. Anatomy of the lymph capillaries and pre-collectors of the skin. In the initial lymphatics. Bollinger A, Partsch H, Wolfe JN, editors. Stuttgart: *Georg Thieme Verlag*, 1985:66-74

11) Smith RO. Lymphatic contractillity: a possible mechanism of lymphatic vessels for the transport of lymph. *J Exp Med* 1949;90:497-509

12) Roddie IC. Lymph transport mechanisms in peripheral lymphatics. *New Physiol Sci* 1990;5:85-9

13) Thiadens SR. Current Status of Education and Treatment Resources for Lymphedema. *Cancer* 1998; 83(12) 2864-2868

14) Paci E, Cariddi A, Barchielli A, Bianchi S, Cardona G, Distante V, et al. Long-term sequelae of breast cancer surgery. *Tumori* 1996;82:321-4

15) Tengrup I, Nittby LT, Christiansson I, Laurin M. Problems with arms are common after breast surgery. Lymphedema is a frequent complication in elderly women treated for breast cancer. *Lakartidningen* 1999;96(46):5089-91

16) Petrek, Heelan M. Incidence of Breast Carcinoma-Related Lymphedema. *Cancer* 1998;83(12):2776-81

17) Ferrandez JC, Serin D, Bouges S. Frequence des lymphoedemes du membre superieur apres traitement du cancer du sein. Facturs de risque. A propos de 683 observations. *Bull Cancer* 1996;83:989-95

18) Tobin MB, Lacey HJ, Meyer L, Mortimer PS. The psycological morbidity of breast cancer related arm swelling. *Cancer* 1993;72:3248-52.

19) Veikkola Tanja and Alitalo Kari. Dual role of Ang2 in postnatal Angiogenesis and lymphangiogenesis. Developmental Cell 2002;3:302-304

20) Niklason LE and Langer R. Advances in tissue engineering of blood vessels and other tissues. Trans Immunol 1997;5:303-306

21) Peppas NA. Hydrogels in Medicine and Pharmacy. Ed. CRC (Boca Raton, FL) 1986;Vol I,II and III.

22) Dornish M, Kaplan D, Skaugrud O. Stadards and guidelines for biopolymers in tissue-engineered medical products: ASTM alginate and chitosan standard guides. Ann NY Acad Sci 2001;944:388-97.

23) Gutowska A, Jeong B, and Jasionowski M. Injectable Gells for tissue engineering. The Anatomical Record 2001;263:342-349

24) Slavin SA, Van den Abbeele AD, Losken A, Swartz MA. Return of Lymphatic Function After Flap Transfer for Acute Lymphedema. *Ann Surg* 1999;229(3): 421-27

Appendices:

NA on this report.