14:25:40 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 05/08/91

Active

Project #: E-25-612 Cost share #: E-25-347 Rev #: 13
Center # : R6585-0A0 Center shr #: F6585-0A0 OCA file #:
Work type : RES
Contract#: ECS-8815656 Mod #: ADMIN REV Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : Y
Main project #:

Project unit: MECH ENGR Unit code: 02.010.126
Project director(s):
NEREM R M MECH ENGR (404)894-2768

Sponsor/division names: NATL SCIENCE FOUNDATION / GENERAL
Sponsor/division codes: 107 / 000

Award period: 880901 to 920229 (performance) 920531 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	611,250.00
Funded	0.00	611,250.00
Cost sharing amount		5,957.00

Does subcontracting plan apply ?: N

Title: MECHANICAL STRESS AND VASCULAR CELL GROWTH

PROJECT ADMINISTRATION DATA

OCA contact: Mildred S. Heyser 894-4820

Sponsor technical contact

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Sponsor issuing office

NICK NAYAK
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NATIONAL SCIENCE FOUNDATION
DGC/ENG
WASHINGTON, D.C. 20550

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NSF supplemental sheet
Equipment title vests with: Sponsor GIT X
NONE PROPOSED
Administrative comments -
TO DELETE BUDGET REVISION NO. 10 DATED 2/21/91.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 07/27/92

Project No. E-25-612 Center No. R6585-0A0

Project Director NEREM R M School/Lab MECH ENGR

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Contract/Grant No. ECS-8815656 Contract Entity GTRC

Prime Contract No. _____

Title MECHANICAL STRESS AND VASCULAR CELL GROWTH

Effective Completion Date 920229 (Performance) 920531 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other _____	N	

Comments BILLING VIA LINE OF CREDIT; 98A SATISFIES REQUIREMENT FOR PATENT REPORT.

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT (SUBPROJECTS)

Closeout Notice Date 07/27/92

Project No. E-25-612 Center No. R6585-0A0

Project Director NEREM R M School/Lab MECH ENGR

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Project # E-25-M04 PD NEREM R M Unit 02.010.126 T
GRANT # ECS-8815656 MOD# BR 6/22/91 MECH ENGR *
Ctr # 10/24-6-R6585-0A1 Main proj # E-25-612 OCA CO MSH
Sponsor-NATL SCIENCE FOUNDAT /GENERAL 107/000
MECHANICAL STRESS AN
Start 880901 End 910228 Funded 15,250.00 Contract 15,250.00

LEGEND
1. * indicates the project is a subproject.
2. I indicates the project is active and being updated.
3. A indicates the project is currently active.
4. T indicates the project has been terminated.
5. R indicates a terminated project that is being modified.

E-25-612

ANNUAL PROGRESS REPORT ON NSF GRANT EET-8815656
ENTITLED "MECHANICAL STRESS AND VASCULAR GROWTH"

Period: September 1, 1988 - May 31, 1989

Principal Investigators: Robert M. Nerem, Ph.D.
R. Wayne Alexander, M.D., Ph.D.

Work on this project focuses on vascular endothelial cells (EC) and the engineering of the cell culture environment so as to provide conditions which are more physiologic. The unique aspects of this project are its (i) systematic study of mechanical stresses of various types, (ii) use of the most advanced image analysis system available for cellular studies, (iii) emphasis on subconfluent monolayers and a variety of different mammalian cell types, and (iv) emphasis on the growth characteristics and growth program of cells. In each of these areas significant progress has been made as is summarized below.

With regard to the systematic study of mechanical stresses of various types, two new systems have been designed and are being tested. One of these is a uniaxial, cyclic stretching device where cells are grown on a compliant membrane which can be stretched with a sinusoidal motion at a frequency in the range of 0.5 to 3 Hz. Initial results on endothelial cell morphology have been obtained at 1 Hz and a modified design is now being constructed in our machine shop. The other new system is a pulsatile flow system which can be used in one of three modes: (1) non-reversing flow, (2) reversing flow, and (3) oscillatory flow (where there is zero mean flow). This system is currently in the final stages of testing.

Our image analysis work uses the Perceptics Biovision system which is based on a

DEC Microvax computer and which was purchased with institutional funds. This system was delivered in mid-December 1988 and over the past five months considerable operating experience has been gained. Additional software also has been written, and the Biovision system provides the capability for multiprobe fluorescence image analysis. In addition to its application in cell shape/motion studies, it thus is now being used to conduct Fura-2 intracellular calcium measurements.

Although most of our work to date and the focus of this project is vascular EC, we also want to investigate whether the response of vascular EC to mechanical stress is unique or whether it can be generalized to other cell types. Thus, we have initiated studies both with 3T3 fibroblasts and MDCK cells. Also, since all of our previous work has been carried out with bovine aortic EC, we also have initiated studies with human microvascular EC and human umbilical vein EC. Studies are being conducted both for confluent and subconfluent monolayers. The latter is of interest not only because the cells are in a growth mode, but also because one can focus on the response of a single cell which is uninfluenced by its neighbors.

A major objective of this research program is to define the influences of mechanical stress on the intrinsic and extrinsic growth programs of cells. In addition to measurements of cell growth, we also are interested in the expression and influence of growth promoting substances. As a baseline study, we have initiated experiments on the influence of hydrostatic pressure on cell proliferation. In this study we have measured both 3-H thymidine incorporation and IGF-I production by bovine aortic endothelial cells (BAEC)

in culture. Thymidine incorporation studies were carried out on confluent BAEC that were growth arrested in 0.4% calf serum for 48 hours prior to the experiment. The BAEC then were placed in either 0%, 0.4%, 2% or 10% calf serum, and the cultures were then exposed to either atmospheric pressure or a pressure of 120 mmHg for 24 hours. Preliminary results have shown a decrease in thymidine incorporation with normal stress.

Preliminary evidence indicates that there is secretion of a immunoreactive Insulin-like Growth Factor (IGF-I) by cultured rat aortic smooth muscle cells, bovine aortic endothelial cells and human umbilical vein endothelial cells. IGF-I levels are markedly higher in younger passage cells, and use of a ^{32}P labeled antisense RNA probe to detect specific IGF-I mRNA species has revealed several transcripts in cultured bovine aortic endothelial cells, namely the 4.9kb, 1.9kb and 1.3kb messages. We also have measured IGF-I production by BAEC exposed to normal stress. The 24 hour production of IGF-I by BAEC exposed to normal stress was increased by 332% over cells exposed to atmospheric pressure. We currently have no explanation for why cells exposed to higher levels of IGF-I have a lower rate of thymidine incorporation. Obvious possibilities to investigate include alterations in IGF-I receptor number or binding properties, production of other factors with negative mitogenic effects, and alterations in receptor processing and signal transduction.

A major hypothesis to be tested is that the membrane signals generated by mechanical stress, e.g. a fluid-imposed shear stress, are not generalized throughout the cell but are localized and thus account for the characteristic elongation and orientation of the cell

which are observed to occur. In testing this hypothesis, our primary tool will be the Biovision image analysis system. One application of this system is the measurement of intracellular calcium, and we already have demonstrated our ability to load cells with Fura-2 and obtain images at two wavelengths, which is necessary to perform the image ratioing required in the measurement of intracellular calcium. Flow studies are now commencing.

Related to the testing of this hypothesis are our studies of the role of protein kinase C in EC responses to shear stress. Preliminary measurements of PKC activity in solubilized homogenates of EC have been performed by analysis of Ca^{2+} and phospholipid-dependent phosphorylation of histone I. We found that 63% of total PKC activity was in the membrane fraction and 37% in the cytosol of EC grown under static conditions. Exposure to a shear stress of 30 dynes/cm^2 for 20 min. caused a decrease in cytosolic PKC activity consistent with translocation of PKC to the membrane.

We have optimized conditions for loading and imaging EC with dansyl-13-TPA. In this there was intense fluorescence of the cytoplasm but not the nucleus. When the fluorescence intensity was mapped with a "pseudo-color" scale, it is readily seen that the perinuclear localization is punctate and morphologically resembles Golgi. When the threshold was lowered, resulting in a larger pseudo-color scale, a topographical pseudo-color map was then generated by use of a pixel by pixel histogram, and this clearly shows the intense perinuclear localization and the very high concentration relative to other parts of the cytoplasm.

Finally, the active release of adenine nucleotides (AdN) from EC may be important in their response to stimuli. Purinergic receptors in fact are found on vascular smooth muscle and endothelial cells, yet the origins and mechanisms of adenine nucleotide release have not yet been fully defined. We have attempted to determine whether BAEC are able to actively release AdN. BAEC were incubated with 3-H Adenosine for 2 hours to label the intracellular AdN pool. Cells were then placed in a parallel plate flow device to precisely control the flow conditions. Aliquots of the perfusate were analyzed for radio-labeled AdN content using HPLC. Under constant flow conditions, AdN were found in the perfusate in the following distribution: 65% AMP, 20% ADP and 15% ATP. Cell morphology was unchanged and LDH was undetectable, indicating that there was not significant cell lysis. To ascertain whether this release of AdN is an active process, the same experiments were carried out at 4°C. Under these conditions AdN were barely detectable. Warming the perfusate resulted in an approximately 325% increase in total AdN in the perfusate after 1 minute. These results show that AdN are actively released by BAEC and suggest that endothelial cells are a potentially important source of AdN.

After only nine months, we feel quite encouraged by the progress we are making. It should be emphasized that this project is a joint effort of the Vascular Biology Laboratory at Emory University School of Medicine and the Biomechanics Laboratory at Georgia Tech. It is a project that neither could do alone. Furthermore, the application of the results to be obtained goes beyond the stated goal of engineering the cell culture environment to make it more physiologic and includes the development of in vitro tissue

for the replacement of vascular components and the logical extension to the development of functional cultured organs.

Finally, there has been one publication to date. This is:

Levesque, M.J. and Nerem, R.M., "The Study of Rheological Effects of Vascular Endothelial Cells in Culture. Biorheology, Vol. 26, No. 2, 1989, pp. 345-357.

There also has been two presentations while the Principal Investigator was a visiting professor in Japan. These are:

Nerem, R.M., "Cellular Biomechanics," invited lecture at the Annual Meeting of the Japan Society of Mechanical Engineers, Tokyo, Japan, April 3-5, 1989.

Nerem, R.M., "Biomechanics and the Endothelial Cell," plenary lecture at the Annual Meeting of the Japanese Society of Biorheology, Tsukuba, Japan, July 5-7, 1989.

Georgia Tech

Georgia Institute of Technology
Atlanta, Georgia 30332-0405

E-25-612

Robert M. Nerem, Ph.D.
Parker H. Petit Chair
for Engineering in Medicine
School of Mechanical Engineering
(404) 894-2768

June 4, 1990

Dr. Paul Werbos
Division of Electrical and
Communications Systems, Rm 1151
National Science Foundation
1800 G Street
Washington, D.C. 20550

Dear Paul:

Enclosed is a third year budget and three copies of a progress report for the grant entitled "Mechanical Stress and Vascular Cell Growth", on which Dr. R. W. Alexander and myself serve as Principal Investigators.

The third year budget has been revised from an amount of $200,000 to $196,000 as you suggested over the phone. Please let me know if I can be of any other assistance to you.

Sincerely,

Robert M. Nerem

RMN/kb

ANNUAL PROGRESS REPORT ON NSF GRANT EET-8815656

ENTITLED "MECHANICAL STRESS AND VASCULAR GROWTH"

Period: September 1, 1988 - May 31, 1990

Principal Investigators: Robert M. Nerem, Ph.D.

R. Wayne Alexander, M.D.,Ph.D.

The issue of mammalian cell growth and the factors which influence both the process of growth and the intrinsic and extrinsic growth program of cells is a critical one to tissue engineering. In the particular research effort supported by this grant, the focus is on vascular cell growth and includes both vascular endothelial cells (EC) and smooth muscle cells (SMC). Although the results to be obtained will have application in general to vascular biology and pathobiology, in the context of tissue engineering the goals are more focused.

First, in the use of cell culture to study vascular cell behavior, there is a critical need to engineer the cell culture environment so as to make it more physiologic. An important part of this is to engineer such culture systems so as to study vascular EC and SMC under the active stress conditions in which they reside in vivo. These conditions are due to the hemodynamics of the vascular system which imposes a force on the arterial wall and thus provides the mechanical stress environment for the cellular components of the wall. This stress can be decomposed into a frictional,

tangential stress, i.e. shear stress, and a normal stress, i.e. pressure. The latter not only acts directly on the endothelial cell inner lining of a blood vessel, but in distending the wall, it also serves to induce stresses within the arterial wall. Thus, in studying in culture the active conditions of stress associated with the in vivo physiologic environment, it is important to separate out the effects of shear stress, pressure, and cyclic stress. This is the goal of this project.

A second goal of this project is to develop the foundation of knowledge necessary to construct vascular grafts use in vitro for bypass surgery. Initially, such grafts will be of a hybrid type, i.e. a graft made of a synthetic material but with an inner lining of pre-seeded, cultured endothelial cells. However, it should be possible to grow entire arteries composed of cultured smooth muscle cells, endothelial cells, and adhesive proteins.

Work on this project is focused on vascular endothelial cells (EC), although some studies of smooth muscle cells also are being carried out. The unique aspects of this project are its (i) systematic study of mechanical stresses of various types, (ii) use of the most advanced image analysis system available for cellular studies, (iii) emphasis on subconfluent monolayers and a variety of different mammalian cell types, and (iv) emphasis on the growth characteristics and growth program of cells. In each of these areas significant progress has been made as is summarized below.

With regard to the systematic study of mechanical stresses of various types, two new systems have been designed and are being used. One of these is a uniaxial, cyclic stretching device where cells are grown on a compliant membrane which can

be stretched with a sinusoidal motion at a frequency in the range of 0.5 to 3 Hz. Initial results for both endothelial cells and smooth muscle cells have been obtained at 1 Hz, and a series of experiments designed to investigate the influence of cyclic stress on the secretion of Insulin-like Growth Factor-1 (IGF-1) will be initiated this summer. This study will be complementary to a study of pressure effects recently completed which showed a direct effect of hydrostatic pressure on IGF-1 secretion by endothelial cells, but not by smooth muscle cells (see Abstract No. 1).

The other new system developed is a pulsatile flow system which can be used in one of three modes: (1) non-reversing flow, (2) reversing flow, and (3) oscillatory flow (where there is zero mean flow). Initial results indicate that the endothelial cell has the ability to not only discriminate in its recognition of different flow environments, but also to transduce that difference in recognition into differing changes in structure and function (see Abstract No. 2, to be presented at the World Congress of Biomechanics).

An important aspect of this project is our image analysis work which uses the Perceptics Biovision system. This system is based on a DEC Microvax computer and was purchased with institutional funds. The system was delivered in mid-December 1988, and over the past 18 months considerable operating experience has been gained. Additional software has been written, and the Biovision system provides the capability for multiprobe fluorescence image analysis.

A major hypothesis to be tested using this system is that the membrane signals generated by mechanical stress, e.g. a fluid-imposed shear stress, are not generalized

throughout the cell but are localized and thus account for the characteristic elongation and orientation of the cell which are observed to occur. In testing this hypothesis, a primary measurement is that of intracellular calcium, and we have demonstrated our ability to load cells with Fura-2 and obtain images at two wavelengths, which is necessary to perform the image ratioing required in the measurement of intracellular calcium. Flow studies have been initiated, and results to date have proved exciting in exhibiting a spatial variation which is time dependent. A poster on these initial observations was presented at the 23rd Annual Hugh Lofland Conference, held in San Antonio, Texas, May 23-26 of this year; however, there is still much more work to be done.

Related to the testing of this hypothesis are our studies of the role of protein kinase C (PKC) in EC responses to shear stress (see Abstract No. 3). Measurements of PKC activity in solubilized homogenates of EC have been performed, and we have found that 63% of total PKC activity was in the membrane fraction and 37% in the cytosol for EC grown under static conditions. Exposure to a shear stress of 30 dynes/cm^2 for 20 min. caused a decrease in cytosolic PKC activity consistent with translocation of PKC to the membrane. There also is evidence of a PKC-dependent phosphorylation of vimentin. Finally, the use of PKC inhibitors blocks the process of endothelial cell elongation and orientation, which is part of that cell's response to shear stress. This points to PKC as an important second messenger in the intracellular transduction of a mechanical signal and in the resulting cytoskeletal and extracellular matrix changes (see Abstract No. 4).

Finally, the active release of adenine nucleotide (AdN) from EC may be important in their response to stimuli. Purinergic receptors in fact are found on vascular smooth muscle and endothelial cells, yet the orgins and mechanisms of AdN release have not yet been fully defined. We have attempted to determine whether BAEC are able to actively release AdN, and our data not only suggests that endothelial cells are a potentially important source of AdN, but that this pathway may be flow mediated. These studies are continuing.

A major objective of this research program is to define the influences of mechanical stress on the intrinsic and extrinsic growth programs of cells. In addition to measurements of cell growth, we also are interested in the expression and influence of growth promoting substances. With regard to the former, we have shown an effect of shear stress on the rate of endothelial cell proliferation and that this decrease is associated with an effect on cell cycle where cells are held up in G_0/G_1. Manuscripts on these findings are now under preparation. In a separate study we have investigated the influence of the flow environment on the process of cell division itself, including the various phases of mitosis and the process of cytokinesis. These results will be presented at the World Congress on Biomechanics in August of this year (see Abstract No. 5).

We also have studied the influence of shear stress on endothelial cells at the level of gene expression. We have shown that endothelial cells after exposure to shear stress exhibit a reduced expression of proto-oncogenes in response to stimulation by α-thrombin (see Abstract no. 6).

In regard to the expression of growth factors, our study on the influence of pressure on endothelial cell secretion of IGF-1 has already been noted. These studies are continuing with the next step being to investigate the effect of cyclic stretch. An additional set of experiments on the influence of shear stress on the expression of platelet-derived growth factor (PDGF) by endothelial cells is being initiated.

Finally, although most of our work to date and the focus of this project is vascular EC, we also want to investigate whether the response of vascular EC to mechanical stress is unique or whether it can be generalized to other cell types. Thus, we have initiated studies both with 3T3 fibroblasts and MDCK cells. Also, since all of our previous work has been carried out with bovine aortic EC, we also have initiated studies with human microvascular EC and human umbilical vein EC. Studies are being conducted both for confluent and subconfluent monolayers. The latter is of interest not only because the cells are in a growth mode, but also because one can focus on the response of a single cell which is uninfluenced by its neighbors.

After only twenty months, we do feel quite encouraged by the progress we are making. It should be emphasized that this project is a joint effort of the Vascular Biology Laboratory at Emory University School of Medicine and the Biomechanics Laboratory at Georgia Tech. It is a project that neither could do alone. Furthermore, the application of the results to be obtained goes beyond the stated goal of engineering the cell culture environment to make it more physiologic and

includes the development of in vitro tissue for the replacement of vascular components and the logical extension to the development of functional cultured organs.

Publications and Presentations on Grant EET-8815656

There have been two publications.

> Levesque, M.J. and Nerem, R.M., "The Study of Rheological Effects of Vascular Endothelial Cells in Culture". Biorheology, Vol.26,no.2, 1989.pp.345-357.
> Levesque, M.J. Sprague, E.A., and Nerem, R.M., "Vascular Endothelial Proliferation in Culture and the Influence of Flow." J. Biomaterials (in press).

In addition there have been a number of invited presentations at conferences.

> Nerem, R.M., "A Mammalian Cell's Response to a Fluid Flow Environment," plenary lecture at the 41st Annual Meeting of the Division of Fluid Dynamics, American Physical Society, Buffalo, NY, November 20-22, 1988
>
> Nerem, R.M., "Cellular Biomechanics," invited special lecture at the Annual Meeting of the Japan Society of Mechanical Engineers, Tokyo, Japan, April 3-5, 1989.
>
> Nerem, R.M., "Biomechanics and the Endothelial Cell," plenary lecture at the Annual Meeting of the Japanese Society of Biorheology, Tsukuba, Japan, July 5-7, 1989.
>
> Nerem, R.M., P.R. Girard., Doty, S.D., Helmlinger, G., and Zeigler, T., "Endothelial Cell Dynamics and Flow," Joint Session, UCLA Symposium on the Endothelial Cell and on Tissue Engineering, Keystone CO, April 6-12, 1990.
>
> Nerem, R.M., "The Vascular Endothelial Cell and Its Physical Environment," Symposium on Polymers as Biomaterials Honoring R.S. Langer, 199th National Meeting of the American Chemical Society, Boston, MA, April 22-27, 1990

Finally, there also have been a number of contributed papers as listed below:

Nerem, R.M., "Mechanical Influences on Living Cells," Bulgarian Fifth National Conference on Biomedical Physics and Engineering, Sofia, Bulgaria, October 14-16, 1988.

Nerem, R.M., "Shear Stress Effects on Anchorage-Dependent Mammalian Cells," NASA/AIBS Workshop on Gravity and the Cell, Washington, D.C., December 1-3,1988.

Nerem, R.M., "Active Motion and Deformation in Vascular Endothelial Cells Exposed to Flow," NATO Advanced Study Institute on Biomechanics of Active Movement and Deformation of Cells, Istanbul, Turkey, September 3-13, 1989.

R.M. Nerem, "Hydrodynamic Effects on Single Cells and their Structure-Function Relationship," Panel Discussion on National Interfaces Between Biomedical and Biochemical Engineering, 21st Annual Meeting of Biomedical Engineering Society, FASEB Meeting, Washington,D.C., April 1-5,1990.

P.R. Girard and R.M. Nerem, "Role of Protein Kinase C in the Transduction of Shear Stress to Alterations in Endothelial Cell Morphology." UCLA Symposium on The Endothelial Cell, Keystone, CO, April 6-12, 1990.

R.V. Geiger, B.C. Berk, R.W. Alexander, and R.M.Nerem, "Single Cell Imaging of Intracellular Calcium in Cultured Vascular Endothelial Cells Exposed to Shear Stress: "Preliminary Observations," 23rd Hugh Lofland Conference on Arterial Wall Metalsolism, San Antonio, TX, May 23-26, 1990.

63rd Scientific Sessions Abstract Form

Medical Research
Nursing Research

American Heart Association

November 12–15, 1990
Dallas Convention Center, Dallas, Texas

Number_____
Please do not fill in!

Avoid disqualification.
Read instructions.

Deadline: Abstracts must be received by Friday, May 11, 1990.

In the box below, assign this abstract a grading category number from the list on pages 7 and 8:

Category Number 12

Corresponding author:

W. Robert Taylor
First Name Middle Initial Last Name
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1. W. Robert Taylor
2. Patrick Delafontaine
3. Kathy K. Griendling
4. Robert M. Nerem
5. R. Wayne Alexander
6.
7.
8.
9.
10.
11.
12.

Select two numbers and key words or phrases from the abstract indexing list on pages 13 and 14 to be used for indexing in the *Supplement to Circulation*.

	Numbers	Key words/phrases
1.	1255	Growth Factors
2.	1300	Hypertension

Pressure Induces Insulin-like Growth Factor-1 Secretion by Endothelial Cells

W. Robert Taylor, Patrick Delafontaine, Kathy K. Griendling, Robert M. Nerem, and R. Wayne Alexander. Cardiology Division, Emory University, Atlanta, GA 30322

Hypertrophy or hyperplasia of vascular smooth muscle (VSMC) is a prominent arterial response to increased blood pressure. VSMC growth may be controlled by factors intrinsic to the arterial wall, such as insulin-like growth factor-1 (IGF-1), a mitogenic peptide secreted by both endothelial (EC) and vascular smooth muscle cells. To assess whether hydrostatic pressure will increase IGF-1 secretion by vascular cells, cultured rat VSMC and porcine EC were exposed to pressure in culture media supplemented with 10% serum. After 24 h, cells were washed and incubated in serum-free media for 3 h, and radioimmunoassayed for IGF-1. In EC basal secretion of IGF-1 was 0.41 ± 0.03 pg/μg protein. Hydrostatic pressure induced a dose-dependent increase in IGF-1 secretion which was maximal at 200 mm Hg (184 ± 15% increase over basal, $p < 0.05$). Basal secretion in VSMC was 0.52 ± 0.09 pg/μg protein, and showed no increase with pressure (100 ± 3% basal at 200 mm Hg). We conclude that EC but not VSMC respond to pressure with IGF-1 secretion. Secretion of IGF-1 by EC represents a novel paracrine mechanism for the control of VSMC growth, which may be important in the pathogenesis of hypertension and atherosclerosis.

The author affirms that the material herein will not have been published as a manuscript prior to presentation at the American Heart Association meeting or presented or published as an abstract at any national meeting or world congress held in the United States, that any animal studies conform with the "Position of the American Heart Association on Research Animal Use" *(Circulation* 1985;71:849) style, and that any human experimentation has been conducted according to a protocol approved by the institutional committee on ethics of human investigation or — if no such committee exists — that it conforms with the principles of the Declaration of Helsinki of the World Medical Association *(Clinical Research* 1966;14:103).

The submitting author also certifies that all authors named in this abstract have agreed to its submission for presentation at the AHA Scientific Sessions and are familiar with the 10-author rule (see "Rules for Submission of Abstracts").

Submitting author's signature

THE EFFECT OF A PULSATILE LAMINAR SHEAR STRESS ON CULTURED VASCULAR ENDOTHELIAL CELL SHAPE AND ORIENTATION.

G. Helmlinger, S. Schreck, and R. M. Nerem. Biomechanics Laboratory, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Ga 30332-0405 USA.

Confluent monolayers of bovine aortic endothelial cells (BAEC) were exposed to pulsatile laminar flow, using a parallel plate flow chamber. Two types of pulsatile flows were investigated: (i) a 1-Hz sinusoidal non-reversing flow with a positive mean shear stress value, and (ii) a 1-Hz sinusoidal flow with a positive mean shear stress value, but with reverse flow during some portion of the cycle. Steady flows corresponding to the mean and maximum shear stress values of the pulsatile flow were run in parallel as controls. In comparing the effect of a non-reversing pulsatile flow to that of a steady flow, it is observed that the BAEC tend to elongate and orient slightly faster in the pulsatile environment and after 30 hours are much more elongated than BAEC either in a steady flow corresponding to the mean value or that corresponding to the maximum value of the shear stress for the pulsatile flow. In contrast, for a reversing pulsatile flow, BAEC initially orient and elongate at a slower rate than BAEC in steady flow controls or in a pulsatile non-reversing flow with the same mean shear stress. Inspite of this slower initial rate, by 24 hours BAEC are aligned and are as elongated as in a steady flow control with a shear stress equal to the maximum of the reversing pulsatile flow. However, for longer durations of exposure to a reversing pulsatile flow, the effect of shear stress is altered with BAEC reverting back to a rounder shape.

ROLE OF PROTEIN KINASE C IN THE TRANSDUCTION OF SHEAR STRESS TO ALTERATIONS IN ENDOTHELIAL CELL MORPHOLOGY. P.R. Girard and R.M.Nerem, Bioengineering Center, Georgia Institute of Technology, Atlanta, Georgia 30332-0405. Endothelial cells exposed to a fluid-imposed shear stress both in vivo and in vitro show an alignment with the direction of flow and an elongation of cells with a concomitant reorganization of the cytoskeletal network. The phosphorylation of cytoskeletal proteins is thought to play a role in the regulation of cytoskeletal structure. We have implicated protein kinase C (PKC) in shear stress-induced morphological alterations based on several criteria. In control, static cultures of EC, approximately 75% of PKC was localized in the cytosol. In EC subjected to a shear stress of 30 dynes/cm^2 for 60 min, there was a 45% decrease in cytosolic levels concomitant with a 47% increase in membrane levels of PKC. Flow-induced morphological changes were inhibited when EC were preincubated with the PKC inhibitor, sphingosine (25 μm), for 90 min prior to exposing the cells to a shear stress of 30 dynes/cm^2 for 24 hr. Immunofluorescent studies of the intermediate filament protein, vimentin, indicated a reorganization of this network in response to shear stress. EC preincubated with ^{32}P and subsequently exposed to shear stress or treated with the PKC activating phorbol ester, TPA, showed an increased level of phosphorylation of vimentin compared to controls. This suggests that PKC-dependent phosphorylation of specific cytoskeletal proteins may be responsible, at least in part, for the dynamic changes in cytoskeletal structure and cell morphology which occur during exposure to a flow environment.

FLUID SHEAR STRESS EFFECTS ON FIBRONECTIN AND CYTOSKELETAL STRUCTURE IN CULTURED VASCULAR ENDOTHELIAL CELLS

P.R. Girard, S.D. Doty, O. Thoumine, and R.M. Nerem, School of Mechanical Engineering, Biomechanics Laboratory, Georgia Institute of Technology, Atlanta, GA 30332-0405 USA

In vivo studies suggest that the hemodynamic environment of the arterial wall plays an important role in atherogenesis. Vascular endothelial cells (EC) exposed to a fluid-imposed shear stress in vivo and in vitro show alterations in cell shape, orientation, and cytoskeletal structure. Fibronectin (FBN), an extracellular matrix protein (ECM) which interacts with the cytoskeleton via focal contacts, may modulate cytoskeletal structure and hence cell shape and function. In order to examine ECM-cytoskeletal responses to a fluid dynamic environment, bovine aortic EC were subjected to shear stress in vitro using a parallel plate, flow chamber. Following exposure to a shear stress in the range of 30-50 dynes/cm^2 for 24 hr, EC were elongated, and both FBN and the focal contact protein, vinculin, showed structural rearrangements concomitant with actin microfilament reorganization, including an alignment of these cytomatrix proteins with the direction of flow. A double-label immunofluorescence study indicated that vinculin, visualized as discrete dots along cell-cell contact areas in confluent static cultures of EC, appeared as discontinuous streaks co-localized along axially-aligned actin stress fibers in cells exposed to shear stress. During the initial phase of cell elongation, vinculin, although distributed throughout the cell, appeared to be more highly concentrated in the proximal (relative to flow direction) cell regions. In addition to the flow-induced reorganization of cell-associated fibronectin, there was also an initial decrease in the amount of fibronectin followed by an increase after longer times of exposure to shear stress compared to static cultures. This biphasic response may allow cell morphological changes to occur with a subsequent increase in cell adhesion to reduce the possibility of cell detachment due to shear forces. These findings suggest that shear stress-induced alterations in cell-associated fibronectin may modulate cell behavior in vitro and that vinculin in focal contacts may serve as part of a transducing network responsible for the transmission of information from the extracellular matrix to the cell's internal cytoskeletal structure.

THE INFLUENCE OF SHEAR STRESS ON CELL DIVISION IN CULTURED VASCULAR ENDOTHELIAL MONOLAYERS

T. Ziegler, P. R. Girard and R. M. Nerem. Biomechanics Laboratory, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta , Ga 30332-0405.

Subconfluent monolayers of actively dividing bovine aortic endothelial cells (BAEC) were exposed to a laminar shear stress using a parallel plate flow chamber. Cells were videotaped at 400X magnification in order to visualize and quantify the process of mitosis and cytokinesis in both sheared and static cultures. BAEC in static culture round up when they divide, and it was observed that as part of this process there was (i) partial detachment of the cell during the prophase, (ii) complete rounding between metaphase and anaphase and (iii) reattachment and spreading of the cell during and after cytokinesis. The total time duration for cell division was 33.7 +/- 7.5 min. When BAEC were exposed to a shear stress of 70 dynes/cm^2 for 2 hours, the cell shape was unchanged. During cell division, processes (i) and (ii) as noted above were observed; however, the process of reattachment and spreading was more intense. Finally, when BAEC were exposed to the same shear stress but for a duration of 15 hours, they were found to elongate and align with flow direction. Here, during cell division, our observations indicate (i) a slight detachment during prophase (as for static culture), (ii) conservation of the elongated shape during the entire duration of cell division, and (iii) after cytokinesis, an active elongation of the daughter cells. The duration of cell division for such shear-stress exposed cells was 44.7 +/- 9.9 min. Following cytokinesis, the process of further elongation of the daughter cells extended over a period of 1 to 2 hours.

SHEAR STRESS ALTERS THE GENETIC GROWTH PROGRAM OF CULTURED ENDOTHELIAL CELLS

B.C. Berk, P. Girard*, M. Mitsumata*, R.W. Alexander, and R. Nerem*,
Cardiovascular Division, Emory University School of Medicine, Atlanta, Georgia 30322 USA, and School of Mechanical Engineering*, Georgia Institute of Technology, Atlanta, Georgia USA

Endothelial cells (EC) are uniquely situated in the cardiovascular system for physiological response to alterations in fluid flow and shear stress. In vivo, the EC mitogenic rate is normally < 0.01%/day, while in subconfluent culture the rate is 50-100%/day. We hypothesized that the increased proliferation in static culture is due to the absence of shear stress. To study the effect of shear stress on EC growth state we compared growth-related intracellular events in EC grown in static culture with those exposed to shear stress. Bovine aortic EC were grown on 200 cm^2 Mylar sheets in 10% fetal calf serum until 95% confluent, and then exposed for 24 hr to 30 dynes/cm^2 in a parallel plate flow chamber. Cell alignment in the direction of flow was documented in all cultures. Shear stress markedly inhibited EC growth in response to 10% serum. DNA synthesis, measured by ^{3}H-thymidine incorporation, was decreased from 126 $\pm$ 21 cpm/ug in static to 31 $\pm$ 4 cpm/ug in shear stress EC. Alterations in EC growth state were further studied by analysis of the cell cycle-dependent accumulation of the mRNA for the proto-oncogene c-myc. In response to the potent agonist α-thrombin (13 nM), c-myc levels at 1 hr increased 28 $\pm$ 8 fold in CON EC, but only 4 $\pm$ 2 fold in shear stress EC. These data suggest that physiological shear stress alters EC cell-cycle kinetics and expression of EC early growth response genes, and thereby regulates EC growth state.

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)

Third Year

SUMMARY PROPOSAL BUDGET

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (MONTHS) Proposed	Granted
AWARD NO.		

ORGANIZATION: Georgia Tech Research Corporation

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: Robert M. Nerem

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)	NSF FUNDED PERSON-MOS. CAL.	ACAD	SUMR	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT)
1. Robert M. Nerem, P.I. ($10,099/mo.)	1.5			$15,149	$
2. R. Wayne Alexander, Co-P.I.				-0-	
3. M.J. Levesque ($4,043/mo.)	2.5			10,108	
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. ( ) TOTAL SENIOR PERSONNEL (1-5)					
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. (2) GRADUATE STUDENTS (24 mo. x $1100/mo.)				26,400	
4. ( ) UNDERGRADUATE STUDENTS					
5. (1) SECRETARIAL-CLERICAL (2 mo. x $2,172/mo)				4,344	
6. ( ) OTHER					
TOTAL SALARIES AND WAGES (A+B)				56,001	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) 27.6%				7,785	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				63,786	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000:)					
TOTAL PERMANENT EQUIPMENT					
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS) (trips for 2 scientific mtgs)				1,654	
2. FOREIGN					
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
TOTAL PARTICIPANT COSTS					
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				11,290	
2. PUBLICATION COSTS/PAGE CHARGES				1,500	
3. CONSULTANT SERVICES					
4. COMPUTER (ADPE) SERVICES					
5. SUBCONTRACTS					
6. OTHER Subcontracts to Emory University School of Medicine				68,876	
TOTAL OTHER DIRECT COSTS					
H. TOTAL DIRECT COSTS (A THROUGH G)				147,106	
I. INDIRECT COSTS (SPECIFY) 62.5% of MTDC					
TOTAL INDIRECT COSTS				48,894	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				196,000	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 196,000	$

PI/PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY — INDIRECT COST RATE VERIFICATION: Date Checked	Date of Rate Sheet	Initials DGC / Program
INST. REP. TYPED NAME & SIGNATURE*	DATE			

NSF Form 1030 (1-87) *Supersedes All Previous Editions*

*SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPM 233)

E25-612

OMB Number 345-0058

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WASHINGTON, DC 20550

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PI/PD Name and Address

Robert M. Nerem, Ph.D.
School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0405

NATIONAL SCIENCE FOUNDATION
FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

1. Program Official/Org. George A. Hazelrigg

2. Program Name ENG/ECS

3. Award Dates (MM/YY) **From:** 9/1/88 **To:** 2/29/92

4. Institution and Address

Georgia Tech Research Corporation
Atlanta, GA 30332

5. Award Number ECS-8815656

6. Project Title

Mechanical Stress and Vascular Cell Growth

This Packet Contains
NSF Form 98A
And 1 Return Envelope

NSF Grant Conditions (Article 17, GC-1, and Article 9, FDP-II) require submission of a Final Project Report (NSF Form 98A) to the NSF program officer no later than 90 days after the expiration of the award. Final Project Reports for expired awards must be received before new awards can be made (NSF Grants Policy Manual Section 677).

Below, or on a separate page, provide a summary of the completed projects and technical information and attach it to this form. Be sure to include your name and award number on each separate page. See below for more instructions.

PART II - SUMMARY OF COMPLETED PROJECT (for public use)

The summary (about 200 words) must be self-contained and intelligible to a scientifically literate reader. Without restating the project title, it should begin with a topic sentence starting the project's major thesis. The summary should include, if pertinent to the project being described, the following items:

- **The primary objectives and scope of the project**
- **The techniques or approaches used only to the degree necessary for comprehension**
- **The findings and implications stated as concisely and informatively as possible**

See attached.

PART III - TECHNICAL INFORMATION (for program management use)

List references to publications resulting from this award and briefly describe primary data, samples, physical collections, inventions, software, etc. created or gathered in the course of the research and, if appropriate, how they are being made available to the research community.

See attached.

	6/19/92
Principal Investigator/Project Director Signature	**Date**

IMPORTANT:
MAILING INSTRUCTIONS
Return this *entire* packet plus all attachments in the envelope attached to the back of this form. Please copy the information from Part I, Block I to the *Attention line* on the envelope.

PART IV — FINAL PROJECT REPORT — SUMMARY DATA ON PROJECT PERSONNEL

(To be submitted to cognizant Program Officer upon completion of project)

The data requested below are important for the development of a statistical profile on the personnel supported by Federal grants. The information on this part is solicited in response to Public Law 99-383 and 42 USC 1885C. All information provided will be treated as confidential and will be safeguarded in accordance with the provisions of the Privacy Act of 1974. You should submit a single copy of this part with each final project report. However, submission of the requested information is not mandatory and is not a precondition of future award(s). Check the "Decline to Provide Information" box below if you do not wish to provide the information.

Please enter the numbers of individuals supported under this grant.
Do not enter information for individuals working less than 40 hours in any calendar year.

	Senior Staff		Post-Doctorals		Graduate Students		Under-Graduates		Other Participants[1]	
	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.
A. Total, U.S. Citizens	1	1			3	1	2	2		
B. Total, Permanent Residents										
U.S. Citizens or Permanent Residents[2]:										
American Indian or Alaskan Native ...										
Asian ...										
Black, Not of Hispanic Origin ...										
Hispanic ...										
Pacific Islander ...										
White, Not of Hispanic Origin ...	1	1			3	1	2	2		
C. Total, Other Non-U.S. Citizens					1	1				
Specify Country										
1. France					1	1				
2.										
3.										
D. Total, All participants (A + B + C)	1	1			4	2	2	2		
Disabled[3]										

☐ Decline to Provide Information: Check box if you do not wish to provide this information (you are still required to return this page along with Parts I-III).

[1]Category includes, for example, college and precollege teachers, conference and workshop participants.

[2]Use the category that best describes the ethnic/racial status for all U.S. Citizens and Non-citizens with Permanent Residency. *(If more than one category applies, use the one category that most closely reflects the person's recognition in the community.)*

[3]A person having a physical or mental impairment that substantially limits one or more major life activities; who has a record of such impairment; or who is regarded as having such impairment. *(Disabled individuals also should be counted under the appropriate ethnic/racial group unless they are classified as "Other Non-U.S. Citizens.")*

AMERICAN INDIAN OR ALASKAN NATIVE: A person having origins in any of the original peoples of North America, and who maintain cultural identification through tribal affiliation or community recognition.

ASIAN: A person having origins in any of the original peoples of East Asia, Southeast Asia and the Indian subcontinent. This area includes, for example, China, India, Indonesia, Japan, Korea and Vietnam.

BLACK, NOT OF HISPANIC ORIGIN: A person having origins in any of the black racial groups of Africa.

HISPANIC: A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.

PACIFIC ISLANDER: A person having origins in any of the original peoples of Hawaii; the U.S. Pacific Territories of Guam, American Samoa, or the Northern Marianas; the U.S. Trust Territory of Palau; the Islands of Micronesia or Melanesia; or the Philippines.

WHITE, NOT OF HISPANIC ORIGIN: A person having origins in any of the original peoples of Europe, North Africa, or the Middle East.

THIS PART WILL BE PHYSICALLY SEPARATED FROM THE FINAL PROJECT REPORT AND USED AS A COMPUTER SOURCE DOCUMENT. DO NOT DUPLICATE IT ON THE REVERSE OF ANY OTHER PART OF THE FINAL REPORT.

SUMMARY -- NSF GRANT ECS-8815656

This grant funded a new collaboration in tissue engineering involving a group in the Bioengineering Center at Georgia Tech and a group in the School of Medicine at Emory University. The focus has been to study in cell culture the influence of mechanical stress on the growth programs of vascular endothelial cells; however, the long term goal is to develop a tissue engineered vascular graft, i.e., a blood vessel substitute engineered from living cells. Studies have included as mechanical environments the direct effect of pressure, the influence of flow and the associated shear stress, and alterations in cells induced by cyclic stretch. The biological end points have included endothelial cell growth, the secretion of growth promoting substances, and the signal recognition and transduction mechanisms involved. From the work on this grant it is clear that the mechanical environment of a cell is important to the engineering of a physiologic cell culture environment. However, it is equally clear that there are other components which need to be included. Paramount among these, we hypothesize, are the cellular interactions which take place between the endothelial cell and the smooth muscle cell, and this will provide a focus for future studies.

FINAL TECHNICAL REPORT ON NSF GRANT ECS-8815656

ENTITLED "MECHANICAL STRESS AND VASCULAR CELL GROWTH"

Period: September 1, 1988-February 28, 1992

Principal Investigators: Robert M. Nerem, Ph.D.
R. Wayne Alexander, M.D., Ph.D.

This grant funded a new collaboration in tissue engineering involving Dr. Nerem's group at Georgia Tech and the Vascular Biology Laboratory at Emory University School of Medicine headed by Dr. Wayne Alexander, Director, Division of Cardiology. The focus of this effort has been to study *in vitro* the influence of mechanical stress on the growth programs of vascular endothelial cells (EC), including signal recognition and transduction mechanisms.

It is recognized that *in vivo* EC exhibit low levels of cell turnover and are in a non-growth promoting mode, while *in vitro* EC have significantly higher levels of cell turnover and release growth promoting substances. This difference is believed to be due to differences between the *in vivo* environment of EC as compared to that in which they reside *in vitro* in cell culture. An important part of this difference is the mechanical stress-free environment associated with static culture, and thus a key element of this proposal has been to engineer the cell culture environment so as to include the types of mechanical stresses encountered *in vivo*.

Studies to date have included the direct effect of pressure on EC which has been found to enhance the secretion of insulin-like growth factor-1 (IGF-1), and these are now being extended to

an investigatiøn of cyclic stretch effects on IGF-1 expression (both mRNA and product) by EC grown on a compliant membrane. In parallel a series of experiments have been conducted on the effects of laminar shear stress on a confluent EC monolayer. These studies have shown that the influence of flow is to reduce the rate of DNA synthesis as indicated by ^{3}H-thymidine incorporation, with flow cytometry measurements providing evidence that EC are prevented from entering S phase and held up in G_0/G_1. Time-lapse video pictures indicate that the flow not only influences cell cycle progression, but also affects the various mitotic phases and the process of cytokinesis. Furthermore, the effect of flow is manifested at the gene expression level.

In regard to signaling, in response to flow intracellular Ca^{2+} is elevated and protein kinase C (PKC) is translocated from cytosol to membrane. As part of this project we have initiated an effort to mathematically model the response of an endothelial cell to flow, including the intracellular second messengers.

We also examined the mechanism for flow-induced PDGF expression, focusing on PKC. In bovine aortic endothelial cells exposed to flow, increases in PDGF B-chain, but not PDGF A-chain, were observed. PKC appeared to be involved, because phorbol 12-myristate 13-acetate (PMA) induced PDGF B-chain mRNA. Activation of PKC alone, however, was insufficient to induce PDGF mRNA. A PKC-independent pathway was suggested by the fact that inhibition of PKC failed to block PMA or flow-induced PDGF B-chain expression. These results demonstrate flow-induced PDGF B-chain expression in endothelial cells that appears to be mediated by

both PKC-dependent and -independent pathways.

An important advance on this project has been the development of a laminar pulsatile flow system where endothelial monolayers can be exposed to a variety of different, but physiologically realistic pulsatile flow environments. This provides us with a rather unique capability, and it is clear from our results on EC morphology and F-actin localization that EC recognize and distinguish between different flow environments. Specifically, we have demonstrated that EC respond differently to a non-reversing pulsatile flow as compared to a reversing pulsatile flow or as opposed to a purely oscillatory flow. Since all three of these are physiologically relevant, this has important implications for the study of vascular biology and the use of cell culture in such studies.

It should be noted that seven graduate students worked on this project. The doctoral students were: R. V. Geiger, who received his Ph.D. in December 1991; G. Helmlinger; K. Schnetzer; T.F. Wiesner; and T. Ziegler. Four students received their M.S. degrees based on work conducted with support of this project. These are: G. Helmlinger, L. Hill, C. Vanhee, and T. Ziegler. Finally, during the summers a number of undergraduate students worked on this project, some with NSF support. The latter includes T. Miller and A. Saylor, the former being from Duke University and the latter a female from Mercer University, who worked during summer 1990. E. Hutchinson and K. Nork, the latter a female biology student at Georgia Tech, worked on the project during the summer of 1991.

The unique interdisciplinary environment associated with the conduct of this project's activities also should be noted. Not only is this a cooperative effort between an engineering school and a medical school, but it is one which is strongly integrated. As part of this we both have acquired identical light microscope image analysis systems (Perceptics Biovision). This allows us to readily move data back and forth. More importantly, as part of this integration there are students from Georgia Tech working in the laboratories of Emory University School of Medicine and researchers from Emory working in the laboratories of Georgia Tech. An example of the latter is that there were two M.D.'s (T. Jordan and M. Wyatt) who did research at Georgia Tech as part of their advanced training at Emory.

From our work to date it is clear that the mechanical environment of a cell is important to the engineering of a physiologic cell culture environment. However, it is equally clear that there are other components which need to be included. Paramount among these, we hypothesize, are the cellular interactions which take place between the endothelial cell and the smooth muscle cell. This has important implications not only to the conduct of research in cell culture, but also for the development of tissue-engineered vascular prostheses.

Publications and Presentations: Journal publications and meeting presentations through May 31, 1992 are listed below.

Journal Articles

Levesque, M.J. and Nerem, R.M., "The Study of Rheological Effects of Vascular Endothelial Cells in Culture, " Biorheology, Vol. 26, No. 2, 1989, pp. 345-357.

Nerem, R.M., "Influence of Flow on Vascular Cell Functions, " Journal of Japan Atherosclerosis Society, Vol. 18, No. 6, 1990, pp. 631-633.

Levesque, M.J., Nerem, R.M., and Sprague, E.A., "Vascular Endothelial Cell Proliferation in Culture and the Influence of Flow," Biomaterials, Vol. 11, No. 4, 1990, pp. 702-707.

Nerem, R.M. and Girard, P.R., "Hemodynamic Influences on Vascular Endothelial Biology," Toxicological Pathology, Vol. 18, No. 4, Part 1, 1990, pp. 572-582.

Helmlinger, G., Geiger, R.V., Schreck, S., and Nerem, R.M., "Effects of Pulsatile Flow on Cultured Vascular Endothelial Cell Morphology," J. Biomech. Engr. , Vol. 113, No. 2, 1991, pp. 123-131.

Geiger, R.V., Berk, B.C., Alexander, R.W., and Nerem, R.M., "Flow-Induced Calcium Transients in Single Endothelial Cells: Spatial and Temporal Analysis," American Journal of Physiology: Cell Physiology (in press).

Nerem, R.M., "Tissue Engineering in the U.S.A.," Medical and Biological Engineering and Computing (in press).

Nerem, R.M., "Vascular Fluid Mechanics, The Arterial Wall, and Atherosclerosis," ASME J. Biomechanical Engineering (in press).

Nerem, R.M., Harrison, D.G., Taylor, W.R., Alexander, R.W., "Hemodynamics and Vascular Endothelial Biology," J. Cardiovascular Pharmacology (in press).

Mitsumata, M., Nerem, R.M., Berk, B.C., "Fluid Shear Stress Stimulates Platelet-Derived Growth Factor Expression in Endothelial Cells," Americal Journal of Physiology: Heart and Circulatory Physiology (submitted for publication).

Presentations and Abstract

Nerem, R.M., "Mechanical Influences on Living Cells," Bulgarian Fifth National Conference on Biomedical Physics and Engineering, Sofia, Bulgaria, October 14-16, 1988.

Nerem, R.M., "A Mammalian Cell's Response to a Fluid Flow

Environment," plenary lecture at the 41st Annual Meeting of the Division of Fluid Dynamics, American Physical Society, Buffalo, NY, November 20-22, 1988.

Nerem, R.M., "Shear Stress Effects on Anchorage-Dependent Mammalian Cells," NASA/AIBS Workshop on Gravity and the Cell, Washington, D.C., December 1-3, 1988.

Nerem, R.M., "Cellular Biomechanics," invited special lecture at the Annual Meeting of the Japan Society of Mechanical Engineers, Tokyo, Japan, April 3-5, 1989.

Nerem, R.M., "Influence of Flow on Cell Function," Japan Atherosclerosis Society, Tokyo, Japan, June 8-9, 1989.

Nerem, R.M., "Biomechanics and the Endothelial Cell," plenary lecture at the Annual Meeting of the Japanese Society of Biorheology, Tsukuba, Japan, July 5-7, 1989.

Nerem, R.M., "Endothelial Cell Responses to Shear Stress: Implications in the Development of Endothelialized Synthetic Vascular Grafts," 2nd IFMBE Pan-Pacific Symposium, Melbourne, Australia, July 24-26, 1989.

Nerem, R.M., "Active Motion and Deformation in Vascular Endothelial Cells Exposed to Flow," NATO Advanced Study Institute on Biomechanics of Active Movement and Deformation of Cells, Istanbul, Turkey, September 3-13, 1989.

Nerem, R.M., "Influence of Shear Stress on Endothelial Cell Structure and Function," Argentina-U.S. Workshop on Mass Transfer in Biomedical Engineering, Buenos Aires, Argentina, October 18-19, 1989.

Vanhee, C., Ziegler, T., Girard, P.R. and Nerem, R.M., "Influence on Flow on the Proliferation of 3T3 Fibroblasts," Federation of American Societies for Experimental Biology 75th Annual Meeting, Atlanta, GA, April 21-25, 1991.

Nerem, R.M., "Cellular Engineering." ALZA Distinguished Lecture, Biomedical Engineering Society, FASEB Annual Meeting, Atlanta, GA, April 21-25, 1991.

Nerem, R.M., "Endothelial Cell Dynamics and Flow," Festschrift for Colin G. Caro, Imperial College of Science, Technology, and Medicine, London, England, May 16-17, 1991.

Geiger, R.V., Berk, B.C., Alexander, R.W., and Nerem, R.M., "Temporal and Spatial Analysis of Intracellular Calcium in Cultured Endothelial Cells Exposed to Flow," ASME Applied Mechanics and Biomechanics Summer Conference, Columbus, OH, June 16-19, 1991.

Nerem, R.M., "Engineering Applications in Molecular and Cell

Biology: An Introduction," World Congress on Medical Physics and Biomedical Engineering, Kyoto, Japan, July 7-12, 1991.

Nerem, R.M., "The Influence of Flow on Vascular Endothelial Proliferation," The Yamanashi Blood Flow and Arterial Diseases Meeting, Yamanashi, Japan, July 15, 1991.

Geiger, R.V., Helmlinger, G., Berk, B.C., Alexander, R.W., and Nerem, R.M., "Real-Time Imaging of Flow-Induced Calcium Signals in Cultured Vascular Endothelial Cells," U.S.A.-China-Japan Conference on Biomechanics, Atlanta, GA, August 25-29, 1991.

Nerem, R.M., "Cellular Engineering," IV International Symposium on Biomedical Engineering," Peniscola, Spain, September 17-20, 1991.

Nerem, R.M., Berk, B.C., Mitsumata, M., Ziegler, T., and Alexander, R.W., "Vascular Endothelial Cell Proliferation in the Presence of Flow," Workshop on Hemodynamics and Thrombotic Factors in Atherogenesis, 9th International Atherosclerosis Symposium, Rosemont, IL, October 6-11, 1991.

Helmlinger, G., Geiger, R.V., Berk, B.C., Alexander, R.W., and Nerem, R.M., "Temporal Oscillations of Intracellular Calcium $\{Ca^{2+}\}$, in Bovine Aortic Endothelial Cells (BAECs) Subjected to Fluid Flow," Annual Fall Meeting of the Biomedical Engineering Society, Charlottesville, VA, October 1214, 1991.

Ziegler, T. and Nerem, R.M., "The Effect of a Laminar Shear Stress on Human Dermal Microvascular Endothelial Cells," Tenth Southern Biomedical Engineering Conference, Atlanta, GA, October 18-21, 1991.

Vanhee, C., Nerem, R.M., and Girard, P.R., "Influence of Fluid-Induced Shear Stress on Cell Proliferation in NIH 3T3 Fibroblasts," ASME Winter Annual Meeting, Atlanta, GA, December 1-6, 1991; also, Bioprocess Engineering Symposium, BED-Vol. 21, pp. 27-28, 1991.

Nerem, R.M., "Fluid-Imposed-Shear Stress Effects on the Growth Program of Cultured Vascular Endothelial Cells," Subgroup Meeting on the Role of Mechanical Stress Factors in Controlling Biological Processes, 31st Annual Meeting of the American Society for Cell Biology, Boston, MA, December 8-13, 1991.

Nerem, R.M., "Tissue Engineering a Blood Vessel: Flow Effects on Vascular Endothelial Cell Growth," Cell Culture Engineering III, Palm Coast, FL, February 2-7, 1992.

Nerem, R.M., Berk, B.C., Mitsumata, M., Ziegler, T., Alexander, R.W., "Mechanical Effects on Vascular Cell Growth," invited lecture at Keystone Symposia on Tissue Engineering, Keystone, CO, April 3-10, 1992.