16:57:48 OCA PAD INITIATION - PROJECT HEADER INFORMATION 07/13/95

Active

Project #: E-25-5A4 Cost share #: Rev #: 0
Center # : 10/11-6-P5096-5A0 Center shr #: OCA file #:
Work type : INST
Contract#: 5 T32 GM08433-05 Mod #: Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: 93.859
Main project #: PE #:

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

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 950701 to 960630 (performance) 960930 (reports)

Sponsor amount	New this change	Total to date
Contract value	75,368.00	75,368.00
Funded	75,368.00	75,368.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: CELLULAR ENGINEERING TRAINING PROGRAM

PROJECT ADMINISTRATION DATA

OCA contact: E. Faith Gleason 894-4820

Sponsor technical contact

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SAME

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
INITIATION OF 5TH YEAR OF NRSA GRANT
THIS GRANT IS EXCLUDED FROM EXPANDED AUTHORITIES.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 11/05/96

Project No. E-25-5A4_____ Center No. 10/11-6-P5096-5A0_

Project Director NEREM R M_____ School/Lab MECH ENGR_____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

Contract/Grant No. 5 T32 GM08433-05_____ Contract Entity GTRC

Prime Contract No. _____

Title CELLULAR ENGINEERING TRAINING PROGRAM_____

Effective Completion Date 960630 (Performance) 960930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
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_____

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

E-25-5A4
#1

Georgia who enroll in graduate studies, support is available from the Patricia Roberts Harris Fellowships and the Regents' Opportunity Scholarship Program, both of which began in 1978. These programs have been quite effective in enrolling black Americans and women. The engineering college also participates in the National Consortium for Graduate Degrees for Minorities in Engineering, Incorporated (GEM) and Georgia Tech is a charter member of the National Consortium for Educational Access (NCEA), the goal of which is to increase the number of black Ph.D.'s. NCEA, which is located on the Georgia Tech campus, helps identify and refer students to Georgia Tech and provides a fellowship supplement to those who are accepted into the doctoral program and receive assistantships from the school. In 1987 Georgia Tech established the President's Minority Fellowships to assist promising minority students in their doctoral studies. The award consists of a $4,000 supplement to the normal assistantship stipend, and is renewable for up to four years. In 1990 the National Science Foundation awarded a five-year grant with total funds of $1,000,000 to Georgia Tech. The title of this grant is "Graduate Engineering Education for Women, Minorities, and Persons with Disabilities at Georgia Tech." Finally, Georgia Tech has just received a $1.1 million award from the Sloan Foundation. The purpose of this award is to provide the support necessary for programs that will enable Georgia Tech to more than double its production of Ph.D.'s who are underrepresented minorities.

The result of these programs is that there currently are 87 underrepresented minority and 145 woman students at Georgia Tech pursuing a Ph.D. in engineering. Although current enrollments still are far short of Georgia Tech's goal, it does represent considerable progress. With the continued assistance of fellowships and other types of financial support and the additional efforts by the Institute, the substantial recruiting activities for minorities and women at the graduate level will continue to increase the enrollment, retention, and graduation of these groups.

In our bioengineering program and as indicated in the Progress Report which follows, we have been successful in the recruitment of women into our program. This is true both of bioengineering in general and the specific area of cellular engineering. We also have been somewhat successful in the recruitment of minorities; however, this must continue to be a priority in the future. In this it will be to our advantage to more aggressively use the existing programs which have been developed at Georgia Tech to recruit minority and women students. An important part of this will be increased personal contact by bioengineering faculty with both women and minority undergraduates at Georgia Tech and with applicants to graduate school.

D. PROGRESS REPORT

In the four years since the proposal was submitted which led to our current Cellular Engineering Training Program Grant (GM 08433), our program has undergone an enormous expansion. This is shown in Table 1 below which provides information on the demographics of both our total bioengineering program and our cellular engineering program.

Several things should be noted. First, in the four years represented by these data, there has been a significant expansion in the number of predoctoral students in our Cellular Engineering Program. This program has grown by more than 50 percent; more importantly, both the number of women and the number of under-represented minorities in our

Cellular Engineering Program have increased dramatically. In four years, the number of women has gone from 3 to 13 and the number of underrepresented minorities from 1 to 5, with three of these being women. Thus there currently are a total of 15 women/underrepresented minorities out of total predoctoral student enrollment of 26, 50 percent of the total number of students, with 15 percent being underrepresented minorities.

We believe that this is a major accomplishment. Although our Cellular Engineering Training Grant provides support for only 4 of the 26 students in our program, this Training Grant has been a critical foundation to the expansion of our activities. Furthermore, it has served to add motivation and reinforcement of our efforts to recruit women and underrepresented minorities. We have placed an emphasis on both of these sections of the population since in general engineering student bodies have insufficient numbers of both women and underrepresented minorities.

Table 7 lists all trainees who have been supported by our current training grant. This includes a total of seven students, four of whom were women, with one of these being an under-represented minority. Table 8 lists publications resulting from the training of these seven students. The progress of each of these seven students in their research is described as follows.

Jill Dyken's dissertation project was entitled "Effects of Ammonium Ion and Culturing Mode on Protein Production from Endocrine Cell Cultures." Endocrine cells have the ability to release biologically active hormones in a regulated fashion. In order to optimize designs of industrial and medical systems exploiting this property, it is necessary to study how different culturing environments, inherent in those systems, affect the protein trafficking events leading to secretion of bioactive hormones. This research showed how protein processing and secretion are affected by elevated levels of the metabolite ammonium and by culturing cells in different morphologies. As part of this she demonstrated that ammonium had a strong, selective inhibitory effect on the regulated pathway of secretion in two endocrine cell lines, ßTC3 and AtT-20. Finally, she examined the effect of ammonium on indicators of cellular metabolism and developing a comprehensive, quantitative model that described the effect of ammonium on endocrine cells. Ms. Dyken graduated with her Ph.D. this past summer and took a postdoctoral position at the Westinghouse Savannah River Site.

The project on which Mr. James W. Piper has been working is the *in vitro* study of the functional activities of the cell adhesion receptor, E-selectin. The objective is to elucidate the role of E-selectin on the adhesion of tumor cells to vascular endothelial cells. The research involves a collaboration with Dr. Robert A. Swerlick in the Department of Dermatology, Emory University School of Medicine. A protocol for using a simplified system to examine binding of cells via the E-Selectin molecule was developed. The protocol anchors a LEC-EGF fragment of the E-Selectin molecule to the wells of a plastic 96-well plate. These anchored fragments possess the binding functionality and specificity of the complete E-Selectin molecule. With this system, the site density of E-Selectin, magnitude of separation force, and duration of separation force can be controlled. Experiments examining he ability of E-Selectin to mediate tumor cell lines (Colo-205 and HL-60) were completed using a centrifugation technique. Results show the quantitative relationship among site density of E-Selectin, the magnitude and duration of the

imposed dislodging force, and the binding of the tumor cells. Mr. Piper has completed all his courses; however, in this past year he did take two public policy courses. He has also passed his Ph.D. qualifiers and thesis proposal and is working full time on his dissertation. It is expected that he will graduate in 1996.

Marena Gatewood Brown's thesis work is aimed at determining the role of sickle erythrocyte membrane components on the upregulation of endothelial cell adhesion molecule expression leading to increased adhesion of sickle erythrocytes to the endothelium. This research will contribute to a further understanding of the mechanisms of blood vessel damage and blood vessel occlusion associated with sickle cell disease. To date she has demonstrated that incubation of cultured endothelial cells with sickle (and not normal) red cells upregulates endothelial cell ICAM-1, VCAM-1, and ELAM-1 expression. All work has been performed using human umbilical vein endothelial cells (HUVEC). The key observations are as follows: sickle cells promote endothelial CAM expression in a time-and dose-dependent manner, promoting greater levels of CAM expression than controls; the endothelial response elicited by the sickle sample is similar to that promoted by cytokines, such as TNF-α; endothelial activation occurs via a humoral mechanism, with the soluble factor that acts upon the endothelium is generated *in situ;* and leukocytes are the effector (mediating) cells in the observed phenomenon. Specifically, she has shown that cytokines secreted by leukocytes induce the cell adhesion molecule expression. Ms. Brown is currently wirting her dissertation and is expected to graduate this summer.

Linda Greer's thesis work is focused on tissue engineering a blood vessel substitute. This is being done using a co-culture system. Her early work was with a model which was based on a collagen gel, in which vascular smooth muscle cells were incorporated and on top of which an endothelial monolayer is plated. In these initial studies she focused on measuring the mechanical properties of such gels, including the influence of smooth muscle cell density. More recently she has begun to work with an endothelial cell-fibroblast co-culture, one which also includes a biodegradable scaffold. Her interest in this system came from spending the summer of 1993 as an intern at Advanced Tissue Sciences, Inc., one of the leading tissue engineering companies in the world, located in La Jolla, California. She has passed her Ph.D. qualifiers, will present her dissertation proposal during Spring Quarter 1995, and now has moved off of the training grant and is supported by a Patricia R. Harris Fellowship.

Scott Chesla's research is examining the influence that structural variations in the FcγR III (CD16) molecule has on cellular adhesion during both macroscopic contacts (i.e. many bonds, large contact area) and focal contacts (i.e. single bond, point contacts). He is using CD16 as an experimental model receptor because it naturally occurs in various isoforms (membrane anchor variations) and alloforms (extracellular domain variations). He has previously examined how structure influences the receptor properties of mobility and affinity which in turn affect cellular adhesion. His current research deals with focal contacts and utilizes the micropipette technique to evaluate how receptor structural variations affect the detachment mechanism (receptor extraction or specific binding site dissociation) during detachment. This is of interest because to date the idea of different detachment mechanisms has scarcely been cited in literature and correlations to receptor affinity or membrane anchorage is nonexistent. Scott Chesla presented his dissertation proposal last year. He was an intern at Advanced Tissue Sciences, Inc. during

summer 1994.

Karen Schnetzer's research has focused on the investigation of cyclic stretch effects on the regulation of gene expression in vascular smooth muscle cells. She designed her own cyclic stretch device using a silicon membrane and developed several new approaches, including the use of chitosan as an extracellular matrix on the silicon membrane. She has investigated gene regulation at the mRNA level with a modified RNase protection assay utilizing biotin-labeled RNA probes. The particular genes on which she has focused include MCP-1 and the cell membrane tyrosine kinase receptor, IGF-1R, associated with insulin-like growth factor I (IGF-1). As part of her training, Ms. Schnetzer spent six weeks at the Marine Biology Laboratory in Woods Hole, MA as a student in the cell physiology summer course. Ms. Schnetzer originally was supported by an NSF Graduate Fellowship and then by a grant from the American Association of University Women. More recently she has been supported by our training grant, and she is expected to finish this summer.

Tom Williams is investigating the mechanical properties of cells during the process of adhesion formation and detachment. This research combines experimentation with mathematical modeling on two length scales -- the scale of the whole cell and the scale of the adhesion molecules. On the cellular scale, he hypothesizes that the observed deformation of an adherent cell during micropipette-assisted detachment can be used to quantify the strength of the adhesive contact, as well as structural properties of the cell itself, such as membrane tension, cytosolic pressure and viscosity. Preliminary results using nucleated cells indicate a possible force resolution for this technique well below 10^{-5} dynes when using computer-based image processing. This work is significant in that it allows for the study of adhesion as a dynamic process rather than a static state. On the molecular scale, the modeling will incorporate proposed roles for adhesion receptor-ligand kinetics, strength, and diffusion, as well as non-specific colloidal interactions. Experimental validation will use dual micropipette manipulation of synthetic lipid vesicles undergoing biotin-avidin mediated adhesion.

We believe that all of our Cellular Engineering Program students, including the seven who have been supported by our Training Grant, and whose progress has been discussed in this section, are receiving a unique, interdisciplinary education. The Whitaker Award, which Georgia Tech received in 1993, has provided a foundation for the further expansion of our cellular engineering activities. With this institutional development grant, one of only five such awards given since the inception of this particular program in 1988, Georgia Tech will be adding six new faculty over a four year period, with the first two being Drs. Cargill and Prausnitz. The focus of this faculty recruitment will be the area of cellular and tissue engineering, and in addition to the six faculty to be added at Georgia Tech, Emory University School of Medicine as a partner in this award will add two new faculty. Some of the Whitaker funds are being used to provide traineeships to first year graduate students. A part of the award, i.e. $1 million, will go into the construction of a new addition to our present building which will provide an additional 35,000 ft^2 of assignable space. Thus, the resources exist to expand our program in terms of the number of faculty and space and startup funds required for the new faculty. It is with this in mind that we have asked for a significant increase in predoctoral training positions in this competing continuation proposal of our current NIH training grant.