09:58:18 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 01/18/96

Active

Project #: E-25-X15 Cost share #: Rev #: 6
Center # : 10/24-6-R7466-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: LTR DTD 920414 Mod #: LTR DTD 1-2-96 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : Y CFDA: N/A
Main project #: PE #: N/A

Project unit: MECH ENGR Unit code: 02.010.126
Project director(s):
ZHU C MECH ENGR (404)894-3269

Sponsor/division names: WHITAKER FOUNDATION /
Sponsor/division codes: 500 / 057

Award period: 920701 to 951231 (performance) 960229 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	180,000.00
Funded	0.00	180,000.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: MECHANISM OF MELANOMA CELL-ENDOTHELIAL CELL ADHESION

PROJECT ADMINISTRATION DATA

OCA contact: Jacquelyn L. Bendall 894-4820

Sponsor technical contact

MILES J. GIBBONS, JR.
(717)763-1391

THE WHITAKER FOUNDATION
4718 OLD GETTYSBURG ROAD, SUITE 405
MECHANICSBURG, PA 17055-4380

Sponsor issuing office

MILES J. GIBBONS, JR.
(717)763-1391

THE WHITAKER FOUNDATION
4718 OLD GETTYSBURG ROAD, SUITE 405
MECHANICSBURG, PA 17055-4380

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

Administrative comments -
ISSUED TO REVISE DELIVERABLE SCHEDULE TO REFLECT EXTENDED REPORT DATE
INDICATED IN LETTER FROM SPONSOR DATED 1-2-96.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 01/29/96

Project No. E-25-X15 Center No. 10/24-6-R7466-0A0

Project Director ZHU C School/Lab MECH ENGR

Sponsor WHITAKER FOUNDATION/

Contract/Grant No. LTR DTD 920414 Contract Entity GTRC

Prime Contract No.

Title MECHANISM OF MELANOMA CELL-ENDOTHELIAL CELL ADHESION

Effective Completion Date 951231 (Performance) 960229 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	Y	
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

NOTE: Final Patent Questionnaire sent to PDPI.

E25-X15

#1

PROGRESS REPORT

During the current grant year our research project has been proceeding according to the time table outlined in the original proposal. Progress in centrifugation assays, deformability assays, and micromanipulation assays as well as in mathematical modeling are summarized below.

Centrifugation Experiments. We have focused our effort on dissecting the contribution from a particular cell adhesion molecule (CAM), i.e. E-selectin. Through a collaborative arrangement, we have been able to obtain soluble E-selectin and its antibody from Dr. David H. Presky (Hoffmann-La Roche, Nutley, NJ). We have established the experimental conditions, including the optimal concentrations for various reagents, the most sensitive window for binding variations, and the number density (molecular sites per unit area) of E-selectin immobilized on the surface. Some of the results are shown in Fig. 1. Coating of soluble E-selectin on 96-well plates with variable densities was demonstrated using ELISA. Adherence assays using centrifugation technique demonstrated that HL-60 cells bound to plastic surfaces coated with E-selectin and that the increased binding very closely correlated with the increasing concentrations of the coating E-selectin solutions (Fig. 1A). Figs. 1B and 1C show the number of E-selectin molecules per unit area of surface as a function of the concentration of E-selectin solutions added to coat the surface and of the concentration of the capture antibody. Combining the data in Figs. 1A and 1B, it can be seen that the percent of HL-60 binding falls off rapidly at an E-selectin surface number density of around 200 molecules/μm^2. These reagents add powerful tools to our experimental capability. Not only will such a well-defined system allow for detailed experimental studies of the functionality of E-selectin, but it also is ideal for experimental validation our mathematical models. The use of a flat, rigid, and smooth plastic surface coated with predetermined density of a single species of CAM with restored functional activities for melanoma cell binding greatly simplifies the model, removes many uncertainties, and provides direct measurements for a number of model parameters. We anticipate that we will be able to generate large amount of data during the next granting year.

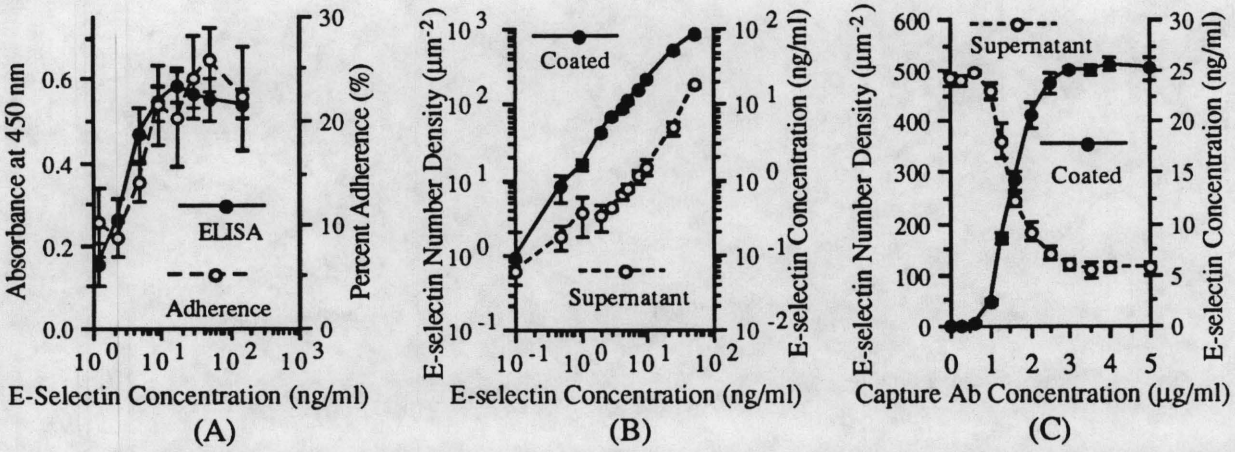


Fig. 1. (A) Amount of E-selectin coated on plastic surfaces as assessed by ELISA and percent adherence of HL-60 cells to the E-selectin coated surfaces as functions of the concentration of soluble E-selectin used to coat the surfaces. The capture antibody concentration was 5 μg/ml. (B) Number density of E-selectin coated on plastic surface and E-selectin concentration at the supernatants as functions of the concentration of soluble E-selectin added to coat the surfaces. The capture antibody concentration was 5 μg/ml. (C) Number density of E-selectin coated on plastic surface and E-selectin concentration at the supernatants as functions of the concentration of capture antibody. The concentration of soluble E-selectin added to coat the surfaces was 25 ng/ml. Experiments for (B) and (C) were repeated four times with very similar results. Shown here are data of a typical experiment (presented as mean ± standard deviation of measurements from at least quadruplicate wells).

Deformability Experiments. Our micropipette system has been operational since early 1992. Using a computer program (provided by Dr. Richard Skalak, University of California, San Diego) based on the model of Dong et al. (1988), the mechanical properties of melanoma cells and endothelial cells have been derived from the deformability measurements. Fig. 2 shows four sequential photomicrographs of a typical experiment, and Fig. 3 shows representative results. After automation of the data acquisition from tape-recorded images, deformability measurements will become a routine in our lab next year.

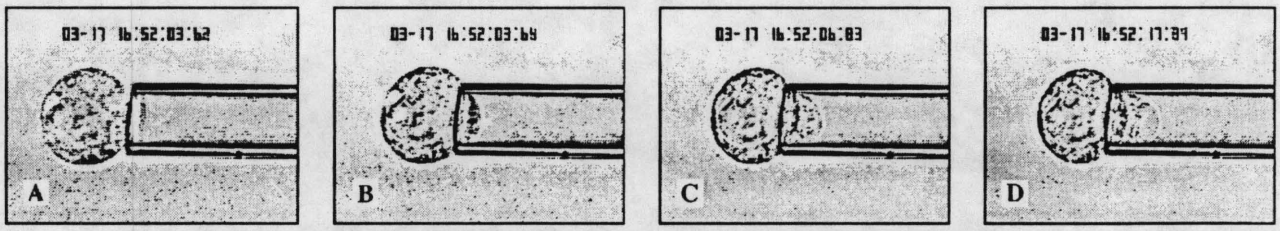


Fig. 2. Sequential photomicrographs of a deformability experiment. The tape-recorded images were digitized and processed using the Macintosh Quadra 950 to allow direct printing from a laserwriter. The numbers on the top of each photo represent, in order, month-day and hours:minutes:seconds:tens-of-milliseconds. (A) The micropipette with zero pressure was positioned to a WM-266-4 melanoma cell. (B) 40 milliseconds later, the melanoma cell "jumped" into the pipette in response to a step aspiration pressure. (C) and (D) After the initial elastic response, the cell continued to deform in a viscoelastic creep and the length of the cell "tongue" into the pipette increased in time.

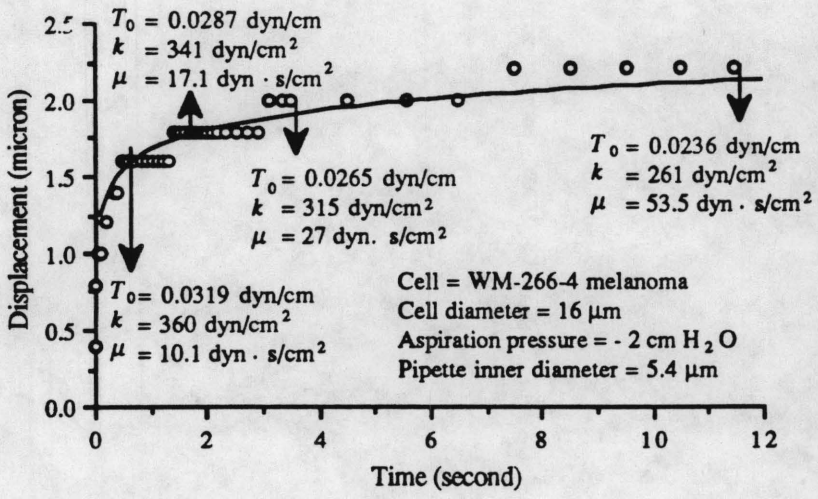


Fig. 3. Time course of the displacement of the cell "tongue" into the pipette in response to a step aspiration pressure. The mechanical properties are represented by three model parameters: the prestressed tension T_0 of the cortex, and the elasticity k and viscosity μ of the cytoplasm. Their values are derived by fitting the theoretical prediction (solid curve) with the experimental measurements (open circles). The parameter values vary slightly with the duration of the time course used for curve fitting, suggesting that these are apparent values only.

Micromanipulation Experiments. We also have begun preliminary cell adhesion experiments with the micropipette system. Fig. 4 shows four sequential photomicrographs of a micromanipulation assay. A practical problem of this protocol is that, under the optical microscope, it is very difficult to distinguish the bright field images of melanoma cells from those of endothelial cells after they are detached from the culture flasks and suspended in the cell chamber, as can be seen in Fig. 4. Another concern is that detaching the anchorage-dependent endothelial cells might alter their adhesive properties because adhesion molecules might not be distributed uniformly on the basal and apical surfaces of an endothelial cell. We have used several approaches to address these issues. With the approval of the Whitaker Foundation, we have purchased another microscope workstation (Ziess) with fluorescence accessories. Labeling melanoma cells and endothelial cells with different fluorescent dyes will allow us to readily distinguish the two types of cells under the microscope. We also developed two modified protocols for the micromanipulation assay. Instead of detaching the endothelial cells from a culture flask and suspending into the cell chamber, they are directly subcultured in the cell chamber. In the first protocol, the cell chamber with endothelial cells grown on its bottom surface is mounted on the microscope stage for experiment. The melanoma cells detached from a culture flask are introduced into the cell chamber and allowed to adhere to the apical surfaces of the endothelial cells whose basal surfaces remain attached to the substrate. In the second protocol, microcarrier beads

are added to the subcultures to allow the endothelial cells to grow over their surfaces. During the experiment, a melanoma cell is picked up by a micropipette and placed onto an endothelial cell grown on the bead to allow adhesion to occur. The manipulation procedures then follow and the critical force required to separate the melanoma cell from the endothelial cell is measured. These two modified protocols are shown in Fig. 5. The suspending melanoma cells mimic their circulating counterpart in metastasis while the attachment of endothelial cells to glass surfaces or microcarrier beads resembles their resting on a basement membrane. An added advantage of the new protocols is that the degree of complexity of the experiment is greatly reduced because only a single micropipette is used instead of two. Therefore, much more measurements can be made in a two-hour experiment using the new protocols as compared to the old one. The second new protocol also allows us to evaluate the effects of different modes of detachment by comparing the magnitude of detachment forces between that applied in the direction perpendicular to the adhesion plane with that applied in the direction tangential to the contact plane. Systematic experiments based on these protocols are expected for the next granting year, including the regulation of adhesion by biologic response modifiers and the inhibition of adhesion by blocking antibodies.

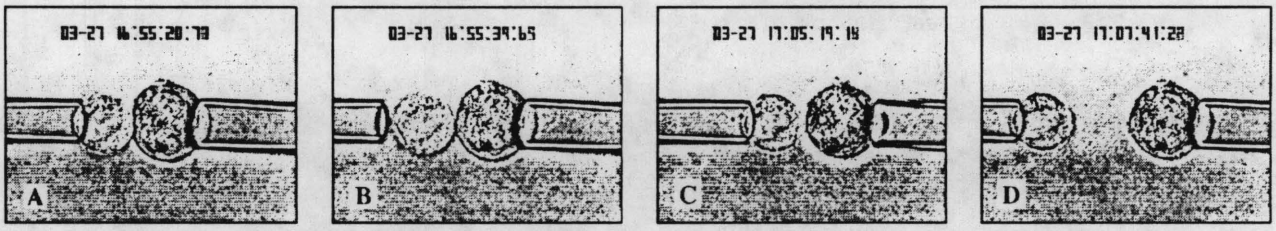


Fig. 4. Sequential photomicrographs of a micromanipulation experiment. The smaller cell on the left was an endothelial cell and the larger cell on the right was a melanoma cell. (A) The two cells were aligned and brought into direct contact. (B) The endothelial cell was released to allow for adhesion with the melanoma cell free of applied forces. (C) The endothelial cell was recaptured later and the two cells were gradually pulled away from each other. (D) The two cells were separated when the aspiration pressure exceeded the critical value.

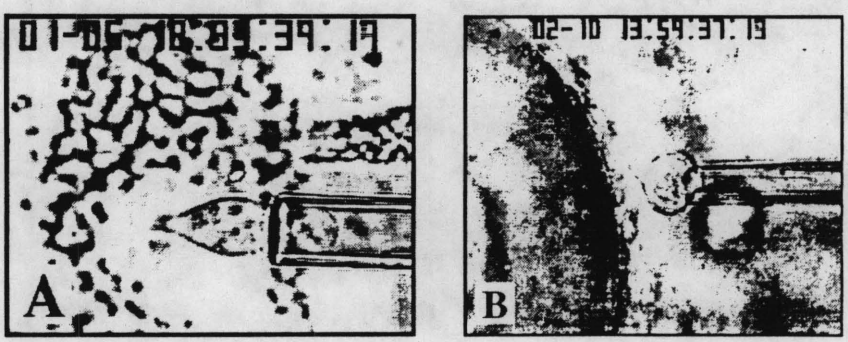


Fig. 5. Illustration of the two modified protocols for micropipette manipulation experiments. (A) The achorage-dependent cells were subcultured on the bottom glass surface of the cell chamber. (B) The achoragedependent cells were sub-cultured on the surfaces of microcarrier beads.

Mathematical Modeling. Image acquisition and processing is the first step of any analysis, since the raw data from the deformability and micropipette adhesion experiments are obtained in the form of images and stored in video tapes (Figs. 2 and 4). The data shown in Fig. 3 were obtained by measuring distances manually on images displayed on the monitor by single frame playback of the video tape, which was very slow and labor-intensive. We have purchased a Macintosh Quadra 950 equipped with a Neotech image grabber (Advent Computer Products, Encinitas, CA), an Ultimage Concept VI software (GTFS, Santa Rosa, CA), a LabView 2 software (National Instruments, Austin, TX), and a Super-VHS computer-controlled VCR (NEC Technologies, Wood Dale, IL). A computer program for automated image acquisition and processing are currently being developed, and we expect this to be done during the next granting year. The Quadra 950 computer also is being used for mathematical modeling. We have focused our effort on the phase of spontaneous adhesion during the current granting year. Modeling equations describing the time course of adhesion area are being solved using a series expansion method and an integral method. We anticipate that these calculations will be finished during the next granting year, and we will proceed with the modeling of forcible separation.

E-25-X15
2

Georgia Tech

THE GEORGE W. WOODRUFF SCHOOL OF
MECHANICAL ENGINEERING

Georgia Institute of Technology
Atlanta, Georgia 30332-0405

June 10, 1994

Dr. Karen M. Mudry, Program Director
The Whitaker Foundation
901 15th Street, N. W., Suite 1000
Washington, DC 20005

Dear Dr. Mudry:

I am enclosing three copies of the progress report for the current granting year (July 1, 1993 - June 30, 1994). Please let me know if anything else is required.

Sincerely,

Cheng Zhu, Ph. D.
Assistant Professor

Encl.

PROGRESS REPORT

Progress in various aspects of our research project during the current grant year is summarized below.

Centrifugation Experiments and Related Analyses. We have again focused our effort on dissecting the contribution from a particular cell adhesion molecule (CAM), i.e., E-selectin. Our strategy consists two steps. In the first step, a simplified system of E-selectin coated plate has been used to systematically quantify the influences of several physiochemical parameters on tumor cell adhesion. The strength of the E-selectin coated plate system lies in its simplicity and reliability (much less biological variability) which allows for quick generation of large amount of data. The quantitative relationships developed in the first step would then be verified using plates cultured with endothelial cells in the second step. Key results of the first step experiments are summarized in Fig. 1. Tumor cell adhesion to E-selectin coated plate has been shown to be independent of the duration of spinning (Fig. 1A), to decrease with the increasing relative centrifugal force (RCF) (Fig. 1B), and to increase with the increasing E-selectin density (Fig. 1C).

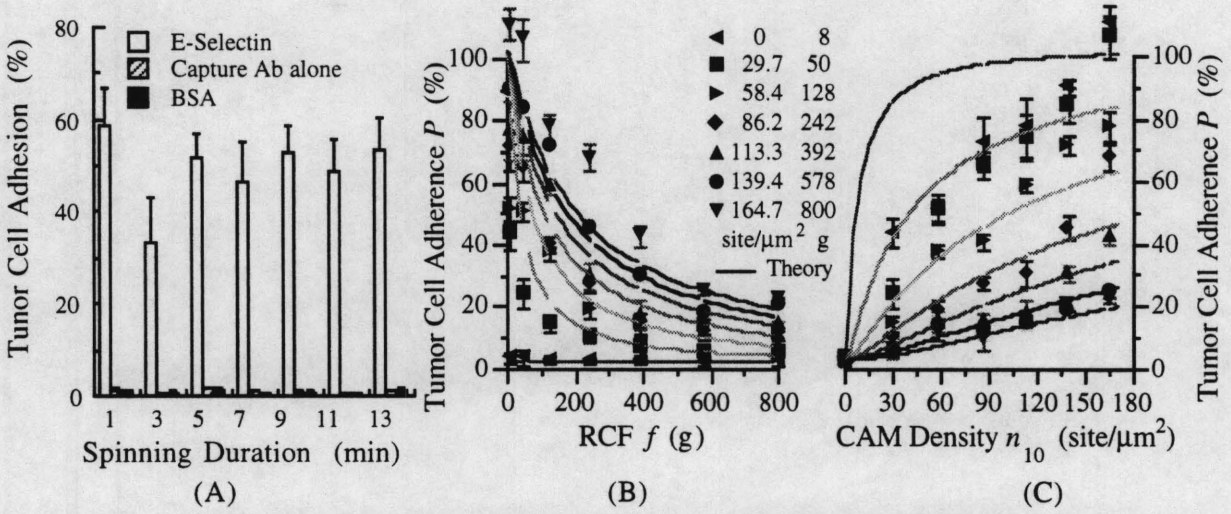


Fig. 1. Percentage of tumor cell (colon carcinoma cell line Colo 205) adherence to E-selectin coated surfaces as a function (A) of the duration of application of the centrifugal force, (B) of the relative centrifugal force (RCF) at various site densities of E-selectin, and (C) of the site density of E-selectin at various RCFs. Binding was specifically mediated by E-selectin because tumor cells did not adhere to the control surfaces coated with BSA or capture antibody alone. Specificity also was confirmed using monoclonal antibodies directed against E-selectin and its ligands (data not shown). Experiments were repeated three times with very similar results. Shown here are data of a typical experiment (presented as mean ± standard deviation of measurements from at least quadruplicate wells). The solid curves are model predictions (see text).

We found that, except for the very low RCF case (8g), the families of data shown in Fig. 1B or 1C lined up along a single curve if the percentage of tumor cell adherence P was plotted against f/n_{10} (Fig. 2A), the ratio of the relative centrifugal force f and the E-selectin site density n_{10}, or against its reciprocal n_{10}/f (Fig. 2B). This allowed us to identify two stochastic processes, one for attachment (the very low RCF case) and the other for detachment (the rest of data). The percentage of adherent for a cell population is related, in the former case, to the probability of a single cell to form at least one bond and in the latter case, to the probability of a single cell to have an adhesion strength greater than the detachment force. The attachment process requires further experimental studies to be conducted in the next grant year. The data for the detachment process enabled us to improve our stochastic model for cell detachment by centrifugal force as described in the original proposal. The ratio n_{10}/f represents a similarity variable the existence of which suggests a mathematical structure of the detachment condition for an individual cell, i.e., $n_{10}/f < x$. We further found that the single cell property x was lognormally distributed among the cell population as shown in Fig. 2C. Thus, not only can the data presentation be much better organized, but these families of data can be predicted through a known functional form using only two parameters (Figs. 1B and 1C). We also were able to determine that the cell mass was a most

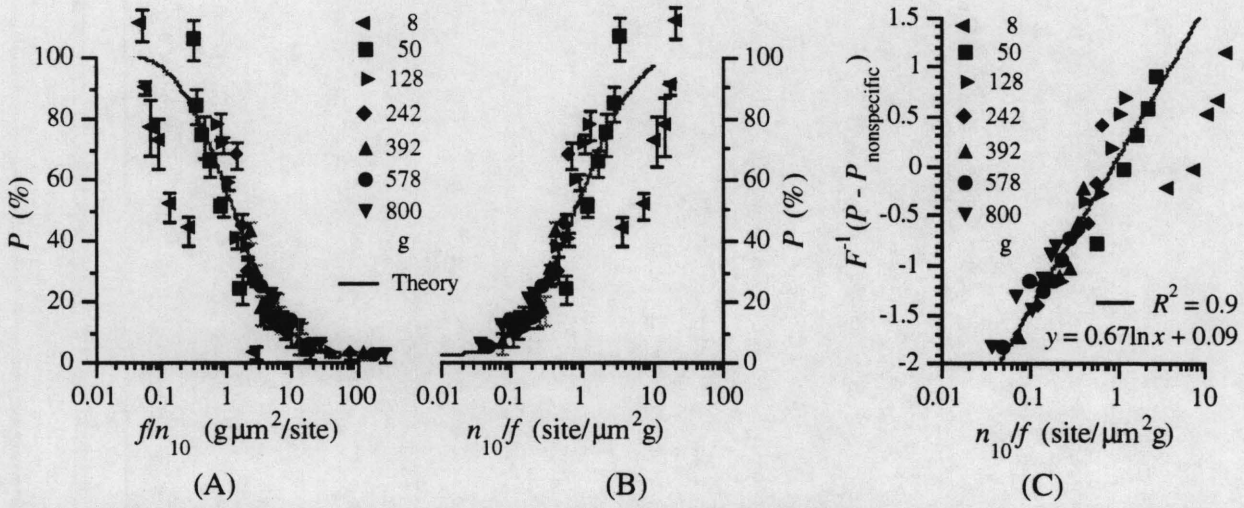


Fig. 2. Data from Figs. 1B and 1C replotted using the similarity variable. P as a function of the ratio f/n_{10} (A) or of its reciprocal n_{10}/f (B). Except for the very low RCF case (8g), all the data line up along a straight line in the log scale when the ordinate is transformed using the inverse cumulative normal distribution $F^{-1}(\bullet)$ (C). Thus, the functional form of such relationship is determined to be a lognormal distribution. $P_{\text{nonspecific}}$ is the nonspecific binding to the control surfaces coated with the capture antibody alone.

likely candidate for this random variable while the expression of the counter-receptor (SLe^x and SLe^a) on Colo 205 cells was not. The latter implies that the ligand density is in excessive amount as compared to the coated E-selectin density used in our experiments. Independent verification of the size and density distributions for Colo 205 cells is underway. Upon completion of these experiments, we will be able to predict the bonding force of the receptor-ligand interaction.

To relate the data generated using the E-selectin coated plates to the more physiologic system of cultured endothelial cells, as part of the second step, experiments have been conducted to quantify the site density of E-selectin expressed on cultured endothelial cell monolayers upon stimulation by various concentrations of biological response modifiers. This was done using ^{125}I-labeled anti-E-selectin monoclonal antibody (1D6) because this method allowed for quantitation of the absolute site density as opposed to relative expression levels determined using ELISA as originally proposed. The method consisted of measurement of the binding affinity of 1D6 for E-selectin via Scachard analysis (Fig. 3A) and, based on the measured K_D, determination of the E-selectin density from the radioactivity of the ^{125}I-labeled 1D6 that was bound to the E-selectin expressed on the endothelial cells (Fig. 3B). Parallel adhesion assays are underway to validate the detailed quantitative relationships as shown in Fig. 2.

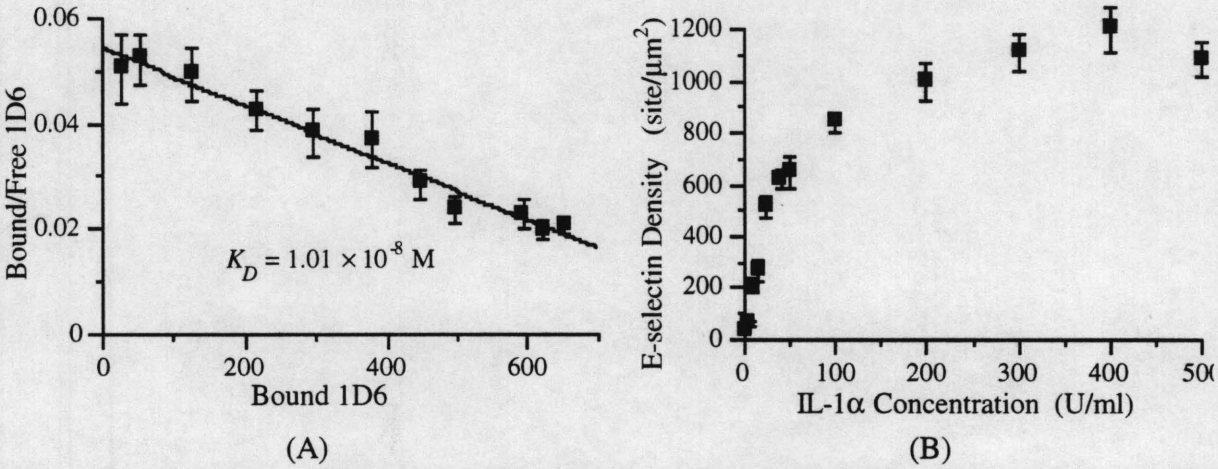
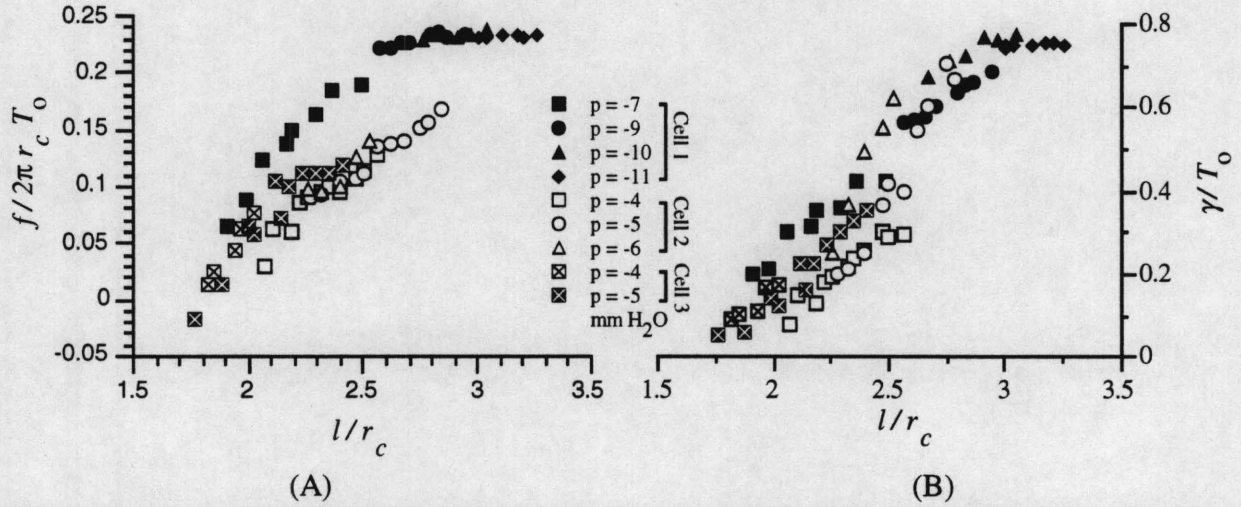


Fig. 3. (A) Scachard analysis for the determination of the binding affinity of 1D6 mAb for E-selectin expressed on endothelial cells. (B) Site density of E-selectin expressed on cultured endothelial cell monolayer as a function of the concentration of interleukin-1α used to stimulate the cells. Experiments were repeated four times with similar results. Shown here are data of a typical experiment (presented as mean ± standard deviation of measurements from at least quadruplicate wells). The solid line in (A) is the best fit to the data the slope of which enables the calculation of the dissociation constant K_D.

Micropipette Experiments and Related Modeling. Our strategy has been to use the centrifugation assay to assess the effects of multiple experimental conditions and to use the micropipette assay to quantify detailed adhesion force and energy at the single cell level, since the former is a more effective screening method while the strength of the latter lies in its sophistication and precision. As shown in Fig. 4, the adherent tumor cell deformed as it was being aspirated and pulled by the micropipette. We have been developing a series of mathematical models to extract the information encoded in the shape change, including the histories of the adhesion force (Fig. 5A) and energy (Fig. 5B) during the entire process of separation as well as the mechanical properties of the very same cell. This is in contrast to the previous protocol discussed in the original proposal which could only obtain the end-point value of the force required to separate the cell pair.

Fig. 4. Computer-processed image of an adherent tumor cell overlain by the computed cell shape (dashed curve) to best fit a set of measured coordinates of the cell outline (dots). The fitting of the observed to the predicted cell shape allowed for the evaluation of the adhesion force and energy at the very moment the image was recorded. Also computed by the model are the mechanical properties of the cell.



(A) (B)

Fig. 5. Dimensionless axial force resultant (A), and surface adhesion energy density (B) as functions of the dimensionless pipette location at various levels of aspiration pressure (in mm H_2O). Each datum point in Fig. 5A or 5B was computed from measurements from one image of a deformed cell, such as those shown in Fig. 4. Data for three cells were shown, and tens of deformation states were analyzed for each cell.

This research combines the modeling of cell adhesion with that of cell deformations. To be able to simultaneously evaluate the mechanical properties and the adhesive properties for the same cell is important for two reasons. First, the basis of our approach is to use the cell itself as a mechanical transducer and therefore the accuracy of the calculated adhesive force and energy values depends critically on those of the mechanical properties used in the calculation. Secondly, comparison of the mechanical property values computed from multiple deformed states allows us to validate the the appropriateness of the mechanical model for the cell.

The cell was modeled as a liquid drop enclosed by a prestressed cortical shell. The simplest model in our series assumes that the cellular deformation is quasi-static. The cortical tension and transcortical pressure computed based on this model is shown in Fig. 6. The data implies that the cortical tension is indepedent of the degree of stretch (as a true mechanical property should be) but strongly depends on the aspiration pressure (which contradicts the concept of mechanical property). We believe that such unrealistic results is due to the incorrect quasi-static assumption. We are currently modifying the model to remove this assumption by including cytoplasmic flow. We will also model the molecular dynamics of adhesive receptors to relate the molecular properties to the adhesive properties evaluated from cellular deformation.

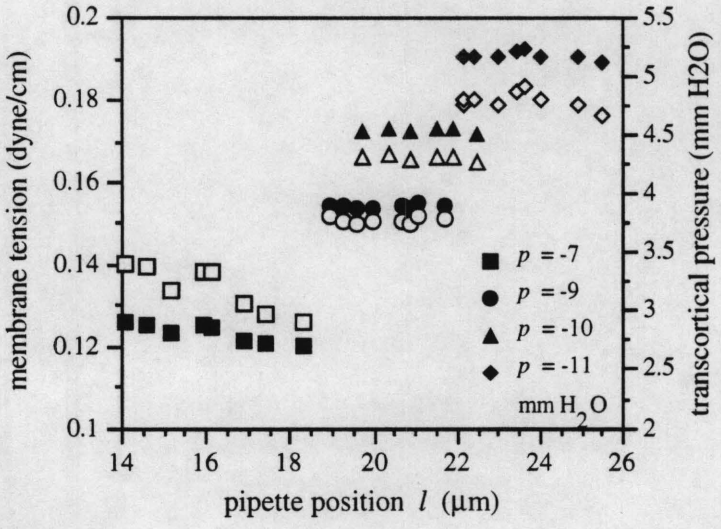


Fig. 6. Model prediction of the prestressed cortical tension (closed symbols), and the transcortical pressure (open symbols) as functions of the pipette position, l, at several levels of aspiration pressure, p. Each pair of datum points was computed from measurements from one image of a deformed cell, such as those shown in Fig. 4.

E25 - X15
N/A

Georgia Tech

Office of Grants and Contracts Accounting

Georgia Institute of Technology
190 Bobby Dodd Way
Atlanta, Georgia 30332-0259
USA
404•894•4624; 2629
Fax: 404•894•5519

November 8, 1993

The Whitaker Foundation
Attn: Mr. Miles J. Gibbons, Jr.
Suite 405
4718 Old Gettysburg Road
Mechanicsburg, PA 17055-8411

RE: "Mechanism of Melanoma Cell-Endothelial Cell Adhesion"
Dr. Cheng Zhu
E25-X15/R74660A0

Dear Mr. Gibbons:

Enclosed is the Annual Financial Report for the first year on the above research project, as required by the terms of the letter of agreement covering the grant.

We appreciate the support the Whitaker Foundation gives for research at Georgia Tech.

If you have any questions or if we can be of further assistance, please call either Ellen Scott at (404) 894-6757 or me at (404) 894-2629.

Sincerely,

David V. Welch
Director

DVW/ers

Enclosure

c: Dr. Cheng Zhu, ME 0405
Mr. Pete Dawkins, ME 0405
OCA/Reports 0420
File

Georgia Institute of Technology
Grants & Contracts Accounting
Atlanta, Georgia 30332-0259

The Whitaker Foundation
Suite 405
4718 Old Gettysburg Road
Mechanicsburg, PA 17055-4380

E25-X15/R74660A0
November 8, 1993

Annual Financial Statement

Mechanism of Melanoma Cell-Endothelial Cell Adhesion
Project Director: Dr. Cheng Zhu

July 1, 1992 through June 30, 1993

	Personal Services	Fringe Benefits	Materials & Supplies	Equipment	Travel	Sub-Contracts	Overhead	Total
Budget	27,045.00	4,167.00	2,400.00	15,281.00	1,000.00	11,090.00	6,922.00	67,905.00
Actual	27,164.83	4,159.57	2,653.55	14,822.67	941.95	11,090.00	6,983.98	67,816.55
Balance	-119.83	7.43	-253.55	458.33	58.05	0.00	-61.98	88.45

E-25-X15 N/A

Georgia Tech

Office of Grants and Contracts Accounting

Georgia Institute of Technology
190 Bobby Dodd Way
Atlanta, Georgia 30332-0259
USA
404•894•4624; 2629
Fax: 404•894•5519

November 9, 1994

The Whitaker Foundation
Attn: Mr. Miles J. Gibbons, Jr.
Suite 405
4718 Old Gettysburg Road
Mechanicsburg, PA 17055-8411

RE: "Mechanism of Melanoma Cell-Endothelial Cell Adhesion"
Dr. Cheng Zhu
E-25-X15/R74660A0

Dear Mr. Gibbons:

Enclosed is the Annual Financial Report for the second year on the above research project, as required by the terms of the letter of agreement covering the grant.

We appreciate the support of the Whitaker Foundation to research at Georgia Tech.

Should you have questions or require further assistance, please call either Dale Turner (404) 894-5521 or me at (404) 894-2629.

Sincerely,

David V. Welch
Director

DVW/dct

Enclosure

c: Dr. Cheng Zhu, ME 0405
Mr. Pete Dawkins, ME 0405
OCA/Reports 0420
File

GEORGIA INSTITUTE OF TECHNOLOGY
GRANTS AND CONTRACTS ACCOUNTING
ATLANTA, GEORGIA 30332-0259

The Whitaker Foundation
Suite 405
4718 Old Gettysburg Road
Mechanicsburg, PA 17055-4380

E-25-X15/R74660A0
November 9, 1994

Annual Financial Statement
Mechanism of Melanoma Cell-Endothelial Cell Adhesion
Project Director: Dr. Cheng Zhu

July 1, 1993 through June 30, 1994

	Personal Services	*Fringe Benefits*	*Materials & Supplies*	*Capital Outlay*	*Travel*	*Sub-Contracts*	*Overhead*	***Total***
Budget	13,745.00	288.00	2,400.00	22,488.00	1,000.00	11,456.00	3,487.00	54,864.00
Actual	13,820.95	287.64	3,488.65	894.51	19,070.44	13,562.44	3,739.37	54,864.00
Balance	(75.95)	0.36	(1,088.65)	21,593.49	(18,070.44)	(2,106.44)	(252.37)	0.00

E-25-X15 4/9

Georgia Tech

Office of Grants and Contracts Accounting

Georgia Institute of Technology
190 Bobby Dodd Way
Atlanta, Georgia 30332-0259
USA
404•894•4624; 2629
Fax: 404•894•5519

September 7, 1995

Mr. Miles J. Gibbons, Jr.
Whitaker Foundation
901 15th Street, N. W.
Suite 1000
Washington, DC

RE: "Mechanism of Melanoma Cell-Endothelial Cell Adhesion"
Dr. Cheng Zhu
E-25-W31/R74660A0

Dear Mr Gibbons:

Attached is the Annual Financial Report for the third year on this above research project, as required by the terms of the letter of agreement covering the grant. There will also be a final financial report submitted after the 6 month no cost extension period has ended.

We appreciate the support of the Whitaker Foundation research at Georgia Tech.

Should you have any questions or require further assistance, please call either Latonda Milner (404) 894-3499 or me at (404) 894-4624.

Sincerely,

David V. Welch
Director

DVW/lem

Enclosure

cc: Dr. Cheng Zhu, ME 0405
Mr. Pete Dawkins, ME 0405
OCA/Reports 0420 (E-25-X15 main project)
File

GEORGIA INSTITUTE OF TECHNOLOGY
GRANTS AND CONTRACTS ACCOUNTING
ATLANTA, GEORGIA 30332-0259

The Whitaker Foundation
901 15th Street, N.W.
Suite 1000
Washington DC 20005
7-Sep-95
E-25-W31/R74660A1

Annual Financial Statement
Mechanism of Melanoma Cell-Endothelial Cell Adhesion
Project Director: Dr. Cheng Zhu

July 1, 1994 through June 30, 1995

	Personal Services	*Fringe Benefits*	*Materials & Supplies*	*Capital Outlay*	*Travel*	*Sub-Contracts*	*Overhead*	*Total*
Budget	29,817.00	4,595.00	2,410.00	0.00	1,000.00	11,844.00	7,565.00	57,231.00
Actual	18,780.24	1,346.23	2,045.96	1,369.00	320.00	11,844.00	4,498.49	40,203.92
Balance	11,036.76	3,248.77	364.04	(1,369.00)	680.00	0.00	3,066.51	17,027.08

*Note: Balance from year two budget expended on GRA
James Piper for the summer quarter 1994

E-25-X15
#3

FINAL REPORT

Project Title: **Mechanism of Melanoma Cell-Endothelial Cell Adhesion**
Principal Investigator: **Dr. Cheng Zhu**

The general goal of this project is to elucidate the mechanisms of adhesion of tumor cells to endothelial cells as related to cancer metastasis. Melanoma is a highly malignant skin cancer which is characterized by a high incidence of metastasis associated with relatively small primary tumors. After leaving the primary tumor, a circulating tumor cell needs to adhere to an endothelial cell of the blood vessel wall in order to exit and to grow in a target organ. Thus, the adhesion of tumor cells to endothelial cells is a critical step in the metastatic process.

This project was undertaken to advance our understanding of tumor cell endothelial cell adhesion. Experiments were performed to systematically quantify the effects on tumor cell adhesion of several physicochemical parameters. The data were analyzed using mathematical models that were developed based on concepts and principles of physics and chemistry. Not only were we able to validate the mathematical models with the experimental data, but we were also able to evaluate model parameters by comparing the predictions with measurements. We now have developed a theoretical framework that identifies the important factors and predicts how these factors influence the outcome. We also have developed a simple method to evaluate these parameters for any given system. These can then be used to design therapeutic approaches for preventing metastasis by altering adhesiveness. Highlights of our findings are listed below.

- Developed a detailed mechanical property model of HL-60 tumor cell and experimental measurements of these properties (cortical tension and cytoplasmic viscosity). This enables us to use the cell itself as a mechanical transducer to measure the force and energy of adhesion from the observed deformation of the cell.

- Systematically investigated the quantitative relationship among adhesion strength, receptor and ligand desities, and binding affinity in a series of experiments using a simplified system of sialyl-Lewis X (sLe^x) expressing tumor (Colo-205) cells adherent to a plastic surface coated with a construct of E-selectin.

- Quantified the E-selectin site density (molecule per unit cell surface area) expressed on the endothelial cells and how such expression changes in response to cytokine stimulation. This allowed us to relate the data generated using the simplified system of adhesion to plastic surface coated with E-selectin with the more physiologic system of adhesion to endothelial cells.

- Constructed a stochastic model of chemical kinetic for small systems, obtained a closed-form solution to the equilibrium case, and used it to successfully predict the experimental data.

- Tested different relationships between binding affinity and bond force that have been suggested in the literature (for the first time, only possible with the use of our model), and identified the formulation the best describe our data.

- Developed a general experimental and analytical procedure to determine the properties of interactions between surface-bound receptors and ligands and used this to evaluated such properties in our experimental system. No other methods currently exist to measure the binding affinity of surface-bound receptors and ligands. The existing methods can measure the binding affinity of receptors and ligands in liquid phase. Such property is relevant to binding of soluble ligand but not to binding of surface-bound ones. Receptor-ligand binding that mediates cell adhesion are surface-bound.

PAPERS RESULTING FROM THIS AND RELATED PROJECTS

1. Yang, J., Xu, Y., Zhu, C., Hagan, M.K., Lawley, T.J. and Offermann, M.K., "Regulation of adhesion molecule expression in Kaposi's sarcoma cells," J. Immunol., vol. 152, pp. 361-373, January 1994.

2. Nagarjan, S., Cobern, L., Anderson, P., Zhu, C. and Selvaraj, P., "Ligand binding and phagocytosis by CD16 (FcγRIII) isoforms: Phagocytic signaling by associated ς and γ subunits in CHO cells," J. Biol. Chem., vol. 270, no. 43, pp. 25762-25770, October 1995.

3. Ku, D.N. and Zhu, C., "The Mechanical Environment of the Artery," in Role of Hemodynamic Forces in Modulating Vascular Cell Biology, pages 1-23, (B. E. Sumpio, Ed.) Boca Raton, FL: CRC Press, 1993.

4. Zhu, C., Williams, T.E., Delobel, J., Xia, D. and Offermann, M.K., "A Cell-Cell Adhesion Model for the Analysis of Micropipette Experiments," in Cell Mechanics and Cellular Engineering. pages 160-181, (V. C. Mow, F. Guilak R. Tran-Son-Tay and R. M. Hochmuth, Eds.) New York: Springer-Verlag, 1994.

5. Zhu, C., "Biomechanics and Thermodynamics of Cell Adhesion," in Principles of Cell Adhesion. pages 23-39, (M. Steiner and P. D. Richardson, Eds.) Boca Raton, FL: CRC Press, 1995.

6. Delobel, J., Yang, J., Offermann, M.K. and Zhu, C., "Mechanical properties of membrane tethers mediating the cell adhesion of Kaposi's sarcoma," in 1992 Advances in Bioengineering, BED-Vol. 22, pp. 391-394, (M.W. Bidez, Ed.), November, 1992.

7. Zhu, C., Delobel, J., Ferguson, L. and Offermann, M.K., "Quantitation of adhesion forces of cultured Kaposi's sarcoma cells for leukocyte cell lines," 1993 Advances in Bioengineering,, 1993, BED-Vol. 26, pp. 351-354, (J.M. Tarbell, Ed.), November, 1993.

8. Zhu, C. and Chesla, S.E., "Detachment forces and mechanisms of Fcγ receptor III (CD16) isoforms from ligands at the single crossbridge level," Proceedings of the 4th China-Japan-U.S.A.-Singapore Conference on Biomechanics, May, 1995.

9. Chesla, S.E., Selvaraj, P. and Zhu, C., "The mechanics of single molecular interactions between Fcγ receptor III (CD16) isoforms and their ligands," Proceedings of the 1995, Bioengineering Conference, BED-Vol. 29, pp. 455-456, (R.M. Hochmuch, N.A. Langrana and M.S. Hefzy, Eds.), June, 1995.

10. Williams, T.E., Delobel, J., Xia, D. and Zhu, C., "A cell deformation model for analysis of adhesion experiments," Carolina Conference in Biomedical Engineering, Chapel Hill, NC, February 4-5, 1994.

11. Zhu, C., Williams, T.E., Delobel, J. and Xia, D., "Mechanical analysis of cell adhesion in micropipette experiments," Keystone Symposia on Molecular and Cellular Biology, Taos, NM, February 20-26, 1994.

12. Zhu, C., Williams, T.E., Delobel, J., Xia, D. and Offermann, M.K. "A continuum model of cell adhesion for the analysis or micropipette experiments," The Second World Congress of Biomechanics, Amsterdam, the Netherlands, July 10-15, 1994.

13. Zhu, C. and Williams, T.E., "A mechanical analysis of cell-cell adhesion as applied to the micropipette experiment," Annual Fall Meeting of the Biomedical Engineering Society, Tempe, AZ, October 14-16, 1994.

14. Li, Y. H. and Zhu, C., "In vitro penetration of endothelial cell monolayer and invasion of matrigel barrier by human lung carcinoma cell," Keystone Symposium on Cancer Cell Invasion and Motility, Tammarron, CO, February 5-11, 1995.

15. Zhu, C., Williams, T.E. and Xia, D., "A mechanical analysis of cell-cell adhesion for the micropipette experiments," Biophysical Society Annual Meeting, San Francisco, CA, February 12-16, 1995.

16. Xia, D., Williams, T.E. and Zhu, C., "An automated image acquisition, processing and analysis system for high accuracy cell membrane detection," Experimental Biology Annual Meeting, Atlanta, GA, April 9-13, 1995.

17. Chesla, S.E., Li, P., Selvaray, P. and Zhu, C., "Separation forces and detachment mechanisms of FcγRIII isoforms from ligands at the single bond level," Experimental Biology Annual Meeting, Atlanta, GA, April 9-13, 1995.

18. Li, Y.H. and Zhu, C., "In vitro studies on interaction of lung carcinoma cells with endothelial cell monolayers by video cinemicroscopy," Experimental Biology Annual Meeting, Atlanta, GA, April 9-13, 1995.

19. Zhu, C. and Li, Y.H., "In vitro studies of tumor cell transmigration across cultured endothelial cell monolayers," Joint 9th International Congress of Biorheology and 2nd International Congress on Clinical Hemorheology, Big Sky, Montana, July 23-28, 1995.

20. Zhu, C., Chesla, S.E. and Selvaraj, P., "Separation forces and detachment mechanisms of CD16 (FcγRIII) isoforms from ligands at the single bond level," Joint 9th International Congress of Biorheology and 2nd International Congress on Clinical Hemorheology, Big Sky, Montana, July 23-28, 1995.

21. Zhu, C., Chesla, S.E. and Selvaraj, P., "Molecular biolmechanics of receptor-ligond binding at the signle bond level," 2nd International Conference on Cellular Engineering, La Jolla, CA, August 19-22, 1995.

22. Zhu, C., Chesla, S.E. and Selvaraj, P., "Detachment forces and mechanisms of cell-cell adhesion mediated by FcγRIII (CD16) isoforms at the single bond level," Annual Fall Meeting of the Bioengineering Society, Boston, MA, October 6-8, 1995.

23. Zhu, C. and Chesla, S.E., "A mechanical analysis of a micropipet aspirated red blood cell loaded by a point force," Biophysical Society Annual Meeting, Baltimore, MD, February 17-21, 1996.

24. Zhu, C., Piper J.W. and Swerlick, R.A., "A stochastic model of cell-substrate detachment via centrifugation," Biophysical Society Annual Meeting, Baltimore, MD, February 17-21, 1996.

25. Li, Y.H. and Zhu, C., "In vitro characterization of dynamic interactions of human tumor cells with endothelial cell monolayer," Annual Meeting of the American Association for Cancer Research, Washington, DC, April 20-24, 1996.

26. Zhu, C., Piper J.W. and Swerlick, R.A., "A mathematical model of cell detachment via centrifugation," Colloid and Surface Science Symposium, Potsdam, NY, June 16-19, 1996.

27. Piper, J.W., Swerlick, R.A., and Zhu, C., "Determination of affinity and characteristic stretch of surface-Bound receptor/ligand binding under force," Annual Fall Meeting of the Biomedical Engineering Society, University Park, PA, October 4-6, 1996.

GRANTS RECEIVED BY THE PI AFTER THE RECIPIENT OF THE WHITAKER AWARD

Title (classification)	Organization Funding	Amount	Date
Modeling of Transient Processes in Cell Adhesion (related)	National Science Foundation	$ 80,000	9/1/92-2/28/95
Regulation of Cell Surface Adhesion in Kaposi's Sarcoma (related)	Emory/Georgia Tech Biomed Tech Res Ctr	$ 30,000	7/1/92-6/30/93
Effect of HIV-1 Tat AIDS-Associated on Cell Adhesion in Kaposi's Sarcoma (new)	Georgia Tech Research Council Interdisciplinary Research Program	$ 20,000	9/1/92-6/30/93
Presidential Faculty Fellow Award: Cell Adhesion and Cell Locomotion (related)	National Science Foundation	$510,000	9/1/93-2/28/99
Regulation of Cell Adhesion in Kaposi's Sarcoma (related)	Emory/Georgia Tech Biomed Tech Res Ctr	$ 30,000	7/1/93-6/30/94
Quantitation of Ligand Binding and Cell Adhesion by CD16 Isoforms (related)	Emory/Georgia Tech Biomed Tech Res Ctr	$ 29,000	7/1/94-6/30/95
Ligand Bonding Force and Cell Adhesion of CD16 Isoforms (related)	National Institutes of Health	$500,500	9/1/95-8/31/00
A Novel Tumor Cell-Endothelial Cell Interaction (related)	GIT/MCG Biomed Res & Education Program	$ 30,000	7/1/95-6/30/96
Determination of Phagoeytic Activity in Single Cells Using Micropipets and Chemically Modified Spheres (new)	Georgia Tech IBB New Initiative Seed Grant Program	$ 9,000	11/1/95-6/30/96

Final Report
Principal Investigator Status Form

PI's Name: Dr. Cheng Zhu

PI's Institution: Georgia Institute of Technology

PI's Department: Mechanical Engineering

PI's Current Title: Assistant Professor

☐ Tenured ☒ Tenure track ☐ Non-Tenure track

Promotions received during Whitaker Foundation support:

☐ Research Associate to Research Assistant Professor
☐ Instructor to Assistant Professor
☐ Assistant Professor to Associate Professor
☐ Associate Professor to Full Professor
☐ Other (list) _____

Have you changed institutions since receiving Whitaker Foundation support?
☐ Yes ☒ No

Awards received during Whitaker Foundation support:
1993 Presidential Faculty Fellows Award (NSF)
1995 First Independent Research Support and Transition Award (NIH)
1992 Y. C. Fung Young Investigator Award (ASME, Bioengineering Division)

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