Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Allegheny-Singer Research Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 06/30/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Rebecca E. Pfeifer

4. **Grant Contact Person's Telephone Number:** 412-359-3137

5. **Grant SAP Number:** 4100047623

6. **Project Number and Title of Research Project:** 04 – Myofibroblast Inhibition in Dupuytren's Contracture

7. **Start and End Date of Research Project:** 01/01/2009 – 06/30/2010

8. **Name of Principal Investigator for the Research Project:** Latha Satish, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 36,647

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Satish	Principal Investigator	10%	8,761.17

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement

below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We plan to submit an NIH R21 application to continue this research project.

12. Future of Research Project. What are the future plans for this research project?

We will continue the project with NIH funding. An R21 application will be submitted in the upcoming cycle.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	2			
Unknown				
Total	**2**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown	2			
Total	**2**			

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black	2			
Asian				
Other				
Unknown				
Total	**2**			

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The experimental plans for the research project have been expanded through the funding obtained from the state grant. The funding also provided the resources to train undergraduate students through summer internships. The results obtained through this project have resulted in new ideas for expanding this project for animal studies.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

We have begun a collaboration with Dr. William A. LaFramboise from the Department of Pathology at the University of Pittsburgh.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

We are working with primary cultures of fibroblasts derived from areas of Dupuytren's disease cord (DD cord) from DC patients and small samples of adjacent, phenotypically normal palmar fascia (PF) and palmar fascia from patients undergoing carpal tunnel release (CTR) provided by Dr. David B. O'Gorman of Lawson Health Research Institute, London, Ontario, Canada (Dr. O'Gorman has kindly agreed to serve as a Consultant for this project). Currently, we have frozen stocks of fibroblasts derived from six DD cord-derived cell cultures, six patient-matched PF-derived cell cultures and six PF- obtained from carpal tunnel patients. As Dupuytren's disease cords are surgically resected much more frequently than nodules, we have utilized these abundant and biologically useful tissues to allow us to compare multiple matched pairs of primary cells in this study.

We have in parallel been collecting tissue biopsies from patients presenting to the Allegheny General Hospital (AGH) Division of Hand Surgery with the diagnosis of DC and carpal tunnel syndrome. Primary cultures of fibroblasts are also derived from the tissue biopsies obtained from AGH. Primary cultures isolated by this procedure invariably display a fibroblastic morphology. These tissue biopsies are also available to directly examine in vivo gene expression by immunohistochemistry or in situ hybridization.

Cell Culture

Primary cultures of fibroblasts are maintained in α-MEM-medium (Invitrogen Corporation) supplemented with 10% fetal bovine serum (Gemini Bioproducts) and 1% antibiotic-antimycotic solution (Sigma). Cells were grown in 10cm2 plates and, upon reaching 80% confluence, the cells were lysed using RLT-lysis buffer to extract total RNA (Qiagen). Quality of the RNA was assessed by OD260/280 in an ND-1000 spectrophotometer (Nanodrop Technologies) and by capillary electrophoresis with the Agilent 2100 bioanalyzer (Agilent Technolgies, Inc).

Basal Levels of alpha smooth muscle actin (α-SMA) Expression is Elevated in DC Derived Fibroblasts

Previous studies have reported on the elevated levels of α-SMA in DC, a marker to identify the presence of myofibroblasts. We wished to confirm such differential expression in α-SMA in our fibroblast cell cultures derived from DD, PF and CT release. Taqman probes for α-SMA and control GAPDH were obtained from Applied Biosystems. Primary antibodies for α-SMA and GAPDH was purchased from Abcam. We confirmed an increase in the expression of α-SMA both at the mRNA (measured by qRT-PCR) and protein (measured by Western blot) level in DD cord-derived fibroblasts relative to carpal tunnel and unaffected palmar fascia-derived fibroblasts (Figure 1a & 1b). TGF-β1 has been shown to be an important factor influencing accumulation of myofibroblasts in DC disease. Several authors have reported stimulation of α-SMA expression by TGF-β1 but over different time periods. We chose to stimulate the fibroblasts with 2 ng/ml of TGF-β1 for 24 hr since we observed a good increase in the α-SMA levels at the mRNA and protein levels.

Real Time RT-PCR to Determine the Basal Level Expression of Candidate Genes in DD-cord Derived Fibroblasts

Preliminary findings on mRNA expression levels of Type I, Type III collagen and fibronectin (Figure 2a, 2b, 2c) suggests that the expression level all these three genes are significantly elevated in DD-cord derived fibroblast (DD) and in uninvolved PF-derived fibroblasts (PF) when

compared to CT-derived fibroblasts (CT). This study was mainly designed to ascertain the basal expression of ECM levels in fibrotic tissue in comparison to fibroblasts obtained from non-fibrotic tissues. On the other hand, we found basal mRNA expression levels of MMP-1 (interstitial collagenase) and tissue inhibitor of metalloproteinases-1 (TIMP-1) (Figure 3a and 3b) to be increased in DD –cord derived fibroblasts though it was not a statistically significant difference. Previous studies on MMP1 and TIMP-1 tissue expression on DD- nodule and cord shows that MMP-1 and TIMP-1 was significantly elevated in DD nodules. The expression of MMP-1, MMP-9 and TIMP-1 will be further investigated for its activity and protein levels as mentioned in the research plan.

The main objective of this proposal is to determine if increased adenylyl cyclase levels will have an influence on cAMP levels, which, will ultimately have an effect on myofibroblast formation. As a first step we examined the mRNA expression level of adenylyl cyclase isoform 6 (ACY6) (Figure 4) as this is the isoform, which is predominantly expressed in many cell types (please see Research Plan for references). Our findings show that mRNA levels of AC6 was expressed natively at relatively high levels in DD-cord-derived fibroblasts. This preliminary finding of increased mRNA expression level (the protein levels will also be confirmed by western blots) of ACY6 in DD-cord derived fibroblasts is encouraging and we will also study the ACY6 activation when stimulated with TGF-β1 and forskolin as mentioned in the research plan which is key to this grant proposal.

Our initial studies on mRNA expression levels of fibronectin, Type I and Type III collagen suggest that the expression level all these three genes are significantly elevated in DD-cord derived fibroblast (DD) and in uninvolved PF-derived fibroblasts (PF) when compared to CT-derived fibroblasts (CT). This study was mainly designed to ascertain the basal expression of ECM levels in fibrotic tissue in comparison to fibroblasts obtained from non-fibrotic tissues. On the other hand, we found basal mRNA expression levels of MMP-1 (interstitial collagenase) and tissue inhibitor of metalloproteinases-1 (TIMP-1) to be increased in DD –cord derived fibroblasts though it was not a statistically significant difference. Previous studies on MMP1 and TIMP-1 tissue expression on DD- nodule and cord show that MMP-1 and TIMP-1 was significantly elevated in DD nodules. The expression of MMP-1, MMP-9 and TIMP-1 will be further investigated for its activity and protein levels as mentioned in the research plan.

Forskolin Reduced TGF--β1 Stimulated α-SMA and Fibronectin mRNA expression and protein levels in DD-Cord Derived Fibroblasts

Based on previous reports we chose to use 2 ng/ml of TGF- β1 and 10 μM of forskolin to stimulate DD-cord derived fibroblasts. Preliminary findings show that TGF-β 1 (2 ng/ml) stimulation for 24h resulted in significant increase in basal expression level of α-SMA and fibronectin levels in DD-cord derived fibroblasts. Interestingly, the basal level of α-SMA and TGF-β 1 induced α-SMA levels was reduced after treating with forskolin for 24h in DD-cord derived fibroblasts. Similarly, addition of forskolin resulted in reduction of both basal and TGF-β 1 simulated fibronectin levels in DD-cord derived fibroblasts (Figure 5). This initial observation gives a clue that increase in cAMP by forskolin (cAMP activity will be verified using cAMP assay kit) might play a key role in reducing the myofibroblast transformation during non-induced and growth factor induced state of DD-cord derived fibroblasts. These experiments were performed in two different cell lines of DD-cord derived fibroblast obtained from two different

patients and was performed only once, the experiments will be repeated with all the other four sets of samples to confirm reproducibility and consistency of the results obtained. Cell lysates obtained were also used to determine the changes in the protein levels in DD-cord derived fibroblasts after stimulation with forskolin and TGF-β 1. We found that similar to mRNA levels, the protein levels of α-SMA and Fibronectin was also significantly reduced in TGF-β1 stimulated DD-cord derived fibroblasts (Figure 6a and Figure 6b).

Forskolin Reduced TGF--β1 Stimulated connective tissue growth factor (CTGF-) mRNA Expression in DD-cord Derived Fibroblasts

Connective tissue growth factor (CTGF) is a multicellular protein that plays diverse biological roles in cellular signaling and adhesion. It is known to regulate aspects of cell migration, proliferation, differentiation, angiogenesis, ECM production and cell-matrix interactions. It has also been documented that fibroblasts cultured from hypertrophic scars and keloids show increased basal expression of CTGF. Furthermore, fibroblasts derived from hypertrophic scars when stimulated with TGF-β1 elaborate more CTGF expression. Since Dupuytren's disease is a fibrotic disease we were interested in determining if forskolin would reduce the expression of CTGF in DD-cord derived fibroblasts. Our studies demonstrated that increase in cAMP levels reduced the expression of TGF-β1 induced CTGF levels (Figure 7). Studies are in progress to determine if there is any significant change in the CTGF protein levels as well.

Increase in cAMP Levels Did Not Have Significant Impact on Type I or Type III collagen mRNA levels in DD-cord derived fibroblasts

Since collagen plays a significant role towards the progression of DC we were interested in determining if increase in cAMP levels will reduce Type I (Figure 8) and Type III collagen levels (Figure 9) in DD-cord derived fibroblasts. Surprisingly, our preliminary data show that increase in cAMP levels did not significantly reduce the TGF-β1 elevation of type I or type III collagen mRNA levels. Studies are in progress to confirm these changes in few more cell lines of DD-cord and also to correlate the mRNA changes with the protein levels.

Fibroblast Populated Collagen Lattice (FPCL) Contraction Assay

A major feature of DC is abnormal contractile fibrosis, presumably occurring through the actions of the DC fibroblast/myofibroblast cell populations. A widely used assay to test cell contractility is the fibroblast populated collagen lattice contractile (FPCL) assay. We have investigated whether our primary cultures of DC cells have innate increased contractility relative to control cells. Collagen contraction was carried out using disease fibroblasts from DC-affected palmar fascia (DD), fibroblasts from uninvolved palmar fascia of DC patients (PF), and control fibroblasts obtained at carpal tunnel release from patients without DC (CT); results are shown in Figure 7a and b. Collagen lattices were prepared by mixing cell suspensions with a neutralized solution of collagen type I matrix (8 parts Pure ColTM collagen type I, 2.9 mg/ml, INAMED +1 part 10x α-MEM+1 part HEPES buffer, pH 9.0). The cell-collagen concentrations were adjusted with sterile PBS to attain a final concentration of 2.0 mg/ml and a final cell concentration of 8.6 x 104 cells/ml of matrix. The cell-collagen mixture was then aliquoted into 24 well culture dishes (0.5 ml/well) that were pre-treated with a PBS solution containing 2 % (w/v) BSA. Following FPCL polymerization (1 hr, 37°C) culture medium (0.5 ml) consisting of α-MEM + 10% FBS was added at top of each lattice. After two days of culture the attached FPCLs were mechanically released from the sides of the culture plates. Digital images of the contracting FPCL were

captured at various time points over a 48 hr assay period and the collagen lattice areas were then quantified using Adobe Photoshop v7.0. The images shown here are the representations of 24 hr period as within this time point we observed significant differences in collagen contraction between the cell populations. This assay has been standardized in our laboratory with the protocols obtained from Dr. O'Gorman's laboratory in Canada as mentioned above. Preliminary data showed that diseased FPCL cultures contracted collagen faster and to a greater degree compared to control FPCL cultures (both in CTR fibroblasts and uninvolved palmar fascia fibroblasts) (Figure 10a & 10b). This is in agreement with previous reports that show distinct phenotypic differences between control and diseased primary cultures.

Figure 1

a

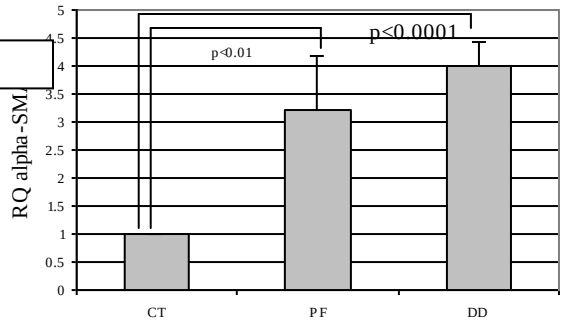
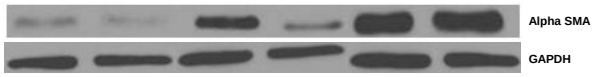


Figure 1a: mRNA expression level of alpha smooth muscle actin determined using real time RT-PCR on six different cultures of fibroblasts derived from carpal tunnel release. PF - represents fibroblasts derived from patient matched unaffected palmar fascia of DD. DD- represents fibroblasts derived from Dupuytren's disease.Values are mean ±SEM of two independent studies each performed in duplicates. Human GAPDH was used as internal control. Statistical analyses were performed using Student's t test.

b

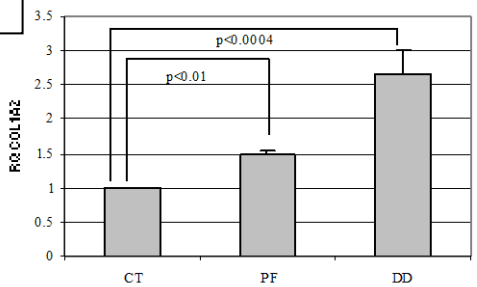


1 2 3 4 5 6

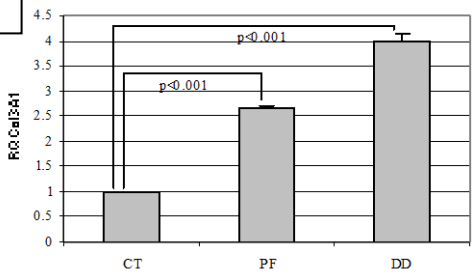
Figure 1b: Western Blots results using α-SMA antibody showed increased expression level of α-SMA proteins in DD-cord derived fibroblasts (Lanes 5-6) in comparison to fibroblasts derived from patient matched unaffected palmar fascia of DD (Lanes 3-4) and fibroblasts derived from carpal tunnel release (Lanes 1-2).

Figure 2

a



b



c

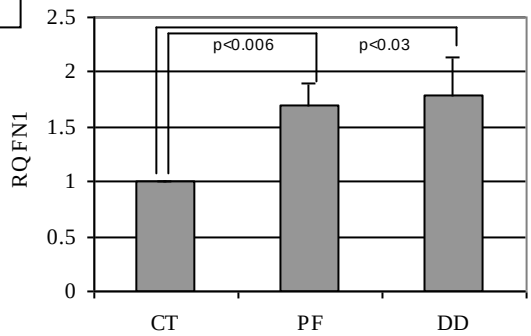


Figure 2 : Real time RT-PCR on COL1A2 (a) Col3A1 (b) and FN1 (c) from RNA isolated from six different cultures of fibroblasts obtained from Dupuytren's Disease (DD) cord, uninvolved palmar fascia of DC patients (PF), and from carpal tunnel patients without DC (CT). Values are mean ± SEM of two independent studies, each performed in duplicates. GAPDH was used as an internal control. Statistical analyses was performed by Student's t test. Relative quantification of gene expression was calculated by comparing δ Ct values between DD, PF and CT-derived fibroblasts.

Figure 3

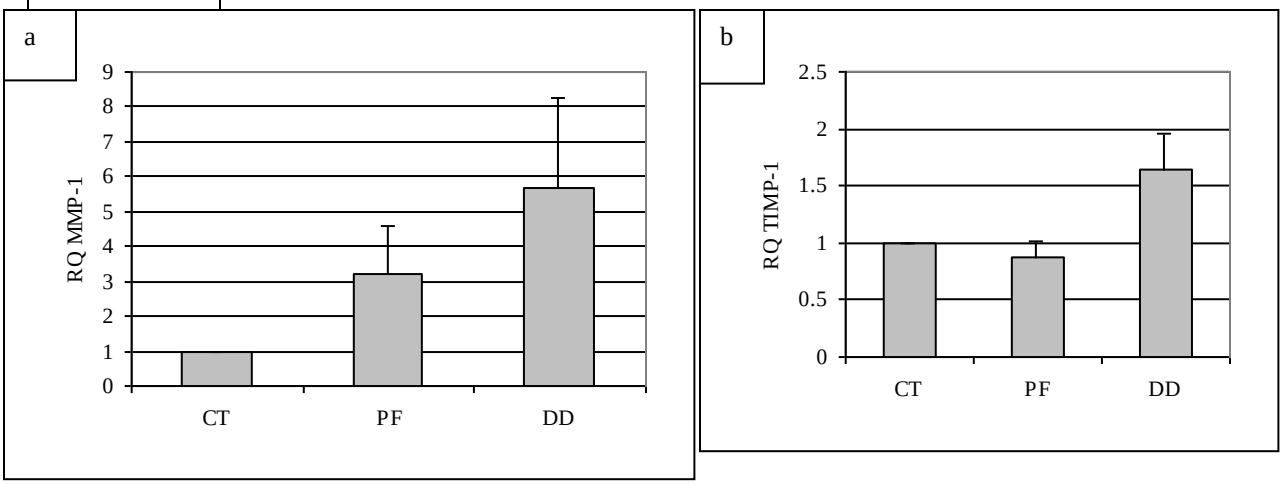


Figure 3 : Real time RT-PCR on MMP-1 (a) and TIMP-1 (b) from RNA isolated from six different cultures of fibroblasts obtained from Dupuytren's Disease (DD) cord, uninvolved palmar fascia of DC patients (PF), and from carpal tunnel patients without DC (CT). Values are mean ± SEM of two independent studies, each performed in duplicates. GAPDH was used as an internal control. Statistical analyses was performed by Student's t test. Relative quantification of gene expression was calculated by comparing δ Ct values between DD, PF and CT-derived fibroblasts.

Figure 4

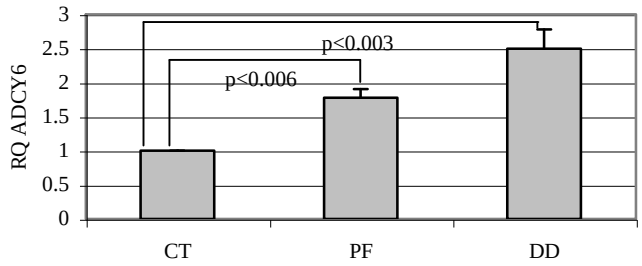


Figure 4 : Real time RT-PCR on ADCY6 from RNA isolated from six different cultures of fibroblasts obtained from Dupuytren's Disease (DD) cord, uninvolved palmar fascia of DC patients (PF), and from carpal tunnel patients without DC (CT). Values are mean ± SEM of two independent studies, each performed in duplicates. GAPDH was used as an internal control. Statistical analyses was performed by Student's t test. Relative quantification of gene expression was calculated by comparing δ Ct values between DD, PF and CT-derived fibroblasts.

Figure 5

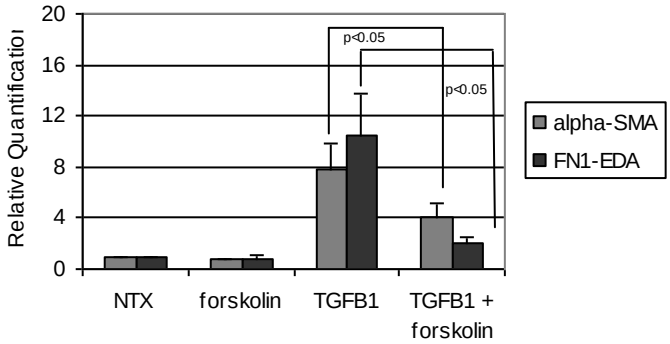


Figure 5: DD-cord derived fibroblasts were grown to confluence and quiesced for 48h. After 48h cells were treated with 2 ng/ml of TGF-β1, 10uM of forskolin for 24h. After 24h cells were lysed with RLT-lysis buffer and RNA was extracted as per maufacturer's instructions. Real time RT-PCR was performed using human α- SMA, fibronectin and GAPDH probes obtained from applied biosystems. Relative quantification of gene expression was calculated by comparing δ Ct values between no treatment and treated samples.

Figure 6a

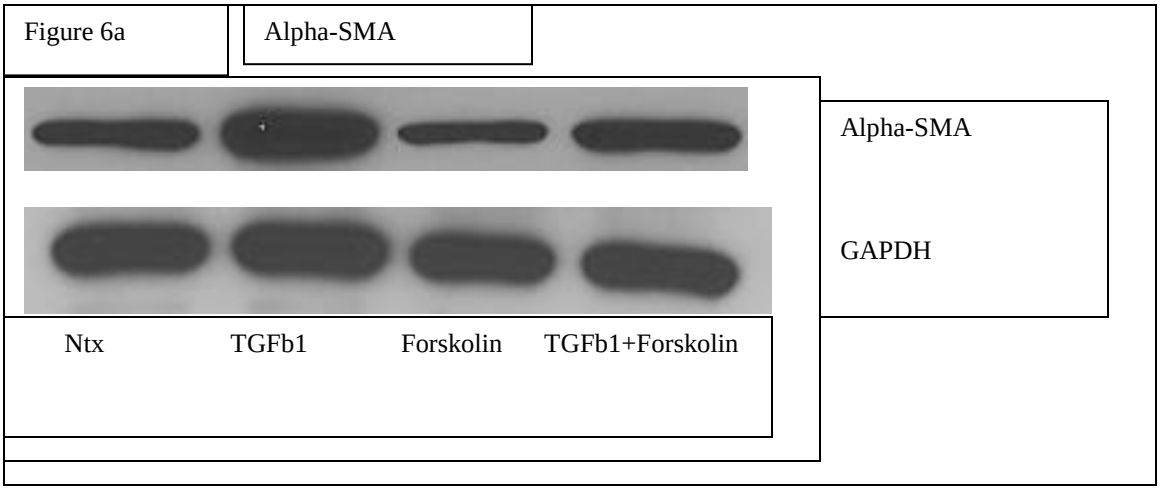


Figure 6b

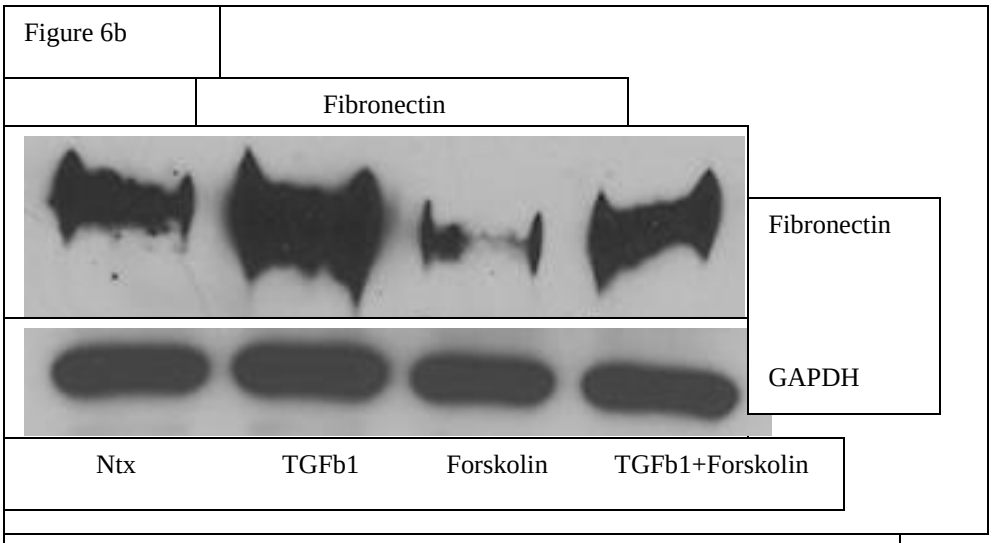


Figure 6a and 6b: Western Blot analysis on cell lysates derived from DD-cord derived fibroblasts showed that alpha-SMA and fibronectin protein levels were significantly reduced in forskolin and forskolin+TGFbeta1 samples. GAPDH was used as loading control. Shown here is the representative immunoblot of three different independent experiments performed on two different cell lines.

Figure 7

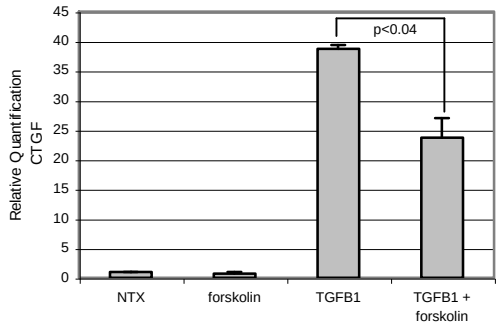


Figure 7: DD-cord derived fibroblasts were grown to confluence and quiesced for 48h. After 48h cells were treated with 2 ng/ml of TGF-β1, 10uM of forskolin for 24h. After 24h cells were lysed with RLT-lysis buffer and RNA was extracted as per maufacturer's instructions. Real time RT-PCR was performed using human CTGFand GAPDH probes obtained from applied biosystems. Relative quantification of gene expression was calculated by comparing δ Ct values between no treatment and treated samples.

Figure 8

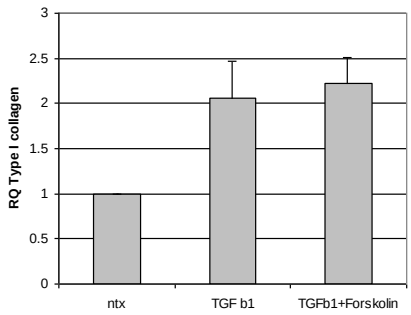


Figure 9

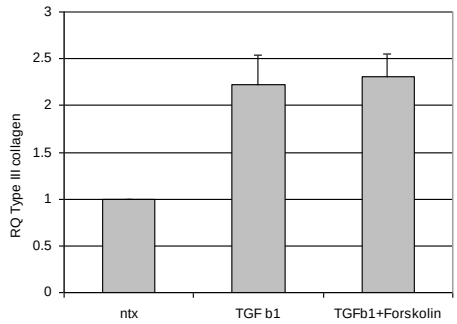


Figure 8 & 9: DD-cord derived fibroblasts were grown to confluence and quiesced for 48h. After 48h cells were treated with 2 ng/ml of TGF-β1, 10uM of forskolin for 24h. After 24h cells were lysed with RLT-lysis buffer and RNA was extracted as per maufacturer's instructions. Real time RT-PCR was performed using human Type I and Type III collagen mRNA and GAPDH probes obtained from applied biosystems. Relative quantification of gene expression was calculated by comparing δ Ct values between no treatment and treated samples.

Figure 10

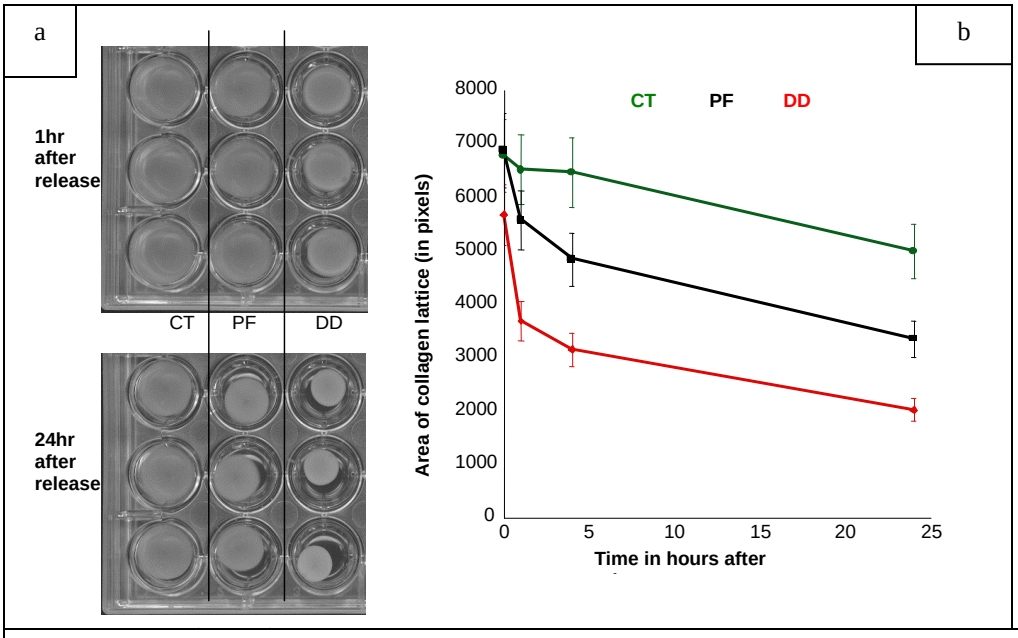
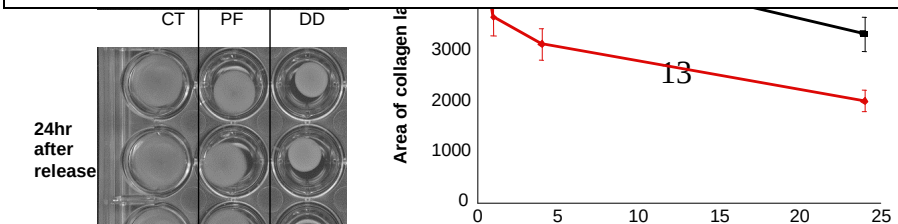


Figure 10: (a) Representative images of contracting FPCL after 1hr and 24 hr release are shown. CT- represents fibroblasts derived from carpal tunnel release. PF - represents fibroblasts derived from patient matched unaffected palmar fascia of DD. DD-represents fibroblasts derived from Dupuytren's disease.(b) Digital images of contracting FPCL cultures were captured and analyzed to quantify collagen contraction. The mean FPCL area (pixels) were plotted over time with each data point representing the mean ± the SDM of two independent studies.



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White

_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We are planning to submit a manuscript to the Journal of Hand Surgery within the next two months.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance

of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Satish, Latha	Research Assistant Professor
eRA COMMONS USER NAME SATISHL	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Madras, Chennai, India	BSc	1990	Zoology
University of Madras, Chennai, India	MSc	1992	Zoology
University of Madras, Chennai, India	M.Phil	1993	Zoology
University of Madras, Chennai, India	PhD	1999	Zoology & Biotechnology
University of Pittsburgh, Pittsburgh, PA	Post Doc	1999-2004	Cell and Molecular Biology

A. Personal Statement

The main aim of this project is to establish the effectiveness of probiotic bacteriotherapy to limit the pathogenic bacterial load in burn wounds. In addition, we will seek to determine if probiotics are effective in reducing inflammation and scarring. I believe that my long experience in the fields of wound healing and burn research (over 17 years) has well-prepared me to successfully pursue this project. My graduate thesis research project was to evaluate the efficacy of an enzyme preparation in the treatment of burn wounds. I had the great opportunity to collaborate with one of the leading Burn Surgeons in India (Dr. Mathangi Ramakrishnan), which provided me an opportunity to interact with patients directly so as to better understand first-hand the problems in burn wound healing and the need for more effective therapies. My post-doctoral years with Dr. Alan Wells focused on signaling mechanisms of various cytokines and chemokines involved in normal and abnormal wound healing (including keloids and hypertrophic scars), so I have a firm understanding of the inflammatory mechanisms relevant to scar formation. I have expanded my studies to other fibroproliferative disorders (eg. Dupuytren's contracture) after joining Dr. Sandeep Kathju at the Center for Genomic Sciences. The present proposal represents an opportunity for me to fuse my extensive background in burns with my ongoing interests in fibrotic and inflammatory disorders, and I believe I can make a strong contribution to its successful prosecution.

B. Positions

1992-93 Post Graduate Student, University of Madras, Chennai, India.
1994-98 Senior Research Fellow, Dept. of Biotechnology, Central Leather Research Institute, Adyar, Chennai, India.
1999-04 Post Doctoral Research Fellow, Department of Pathology, University of Pittsburgh, Pittsburgh, PA.
2005-2006 Research Associate, Center for Genomic Sciences, Allegheny-Singer Research Institute, Pittsburgh, PA.
2007-Present Research Assistant Professor, Center for Genomic Sciences, Allegheny-Singer Research Institute, Pittsburgh, PA.

C. Honors and Awards

1994 Awarded Senior Research Fellowship from Council of Scientific and Industrial Research, Government of India.

1998 Best Paper Award - Vth National Academy of Burns Conference, Chennai, India.

2002 First Place for the Paper presented at the 13th Annual Pathology Research Day Symposium,University of Pittsburgh.

2003 Awarded Pathology Post Doctoral Research Training Grant, University of Pittsburgh.

2003 Awarded Pathology Post Doctoral Travel Grant, University of Pittsburgh.

2003 Third Place for the Paper presented at the 14th Annual Pathology Research Day Symposium, University of Pittsburgh.

2006 Best paper Hardesty Award for the paper presented at the 51st Annual Meeting of the Plastic Surgery Research Council, Danapoint, California.

C. Selected Peer-Reviewed Publications

Most relevant to the current application

1. Latha B, Ramakrishnan M, Jayaraman V and Babu M. 1997. Action of trypsin:chymotrypsin (Chymoral forte D.S.) preparation on acute phase proteins following burn injury in humans. Burns, 23: S3-S7.

2. Latha B, Ramakrishnan M, Jayaraman V and Babu M. 1997. Serum enzymatic changes modulated using trypsin: chymotrypsin preparation during burn wounds in humans. Burns, 7/8: 560-564.

3. Latha B, Ramakrishnan M, Jayaraman V and Babu M. 1998. Efficacy of trypsin: chymotrypsin preparation in the reduction of oxidative damage during burn injury in humans. Burns, 24: 532-538.

4. Latha B, Ramakrishnan M, Jayaraman V, and Babu M. 1999. Physicochemical properties of extracellular matrix proteins in postburn human granulation tissue. Comp Biochem Physiol, 124: 241-249.

5. Latha B and Babu M. 2001. The involvement of free radicals in burn injury: A review. Burns, 27: 309-317.

6. Satish L, Yager D and Wells A. 2003. ELR-negative CXC chemokine IP-9 as a mediator of epidermal-dermal communication during wound repair. J Invest Dermatol, 120: 1110-1117.

7. Tran KT, Rusu SD, Satish L and Wells A. 2003. Aging-related attenuation of EGF receptor signaling is mediated in part by increased protein tyrosine phosphatase activity. Exp Cell Res, 289: 359-367.

8. Glading A, Bodnar RJ, Reynolds IJ, Shiraha H, Satish L, Potter DA, Blair HC and Wells A. 2004. Epidermal growth factor activates m-calpain (calpain II) at least in part, by extracellular signal-regulated kinase-mediated phosphorylation. Mol Cell Biol, 24: 2449-2512.

9. Satish L, Babu M, Tran K, Hebda P and Wells A. 2004. Keloid fibroblasts responsiveness to epidermal growth factor and activation of downstream intracellular signaling pathways. Wound Repair Regen,2: 183-192.

10. Satish L, Kharait S and Bodnar R. 2004. Cell motility as a regulatable target during wound healing. Recent Research Developments in Cell Biology. 2: 37-70 ISBN: 81-7895142-148.

11. Satish L, Blair HC, Glading A and Wells A. 2005. IP-9 (CXCL11) induced cell motility in keratinocytes requires calcium flux-dependent activation of μ-calpain. Mol Cell Biol, 25: 1922-1941.

12. Satish L, Lyons-Weiler J and Wells A. 2006. Preliminary gene expression pattern associated with keloid fibroblasts. Wound Repair Regen, 14: 463-470.

13. Kathju S, Satish L, Rabik C, Rupert T, Oswald D, Johnson S, Hu FZ, Post JC and Ehrlich GD. 2006. Identification of differentially expressed genes in scarless wound healing utilizing PCR suppression subtractive hybridization. Wound Repair Regen, 14: 413-420.

14. Satish L, Abdulally A, Oswald D, Johnson S, Hu FZ, Post JC, Ehrlich GD, Kathju S. 2008. Differential expression of chaperonin containing T-complex polypeptide (CCT) subunits during fetal and adult skin wound healing. Cell Stress Chaperones,13:527-533.

15. Satish L, Hogg J, Johnson S, Oswald D, Post JC, Ehrlich GD and Kathju S. 2010. Expression of RACK1 in healing skin and mucosal wounds. Ann Plast Surg,64: 238-241.

Additional selected publications

16. Wells A, Kharait S, Yates C and Satish L. 2004. Calpain proteases in prostate carcinomas. In: Immunohistochemistry and In Situ Hybridization of Human Carcinomas, Volume 1 (Ed: MA Hayar, Elsevier Science/Academic Press).

17. Satish L, Laframboise WA, O'Gorman DB, Johnson S, Janto B, Gan BS, Baratz ME, Hu FZ, Post JC, Ehrlich GD, Kathju S. 2008. Identification of differentially expressed genes in fibroblasts derived from patients with Dupuytren's Contracture. BMC Med Genomics,Apr 23;1:10.

18. Vi L, Feng L, Zhu RD, Wu Y, Satish L, Gan BS, O'Gorman DB. 2009. Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's Disease and adjacent palmar fascia cells. Exp Cell Res 315:3574-3586.

19. Satish L, Johnson S, Wang JH, Post JC, Ehrlich GD, Kathju S (2010). Chaperonin containing T-complex polypeptide subunit eta (CCT-eta) is a specific regulator of fibroblast motility and contractility. PLoS One. 20;5(4):e10063.

20. Satish L, Johnson S, Abdulally A, Post JC, Ehrlich GD, Kathju S (2010). Cloning and expression of rabbit CCT subunits eta and beta in healing cutaneous wounds. Cell Stress Chaperones Apr 15. [Epub ahead of print].

D. Research Support- Active

W81XWH-08-2-0032 (Russell) Co-I 09/01/2008 – 04/19/2013
University of Pittsburgh (Wake Forest/USA Medical Research Acquisition Activity)
Scarless Wound Healing through Nanoparticle-mediated Molecular Therapies

The goals of this project are to use nanoparticulate means of gene delivery, especially atelocollagen formulations, to manipulate the wound milieu in healing skin, tendon, and burn wounds so as to engineer a scarless repair.

SAP #4100047623
PA Department of Health (Tobacco) PI
01/01/2009-06/30/2010
Myofibroblast inhibition in Dupuytren's Contracture
The major goal of this project is to determine if the formation of myofibroblast could be inhibited to reduce palmar fascia fibrosis.

M2009-0084
Albert B Ferguson M.D. Orthopaedic Fund of the
The Pittsburgh Foundation Grant Co-PI 05/01/2009-04/30/2010
Alterations in extracellular matrix proteins and gene expression patterns in nude rats of Dupuytren's contracture
The goal of this project was to determine the changes in the ECM proteins in the animal model of DC to manipulate the gene expression patterns to prevent recurrence of the disease.

Completed Research Support
M2008-0052
Albert B Ferguson M.D. Orthopaedic Fund of the
The Pittsburgh Foundation Grant Co-PI 05/01/2008-05/01/2009
Gene Expression in naked rats of Dupuytren's Contracture
The main goal of this project is to establish an animal model of DC by determining the changes in the gene expression patterns of genes associated with fibrosis.

(No number)
Wound Healing Foundation's 3M fellowship 06/01/2007-06/01/2008
CCT-eta as a mediator of scarless wound healing
The major goal of this project was to determine if downregulation of CCT-eta could inhibit the formation of scar in adult wounds.

(No number)
Pathology Post Doctoral Research Training Grant – 01/01/2003-01/01/2004
University of Pittsburgh
Keloids responsiveness to ELR-negative CXC chemokine IP-9 - An approach to therapeutic evaluation
The main aim of this project was to determine the role of IP-9 in abnormal scar formation.