Plastic Surgery Foundation

Research Fellowship

Grant Application

Date Submitted: 12/1/2015 10:59:08 PM

Renewal? No

If renewal, current grant:

Resubmission? No | Prior App:

TITLE OF PROJECT *(Titles exceeding 65 characters, including spaces and punctuation, will be truncated.)*

Enhanced Fat Graft Retention Through Novel Stromal Cell Subpopulations

APPLICANT NAME

Blackshear, Charles

HIGHEST DEGREE(S)

M.D.

POSITION TITLE: Postdoctoral Research Fellow

ACADEMIC RANK:

DIVISION:

DEPARTMENT: Reconstructive and Plastic Surgery

E-MAIL ADDRESS:

cblacksh@stanford.edu

Tel: 650-736-1707 Fax: 650-497-8874

APPLICANT'S CURRENT INSTITUTION

Stanford University School of Medicine

MAILING ADDRESS *(Street, city, state, postal code, country)*

257 Campus Drive West
Stanford CA 94305
United States

PROGRAM ELIGIBILITY INFORMATION: *(Responses to selected fields displayed below. For some grant programs this section may be blank.)*

	Response to Solicitation: Yes	Yes
	Solicitation Title: Research Fellowship	Yes
Yes	Previous PSF Applicant?: No	Yes

DATES OF PROPOSED PROJECT *(MM/DD/YYYY)*

From 7/1/2016 Through 6/30/2017

PROPOSED BUDGET

50000.00

Name Stanford University

Address

Research Management Group
3172 Porter Drive
Palo Alto CA 94304
United States

Tel: 650 498 5592 Fax: 650 725-0661

EIN 94-1156365

DUNS 009214214

SIGNING OFFICIAL FOR The Board of Trustees of the Lel

Name Chitty, Maile

Title Research Process Manager/ Institutional Signin

Address

3172 Porter Drive
Palo Alto CA 94304-1212
United States

Tel: 650-736-7645 Fax:

E-MAIL ADDRESS

mchitty@stanford.edu

HUMAN SUBJECTS ☐ No ☒ Yes

Human Subjects Assurance No. FWA00000935

IRB Status: Approved

IRB Date: 11/11/2014

VERTEBRATE ANIMALS ☐ No ☒ Yes

Animal welfare assurance no. A3213-01

IACUC Status: Approved

IACUC Date: 11/21/2014

HUMAN EMBRYONIC STEM CELLS No

Not Applicable

APPLICANT ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF APPLICANT *(In ink. "Per" signature not acceptable.)* DATE

SIGNING OFFICIAL ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with the grantor's terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF SIGNING OFFICIAL *(In ink. "Per" signature not acceptable.)* DATE

SIGNATURE OF DEPARTMENT CHAIR DATE

Applicant: Blackshear, Charles

Application Contacts

Role	*Admin Official To Be Notified If Awarded			Role	*Department Head		
Name	Chitty, Maile			Name	Hawm, Mary		
Institution	The Board of Trustees of the Leland Stanford Junior			Institution	The Board of Trustees of the Leland Stanford J		
Title	Research Process Manager/ Institutional Signing Off			Title	Professor and Chair, Stanford Department of S		
Division	Research Management Group			Division			
Dept	School of Medicine			Dept			
Address	3172 Porter Drive Palo Alto CA 94304-1212 United States			Address	Stanford University School of Medicine Department of Surgery Alway Building, Room M121 300 Pasteur Drive		
Tel:	650-736-7645	Fax:		Tel:	650-498-7387	Fax:	
E-mail	mchitty@stanford.edu			E-mail	mhawn@stanford.edu		
Role	*Financial Officer			Role	Mentor		
Name	Reuter, Mr. Timothy Edward			Name	Wan, Derrick		
Institution	The Board of Trustees of the Leland Stanford Junior			Institution			
Title	Director Post Award			Title	Associate Professor of Surgery		
Division				Division			
Dept	Office of Sponsored Research			Dept	Plastic and Reconstructive Surgery		
Address	3160 Porter Drive, Room 136 Palo Alto CA 94304-8445 United States			Address			
Tel:	650-721-1758	Fax:	650-725-0093	Tel:	(650) 738-1707	Fax:	
E-mail	treuter@stanford.edu			E-mail	dwan@stanford.edu		
Role	Mentor			Role			
Name	Longaker, Michael			Name			
Institution	Stanford University			Institution			
Title	Director, Children's Surgical Research			Title			
Division	Division of Plastic and Reconstructive Surgery			Division			
Dept	Department of Surgery			Dept			
Address	Stanford CA 94305 United States			Address			
Tel:	(650) 736-1707	Fax:	(650) 736-1705	Tel:		Fax:	
E-mail	longaker@stanford.edu			E-mail			
Role				Role			
Name				Name			
Institution				Institution			
Title				Title			
Division				Division			
Dept				Dept			
Address				Address			
Tel:		Fax:		Tel:		Fax:	
E-mail				E-mail			

Plastic Surgery Foundation

Research Fellowship

Grant Application

Date Submitted: 11/24/2015 8:26:26 AM

Renewal? No

If renewal, current grant:

Resubmission? No | Prior App:

TITLE OF PROJECT *(Titles exceeding 65 characters, including spaces and punctuation, will be truncated.)*

Enhanced Fat Graft Retention Through Novel Stromal Cell Subpopulations

APPLICANT NAME Blackshear, Charles	HIGHEST DEGREE(S) M.D.
POSITION TITLE: Postdoctoral Research Fellow	APPLICANT'S CURRENT INSTITUTION Stanford University School of Medicine
ACADEMIC RANK:	MAILING ADDRESS *(Street, city, state, postal code, country)*
DIVISION:	257 Campus Drive West Stanford CA 94305 United States
DEPARTMENT: Reconstructive and Plastic Surgery	
E-MAIL ADDRESS: cblacksh@stanford.edu Tel: 650-736-1707 Fax:	

PROGRAM ELIGIBILITY INFORMATION: *(Responses to selected fields displayed below. For some grant programs this section may be blank.)*

	Response to Solicitation: No	Yes
	Solicitation Title:	Yes
Yes	Previous PSF Applicant?: No	Yes

DATES OF PROPOSED PROJECT *(MM/DD/YYYY)*		PROPOSED BUDGET
From	Through	
7/1/2016	~~7/1/2017~~ 6/30/2017 MDC	50000.00

Name Stanford University School of Medicine Address 269 Campus Drive West Stanford CA 94305 United States	SIGNING OFFICIAL FOR Name Maile Chitty Title Research Process Manager/ Institutional Official Address Research Management Group 3172 Porter Drive Palo Alto, CA 94304-1212
Tel: 650 723 7887 Fax: 650 725 1958 EIN DUNS	Tel: 650-736-7645 Fax: E-MAIL ADDRESS mchitty@stanford.edu
HUMAN SUBJECTS ☐ No ☒ Yes Human Subjects Assurance No. IRB Status: Approved IRB Date: 11/11/2014	VERTEBRATE ANIMALS ☐ No ☒ Yes Animal welfare assurance no. IACUC Status: Approved None IACUC Date: 11/21/2014
HUMAN EMBRYONIC STEM CELLS	

APPLICANT ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.	SIGNATURE OF APPLICANT *(In ink. "Per" signature not acceptable.)* MD	DATE 12/1/15
SIGNING OFFICIAL ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with the grantor's terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF SIGNING OFFICIAL *(In ink. "Per" signature not acceptable.)* Maile Chitty	DATE 12/1/15
	SIGNATURE OF DEPARTMENT CHAIR Mary Hu MD	DATE 12/1/15

Applicant: Blackshear, Charles

Application Contacts

Role	*Admin Official To Be Notified If Awarded		Role	*Department Head	
Name	Chitty, Maile		Name	Hawm, Mary	
Institution	The Board of Trustees of the Leland Stanford Junior		Institution	The Board of Trustees of the Leland Stanford	
Title	Research Process Manager/ Institutional Signing Off		Title	Professor and Chair, Stanford Department of	
Division	Research Management Group		Division		
Dept	School of Medicine		Dept		
Address	3172 Porter Drive Palo Alto CA 94304-1212 United States		Address	Stanford University School of Medicine Department of Surgery Alway Building, Room M121 300 Pasteur Drive	
Tel:	650-736-7645	Fax:	Tel:	650-498-7387	Fax:
E-mail	mchitty@stanford.edu		E-mail	mhawn@stanford.edu	
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Name	Reuter, Mr. Timothy Edward		Name		
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Title	Director Post Award		Title		
Division			Division		
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Address	3160 Porter Drive, Room 136 Palo Alto CA 94304-8445 United States		Address		
Tel:	650-721-1758	Fax: 650-725-0093	Tel:		Fax:
E-mail	treuter@stanford.edu		E-mail		
Role			Role		
Name			Name		
Institution			Institution		
Title			Title		
Division			Division		
Dept			Dept		
Address			Address		
Tel:		Fax:	Tel:		Fax:
E-mail			E-mail		
Role			Role		
Name			Name		
Institution			Institution		
Title			Title		
Division			Division		
Dept			Dept		
Address			Address		
Tel:		Fax:	Tel:		Fax:
E-mail			E-mail		

Stanford MEDICINE | Department of Surgery

MARY T. HAWN, MD, FACS
Professor and Chair
Stanford Medicine Professor in Surgery

November 20, 2015

Plastic Surgery Foundation
444 East Algonquin Road
Arlington Heights, IL 60005-4664

Re: Charles P. Blackshear, M.D. Application for the Plastic Surgery Foundation Research Fellowship Grant

Dear Colleagues,

Charles P. Blackshear, M.D. is a Postdoctoral Research Fellow at Stanford University where he is pursuing a full-time, two year research fellowship where he is free of any clinical duties. He has guaranteed financial support from his lab PI, Dr. Michael Longaker.

Stanford University is able to provide the necessary facilities, equipment and resources which Dr. Blackshear will need to carry out his proposed research. We are committed to the advancement and development of Dr. Blackshear's career throughout his two year research fellowship here at Stanford University.

Sincerely,

Mary T. Hawn, M.D.

MTH/jw

Alway Building, Room M121 | 300 Pasteur Drive, MC 5115 | Stanford, CA 94305-2200
t: 650.498.4292 | f: 650.725.3918 | e: mhawn@stanford.edu
surgery.stanford.edu

Dr. Michael T. Longaker Curriculum Vitae

NAME Longaker, Michael T., M.D., M.B.A.	POSITION TITLE Deane P. and Louise Mitchell Professor; Director, Children's Surgical Research Laboratories; Co-Director, Institute of Stem Cell Research and Regenerative Medicine; Director, Program in Regenerative Medicine
eRA COMMONS USER NAME LONGAKER.MICHAEL	

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
Michigan State University, East Lansing, MI	B.S.	1976-1980	Physiology
Harvard Medical School, MA	M.D.	1980-1984	Medicine
UC San Francisco, CA	Post-Doctoral	1987-1991	Fetal Surgery
Columbia University, NY	M.B.A.	2001-2003	Business

A. Personal Statement

The goal of the proposed research is to identify the mechanisms of competition for angiogenic cells between tumors and wounds. I possess the skills and expertise necessary to lead this project. I am a surgeon by background, with Board Certification in General Surgery, Plastic Surgery, and a fellowship in Craniofacial Surgery. As a surgeon-scientist, I possess an understanding of the clinical problems that currently face surgical practice, and strive to develop novel cell-based strategies that can fill the current gaps in medical and surgical therapies for these diseases. In addition to my clinical training, I have been a productive surgeon-scientist investigating cranial suture biology, wound repair and bone tissue engineering in mice for the last 17 years. In addition, I have directed large research programs at Stanford University for the past 10 years. I am the Director of the Campus-wide Program in Regenerative Medicine with over 150 faculty, and I am the Co-Director of the institute of Stem Cell Biology and Regenerative Medicine. My group collaborates extensively with investigators across the campus, particularly in the Departments of Developmental Biology and Bioengineering. I have the administrative and leadership skills to successfully lead the proposed project. I am aware of the importance of frequent communication among the members of my group and with collaborators, constructing a realistic research plan, timeline, and managing a budget. In summary, I have demonstrated a record of successful and productive research projects in the area of stem cell and skeletal biology along with bone tissue engineering. My experience and expertise have prepared me to lead the proposed project.

B. Positions and Honors

Positions and Employment

1984-1987	Intern and Junior Resident, Department of Surgery, UC San Francisco
1991-1993	Senior and Chief Resident, Department of Surgery, UC San Francisco
1993-1995	Senior and Chief Resident in Plastic Surgery, Department of Surgery, NYU
1995-1996	Fellowship in Craniofacial Surgery, Department of Surgery, UC Los Angeles
1996-2000	John Marquis Converse Professor of Plastic Surgery, New York University
2000-Present	Deane P. and Louise Mitchell Professor of Surgery, Stanford University
	Director, Children's Surgical Research Laboratories
	Co- Director, Institute of Stem Cell Research and Regenerative Medicine
2005-Present	Director, Program in Regenerative Medicine

Other Experience and Professional Memberships

1993-1995	Clinical Instructor in Surgery, New York University
1995-1996	Clinical Instructor in Surgery, UC Los Angeles
1996-2000	Director of Surgical Research, New York University

Selected Honors

1976	Valedictorian, Warren High School, Warren, Michigan
1979	Member of NCAA Men's Championship Basketball Team
1980	Rhodes Scholar National Finalist
1980	Highest Honors, Michigan State University
1996	Clifford C. Snyder Past Chairman Award, Plastic Surgery Research Council
1997-1999	Academic Scholar, American Association Plastic Surgeons

2004	American Society for Clinical Investigation
2007	Institute of Medicine of the National Academies of Science
2008	Association of American Physicians
2009	I.S Ravdin Lecture in Basic Medical Sciences, American College of Surgeons
2011	Flance-Karl Award, American Surgical Association
2012	Sheen Award, Bank of America / American College of Surgeons

C. Selected peer-reviewed publications (Selected from over 1100 total)

1. Warren, S.M., Brunet, L.J., Harland, R.M., Economides, A.N. and **MT Longaker**. The BMP antagonist noggin regulates cranial suture fusion. Nature. 422(6932):625-629. 2003
2. Cowan, CM, Quarto, N, Warren, SM, Salim, A, and **MT Longaker**. Age-related changes in the biomolecular mechanisms of calvarial osteoblast biology affect FGF-2 signaling and osteogenesis. J Biol Chem. 278(34):32005-13. 2003
3. Cowan CM, Shi YY, Aalami OO, Chou YF, Mari C, Thomas R, Quarto N, Contag CH, Wu B, **Longaker MT**. Adipose-derived adult stromal cells heal critical sized mouse calvarial defects. Nature Biotechnology. 22(5):560-567. 2004
4. Salim A, Nacamuli RP, Morgan EF, Giaccia AJ, **Longaker MT**: Transient changes in oxygen inhibit osteogenic differentiation and Runx2 expression in osteoblasts. J Biol Chem. 279(38): 40007-16. 2004
5. Wan DC, Pomerantz, JH, Brunei LJ, Kim JB, Chou YF, Wu BM, Harland R, Blau HM, **Longaker MT:** Noggin Suppression Enhances *In vitro* Osteogenesis and Accelerated *In Vivo* Bone Formation. J Biol Chem. 282:26450-9, 2007
6. Wan DC, Shi YY, Nacamuli RP, Quarto N, Lyons KM, **Longaker MT**. Osteogenic differentiation of mouse adipose-derived adult stromal cells requires retinoic acid and bone morphogenetic protein receptor type IB signaling. Proc Natl Acad Sci USA. 2006 Aug 15; 103(33): 12335-40.
7. Liu KJ*, Arron JR*, Stankunas K, Crabtree GR, **Longaker MT**. Chemical rescue of cleft palate and midline defects in conditional GSK-3beta mice. Nature. 446:79-82. 2007
8. **Longaker, MT**, Baker, L.C. & Greely, H.T. Proposition 71 and CIRM--assessing the return on investment. Nature Biotechnology. 25: 513-521. 2007
9. Gurtner GC, Werner S, Barrandon Y, **Longaker MT:** Wound repair and regeneration. Nature. 453:314-321, 2008
10. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry A, Robbins RC, ***Longaker MT**, *Wu JC. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci U S A. 106:15720-15725. 2009 *corresponding authors
11. Behr B, Leucht P, ***Longaker MT**, *Quarto N. FGF-9 is required for angiogenesis and osteogenesis in long bone repair. Proc Nal Acad Sci 107:11853-11858, 2010 *corresponding authors
12. Jia F, Wilson KD, Sun N, Gupta D, Huang M. Li Z, Panetta NJ, Robbins RC, Kay MA, , ***Longaker MT**, *Wu JC.: A novel minicircle vector for deriving human iPS cells. Nature Methods. 7:197-199. 2010 *corresponding authors
13. Kwan MD, Sellmyer MA, Quarto N, Ho AM, Wandless TJ, **Longaker MT**. Chemical control of FGF-2 release for promoting calvarial healing with adipose stem cells. J Biol Chem. 286:11307-11313, 2011
14. Levi B, Wan DC, Glotzbach JG, Hyunh J, Januszyk M, Montoto D, Sorkin M, James AW, Nelson ER, Li S, Quarto N, Lee M, Gurtner GC, **Longaker MT**. CD105 protein depletion enhances human adipose-de rived stromal cell Osteogenesis through reduction of Transforming growth factor-B1 (TGF-B1) Signaling. J Biol Chem. 286(45):39497-509. 2011
15. Levi B, Nelson ER, James AW, Hyun JS, Montoro DT, Glotzbacj JP, Lee M, Commons GW, **Longaker MT**. Dura mater promotes human adipose-derived stromal cells to undergo osteogenic differentiation in mouse calvarial defects. Stem Cells. 29(8):1241-55. 2011
16. Rinkevich Y, Lindau P, Ueno H, **Longaker MT**, Weissman IL: Germ Layer and Lineage Restriction of Stem and Progenitor Cells Underlie Regeneration of the Mouse Digit Tip. Nature 476: 409-413, 2011
17. Quarto N, Leonard B, Li S, Marchand M, Anderson E, Behr B, Francke U, ,Reijo Pera R, Chiao E, **Longaker MT**. Skeletogenic Phenotype of Marfan embryonic stem cells faithfully phenocpied by patient- specific iinduced pluripotent stem cells. Proc Natl Acad Sci USA. 109(1):215-20. 2012
18. Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez OJ, Navarrete EG, Hu S, Wang L, Lee A, Pavlovic A, Lin S, Chen R, Hajjar RJ, Snyder MP, Dolmetsch RE, Butte MJ, Ashley EA, **Longaker MT**, Robbins RC, Wu JC. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. Science Translational Medicine. 4(130):130ra47. 2012
19. Levi B, Hyun JS, Montoro DLo D, Chan CK, Hu S, Sun n,Lee M, Grova M, Connolly AJ, Wu JC, Gurtner GC, Weissman IL, **Longaker MT:** *In vivo* directed Differentiation of Pluripotent stem cell for skeletal regeneration. Proc Natl Acad Sci 109(50):20379-84, 2012
20. Chan CFK, Lindau P, Jiang W, Chen JY, Zhang LF, Chen CC, Seita J, Sahoo D, Kim JB, Lee A, Park S, Nag D, Gong Y, Kulkami S, Luppen CA, Theologis AA, Wan DC, DeBoer A, Vincent-Thompkins JD, Seo W, Loh K, Walmsley G, Kraft, DL, Wu JC, **Longaker MT*,** Weissman IL*. Clonal precursor of bone, cartilage, and hematopoietic niche stromal cells. Proc Natl Acad Sci. 2013 Jul 30;110(31):12643-8. *corresponding authors

D. Research Support

WFUHS441011 (Gurtner) Longaker Co-PI
09/23/13 - 09/22/18
0.30 calendar
Armed Forces Institute of Regenerative Medicine
Targeted Delivery of a Small Molecule Focal Adhesion Kinase Inhibitor for ScarIsolation and Expansion of Native Vascular Networks for Kinase Inhibitor for Scarless Wound Healing.
The goal of this project is to manipulate the material properties of skin utilizing a FAK inhibitor to eliminate fibrosis and scar formation following burns and traumatic injuries.
$170,240
OVERLAP: None.

WFUHS441013 (Gurtner) Longaker Co-PI
09/23/13 - 09/22/18
0.30 calendar
Armed Forces Institute of Regenerative Medicine
Biomimetic Stem Cell Dressing for Skin Regeneration. The major goal of this project is a Device to Actively Control Mechanobiology During Wound Healing And Prevent Scaring.
$171,947
OVERLAP: None

5R01EB00571804 (Gurtner) 06/01/10 - 03/31/15 1.2 calendar
Inside-Out Tissue Engineering for Organ Fabrication
The goals of this project are to use explanted microcirculatory beds combined with mesenchymal stem cells to generate vascularized neo-organs ex vivo to replace a physiologic role in vivo.
$203,943
OVERLAP: None

5R01AR05445806 (Daldrup-Link) 08/01/12 - 07/31/17 0.12 calendar
Monitoring of Stem Cell Engraftment in Arthritic Joints With MR imaging
The major goal of this project is to develop a clinically applicable MR imaging technique, which provides an early diagnosis of complications of the engraftment process of matrix associated stem cell transplants (MASI).
$253,113
OVERLAP: None

1R24HL11775601A1 (Wu) Longaker Co-PI 04/15/14-03/31/19 03/31/15 0.60 calendar
Biorepository of Human iPSCs for Studying Dilated And Hypertrophic Cardiomyopathy
The major goals of this project are to establish a biorepository to allow for iPSC studies of cardiomyopathy.
$1,032,818
OVERLAP: None

5U01HL09977606 (Wu) 09/30/09 - 04/30/16 0.60 calendar
Induced Pluripotent Stem Cells in the Understanding and treatment of Heart Disease.
The major goal of this project is to study and utilize human-derived iPS cells for the treatment of cardiac disease
$728,015
OVERLAP: None

1R01DE021683 (Longaker) 09/03/12 – 08/31/171.80 calendar
Osteogenic enrichment of adipose-derived stromal cells
Lorenz, Hermann Peter - Project 36212
$239,999

National Institutes of Health
Skin Regeneration: Cellular & Molecular Mechanisms 07/01/14 - 06/30/19 .80 calendar
$467,3811
OVERLAP None

Longaker, Michael T. - Project 114291
07/01/14 - 06/30/15 .12 calendar
Plastic Surgery Educational Foundation
Effects of aging on the skeletal system at the stem cell level
$10,000
OVERLAP None

Longaker, Michael T. - Project 114297
Plastic Surgery Educational Foundation
07/01/14 - 06/30/15 .12 calendar
Mechanisms involved in Diabetic Fracture Healing…
$10,000
OVERLAP: None

Longaker, Michael T. - Project 114298
Plastic Surgery Educational Foundation
07/01/14 - 06/30/15 .12 calendar
Enhancing Bony Reconstruction Through Gene Manipulation of ASCs
$10,000
OVERLAP None

1R21DE02423001 (Longaker,) 05/22/14 – 04/30/16 1.80 calendar
Enhancing Bcl-2 Expression for Bone Regeneration
The goal of this project is to identify and enrich osteogenic subpopulations within adipose-derived stromal cells.
$150,000
OVERLAP None

TR1-01249 (Helms,)
California Institute for Regenerative Medicine
05/01/14 - 04/30/15 .60 calendar
Enhancing healing via Wnt-protein mediated activation of endogenous stem cells
The major goal of this project is to promote the ability for endogenous stem cells to enhance healing through Wnt mediated pathways
$309,685

OVERLAP None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Derrick C. Wan, MD

eRA COMMONS USER NAME (credential, e.g., agency login): WAN002

POSITION TITLE: Associate Professor of Surgery

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
UC San Diego	N/A	08/94-08/95	Biochemistry
Stanford University	B.S.	09/95-06/97	Biology
Columbia University	M.D.	08/97-05/01	Medicine
Stanford University	N/A	06/04-06/06	Post-Doctoral Research

A. Personal Statement

The goal of the proposed research is to investigate the role of scaffolding to enhance wound regeneration. Specifically, we plan to investigate the ability for bioengineered scaffolding to facilitate cellular migration and re-epithelialization for full-thickness wounds. By way of background, I am a board certified Plastic and Reconstructive Surgeon and have completed additional fellowships in both Craniofacial Surgery and Microsurgery. As a practicing physician at Stanford University, I treat a variety of patients including children with craniofacial deformities, as well as adults with soft tissue defects following trauma or tumor exenteration. In addition, I have been running a research laboratory studying soft tissue reconstruction and wound healing for the last four years. As a result of my experiences guiding numerous postdoctoral research fellows, I have developed administrative skills necessary to help oversee this proposal. My laboratory and I will be closely collaborating with the laboratory of Paige Fox, as she provides expertise with scaffolding. In addition, my laboratory will provide expertise on the full-thickness wound healing animal model and histological analyses. In summary, my training record and research history make me ideally suited to collaborate on investigations proposed and I will be able to assist with and ensure completion of the entire project.

B. Positions and Honors

Positions and Employment

2001-2000 Department of Surgery, NYU Intern in General Surgery
2002-2007 Department of Surgery, UCSF Resident in General Surgery
2007-2009 Department of Surgery, UCLA Resident in Plastic and Reconstructive Surgery
2009-2010 Department of Surgery, UCLA Clinical Instructor / Craniofacial Surgery Fellow
2010-2011 Department of Surgery, Chang Gung Memorial Microsurgery Fellow
2011-2015 Department of Surgery, Stanford Assistant Professor of Surgery
2015-Present Department of Surgery, Stanford Associate Professor of Surgery

Other Experience and Professional Memberships

2011- Candidate Member, American Society of Craniofacial Surgery

2011- Candidate Member, American Society for Reconstructive Microsurgery
2012- Fellow, American College of Surgeons
2012- Diplomate, American Board of Plastic Surgeons
2012- Member, Plastic Surgery Research Council
2015- Member, American Society of Maxillofacial Surgeons

Selected Honors

1. 1994 Valedictorian, Mission Viejo High School; Mission Viejo, CA
2. 1994 Mission Viejo High School Mathematics Award
3. 1994 Certificate of Merit, Advanced Level; Violin
4. 1994 Bank of America Achievement Award
5. 1994-1997 J.C. Penney Super Scholar Award
6. 1998 McGraw Hill Book Award
7. 2001 Samuel Rover Anatomy Award
8. 2001 Election to Alpha Omega Alpha
9. 2004 Ethicon-SUS Research Fellowship
10. 2005 Most Outstanding Abstract Presentation – 18th Annual J. Engelbert Dunphy Resident Research Symposium, UCSF Department of Surgery
11. 2005 First Place – PSEF Scientific Essay Contest D. Ralph Millard, MD Investigator Award
12. 2005 First Place (Scientific Poster Presentation) – 11th International Congress of the International Society of Craniofacial Surgery
13. 2006 Peter J. Gingrass, MD Memorial Award
14. 2007 ASMS Best Paper Award – Scientific Category
15. 2008 UCLA Plastic Surgery Resident Research Award
16. 2009 UCLA Plastic Surgery Resident Research Award
17. 2010 UCLA Clinical Faculty Teach Award
18. 2012 ASCFS / Komedy*plast* Research Award
19. 2013 Endowed Faculty Scholar, Child Health Research Institute
20. 2015 Best United States Paper in Plastic and Reconstruction Surgery for calendar 2014
21. Singapore-Stanford Biodesign Fellowship Mentor Award

C. Contribution to Science

1. As a post-doctoral research fellow, I began investigating the use of adipose-derived stromal cells for bone tissue engineering. Much of my early work focused on how these cells became committed along an osteogenic lineage. This included studies on bone morphogenetic protein and the mechanisms involved in guiding multipotent cells towards bone formation. I was able to demonstrate the importance of specific receptors and the interplay between BMP agonists and antagonists in regulating downstream signaling. Furthermore, I have published multiple reports describing the heterogeneity among adipose-derived stromal cells and have helped to define functional subsets of these cells with enhanced bone forming capacity. Findings from my studies have been extended to induced pluripotent stem cells, using bone morphogenetic protein to guide these cells toward osteogenic commitment while simultaneously reducing teratoma formation. Collectively, my fundamental discoveries made in bone biology have facilitated multiple translational studies demonstrating the efficacy of cell-based bone tissue engineering.

A. **Wan DC**, Shi YY, Nacamuli RP, Quarto N, Lyons KM, Longaker MT. Osteogenic differentiation of mouse adipose-derived adult stromal cells requires retinoic acid and bone morphogenetic protein receptor type IB signaling. ***Proceedings of the National Academy of Sciences.*** 2006 Aug; 103(33): 12335-40. PMID: 16894153

B. **Wan DC**, Pomerantz JH, Brunet LJ, Kim JB, Chou YF, Wu B, Harland R, Blau HM, Longaker MT. Noggin Suppression Enhances in vitro Osteogenesis and Accelerates in vivo Bone Formation. ***Journal of Biological Chemistry.*** 2007 Sep; 282(36): 26450-9. PMID: 17609215

C. Levi B*, **Wan DC***, Glotzbach JP, Hyun, J, Januszyk M, Montoro D, James AW, Nelson ER, Li S, Quarto N, Lee M, Gurtner GC, Longaker MT. CD105 depletion enhances human adipose-derived stromal cell osteogenesis through reduction of Transforming Growth Factor β1 (TGF-β1) signaling. ***Journal of Biological Chemistry*** 2011 Nov 11; 286(45): 39497-509. doi: 10.1074/jbc.M111.256529. PMID: 21949130

*These authors contributed equally to the manuscript

D. Levi B, Hyun JS, Montoro D, Lo DD, Chan CK, Hu S, SUn N, Lee M, Grova M, Connolly AJ, Wu JC, Gurtner GC, Weissman IL, **Wan DC***, Longaker MT*. In vivo directed differentiation of pluripotent stem cells for skeletal regeneration. ***Proceedings of the National Academy of Sciences*** 2012 Dec 11; 109(50): 20379-84. doi: 10.1073/pnas.1218052109. PMID: 23169671
*These authors share co-correspondence

E. McArdle A, Chung MT, Chan K, Paik KJ, Rennert R, Walmsley GG, Senarath-Yapa K, Hu M, Seo E, Duldulao C, **Wan DC***, Longaker MT*. Positive Selection for Bone Morphogenetic Protein Receptor Type-IB (BMPR-IB) promotes Differentiation and Specification of Adipose-Derived Stromal Cells towards an Osteogenic Lineage. ***Tissue Engineering, Part A*** 2014 May 22. [Epub ahead of print] PMID: 24854876
*These authors share co-correspondence

2. A second area of research focus has been with fat grafting and soft tissue reconstruction. This includes both clinical studies and basic science research. My laboratory has established a novel mouse model for studying human fat grafting, employing the scalp of immunocompromised mice as a recipient site. I have shown that use of this site facilitates interrogation of fat grafts with microCT, allowing real-time measurement of volume retention without having to sacrifice the mouse. In addition, this model has allowed for repeated measurements of the same fat graft over time. This model has now facilitated multiple other studies on human fat grafting, including demonstration of negative effects of shear on fat graft survival. The role stromal cell supplementation plays in fat graft survival has also been studied using the mouse scalp recipient site, and we have begun to define the number of cells necessary and how these cells augment volume retention. Finally, we adapted our novel model to study radiation injury and the regenerative effects of fat grafting. These findings have important implications on development of future technologies for soft tissue reconstruction and treatment of irradiated defects.

A. Tanna N, **Wan DC**, Kawamoto HK, Bradley JP. Craniofacial microsomia soft tissue reconstruction comparison: inframmary extended circumflex flap versus serial fat grafting. ***Plastic and Reconstructive Sugery.*** 2011 Feb; 127(2): 802-11. doi: 10.1097/PRS.0b013e318fed6e4. PMID: 21285784

B. Chung MT, Hyun JS, Lo DD, Montoro DT, Hasegawa M, Levi B, Januszyk M, Longaker MT, **Wan DC**. Micro-CT Evaluation of Human Fat Grafts in Nude Mice. ***Tissue Engineering, Part C*** 2013 Mar; 19(3): 227-32. doi: 10.1089/ten.TEC.2012.0371. PMID: 22916732

C. Atashroo D, Raphel J, Chung MT, Paik KJ, Parisi-Amon A, McArdle A, Senarath-Yapa K, Zielins E, Tevlin R, Duldulao C, Liautaud O, Momeni A, Domecus B, Rimsa J, Gurtner GC, Longaker MT, **Wan DC**. Studies in Fat Grafting: Part II. Effects of Injection Technique on Material Properties of Fat. ***Plastic and Reconstructive Surgery,*** 2014 Jul; 134(1): 39-46. PMID: 25028817

D. Garza RM, Paik KJ, Chung MT, Duscher D, Gurtner GC, Longaker MT, **Wan DC**. Studies in Fat Grafting: Part III. Fat grafting irradiated tissue: Improved skin quality and decreased fat graft retention. ***Plastic and Reconstructive Surgery*** 2014 Aug; 134(2): 249-57. doi: 10.1097/PRS.0000000000000326 PMID: 25068325

E. Garza RM, Rennert RC, Paik KJ, Atashroo D, Chung MT, Duscher D, Januszyk M, Gurtner GC, Longaker MT, **Wan DC**. Studies in Fat Grafting: Part IV. Adipose-derived stromal cell gene expression in cell-assisted lipotransfer. ***Plastic and Reconstructive Surgery.*** [Epub ahead of print] PMID: 25502860

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1ZyBjoyUhZAQN/collections/49156105/public/

D. Research Support

Ongoing Research Support

California Institute of Regenerative Medicine Yang (PI) 4/1/2015-3/31/19
"Injectable Macroporous Matrices to Enhance Stem Cell Engraftment and Survival"
The major goals of this project are to incorporate macroporous matrices to promote survival of implanted cells and augment fat graft volume retention.

Role: Co-PI

K08 DE024267 Wan (PI)
7/02/14 - 6/30/19
NIH/NIDCR
"Defining BMP-Responsive lncRNA for Bone Regeneration"
The major goals of this project are to identify lncRNA involved in regulating transition from pluripotency to osteogenic differentiation and investigate how these may be manipulated to promote bone formation.
Role: PI

Stanford CHRI Wan (PI)
9/01/13 - 8/31/18
"Developing an effective cell-based approach for bone regeneration in children through epigenetic manipulation"
The major goals of this project are to manipulate expression of pro-osteogenic non-coding RNA to enhance bone engineering strategies for facial defects.
Role: PI

Completed Research Support

ACS Franklin H. Martin Faculty Research Fellowship Wan (PI) 7/01/12 - 6/30/14
"Epigenetic Regulation of Adipose-Derived Stromal Cell Differentiation"
The major goals of this project are to investigate epigenetic mechanisms involved in determining adipose-derived stromal cell osteogenesis and adipogenesis.
Role: PI

OCAC Elite Scholarship Wan (PI) 10/01/10 - 6/30/11
“Defining ASC HOX Profiles for Tissue Engineering”
The major goals of this project were to investigate specific HOX transcription factor profiles which favored osteogenic differentiation of ASCs.
Role: PI

Ethicon-SUS Research Fellowship Wan (PI) 7/01/05 - 6/30/07
“Assessment of the Role of Noggin in Cranial Suture Development”
The major goals of this project were to investigate the role of Noggin on the differentiation capacity of osteoblasts and subsequent calvarial suture development.
Role: PI

R01 DE14526 Longaker (PI) 4/01/02 - 3/31/07
NIH/NIDCR
“Cellular and Molecular Mechanisms of Calvarial Healing”
The major goals of this project were to investigate the mechanisms by which immature, but not mature, dura mater is capable of re-engineering calvarial defects.
Role: Co-Investigator

OTHER SUPPORT

Provide active support for all key personnel. Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts and/or institutional awards. Training awards, prizes, or gifts do not need to be included.

NAME OF INDIVIDUAL

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

BLACKSHEAR, C.P.

ACTIVE

Stanford Child Health Research Institute Grant 7/1/2015 – 6/30/2016 5.0 calendar
$50,000

The major goals of this project are to investigate methods for improving autologous fat graft retention in correcting congenital or acquired soft tissue defects in the pediatric population.

OVERLAP

There is scientific overlap between the aims of this grant and of the application under consideration. If both are funded, the budgets will be adjusted appropriately in conjunction with agency staff.

OTHER SUPPORT

Provide active support for all key personnel. Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts and/or institutional awards. Training awards, prizes, or gifts do not need to be included.

NAME OF INDIVIDUAL

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Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

Wan, D.C.

Active

5K08DE02426902 (Wan, Derrick Check - Project 112020) 07/02/2014-06/30/2019 9.00 calendar

National Institutes of Health $117,125

Defining BMP-responsive lncRNA for bone regeneration

Major Goals: The major goals of this project are to identify lncRNA involved in regulating transition from pluripotency to osteogenic differentiation and investigate how these may be manipulated to promote bone formation.

RT3-07804 (Yang, Fan - Project 117009) 07/01/2015-06/30/2018 1.20 calendar

California Institute for Regenerative Medicine $403,530

Injectable Macroporous Matrices to Enhance Stem Cell Engraftment and Survival

Major Goals: The major goals of this project are to incorporate macroporous matrices to promote survival of implante cells and augment fat graft volume retention.

Overlap

None

Longaker, M.T.

Active

Project 119128 09/01/2015-08/31/2020 2.40 calendar

National Institutes of Health $250,000

Optimizing Fat Grafting for Soft Tissue Reconstruction

Major Goals: The major goals of this project are to investigate techniques for fat grafting that increase retention

Overlap

None

RESOURCES

Identify the facilities to be used as listed below. If appropriate, indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Describe only those resources that are directly applicable to the proposed work. Use continuation pages if necessary.

Laboratory:

Research will be conducted primarily at the Hagey Laboratory for Pediatric Regenerative Medicine, a full-featured basic science laboratory housing the combined labs of Drs. Longaker and Wan. Additional studies and collaboration will take place at the adjacent Lokey Stem Cell Institute and the Center for Clinical Sciences. All facilities are in immediate proximity to each other and offer 24-hour access.

Clinical:

The Plastic Surgery Center is a private practice, outpatient surgery center located in Palo Alto. PSC has an established history with the Hagey Lab, and offers adipose tissue collected from their reconstructive procedures contingent on patient consent.

Animal:

24-hour access to research animals and veterinary staff is provided at the Stanford Comparative Medicine Building, Research Animal Facility and the Veterinary Service Center. Around-the-clock animal imaging facilities are offered at the Clark Center. All facilities are in the immediate vicinity of the Hagey Laboratory.

Computer:

All research facilities provide numerous, shared internet enabled Macintosh and Windows workstations. At the Clark Center animal imaging facility, computers linked to the image storage mainframe and running professional radiology software enable three-dimensional reconstruction and post hoc analysis.

Office:

Administrative offices are on-site at the Hagey Building

Other:

Scientific Environment: Describe how the scientific environment in which the research will be done contributes to the probability of success (eg, institutional support, physical resources, and intellectual rapport). In describing the scientific environment in which the work will be done, discuss ways in which the proposed studies will benefit from unique features of the scientific environment or subject populations or will employ useful collaborative arrangements.

The combined experience of the primary investigators, postdoctoral scholars, graduate students and laboratory technicians at the Hagey Laboratory will be the most integral factor in the success of this project. I have the full support of my colleagues as I advance through this research, and as I transition to my role as independent investigator. Moreover, this considerable human resource will be augmented by collaborators in our sister laboratories at the Lokey Stem Cell Institute and the Center for Clinical Sciences, whom we've established long histories of good rapport with. The full range of state-of-the art basic science equipment is available 24-hours a day.

Early Stage Investigators ONLY: Describe institutional investment in the success of the investigator, eg, resources for classes, travel, training; collegial support such as career enrichment programs, assistance and guidance in the supervision of trainees involved with the ESIs project, and availability of organized peer groups; logistical support such as administrative management and oversight and best practices training; and financial support such as protected time for research with salary support.

Respective Contributions

The research training plan was conceived, reviewed and edited in conjunction with my primary mentor, Derrick C. Wan, M.D. As this is an iterative process, we meet daily to refine this research and to review preliminary results as they become available. This feedback is also provided on a scheduled basis twice weekly, during group lab meeting as well as during one-on-one sessions. Dr. Wan empowers me to make decisions regarding the overarching direction of the project, the conduct of the research and the management of the logistics. As necessary, he intervenes to provide guidance and an experienced viewpoint. As we have done in other projects, we will work closely to accomplish the aims of this research, from defining the overall strategy to one-on-one benchside instruction on basic science technique.

Selection of Sponsor & Institution

As a physician who aspires to a career in academic surgery, specifically pediatric craniofacial surgery, I was immediately drawn to the Hagey Laboratory for Pediatric Regenerative Medicine. I was already familiar with Drs. Longaker and Wan from their extensive published body of work. When the time came for selecting a research laboratory, joining the Longaker/Wan group seemed an ideal fit for the reconstructive surgery research I hoped to accomplish, as well as for the career mentoring they could provide. I was excited to be accepted and to start in the laboratory Summer of 2015.

This proposal perfectly leverages two of the core strengths of the laboratory – stem cell research and investigations into improving cell assisted lipotransfer to correct soft tissue defects. Moreover, the resources provided by Stanford are unparalleled, from research and postdoctoral support to career counseling. At Stanford I've found a nurturing environment with abundant human and material resources to help catalyze my success with this project, and to further my goals of a career in academic surgery.

Goals for Fellowship Training and Career

My goal is to pursue a career in academic Plastic Surgery, specifically in the field of craniofacial reconstruction. It has also been a lifelong goal of mine to critically investigate facets of the physical world as a research scientist. Fortunately, surgery residency and research at Stanford University have afforded me opportunities to pursue both tracks.

In my dedicated lab research years, I hope to further develop as a physician-scientist and independent investigator. I will refine the skills necessary to ask critical questions, to formulate hypotheses, to design studies that test these hypotheses and to critically analyze the results. Additionally, I will develop the skill set of a basic scientist and will become well versed in the techniques of bench research and bioinformatics.

I will contribute positively to the field of Plastic Surgery research by writing manuscripts for peer reviewed journals and by presenting my research at professional conferences. In addition to being intellectually gratifying, my research experience also will help me to better understand how advances in basic science inform and shape our clinical practice.

This proposal addresses a fundamental technique of reconstruction, autologous fat grafting, and endeavors to improve on the process through enrichment with targeted stromal cells. This research has the potential to revolutionize our employment of this bedrock procedure. The pursuit of answers will undoubtedly advance my maturation to surgeon-scientist.

Activities Planned

I am authorized by my residency program to dedicate 100% of my time to research, with no clinical obligations. Thus, nearly the entirety of my effort will be directed towards the research proposed. This will be augmented by twice weekly lab meetings, the first with my primary mentor, Dr. Derrick C. Wan, and the second held by my co-mentor and collaborator, Dr. Michael T. Longaker. Additionally, Dr. Wan meets with his researchers separately on a weekly basis for more in depth guidance and counseling.

Learning will be supplemented by formal didactics in the form of weekly Plastic Surgery and General Surgery grand rounds. Pertinent literature is reviewed twice monthly at the Stanford Stem Cell Institute journal club and the Wan Lab journal review.

Moreover, I will avail myself of the myriad medical, research and career planning lectures offered by Stanford University, particularly those coordinated by the Office for Postdoctoral Affairs for new investigators. I will attend the national surgery conferences as time and laboratory finances will allow.

The postdoctoral fellows of the Hagey Laboratory have jointly constructed a course syllabus in order to promote ongoing education and mutual support:

Course Title	Course Description
STEMREM 200: Stem Cell Intensive	Course designed to learn about methods of tissue culture, mouse embryo fibroblast preparation, embryonic stem and induced pluripotent stem cell culture, differentiation, DNA isolation, PCR, sequencing and basic microscopy.
STEMREM 201A: Stem Cells and Human Development	Didactic course designed to learn about developmental biology, pluripotent stem cells, cell sorting, genomics, bioinformatics, imaging.
STEMREM 201B: Stem Cells and Human Development Laboratory	Laboratory course designed to provide hands-on skills working with stem cells, cell sorting, imaging.
STEMREM 202: Stem Cells and Translational Medicine	Course designed to teach fundamentals of stem cell biology and regenerative medicine, focusing on well-studied system of hematopoiesis, molecular pathways of pluripotency and tissue-specific stem cells and aging as related to stem cell dynamics.
STEMREM 250: Regenerative Medicine Seminar Series	Designed as a forum for Stanford researchers to meet, hear about what is going on in Stem Cell Biology and Regenerative Medicine at Stanford, and spark collaborations. Topics include all areas of regenerative medicine, broadly defined, ranging from fundamental biological principles and basic science advances to novel applications in biotechnology, stem cell biology, and human disease.
MED 255: Responsible Conduct of Research	Course designed to engage participants in case-based discussions of ethical issues commonly encountered in, and raised by, current biomedical research, and introduce participants to methods of analysis of ethical issues and policies relevant to the conduct of research.
BIOS 243: Grant Writing Academy	Overview of the fundamentals for writing competitive NIH Kirschtein NRSA fellowships (F31, F32) and K Awards. Topics include developing specific aims; outlining research and career development plans; and reviewing scientific writing.

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DEPARTMENT OF SURGERY

Derrick C. Wan, MD
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Endowed Faculty Scholar, CHRI
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Stanford, CA 94305-5148

Phone: (650) 556-5514
Fax: (650) 736-1705
E-mail: dwan@stanford.edu

November 24, 2015

To Whom It May Concern:

Please find below a detailed outline of the proposed mentorship plan for Dr. Charles Blackshear, a Postdoctoral Research Fellow in my laboratory. I strongly support his work and application for the Plastic Surgery Foundation Research Fellowship Grant, and am uniquely positioned to mentor him in this venture. I am excited for Dr. Blackshear to begin work on this project, and believe that it will produce significant gains in the fields of reconstructive surgery and autologous fat grafting.

The ultimate goal of this project is to improve retention of autologous fat grafts. Autologous fat transfer is a reconstructive procedure designed to treat soft tissue volume loss secondary to congenital (i.e. Treacher Collins, Pierre Robin), or acquired (i.e. cancer resection, irradiation) defects. However, its widespread clinical use has been limited by unpredictable outcomes. Research has led to the development of cell-assisted lipotransfer (CAL), a procedure in which fat is enriched with adipose-derived stromal cells (ASCs) before injection. Studies demonstrate improved graft survival when CAL is employed, primarily through accelerated graft revascularization. The central hypothesis of this project asserts that enrichment with ASCs promotes revascularization of transplanted fat, resulting in increased volume retention. This may be further enhanced through selection of cell subgroups with the greatest potential to augment fat graft survival. This project aims to identify these subpopulations of ASCs with the greatest ability to augment graft retention. And by promoting fat graft survival, a strategy may be developed to better address the damaging physical and psychosocial impact of congenital or acquired soft tissue defects and significantly reduce the operative burden of treating these conditions.

A. Research Support Available

Source	Title	PI	Dates	Amount
NIH	5K08DE02426902 Project 112020 "Defining BMP-responsive lncRNA for bone regeneration"	Wan	07/02/2014 - 06/30/2019	$117,125
NIH	Project 119128 "Optimizing Fat Grafting for Soft Tissue Reconstruction"	Longaker	09/01/2015 - 08/31/2020	$250,000

B. Previous Trainees

Kshemendra Senarath-Yapa, M.B.B.S.
Resident Surgeon
Northwestern Deanery UK - Department of Plastic and Reconstructive Surgery, Magdalene College Cambridge

David Atashroo, M.D.
Co-founder, Copilots in Care

Graham G. Walmsley
Ph.D. Student in Stem Cell Biology and Regenerative Medicine
M.D. candidate, Stanford School of Medicine (expected graduation Spring 2016)

C. Training Plan, Environment, Research Facilities

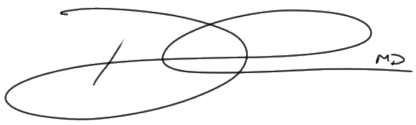
In my laboratory, I work one-on-one with my postdocs on a daily basis, and empower them to make important decisions regarding the conduct and strategic direction of their chosen research. I conduct regularly scheduled group laboratory meetings weekly, and hold a second one-on-one meeting with my trainees later in the week to provide more personalized guidance. Aside from this, Charles will attend monthly building-wide meetings where presentations are made by select postdoctoral fellows in each laboratory in the Hagey building. In the second year of his fellowship, it will be expected that Charles will present at this meeting, where his work will be discussed and data are reviewed and critiqued. Finally, Charles will attend and participate in the Hagey Laboratory journal club, where pertinent articles in the literature are discussed each month.

The facilities at Stanford available to Dr. Blackshear to aid him in his project are second to none and will provide him all the necessary tools to produce world-class research. Dr. Blackshear will have full access to the Hagey Center for Pediatric Regenerative Medicine, the Lokey Stem Cell Institute and the Clark Center for Small Animal Imaging. Furthermore, I will provide any support that he needs beyond what is covered by the provisions of this grant. Dr. Blackshear undoubtedly will exceed all his educational requirements and become a capable independent researcher before he leaves my lab.

During his time in the lab, Dr. Blackshear will have the opportunity to collaborate with top tier researchers across the surgical disciplines, in engineering, and in stem cell biology. He will stay grounded in the basic sciences and maintain his surgical acumen through participation in weekly didactic sessions, through an elective coursework schedule developed by the Hagey postdoctoral research fellows and through attendance and participation at local and national conferences.

D. Applicant's Qualifications and Potential for a Research Career
I am confident that Dr. Blackshear will achieve his goal of becoming a foremost surgeon-scientist as a craniofacial surgeon. His clinical background and operative experience with multiple techniques of adipose aspiration and autologous grafting, make him ideally suited to conduct research into improving this paradigm. Furthermore, Charlie has been a diligent and productive member of every lab he's participated in, as reflected by the high praise given by his former Primary Investigators. Already Dr. Blackshear has demonstrated incredible flexibility and capacity to adapt and learn since starting in my lab last Summer. Finally, Charlie has the direct support of myself, Dr. Longaker and of the entire staff of the Hagey Laboratory as he continues his fellowship. All of these factors portend well for this project and for his time in the lab. This research offers Dr. Blackshear the opportunity to grow personally and professionally, and the completion of it is certain to have a major impact on the treatment of patients with congenital or acquired soft tissue defects.

Sincerely,



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MICHAEL T. LONGAKER, M.D., M.B.A., FACS
Deane P. and Louise Mitchell Professor

Director, Children's Surgical Research
Director, Program in Regenerative Medicine
Deputy Director, Institute for Stem Cell Biology & Regenerative Medicine
Director of Research, Division of Plastic and Reconstructive Surgery
Professor, by Courtesy, Department of Bioengineering

November 30, 2015

RE: Charles P. Blackshear, M.D.
Postdoctoral Scholar
Hagey Laboratory for Pediatric Regenerative Medicine

Dear Selection Committee:

It is my pleasure to write this letter of recommendation for my mentee and collaborator, Charles Blackshear, in strongest support of his application for The Plastic Surgery Foundation Research Fellowship grant. Charlie joined the lab I jointly run with Dr. Derrick Wan in July 2015 after completing two years of General Surgery residency at the Cleveland Clinic Foundation. Under my direction at the Hagey Laboratory for Pediatric Regenerative Medicine at Stanford University, we will cultivate his aspirations of becoming a surgeon-scientist in pediatric craniofacial surgery.

Charlie is a disciplined, motivated, and dedicated physician with excellent potential as a surgeon. His research interests in autologous fat grafting, wound healing, craniofacial biology and the development of new stem cell-based therapies to enhance reconstructive surgical procedures are in keeping with his career goals and the expertise of my laboratory. I meet with Charlie weekly, and use this time to ascertain his progress, provide critical guidance on his projects, and to assist with career planning. He will have the full support of the experienced personnel at Hagey Laboratory, as well as the considerable human and materials resources provided by the adjacent Lokey Stem Cell Research Building.

The goal for his proposed research is to improve autologous fat graft retention by identifying subpopulations of stromal cells that, when used to supplement grafts, improve their survival. The fruits of this research will revolutionize the way we reconstruct disfiguring soft tissue deficits caused by congenital disorders such as Treacher Collins Syndrome, or those acquired from cancer resection or trauma.

Charlie has a well-established reputation as an industrious, hard-working and talented individual and is an extremely attractive candidate for The Plastic Surgery Foundation fellowship. I have the highest expectations for his time here at Stanford and look forward to further honing his potential. I therefore offer my strongest possible recommendation, without reservation, for his grant application.

Sincerely,

Michael T. Longaker, M.D., M.B.A.
Deane P. and Louise Mitchell Professor and Vice Chair
Department of Surgery
Co-Director, Stanford Institute for Stem Cell Biology and Regenerative Medicine
Professor, by Courtesy, Department of Bioengineering
Professor, by Courtesy, Department of Materials Science and Engineering

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Fax: (650) 736-1705
E-mail: dwan@stanford.edu

November 24, 2015

To Whom It May Concern:

Please find below a detailed outline of the proposed mentorship plan for Dr. Charles Blackshear, a Postdoctoral Research Fellow in my laboratory. I strongly support his work and application for the Plastic Surgery Foundation Research Fellowship Grant, and am uniquely positioned to mentor him in this venture. I am excited for Dr. Blackshear to begin work on this project, and believe that it will produce significant gains in the fields of reconstructive surgery and autologous fat grafting.

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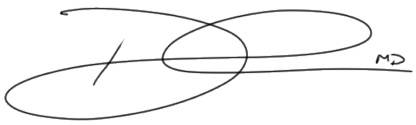
During his time in the lab, Dr. Blackshear will have the opportunity to collaborate with top tier researchers across the surgical disciplines, in engineering, and in stem cell biology. He will stay grounded in the basic sciences and maintain his surgical acumen through participation in weekly didactic sessions, through an elective coursework schedule developed by the Hagey postdoctoral research fellows and through attendance and participation at local and national conferences.

D. Applicant's Qualifications and Potential for a Research Career
I am confident that Dr. Blackshear will achieve his goal of becoming a foremost surgeon-scientist as a craniofacial surgeon. His clinical background and operative experience with multiple techniques of adipose aspiration and autologous grafting, make him ideally suited to conduct research into improving this paradigm. Furthermore, Charlie has been a diligent and productive member of every lab he's participated in, as reflected by the high praise given by his former Primary Investigators. Already Dr. Blackshear has demonstrated incredible flexibility and capacity to adapt and learn since starting in my lab last Summer. Finally, Charlie has the direct support of myself, Dr. Longaker and of the entire staff of the Hagey Laboratory as he continues his fellowship. All of these factors portend well for this project and for his time in the lab. This research offers Dr. Blackshear the opportunity to grow personally and professionally, and the completion of it is certain to have a major impact on the treatment of patients with congenital or acquired soft tissue defects.

Sincerely,



Derrick C. Wan, MD
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MICHAEL T. LONGAKER, M.D., M.B.A., FACS
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Director, Children's Surgical Research
Director, Program in Regenerative Medicine
Deputy Director, Institute for Stem Cell Biology & Regenerative Medicine
Director of Research, Division of Plastic and Reconstructive Surgery
Professor, by Courtesy, Department of Bioengineering

December 1, 2015

Plastic Surgery Foundation
444 East Algonquin Road
Arlington Heights, Illinois 60005

RE: Charles P. Blackshear, M.D.
Applicant: PSF Research Fellowship Grant

Dear Members of the Grant Committee:

This letter is written in most enthusiastic support for Dr. Charles Blackshear, who is applying for the PSF Research Fellowship Grant. A description of my laboratory, my past track record of mentoring trainees, the scientific environment of Stanford University, and his proposed research project are all included herein, along with the enclosed application.

I wish to emphasize that Dr. Blackshear is performing full-time research in my laboratory here at Stanford University, after arriving in July of 2015, and will spend a minimum of two years in the lab. Let me reassure the committee that he is currently, and will continue to be, a full-time research fellow in my laboratory here at Stanford University.

Also, I would like to state that Charles is an outstanding applicant who, without a doubt, will continue to acquire the necessary research tools and skills to become a highly effective plastic surgeon/scientist.

Sincerely,

Michael T. Longaker, M.D., M.B.A.
Deane P. and Louise Mitchell Professor and Vice Chair
Department of Surgery
Co-Director, Stanford Institute for Stem Cell Biology and Regenerative Medicine
Professor, by Courtesy, Department of Bioengineering
Professor, by Courtesy, Department of Materials Science and Engineering

1. Specific Aims

Autologous fat transfer is a widely used procedure for soft tissue reconstruction/augmentation to treat patients with volume loss secondary to disease, trauma, congenital defects, or through the natural process of aging.[1] While first described in the late 19th century, it was not until the last two and a half decades that efforts have been made to introduce standardized, reproducible techniques for both small volume applications in the face and larger volume applications in the trunk and extremities.[2-7] Fat grafting has also increasingly become recognized to possess a regenerative aspect, with the potential to improve tissue abnormalities at recipient sites, as in the case of irradiated skin and open chronic wounds.[8-12] In these scarred, ischemic settings, clinicians have noted improved color/quality of skin or decreased wound depth and erythema along with accelerated closure.[8, 12, 13]

Despite the expanding utility of fat grafting, however, unpredictability in outcomes remains a significant limitation precluding widespread clinical use. Recent studies have reported volume retention to vary from 10-80%.[14, 15] While multiple strategies have been investigated to help overcome this variability, cell-assisted lipotransfer (CAL), the addition of adipose-derived stromal cells (ASCs) to fat prior to injection, has been found to enhance fat graft survival in both preclinical studies and early, small clinical reports.[16-23] How this cellular supplementation of fat results in promotion of graft survival remains poorly understood, but may be related to differentiation of added progenitor cells into adipocytes to augment volume and/or acceleration of graft revascularization to enhance survival of injected fat.[24, 25] Preliminary studies have suggested the later of these two mechanisms to be more significant.

Of equal importance is the known heterogeneity that exists among ASCs and the stromal vascular fraction (SVF) from which they are derived.[26-29] Understanding this heterogeneity and how it relates to cellular function is critical to optimize clinical outcomes with CAL. **Thus, the central hypothesis of this proposal is that supplementation of autologous fat grafts with stromal cells promotes revascularization of transplanted fat resulting in increased volume retention, and this may be further enhanced through selection of cell subgroups with the greatest potential to augment fat graft survival (Figure 1).**

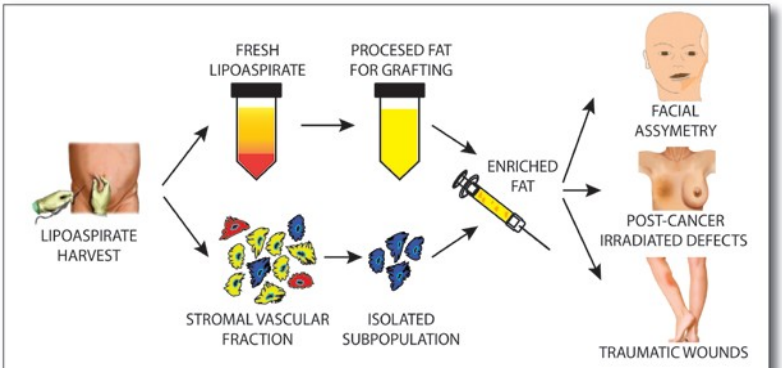

Figure 1. Supplementation of fat with SVF subpopulations can enhance soft tissue reconstruction for facial asymmetry, irradiated defects, and traumatic wounds.

Specific Aim #1: To define stromal cell function in augmentation of fat graft retention in CAL. In highly preliminary data, we have observed that stromal cells added to autologous fat grafts secrete multiple angiogenic factors, and this is associated with enhanced volume retention and vascular density within transplanted fat. In this Aim, we will expand on this finding, using freshly harvested stromal vascular fraction cells and lipoaspirate from the same patient to define how supplemental stromal cells promote graft retention in CAL. Labeled human cells will be added to processed human fat for injection in a novel *in vivo* animal model and evaluation of cellular function and outcome will be made using single-cell RNA sequencing alongside histological analyses of grafted fat.

Specific Aim #2: To determine stromal vascular fraction heterogeneity and identify novel subpopulations with the greatest ability to augment fat graft volume retention. Given significant heterogeneity within the stromal vascular fraction, we will employ single cell transcriptional analysis and correlation of adipogenic and angiogenic gene expression with surface antigen profiles to prospectively isolate novel subpopulations of stromal cells. We will then systematically evaluate these subgroups for ability to enhance fat graft retention with CAL.

2. Research Strategy

A. Significance

Medical Burden of Soft Tissue Defects

Soft tissue deficits following trauma, cancer resection, or secondary to congenital anomalies represent a reconstructive challenge with substantial medical burden.[31] Over 5.6 million such procedures are performed annually in the United States, with the majority related to tumor extirpation and sequelae of adjuvant radiation therapy.[32] Even with intact overlying epithelium, insufficient underlying soft tissue results in visible asymmetry and contour abnormalities, and may also contribute to unstable wounds and inadequate protection of critical organs and structures including bone, implanted hardware, and large vessels.[33-35] Current approaches to address soft tissue defects include local pedicled tissue transfer, microsurgical free-tissue transfer, and synthetic dermal substitutes and/or injectable fillers.[31, 36] However, with a goal of maximizing healing, function, appearance, and patient satisfaction while minimizing donor site morbidity, many of these strategies have proven insufficient or impractical, particularly following cancer resection and radiation where local tissues become fibrotic and hypovascular and vessel depletion limits microvascular reconstructive options.[37-39]

Fat Grafting and Its Limitations

Autologous fat grafting has emerged over the past two decades as an alternative strategy to manage soft tissue defects. Liposuction is associated with minimal trauma, and indications for transfer of fat have expanded beyond treatment of small contour irregularities to larger tissue deficits associated with trauma, cancer resection, or congenital malformations.[7, 34, 40-45] However, clinical outcomes have varied widely, and reported retention of transferred fat has been inconsistent, ranging from 10% to 80%.[14, 15, 46] Avascular fat grafts rely on nutritional diffusion until vascularization from the recipient bed occurs, and early cell death within the central zone results in volume loss and histologic fibrosis and cyst development.[47-49] Fat grafting remains highly unpredictable, necessitating repeated procedures and posing a significant challenge to widespread clinical use.[40]

Cell-Assisted Lipotransfer and Heterogeneity of Stromal Vascular Fraction Cells

To address variability in fat graft retention, enrichment of fat prior to injection with cells from the SVF has been promoted.[21-23] This approach is based on the relative paucity of ASCs found in lipoaspirated fat relative to excised whole fat, which may contribute to long-term atrophy of fat grafts.[16, 54] Furthermore, ASCs within the SVF are capable of adipogenic differentiation and have been shown to secrete angiogenic factors and participate in new vessel formation when placed into ischemic environments.[55-61] Both preclinical and clinical studies have reported enhanced fat graft retention when enriched with ASCs.[16, 20-23] How cellular supplementation promotes graft survival remains largely unknown, but proposed roles have included differentiation into adipocytes and contribution to adipose tissue, endothelial cell formation and release of angiogenic growth factors in response to hypoxia.[16] Determining stromal cell function in enriched fat grafts would help to minimize variability in clinical outcomes.

Heterogeneity among stromal cells also poses a significant challenge to reproducible outcomes with CAL. Subpopulations of ASCs within the SVF have been documented with disparate biologic activity and potentials for lineage differentiation.[26-29, 62] Fully defining this heterogeneity through analyses described in this proposal will guide selection of novel subpopulations within the SVF with the greatest capacity to assist with fat graft retention. This would translate to accelerated recovery and reduced need for secondary fat graft procedures.

B. Innovation

Translational Goals

Our ultimate translational goal is to improve fat grafting outcomes for reconstruction of soft tissue defects. Enrichment of fat with ASCs has already shown promise for enhancing volume retention, and understanding how cellular supplementation results in this observation would facilitate development of safe and reproducible strategies for restoration of tissue deficits. Furthermore, supplementing lipoaspirate with subpopulations of SVF cells isolated using a clinically approved FACS machine, and reinjection of this enriched fat within the operating theater could yield a more effective approach for treating complex soft tissue defects. Importantly, use of freshly harvested SVF cells, as opposed to *ex vivo* cultured ASCs, facilitates translation of this strategy.

Fat Grafting Model

We have pioneered the novel mouse scalp recipient site model for the study of human fat grafting. The

absence of natural subcutaneous fat in this area allows for unambiguous delineation of injected fat.[15, 30, 51] Furthermore, repeated volumetric analysis of grafted fat can be performed over time by microCT without sacrificing the animal. Calculated volumes with this approach have shown very close correlation with actual measurement of explanted samples.[15]

Single-Cell RNA Sequencing

The heterogeneity of SVF cells does not allow for precise, unambiguous determination of cellular activity following implantation with fat using bulk population sequencing methods.[70] Development of targeted microfluidic single-cell transcriptional analysis has begun to yield meaningful information regarding biologic function of individual cells, and this has recently been employed to evaluate SVF cells used to enrich fat.[26, 71] Technological advancements in high-throughput sequencing and single-cell manipulation have since allowed for more complete analysis of transcriptional activity through single-cell RNA sequencing, facilitating annotation of individual cell function through gene ontology and pathway enrichment analysis.[72] We have already applied this novel technique to demonstrate autocrine/paracrine signaling among mouse skeletal stem cells to positively regulate their own expansion.[73] Use of single-cell RNA sequencing to evaluate individual stromal cell transcriptional activity in CAL would enable dissection through SVF heterogeneity and allow for better definition of how these cells enhance fat graft retention. This novel strategy will be pursued in Specific Aim #1 of this application.

C. Approach

Specific Aim #1: To define stromal cell function in augmentation of fat graft retention in CAL.

Rationale: Supplementation of lipoaspirate with stromal cells has been shown to augment fat graft retention. How this enrichment results in enhanced outcomes remains poorly understood, but may be secondary to contribution of progenitor cells to the mature adipocyte population or by promoting graft revascularization. Preliminary studies have begun to evaluate transcriptional activity of isolated SVF cells following implantation with fat, and this strategy will be used to interrogate stromal cell activity in CAL. Single- cell RNA sequencing, in concert with histological and mechanical analyses of grafted fat, will allow for definition of how SVF cell supplementation augments volume retention in grafted fat.

Hypothesis: Addition of stromal cells to enrich fat grafts enhances retention through release of growth factors promoting revascularization of transplanted fat.

Preliminary data in support of Specific Aim #1:

We investigated the mouse scalp as a recipient site for fat grafting.[15] Human lipoaspirate samples were centrifuged to remove fluid and cellular debris, and 200 µl of processed fat was injected beneath the scalp of Crl:NU-*Foxn1*^{nu} mice (**Figure 2A**). MicroCT scans were performed over twelve weeks. The relative absence of natural subcutaneous fat allowed for accurate identification of injected fat (**Figure 2B**). Cubic-spline interpolation was used to reconstruct a 3-dimensional surface for volume calculations (**Figure 2B**). Individual fat graft volume was found to decrease over time, stabilizing by eight weeks (**Figure 2C**), and calculated volume measurements were found to closely correlate with measured volumes on explanted samples (**Figure 2D**). Finally, histologic confirmation of retained human fat was obtained by staining for human specific cannabinoid receptor 1 (**Figure 2E**). These data demonstrate the utility of the mouse scalp as a recipient site and validate microCT as an accurate tool for real-time volume assessment of fat grafts.

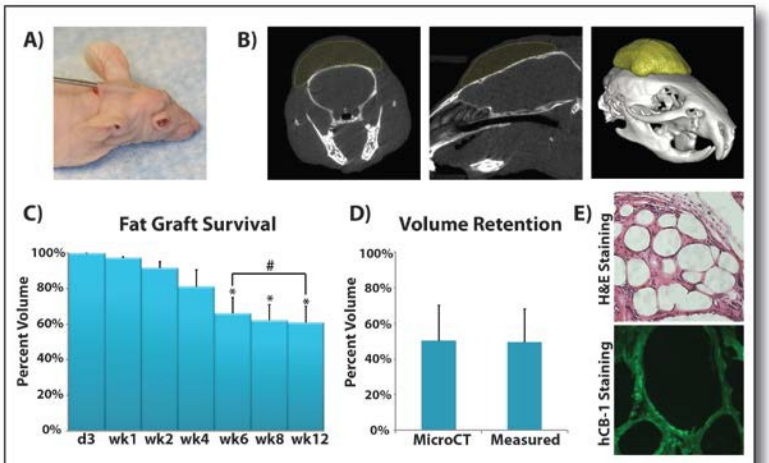

Figure 2. Mouse scalp fat graft model. A) Photograph during fat injection beneath scalp. B) Coronal (left) and sagittal (middle) microCT images of mouse following fat grafting. 3D reconstruction of fat graft (right) based on 2D slices (fat graft in yellow). C) MicroCT revealed fat graft survival decreased over time, stabilizing by eight weeks (*$p < 0.05$; #$p > 0.05$). D) Measured volume of explanted fat grafts (right) closely correlated with microCT volumes (left). E) H&E (top) and immunofluorescent staining for human cannabinoid receptor-1 (bottom) confirmed explanted samples eight weeks after grafting contained healthy human fat.

We have also employed the mouse scalp recipient site for studies on CAL. While supplementation of fat with ASCs found in the SVF have been found to enhance volume retention, the actual number of stromal cells added to fat has varied widely in the literature, undoubtedly contributing to inconsistency in outcomes.[16, 20-23, 25, 75] A titration study was conducted to determine an optimal stromal cell concentration per 200 µl

fat graft. MicroCT scans over eight weeks post- grafting revealed significantly greater volume retention with addition of $1x10^4$ cells compared to all other groups (**Figure 3A**). This was also associated with increased vascularity, as shown by immunofluorescent staining for CD31 (**Figure 3B**).

To define the role additional stromal cells play in CAL, we transduced *ex vivo* cultured cells with copGFP lentiviral particles and a luciferase marker. Subsequent human lipoaspirate samples for fat grafting were enriched with this labeled fat.[71] Bioluminescent imaging following administration of D-Luciferin demonstrated only transient retention of supplemental cells within fat grafts, with minimal signal detected after three weeks (**Figure 4A**). Furthermore, explantation of fat grafts, collagenase digestion, and re-isolation of GFP labeled stromal cells by FACS revealed a dramatic decline in retrievable, viable stromal cells over the course of ten days (**Figure 4B**). Moreover, single-cell microfluidic transcript evaluation by qRT-PCR demonstrated increased vascular endothelial growth factor (VEGF) expression (**Figure 4C**) seen also in pooled populations (**Figure 4D**).

We have also performed RNA-sequencing of stromal cells undergoing adipogenesis to define transcriptome activity during this biological process. We found 3171 total transcripts differentially expressed, of which 2941 were coding. Visualizing data uploaded to the UCSC Genome Browser, we confirmed both peroxisome proliferator-activated receptor gamma (PPAR-γ) and fatty acid binding protein 4 (FABP4), genes involved in adipogenesis, increased over time. Finally, GO-term analysis revealed many of the upregulated transcripts to be involved in fat related biological processes such as lipid biosynthesis, regulation of lipid storage, and fat cell differentiation.

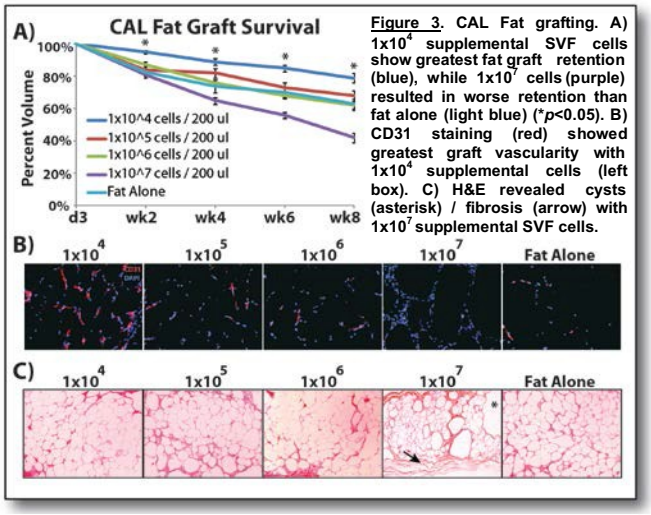

Figure 3. CAL Fat grafting. A) $1x10^4$ supplemental SVF cells show greatest fat graft retention (blue), while $1x10^7$ cells (purple) resulted in worse retention than fat alone (light blue) (*$p<0.05$). B) CD31 staining (red) showed greatest graft vascularity with $1x10^4$ supplemental cells (left box). C) H&E revealed cysts (asterisk) / fibrosis (arrow) with $1x10^7$ supplemental SVF cells.

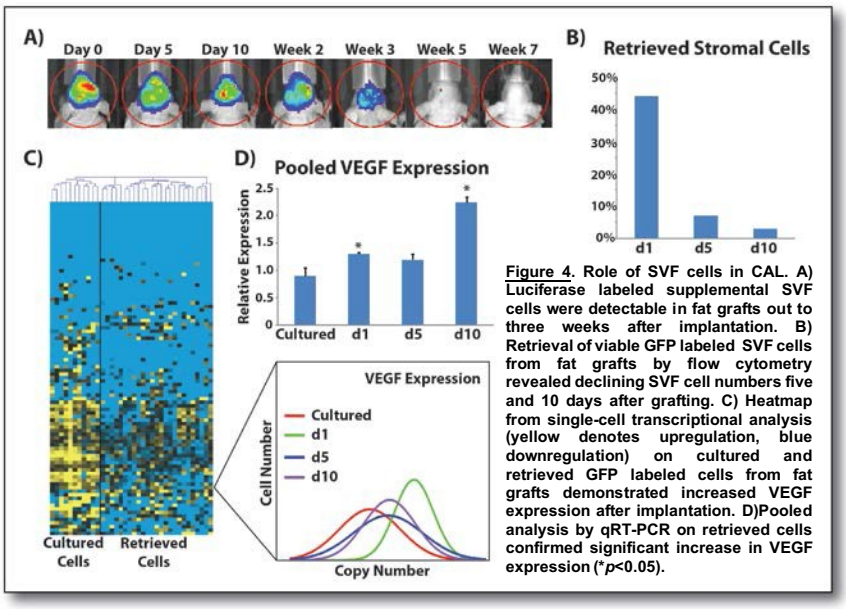

Figure 4. Role of SVF cells in CAL. A) Luciferase labeled supplemental SVF cells were detectable in fat grafts out to three weeks after implantation. B) Retrieval of viable GFP labeled SVF cells from fat grafts by flow cytometry revealed declining SVF cell numbers five and 10 days after grafting. C) Heatmap from single-cell transcriptional analysis (yellow denotes upregulation, blue downregulation) on cultured and retrieved GFP labeled cells from fat grafts demonstrated increased VEGF expression after implantation. D)Pooled analysis by qRT-PCR on retrieved cells confirmed significant increase in VEGF expression (*$p<0.05$).

Overall Experimental Design:

Processing of Lipoaspirate: Fresh lipoaspirate specimens will be obtained from 10 patients using a modified Coleman technique. For fat graft processing, samples will be washed in sterile saline and then centrifuged at 500 *g* for 5 minutes. The oil supernatant and infranatant/cell pellet will be removed to isolate injectable fat. SVF cells will be harvested by digesting lipoaspirate with type II collagenase.[76] SVF cells will be pelleted and then resuspended in Dulbecco's Modified Eagle Medium (DMEM). Rapid labeling of cells to facilitate supplementation of fat with SVF cells from the same patient will be performed using a Qdot® 525 fluorescent probe. Labeled SVF cells will be added to isolated injectable fat at a concentration of $1x10^4$ cells / 200 µl fat.

Fat Grafting: SVF-enriched human fat grafts will be transferred to a 1cc syringe with 16-gauge needle for grafting into 60-day-old Crl:Nu-*Foxn1*^{nu} sex-mismatched mice. A subcutaneous tunnel will first be made by passing the needle antegrade beneath the scalp, and 200 µl of SVF-enriched fat (CAL) will be injected in retrograde fashion into a total of 84 mice (**Table 1**).[15]

Single-Cell RNA Sequencing: Bioluminescent imaging in our preliminary data demonstrated detectable luciferase-labeled stromal cells at the site of injection up to three weeks. Therefore, six animals will be sacrificed at 1, 3, 5, 7, 10, 14 and 21 days following grafting for re-isolation of Quantum Dot labeled SVF cells. Explanted fat grafts will be digested with collagenase type II and a FACSAria flow cytometer (Becton Dickinson, Franklin Lakes, NJ) will be used to identify labeled cells. A 96 well C1 microfluidic system (Fluidigm; South San Francisco, CA) will be used to interrogate cells within each well. Ungrafted, labeled stromal cells will also be captured in single cell wells for comparison to reisolated cells post-grafting. Cell lysis and RNA isolation will be

performed in each well followed by cDNA preparation. Single-cell cDNA size distribution and concentration will be determined on a capillary electrophoresis-based fragment analyzer (Advanced Analytical; Ames, IA). Diluted cDNA in 96-well plates will be used for library construction followed by sequencing.

Processing and Analysis of Single Cell RNA-seq Data: Raw reads will be pre-processed with sequencing-grooming tools, as previously described, [72, 73] and mapped. A minimum 2-fold difference in expression across the time course will be employed as a cut-off for analysis and data will be uploaded to the UCSC genome browser for visualization. Finally, GO-term enrichment analysis will be performed for coding genes to determine which biological processes are upregulated or downregulated in labeled SVF cells following grafting.

Table 1. Specific Aim #1 Determining SVF function in CAL

Groups	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21	Total
RNA-Seq CAL	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	42 animals
Histology CAL	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	42 animals
Histology Non-CAL	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	6 animals	42 animals
Total	18 animals	18 animals	18 animals	18 animals	18 animals	18 animals	18 animals	126 animals

Histological Analyses: Six animals receiving CAL and six animals receiving Non-CAL will be sacrificed at 1, 3, 5, 7, 10, 14, and 21 days following grafting and specimens will be explanted (**Table 1**). Fat grafts will be embedded in formalin and then fixed, dehydrated, and paraffin embedded for histological analyses via haematoxylin and eosin staining and IHC staining to evaluate adipocyte viability and the presence of endothelial cells. Comparison will be made between CAL and Non-CAL fat grafts over multiple time points, and statistical analysis of histological data will be performed on ImageJ (NIH; Bethesda, MD) quantified images.

Pitfalls and Alternative Strategies: We do not anticipate any problems with lipoaspirate harvest and processing as these are routinely performed and extensively published by our laboratory. Quantum Dot nanoparticles are resistant to chemical and metabolic degradation, have long-term photostability, and have been well described to durably label stromal cells for *in vivo* tracking.[77] However, should we encounter difficulties with this approach, alternative lipophilic fluorescent stains including PKH26, DiI, or DiO have been described for SVF labeling and may be used for subsequent cellular isolation through flow cytometry.[78] In addition, should significant membrane transfer of fluorescent dye from labeled cells also be encountered, use of lentiviral GFP transduction may be considered. Single-cell RNA sequencing has already been successfully performed by our group[73], however, if problems arise with this modality, a more targeted approach for expression analysis can also be taken through microfluidic transcript evaluation by qRT-PCR, as described in our preliminary data. Finally, fat grafts may alternatively be implanted into the mouse dorsum should we encounter difficulties with our scalp recipient site.[49, 66]

Specific Aim #2: To determine stromal vascular fraction heterogeneity and identify novel subpopulations with the greatest ability to augment fat graft volume retention.

Rationale: Significant heterogeneity exists among cells comprising the SVF, and distinct populations have been demonstrated through single-cell microfluidic transcriptional analysis and application of Akaike Information Criterion to Fuzzy C-Means algorithm derived partitions.[26, 79] These subgroups comprise functionally different subsets of stromal cells, capable of isolation through correlated surface markers, and have been shown to have implications for bone and soft tissue regenerative strategies.[26, 28, 29] Enrichment of fat grafts with a distinct population of SVF cells rather than a more heterogeneous, nondiscriminatory mix may therefore enhance the effect of supplemental cells to promote fat graft volume retention.

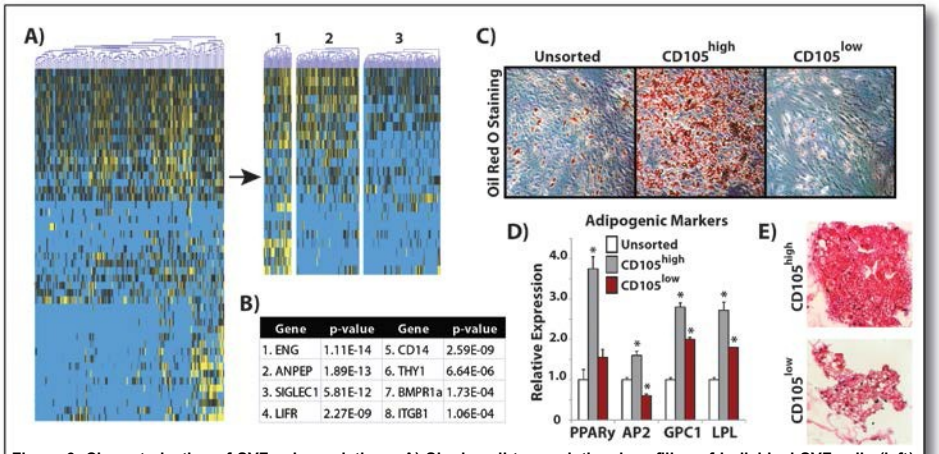

Figure 6. Characterization of SVF subpopulations. A) Single cell transcriptional profiling of individual SVF cells (left) with partitioning into gene expression clusters (right) based on Fuzzy C-Means algorithm. (yellow denotes upregulation, blue downregulation). B) Linear discriminant analysis revealed multiple surface antigens capable of cluster delineation. C) Oil Red O staining demonstrated CD105^{high} stromal cells to have more adipogenic potential (middle) compared to unsorted (left) and CD105^{low} cells (right). D) QRT-PCR for adipogenic markers PPARγ, AP2, GPC1, and LPL showed greater expression in CD105^{high} cells (gray bars) relative to unsorted (white bars) and CD105^{low} stromal cells (red bars) (*p<0.05). E) CD105^{high} cells (top) also formed more adipocytes *in vivo* than CD105^{low} (bottom) when implanted beneath the kidney capsule, as demonstrated by H&E staining.

Hypothesis: Isolation of adipogenic/angiogenic SVF cells based on cell surface markers will augment volume retention when used to enrich fat grafts.

Preliminary data in support of Specific Aim #2:

Individual cells were isolated through flow cytometry and analyzed using single cell transcriptional profiling of select osteogenic/adipogenic genes, as well as multiple cell surface markers. After applying a Fuzzy-C clustering algorithm (**Figure 6A**).[26] and linear discriminant analysis, endoglin (ENG/CD105), emerged as a cluster delineating antigen (**Figure 6B**).[26] $CD105^{high}$ stromal cells demonstrated strong adipogenic potential relative to the $CD105^{low}$ and unsorted fractions, as confirmed by significant lipid accumulation per Oil Red O staining (**Figure 6C**).[26] This was further corroborated by qRT-PCR expression analysis for markers of adipogenic differentiation (**Figure 6D**). Finally, transplantation of cells beneath the kidney capsule of immunocompromised mice resulted in greater *de novo* adipose tissue by $CD105^{high}$ stromal cells compared to the $CD105^{low}$ fraction (**Figure 6E**).

Similar analysis identified Bone Morphogenetic Protein Receptor (BMPR)-IA as another cluster defining gene (**Figure 6B**).[26] Signaling through BMPR-IA has been reported to regulate adipogenesis, and increased expression is associated with human obesity.[80, 81] $BMPR\text{-}IA^{+}$ cells were found to posses greater adipogenic potential than $BMPR\text{-}IA^{-}$ or unsorted cells (**Figure 7A**), and demonstrated enhanced expression of adipogenic markers like PPAR-γ (**Figure 7B**). This was validated *in vivo* through implantation of GFP labeled SVF cells suspended in Matrigel™ into the inguinal fat pads of immunocompromised mice. We noted greater formation of GFP^{+} adipocytes interspersed with host adipocytes by $BMPR\text{-}IA^{+}$ SVF cells compared to unsorted or $BMPR\text{-}IA^{-}$ cells (**Figure 7C**).

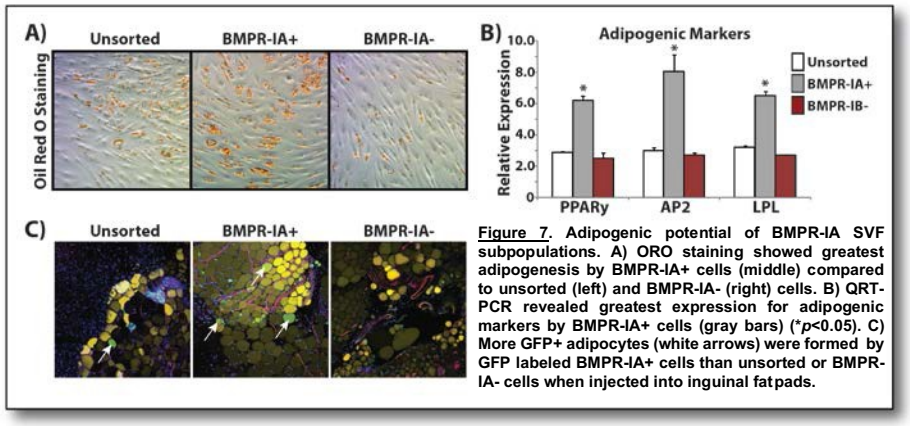

Figure 7. Adipogenic potential of BMPR-IA SVF subpopulations. A) ORO staining showed greatest adipogenesis by BMPR-IA+ cells (middle) compared to unsorted (left) and BMPR-IA- (right) cells. B) QRT-PCR revealed greatest expression for adipogenic markers by BMPR-IA+ cells (gray bars) (*p<0.05). C) More GFP+ adipocytes (white arrows) were formed by GFP labeled BMPR-IA+ cells than unsorted or BMPR-IA- cells when injected into inguinal fatpads.

Paralleling this approach, we have also employed single cell transcriptional profiling of select angiogenic genes, enabling clustering of stromal cells based on expression of VEGF, fibroblast growth factor 2 (FGF2), platelet derived growth factor receptor (PDGFR) α and β. CD248, a member of a novel C-type lectin

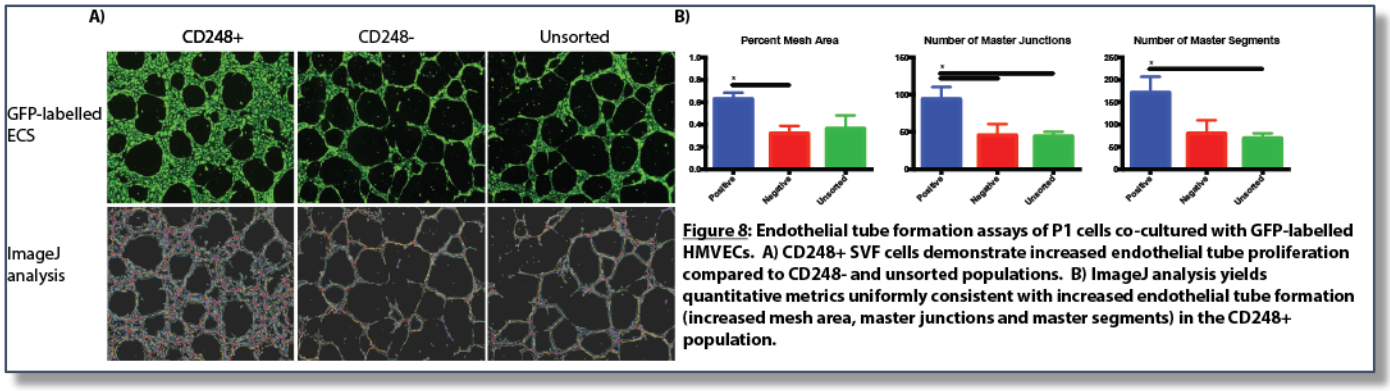

Figure 8: Endothelial tube formation assays of P1 cells co-cultured with GFP-labelled HMVECs. A) CD248+ SVF cells demonstrate increased endothelial tube proliferation compared to CD248- and unsorted populations. B) ImageJ analysis yields quantitative metrics uniformly consistent with increased endothelial tube formation (increased mesh area, master junctions and master segments) in the CD248+ population.

transmembrane receptor family, emerged as a delineating marker. CD248 has been associated with embryologic angiogenesis, post-natal vascular patterning[82-84] and regulation of PDGFRβ signaling and capillary sprouting in skeletal muscle.[85] Isolating subpopulations of SVF based on this surface marker, we noted significantly greater expression of VEGF among $CD248^{+}$ cells compared to unsorted and $CD248^{-}$ cells. Moreover, *in vitro* endothelial tube assays of CD248+ cells demonstrate increased tube proliferation compared to CD248- and unsorted populations (**Figure 8A**). This is apparent visually and is corroborated by ImageJ quantitative analysis (**Figure 8B**).

Overall Experimental Design:

Microfluidic-Based Selective Transcriptional Analysis:

An adipogenic/angiogenic genes of interest list will be constructed by performing a meta-analysis of publicly

available microarray datasets in conjunction with Ingenuity Pathways Analysis gene expression network software (www.ingenuity.com).[86, 87] A RT-PCR reagent mixture will be preloaded into PCR tubes arranged on a 96-well plate. Freshly harvested lipoaspirate will be obtained from five patients and processed for SVF cells. Flow cytometry will be used to sort individual cells into each RT-PCR reagent mixture and PCR tubes will be transferred to a multi-block PCR machine to generate cDNA. The cDNA, combined with PCR Master Mix and individual TaqMan primer/probe assays for each gene of interest, will be loaded into the wells of a 96.96 IFC Chip (Fluidigm) and subject to qPCR. A Fuzzy C-Means clustering algorithm will be employed to soft partition the cells into clusters bearing similarities in gene expression.

Surface Marker Correlation: Linear discriminate analysis will be applied to gene expression data to determine surface markers capable of cluster discrimination. Prospective SVF populations from 10 patients will be sorted based on the five highest correlating surface markers for adipogenic clusters and the five highest correlating surface makers for angiogenic clusters.

Transcriptional Analysis: For each of the five adipogenic cluster defining markers, isolated subgroups will be compared with unsorted cells for transcription of adipogenic genes, including Peroxisome Proliferator-Activated Receptor-γ, Adipocyte Protein 2, and Lipoprotein Lipase. RNA will be harvested from cells and qRT-PCR will be carried. For each of the five angiogenic cluster defining markers, isolated subgroups will be compared with unsorted cells for transcription of angiogenic genes by qRT-PCR, including VEGF, FGF2, PDGFRα and β, Angiopoietin 1 and 2, and stromal cell-derived factor 1. All experiments will be run in triplicate and data will be standardized to GAPDH expression for statistical analysis.

Table 2: Specific Aim #2 CAL with SVF Subgroup

	Groups	MicroCT	Histology	Total
	Non-CAL Fat	6 animals	30 animals	36 animals
	CAL (unsorted SVF)	6 animals	30 animals	36 animals
Adipogenic Marker	CAL (Marker high/+)	6 animals	30 animals	36 animals
	CAL (Marker low/-)	6 animals	30 animals	36 animals
Angiogenic Marker	CAL (Marker high/+)	6 animals	30 animals	36 animals
	CAL (Marker low/-)	6 animals	30 animals	36 animals
Total		36 animals	180 animals	216 animals

Adipogenic Differentiation: For each of the adipogenic cluster defining makers, isolated subgroups will be plated and cultured in adipogenic differentiation medium.[26, 62] Oil red O staining will be performed after seven days and images will be photographed for ImageJ quantification using unsorted SVF cells as controls. Cell-Assisted Lipotransfer with SVF Subgroups: The two cluster defining surface markers capable of identifying cells with either the greatest adipogenic or angiogenic potential will be used for subsequent CAL investigations. Thirty-six 60-day-old Crl:Nu-*Foxn1*^{nu} sex-mismatched mice will undergo fat grafting with no supplemental SVF cells and an additional 36 60-day-old Crl:Nu-*Foxn1*^{nu} sex-mismatched mice under fat grafting with $1x10^4$ unsorted SVF cells per 200 µl fat (**Table 2**). SVF cells will also be separated into two groups based on expression (high/+ or low/-) of the adipogenic cluster defining surface marker identified with *in vitro* assays. Each subgroup of SVF cells will be used to supplement fat for grafting in 36 60-day-old Crl:Nu-*Foxn1*^{nu} sex-mismatched mice (**Table 2**).

MicroCT Volume Measurement: Six animals from each group will undergo microCT imaging three days after fat grafting for baseline, and then at 1, 2, 4, 6, and 8 weeks post-grafting. Coronal and sagittal slices will be used to create a user-defined region of interest and a three-dimensional surface for volume calculations will be generated through cubic- splint interpolation.[15]

Histological Analysis of Fat Grafts: Six animals from each group will be sacrificed at 1, 2, 4, 6, and 8 weeks following grafting (**Table 2**) for histological analysis to evaluate adipocyte viability and endothelial cell proliferation. Statistical analysis will be performed on ImageJ.[30]

Pitfalls and Alternative Strategies: We do not anticipate problems with our microfluidic-based selective transcriptional analysis and surface marker correlation, as this strategy has already been employed and published to successfully identify osteogenic and adipogenic subsets.[26, 79] Preliminary data have also shown this to be successful in identifying stromal cell subsets with greater pro-angiogenic transcriptional activity. Nonetheless, if we encounter difficulty distinguishing "signal" from "noise" or if discordant results are observed between chip runs from the same populations, a relaxation parameter based on the house-keeping gene beta-actin can be implemented to provide more flexibility in cell subset partitioning.

Future Directions

Preliminary studies have demonstrated decreased volume retention for fat grafts when placed into irradiated, ischemic recipient sites.[30] However, reversal of radiation-induced changes to skin following fat grafting has also been noted. The future of the proposed research endeavors to define how CAL may enhance fat graft survival when placed into irradiated recipient sites. We will elucidate the effects of CAL on improving fat graft take and skin quality in the irradiated recipient site, and will characterize improvement to radiation-damaged skin following CAL through histological analysis and mechanical testing.

Human Subjects

Human lipoaspirate samples will be collected in strict accordance to a Stanford University IRB-approved protocol. The IRB regarding this practice and the conduct of the research presented in this proposal has previously been uploaded to Proposal Central. The Plastic Surgery Center (PSC) of Palo Alto has a longstanding relationship with Dr. Longaker and the Hagey Laboratory, and routinely donates lipoaspirate samples (i.e. liposuction, abdominoplasty specimens) to Hagey researchers. This is accomplished through a detailed consent process that begins with the chief surgeon, all of whom at PSC have been briefed on the mission of the Hagey Laboratory, the significance of the research, and the scientific use of the specimens. This is followed by a formal consent obtained by the MD-researcher. Specimens are processed within 24 hours. The patient is made aware that the specimens are anonymized, that no monetary compensation is offered, that any research gleaned from the samples likely will not benefit them directly, and that they may later elect to have their sample removed from the study. Consent forms are archived in the patient charts.

STANFORD UNIVERSITY

Stanford, CA 94305 [Mail Code 5579]

David Spiegel, M.D.
CHAIR, PANEL ON MEDICAL HUMAN SUBJECTS (650) 725-3468

Certification of Human Subjects Approvals

Date: November 11, 2014

To: Michael T. Longaker, MD, MBA, Surgery - Plastic and Reconstructive Surgery
Elizabeth Rosalyn Zielins MD, Ruth Tevlin MD, David Atashroo MD, Derrick Check Wan MD, Anna Luan MD, Adrian Mac Ardail MD, Anne M. Dubin M.D., Charles Kwok Fai Chan BS, Daniel Bernstein, Christopher R. Duldulao BS, Elizabeth Anne Brett, Eric Steven Teasley BS, Fangjun Jia, Irving L. Weissman, Joseph Wu MD PhD, Krysta Biniek PhD, Kevin Jay Hyun Paik, Kshemendra Senarath-Yapa, Matthew B. Kerby PhD, Robert C. Rennert BS, Siny Shailendra DDS, Siddharth Menon BS

From: David Spiegel, M.D., Administrative Panel on Human Subjects in Medical Research

Protocol Tissue Regeneration with Adipose Derived Mesenchymal Cells (SQL 96098)

Protocol ID: 2188 **IRB Number:** 350 (Panel: 3)

The IRB approved human subjects involvement in your research project on 11/11/2014. **'Prior to subject recruitment and enrollment, if this is: a Cancer-related study, you must obtain Cancer Center Scientific Review Committee (SRC) approval;** a CTRU study, you must obtain CTRU approval; a VA study, you must obtain VA R and D Committee approval; and if a contract is involved, it must be signed.'

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Please remember that all data, including all signed consent form documents, must be retained for a minimum of three years past the completion of this research. Additional requirements may be imposed by your funding agency, your department, or other entities. (See Policy 1.9 on Retention of and Access to Research Data at http://doresearch.stanford.edu/policies/research-policy-handbook)

This institution is in compliance with requirements for protection of human subjects, including 45 CFR 46, 21 CFR 50 and 56, and 38 CFR 16.

David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: Dean's Office - Principal Investigator Retention Package - Grant: 1208077-484-EAFGS , SPO: NONE

Expedited Under Category: 5

Assurance Number: FWA00000935 (SU), FWA00000934 (SHC)

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: NIH - Grant: 1 R01 EB005718-01A2 , SPO: 45420

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: NIH - Grant: 1R01DE021683 , SPO: 50008

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From: David Spiegel, M.D., Administrative Panel on Human Subjects in Medical Research

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: NIH - Grant: R21DEO24230-01 , SPO: 111999

Expedited Under Category: 5

Assurance Number: FWA00000935 (SU), FWA00000934 (SHC)

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: Plastic Surgery Foundation , SPO: 114298

Expedited Under Category: 5

Assurance Number: FWA00000935 (SU), FWA00000934 (SHC)

D. Vertebrate Animals

Justification of Animal Use: While human tissue is ideal for experiments, its availability is limited and research is often conducted with mouse tissue as a close substitute. The species of the mice used in our proposed experiments have been used previously by our lab for successful experiments. We will also be using wild type mice animals which were also chosen based on our previous experiments. The amount of mice to be used is the bare minimum needed to run our proposed experiments with a significant amount of data. In Specific Aim #2 we are able to half the amount of mice needed for implantation of cells because we will be using bilateral renal capsules. We are not able to decrease the amount of animals further.

Veterinary Care of Animals: Stanford University has AAALAC accredited veterinary facilities. All mice will be allowed to acclimate for at least one week before participating in experiments. All tissue harvested from both transgenic and wildtype mice will be acquired following euthanasia with CO_2.

Anesthesia and Pain Control: All mice which are operated on will receive the following anesthesia: 20 mg/kg of Ketamine, 15 mg/f of Xylazine, and 0.2 mg/kg of acepromazine maleate. All animals will also be given a preoperative dose of 10 mg/kg cefazolin antibiotic for prevention of infection. Adequacy of anesthesia will be tested with a firm forepaw pinch, watching for minimal reaction. Post-operative analgesia will be achieved with 3 doses of 0.5mg/kg of Buprenorphine, given every 4 hours. If more analgesia medication is required thereafter, it will also be administered.

Euthanasia: Euthanasia will be performed via CO_2 narcosis. CO_2 narcosis is used due to its painlessness. It is an accepted method of euthanasia which is consistent with the recommendations of the American Veterinary Medical Association as well as being recommended by the Administrative Panel on Laboratory Animal Care at Stanford University.

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STANFORD UNIVERSITY

Stanford, CA 94305 [Mail Code 5579]

David Spiegel, M.D.
CHAIR, PANEL ON MEDICAL HUMAN SUBJECTS (650) 725-3468

Certification of Human Subjects Approvals

Date: November 11, 2014

To: Michael T. Longaker, MD, MBA, Surgery - Plastic and Reconstructive Surgery
Elizabeth Rosalyn Zielins MD, Ruth Tevlin MD, David Atashroo MD, Derrick Check Wan MD, Anna Luan MD, Adrian Mac Ardail MD, Anne M. Dubin M.D., Charles Kwok Fai Chan BS, Daniel Bernstein, Christopher R. Duldulao BS, Elizabeth Anne Brett, Eric Steven Teasley BS, Fangjun Jia, Irving L. Weissman, Joseph Wu MD PhD, Krysta Biniek PhD, Kevin Jay Hyun Paik, Kshemendra Senarath-Yapa, Matthew B. Kerby PhD, Robert C. Rennert BS, Siny Shailendra DDS, Siddharth Menon BS

From: David Spiegel, M.D., Administrative Panel on Human Subjects in Medical Research

Protocol Tissue Regeneration with Adipose Derived Mesenchymal Cells (SQL 96098)

Protocol ID: 2188 **IRB Number:** 350 (Panel: 3)

The IRB approved human subjects involvement in your research project on 11/11/2014. **'Prior to subject recruitment and enrollment, if this is: a Cancer-related study, you must obtain Cancer Center Scientific Review Committee (SRC) approval;** a CTRU study, you must obtain CTRU approval; a VA study, you must obtain VA R and D Committee approval; and if a contract is involved, it must be signed.'

The expiration date of this approval is 11/11/2015 at Midnight. If this project is to continue beyond that date, you must submit an updated protocol in advance for the IRB's re-approval. If this protocol is used in conjunction with any other human use it must be re-approved. Proposed changes to approved research must be reviewed and approved prospectively by the IRB. No changes may be initiated without prior approval by the IRB, except where necessary to eliminate apparent immediate hazards to subjects. (Any such exceptions must be reported to the IRB within 10 working days.) Unanticipated problems involving risks to participants or others and other events or information, as defined and listed in the Report Form, must be submitted promptly to the IRB. (See Events and Information that Require Prompt Reporting to the IRB at http://humansubjects.stanford.edu.)

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Please remember that all data, including all signed consent form documents, must be retained for a minimum of three years past the completion of this research. Additional requirements may be imposed by your funding agency, your department, or other entities. (See Policy 1.9 on Retention of and Access to Research Data at http://doresearch.stanford.edu/policies/research-policy-handbook)

This institution is in compliance with requirements for protection of human subjects, including 45 CFR 46, 21 CFR 50 and 56, and 38 CFR 16.

David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: Dean's Office - Principal Investigator Retention Package - Grant: 1208077-484-EAFGS , SPO: NONE

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: NIH - Grant: R21DEO24230-01 , SPO: 111999

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David Spiegel, M.D., Chair

Approval Period: 11/11/2014 THROUGH 11/11/2015

Review Type: EXPEDITED - CONTINUING REVIEW

Funding: Plastic Surgery Foundation , SPO: 114298

Expedited Under Category: 5

Assurance Number: FWA00000935 (SU), FWA00000934 (SHC)