RESEARCH GRANT FINAL REPORT

The final report is due to the IUGA office no later than one month following the completion of the research study time period. This will be 7 months following the interim report (6 months to completion of study period + 1 month). The final report does not replace the mandatory abstract/manuscript which must also be submitted to the IUGA annual meeting and the International Urogynecology Journal (IUJ), respectively.

TITLE OF PROJECT:

Adipose Derived Stem Cell Therapy for Lower Urinary Tract and Pelvic Floor Disorders

PRINCIPLE INVESTIGATOR AND INSTITUTION:

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ALL COINVESTIGATOR(S) AND INSTITUTIONS:

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2. Nestor Gonzalez-Cadavid, Ph.D., Division of Endocrinology and Molecular Medicine, Charles Drew University of Medicine and Science, Los Angeles, California.
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4. Itsvan Kovanecz, Ph.D., LA BioMed at Harbor-UCLA Medical Center, University of California, Los Angeles, California.

I. Provide a detailed report of final results obtained and state whether research aims were met

Since the initial grant approval, we have completed the following objectives:

Aim 1: To isolate, purify, and characterize the adipose-derived stem cells (ADSC) for future implantation. This aim has been completed and the results are described below.

Aim 2: To determine the effects of implanting ADSC into the atrophic levator ani and urethral sphincter of rat models of stress urinary incontinence (SUI) on muscle regeneration and reversion of their atrophy, as well as on the correction of experimental SUI. This aim was partially completed and the experiments of this Aim are currently in progress. This project was not completed within the allotted time and projected budget because it required a higher budget, particularly for the Aim 2. However, the preliminary data generated by this IUGA grant have allowed the PI to successfully obtain funding from several new grants, as described in
section IV below, to continue this investigation. The PI is, therefore, confident that he will be able to complete this project in the next 6 months.

We have prepared adipose-derived stem cells (ADSC) from the inguinal fat utilizing the procedure described in the experimental design of the proposal. We investigated whether ADSC can in our hands differentiate per se into smooth muscle cells (SMC) or they require the paracrine modulation by other SMC, in dual culture incubations. Figures 1 and 3 shows that ADSC upon 2 weeks of incubation expressed per se the markers of SMC, and this expression was only slightly stimulated by the co-culture with SMC on top inserts. Skeletal myogenesis (regeneration of skeletal muscles) is shown in Figure 2.

![Fig 1. ADSC generate smooth muscle cells.](image1)

Stem cells implanted for 2 weeks. **Top left:** blue filter for stained nuclei of implanted stem cells (DAPI stain); **Top right:** Immunohistochemical stain for ASMA, an indicator for smooth muscle formation; **Bottom:** merge of both fields. Amplification: 200X.

![Fig 2. ADSC generate skeletal muscle.](image2)

Some of the stem cell nuclei are visible in cross sections of the myofibers in skeletal muscle. **Top left:** blue filter for stained nuclei of implanted stem cells (DAPI stain); **Top right:** immunostained myofibers of skeletal muscle (MHC-II stained); **Bottom:** merge of both fields. Amplification: 400X.

![Fig 3. ADSC cultures are ongoing and express SMC markers.](image3)

ADSC were seeded in the bottom chambers of dual cultures in DMEM. The top chambers had no cells (--), or smooth muscle cells (SMC) from human corpora (HC), rat corpora (RC), or human aorta (HV). Western blots were performed at 2 weeks for smoothelin (SMO) and alpha smooth muscle actin (ASMA) as SMC markers, and GAPDH as reference gene.
These results indicated that we have successfully isolated and characterized adiposed derived stem cells (ADSC) and these ADSC can differentiate and form smooth muscle as well as skeletal muscle. The *in vivo* studies with animal models for the regeneration of smooth and skeletal muscles in the defected pelvic floor and urethral support are currently in progress.

II. Was an abstract submitted to the IUGA annual meeting? Yes X No ___

1) Abstract at the 2008 IUGA Annual Meeting:

The following abstract has been submitted and accepted as an oral-poster presentation at the 2008 IUGA annual meeting in Taiwan and received the IUGA Award of Excellence for Best Oral Poster Presentation.

Title: TISSUE ENGINEERING WITH MUSCLE-DERIVED STEM CELLS FOR VAGINAL RECONSTRUCTION IN THE RAT MODELS.

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Objective: The objective of this study is to investigate whether tissue engineering with muscle-derived stem cells (MDSC) implanted on a biodegradable graft material can regenerate vaginal tissues. Specifically, we have investigated in the rat models whether: a) MDSC implanted on biodegradable polymeric materials (Vicryl mesh, Polyglactin 910), which serve both as a surgical graft for temporary support and as a scaffold for guiding tissue regeneration, generate *in vitro* and *in vivo* smooth muscle cells (SMC) and other cell types; b) implanted MDSC express specific markers applicable to their detection upon implantation into the defective vagina after hysterectomy and partial vaginectomy; and c) implanted MDSC regenerate smooth muscle and stimulate vaginal tissue repair.

Background: Although surgical techniques for correction of vaginal or pelvic organ prolapse have undergone many modifications, there still remains an unacceptable high reoperation rate for persistent or recurrent prolapse. The role of biological and synthetic grafts in the pelvic reconstruction of vaginal prolapse is controversial. Tissue engineering with MDSC holds promise for providing effective pelvic and vaginal support through tissue repair and regeneration. MDSC are capable to give rise to several cell lineages, including the differentiations into myofibers, smooth muscle cells, endothelial cells, and neural cells. In order to promote muscle/tissue regeneration, stem cells may be implanted directly into the defective tissues. However, direct cell injection therapies are insufficient in the case of pelvic organ prolapse because mechanical support is necessary to restore and maintain the anatomy while new functional muscles/tissues are formed. Tissue engineering with stem cells using biodegradable material, that can serve both as a graft for temporary support and as a scaffold for guiding tissue regeneration, is a novel and promissory approach for pelvic reconstruction.
Methods: MDSC were prepared from skeletal muscle by the pre-plating procedure, grown on monolayer, or on polymeric grafts (Polyglactin 910 Vicryl mesh), under 2.5% or 20% fetal bovine serum with or without TGFβ1, and tested for differentiation by immunocytochemistry (ICC), quantitative western blot, and real time PCR. Putative MDSC markers were screened by DNA microarrays (SuperArray) followed by RT-PCR, ICC, and western blot. MDSC on polymeric grafts were labeled with nuclear marker 4,6-diamidino-2-phenylindone (DAPI) and implanted on the vagina of rats that underwent hysterectomy and partial vaginectomy. Control group with intact vagina and studied group with defective vagina were employed. Vaginal reconstructions were carried out with either polymeric graft alone or polymeric graft and MDSC. Rats were sacrificed after 4, 8, and 12 weeks. Dual immunofluorescence was used to detect MDSC differentiation and hematoxylin/eosin was used for histology.

Results: MDSC, both on monolayer and on grafts, differentiated in vitro into smooth muscle cells (SMC), as determined by α-smooth muscle actin (ASMA), calponin, and smoothelin. For in vivo studies, MDSC expressed Oct-4 and myoglobin, in addition to myst 4, Nanog, Notch 3, Wnt 1, CD63, and muscle creatine kinase are potential markers. Both fluorescent nuclear marker 4,6-diamidino-2-phenylindone (DAPI) and myoglobin antibody detected MDSC implanted in the vagina, and dual DAPI/ASMA indicated their conversion to SMC at 4 weeks. Vaginal tissue repair was stimulated by implanted MDSC on biodegradable polymer graft, as compared to the biodegradable graft alone, by differentiating into SMC and restoring normal histology of the vagina at 4, 8, and 12 weeks.

Conclusions: Tissue engineering with MDSC implanted on the biodegradable polymer (Polyglactin 910) is a novel and promising approach for vaginal reconstruction. The implanted MDSC regenerate smooth muscle and stimulate vaginal repair in a rat model.

Disclosures
Was consent obtained from patients? No
Was this work supported by industry? No
Does the presenter or any of the authors act as a consultant, employee (part time or full time) or shareholder of an industry? No

2) For future IUGA meeting(s):

The preliminary data generated by this IUGA grant have allowed the PI to successfully obtain new grants, which have been awarded recently (please see the description in part IV below), in order to continue and expand the current project. The results of these studies will be submitted for presentation(s) at the next IUGA annual meeting.

III. Was a manuscript submitted to IUJ? Yes No X

1) Submitted paper:

During this report period, a manuscript was accepted for publication in Obstetrics and Gynecology: Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini MG, Bhatia NN, Gonzalez-Cadavid NF. Stimulating Vaginal Repair in Rats through Skeletal Muscle-Derived Stem Cells Seeded on Small Intestinal Submucosal Scaffolds. Obstet Gynecol 2009; 114:300-309.

This paper involved mainly with muscle derived stem cells (MDSC, stem cells derived from skeletal muscles) while the IUGA grant focuses on adipose derived stem cells (ADSC), although
some experiments were overlapped between these projects. The IUGA grant concentrated on ADSC (adult stem cells derived from adipose tissues) and the experiments of Aim 2 is currently in progress. The IUGA project was not completed within the allotted time and projected budget because it required a higher budget, particularly for the Aim 2 experiments. However, the preliminary data generated by this IUGA grant have allowed the PI to successfully obtain funding from several grants, as described in section IV below, to continue this investigation. The PI is, therefore, confident that he will be able to submit the results as 2 manuscripts to IUJ for publications and abstracts to the IUGA 2010 meeting for presentations.

2) In preparation manuscript:

A manuscript entitled “Regeneration of Levator Ani in Defected Pelvic Floor by Stem Cells” is in preparation for IUJ. With the funded grants that we received recently, as described above, we are actively involved with the experiments of Aim 2 and anticipate that another manuscript will be submitted to IUJ in the near future.

IV. Provide a detailed report of expenditures

1) Provide a detailed report of expenditures:

Reagents and supplies (antibodies, culture medium, other reagents and supplies for cell culture, cell characterization, enrichment, differentiation, implantation): $9,400; animal purchase and housing: $6,000; other supplies (disposable lab-ware, general reagents): $4,600; Total: $20,000.

2) Whether the study was completed within the projected budget:

This project was not completed within the allotted years and projected budget because it required a higher budget, particularly for the Aim 2. However, the preliminary data generated by this IUGA grant have allowed the PI to successfully obtain funding from the following funded grants:

5. “Tissue Engineering with Muscle Derived Stem Cells for Pelvic Reconstruction”. Source: Allyson Fellowship Award, June Allyson Foundation of the American Urogynecologic Society. From 07/01/07 to 06/30/08. Awarded amount: $24,000 (direct cost). Role: PI.
6. “Reversion of Levator Ani Atrophy with Muscle Derived Stem Cells in Stress Urinary Incontinence”. Source: Astellas Scholar Award, Astellas-AUGS Foundation of the American
Urogynecologic Society. From 07/01/07 to 06/30/08. Awarded amount: $30,000 (direct cost). Role: PI.

These grants allow the PI to continue the experiments of Aim 2. The preliminary data obtained with this IUGA grant also allow the PI to submit another proposal to the CiRM (California Institute of Regenerative Medicine for $2,500,000 for 5 years). Unfortunately, this proposal was not funded.

We are, therefore, confident that we will be able to complete this project in the next 6 months and the results will be submitted to IUJ for publication(s) and to IUGA 2010 meeting for presentations. The PI would like to express his sincere appreciation to the IUGA for supporting this project.

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