

AD\_\_\_\_\_

Award Number:

W81XWH-07-1-0181

TITLE:

**MODULATION OF STEM CELLS DIFFERENTIATION AND MYOSTATIN AS AN  
APPROACH TO COUNTERACT FIBROSIS IN MUSCLE DYSTROPHY AND  
REGENERATION AFTER INJURY**

PRINCIPAL INVESTIGATOR:

Nestor F. Gonzalez-Cadavid, PhD

CONTRACTING ORGANIZATION:

Charles Drew University  
Los Angeles, CA 90059

REPORT DATE:

March 2009

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>				
1. REPORT DATE (DD-MM-YYYY) 01-03-2009		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 03/1/08 to 02/28/09
4. TITLE AND SUBTITLE  MODULATION OF STEM CELLS DIFFERENTIATION AND MYOSTATIN AS AN APPROACH TO COUNTERACT FIBROSIS IN MUSCLE DYSTROPHY AND REGENERATION AFTER INJURY			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER W81XWH-07-1-0181	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Nestor F. Gonzalez-Cadavid			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Charles Drew University (CDU)  Los Angeles, CA 90059			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Fort Detrick, Maryland 21702-5012			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT During Year 2 we have confirmed that muscle derived stem cells ( <b>MDSC</b> ) obtained from the wild type ( <b>wt</b> ) mouse skeletal muscle have in vitro a long-term myogenic capacity, but restricted to passages 10 to about 40-45 or earlier. The in vitro yield of myotubes from wt MDSC expressing myosin heavy chain II was not modified even at the optimal myotube forming capacity at early passages, by any of the several agents that had previously failed at suboptimal myogenic capacity at late passages, namely myostatin, myostatin antibodies, Mst shRNA, Mst cDNA, T3, follistatin, testosterone or TGFβ1. However, a PDE5 inhibitor that increases cGMP and dimethylsulfoxide, a modulator of embryonic stem cell differentiation, did upregulate the number of myotubes from wt MDSC. These MDSC differentiated in vitro into cells expressing cardiomyocyte markers, and in vivo stimulated the formation of myofibers in the rat skeletal muscle and of smooth muscle and epithelial cells in other organs. However, MDSC from the mdx or the Mst ko mouse did not form myotubes or express MyoD in vitro under optimal myogenic conditions for wt MDSC. Cultures of multipotent cells have also been obtained from the skeletal muscle and other tissues of the Oct-4 Pr-gfp transgenic mouse, visualized by green fluorescence, and are currently been characterized to determine whether they are more efficient in myogenesis than the pP6 MDSC. Their selective loss may explain the myogenic deficiency of mdx and Mstko MDSC. The study of the association of myostatin/follistatin expression in vivo with the fibro-adipogenic degeneration in mdx muscle is ongoing.				
15. SUBJECT TERMS Myostatin, muscle dystrophy, stem cells, myogenesis, Oct-4				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  UU	18. NUMBER OF PAGES  64
a. REPORT u	b. ABSTRACT u	c. THIS PAGE u		
				19b. TELEPHONE NUMBER (include area code)

## Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	12
Reportable Outcomes.....	13
Conclusion.....	14
References.....	15
Appendices.....	50

## INTRODUCTION

The **overall objective** of this grant is:

To investigate in the mdx mouse a novel therapeutic approach for Duchenne's muscular dystrophy (**DMD**) based on the inhibition of myostatin (**Mst**) expression and/or activity, for the alleviation of fibrotic and fatty degeneration of the muscle, that would also facilitate the differentiation of transplanted dystrophin+ (**D+**) muscle-derived stem cells (**MDSC**), in order to ameliorate disease progression.

This will be achieved by: a) comparing the in vitro myogenic and fibrogenic/adipogenic potential of MDSC from D-/Mst+, D+/Mst+ or D+/Mst- mice; b) blocking myostatin expression by gene transfer of myostatin short hairpin RNA (Mst shRNA), or transplantation of D+ MDSC engineered with Mst shRNA, and measuring the myogenic/fibro-adipogenic balance, dystrophin expression, and muscle function; and c) combining this with the inhibition of myostatin activity by follistatin.

Since there were several pitfalls that have delayed the in vitro work, the **specific objectives** to be fulfilled in **Years 1 and 2**, were:

**Objective 1.** To determine in vitro whether the inhibition of myostatin in MDSC from dystrophic and normal gastrocnemius: a) improves the balance between myogenic and fibro-adipogenic differentiation; b) occurs via Smad pathways and involves paracrine interactions.

**Task 1: To assess the influence of dystrophin or myostatin deficiency on the myogenic and fibro-adipogenic potential of stem cell-enriched cultures from gastrocnemius**

The gastrocnemius will be excised from 3 month old mdx mice, and from Mst(+/+) and Mst(-/-) mice (n=8/group, total 24 mice). MDSC will be isolated (pP6 fraction), followed by Sca1+ selection, and tested for myogenic, fibrotic (myofibroblast), and adipogenic differentiation, in monocultures, and in dual cultures for paracrine and juxtacrine interactions with C2C12 myoblasts.

Cell markers throughout this proposal will be compared by immunocytochemistry (ICC) or dual fluorescence, combined with quantitative image analysis (QIA), and/or quantitative western blot and/or real time RT/PCR.

Outcomes: A) Cell type characterization: a) yield and replication rate; b) stem cells (Sca1, CD34) and satellite cells (Pax7); B) TGF $\beta$  family expression: myostatin and TGF $\beta$ 1; C) Lineage commitment: a) myogenesis, by MyoD (early), myogenin (intermediate), and MHC II (late); b) fibrogenesis by alpha smooth muscle actin (ASMA) and collagen I/III ratio; c) adipogenesis by Oil-Red-O count, PPAR $\gamma$ , and C/EBP $\alpha$ .

**Task 2. To assess the influence of blocking myostatin activity and/or expression on the myogenic and fibro-adipogenic potential of stem cell-enriched cultures from dystrophic and wild type gastrocnemius, and whether this process is affected by the Smad pathway**

MDSC from the 1) mdx, and 2) Mst(+/+) groups (Task 1), will be subjected to anti-myostatin treatments and tested for differentiation as in Task 1: a) anti-myostatin antibody; b) follistatin; c) transfection with AdV-Mst shRNA. Controls will be MDSC from the 3) Mst(-/-) group, with either: d) recombinant myostatin (Mst-110); or b) transfection with AdV cDNA for myostatin (AdV-Mst cDNA), with and without plasmid Smad7 cDNA, and tested as in Task 1.

Outcomes: A) TGF $\beta$  family expression: myostatin and TGF $\beta$ 1; B) Lineage commitment: a) myogenesis by MHC II; b) fibrogenesis by ASMA; c) adipogenesis by Oil-Red-O count.; C) Smad pathway: Smad members and their phosphoproteins

**Objective 2.** To compare in vivo the markers of fibro-adipogenic degeneration of gastrocnemius and diaphragm in the mdx mouse, and to ameliorate this process by inhibiting

myostatin expression through gene transfer or implantation of ex vivo engineered MDSC, combined or not with blockade of myostatin activity.

**Task 3. To compare the molecular markers of fibro-adipogenic infiltration of the non-injured and regenerating gastrocnemius and diaphragm in the mdx mouse in relation to the myostatin/follistatin ratio**

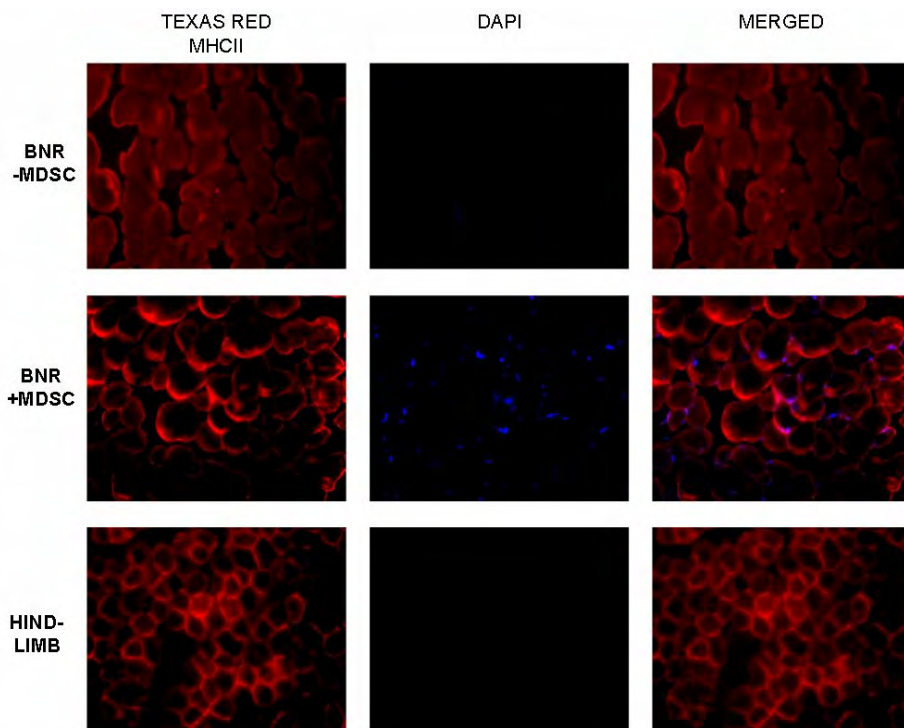
Series 1: 10.5-month old mdx mice will be injected with notexin in the gastrocnemius of one leg; Series 2: mice will receive notexin on the hemi-diaphragm. Mice groups (n=6/sub-group, **total: 66 mice**) will be sacrificed at 2, 5, 7, 14, 28, and 42 days (after intra-tail injection of Evans blue). The 14 and 45 days groups in series 1 will be injected in the gastrocnemius with a collagen I promoter- $\beta$  galactosidase DNA construct 5 days prior to sacrifice. Blood and muscles will be obtained, using contralateral intact muscles as controls.

**Outcomes:** A) TGF $\beta$  family expression and stem cells: myostatin, TGF $\beta$ 1, Sca1; follistatin; B) Myofiber content/ regeneration: MHC II, and muscle weights; number of central nuclei; cross-sectional area of myofibers; and serum creatine kinase; C) Fibrotic infiltration: by ASMA and Masson trichrome (interstitial area)/Evans blue (necrosis) staining, collagen synthesis by luminometry, and content by hydroxyproline; c) Adipogenic infiltration by Oil-Red-O count in frozen not fixed sections.

**The predicted Milestones for Years 1 and 2 are:**

**Year 1: Tasks 1 and 2: 24 mice. Milestones.** A) In vitro comparison of myogenic, fibrogenic and adipogenic potential of stem cells from intact and regenerating muscle from mdx, wt and Mst(-/-) mice; b) effects on these differentiation potentials produced by blocking myostatin and its Smad pathway.

**Year 2: Task 3 and part of 4: 86 mice. Milestones:** A) association of myostatin/follistatin expression in vivo with the fibro-adipogenic degeneration in mdx muscle with and without injury; B) start effects on mdx muscle repair by Mst siRNA and stem cells programmed or not with this construct;.



**Fig. 1. DAPI-labeled MDSC implanted into the rat skeletal muscle differentiated into myofibers.** Top and middle: the levator ani of rats subjected to bilateral nerve resection. Bottom: gastrocnemius of the same rats. 4 weeks after injection

## BODY

### Objectives 1 and 2

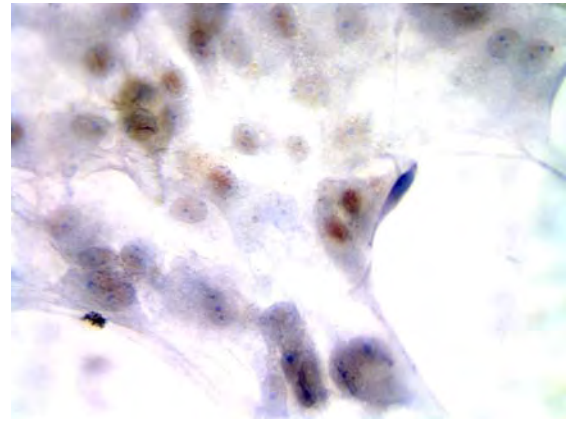
#### Wild type MDSC.

MDSC are the main tool of the current grant, as obtained from the wild type, mdx and Mstko mice. Therefore, despite the study of their in vitro conversion was initially planned to finalize in Year 1, Tasks 1 and 2 have continued during Year 2, and still there are unexpected pitfalls, or rather a behavior towards modulating agents, that need to be resolved.

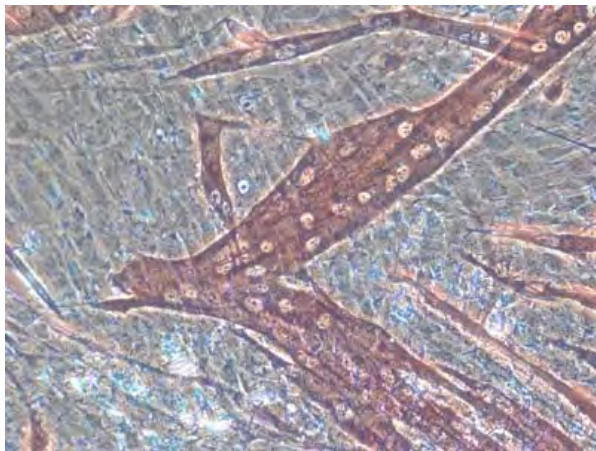
The abstract **A-5** reported during Year 1 as

showing that MDSC (pP6 fraction in the preplate procedure) from the wild type mice (**wt MDSC**) convert in vitro and in vivo into different lineages, including smooth muscle and epithelial cells, by implantation into the injured rat vagina, was now expanded. This includes a quantitative assessment of their ability to combat fibrosis in vivo (**P-4**), very pertinent to their potential effects on the mdx muscle. In turn, other experiments have shown that when they are implanted into the rat levator ani, a sexually dimorphic skeletal muscle, and into the gastrocnemius, they can presumably convert into myotubes. This is presented on **Fig. 1**, where DAPI labeled MDSC implanted into these skeletal muscles integrate within myofibers stained for myosin heavy chain II by immuno-histochemistry with streptavidin-bound Texas red and biotinylated secondary antibody.

The mouse wt MDSC were also tested for their ability to regenerate cardiomyocytes in the heart of rats undergoing myocardial infarction by coronary ligation, both by themselves or in the presence of treatment with a PDE5 inhibitor (sildenafil) that we showed in vitro to stimulate myogenesis (see below) and that we aim to apply in vivo to the mdx mouse. We



**Figure 2. MDSC in vitro convert into cells expressing the cardiomyocyte marker GATA-4.** 4 weeks of incubation in DMEM10% in the presence of DMSO



**Fig. 3. wt MDSC in vitro convert into large polynucleated myotubes.** Staining for MHC-II. Top: 200X; bottom: 400X

gave the mouse MDSC, and we also prepared MDSC from the rat skeletal muscle that are able to generate cardiomyocytes in vitro, as judged by GATA-4 staining in immunocytochemistry (**Fig. 2**). Preliminary results have been obtained just from the first arm of the study with only pharmacological treatment (**A-6**) that confirm the antifibrotic effects of raising cGMP levels, a procedure that also stimulates in vitro myogenesis (see below). The second arm, with the mouse and rat MDSC is ongoing, and we cannot at this moment predict whether this antifibrotic effect will be enhanced by the combinations with stem cells, or exerted by them alone, but this is the new approach that we intend to apply to the mdx mouse based on our in vitro experiments for skeletal myogenesis (see below).

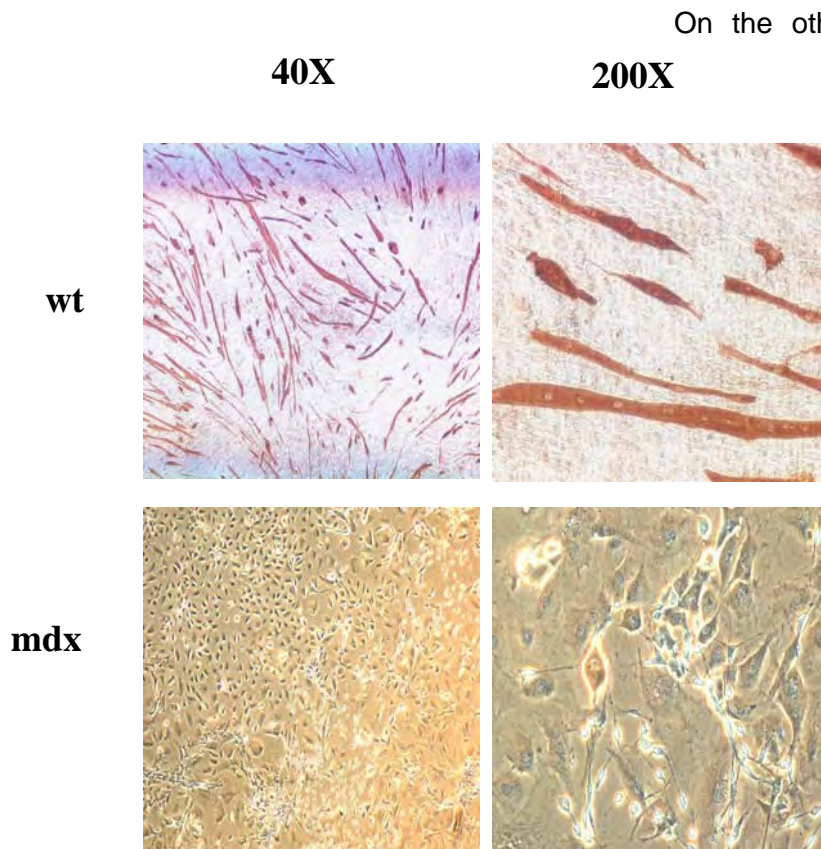
In vitro, wt MDSC have repeatedly been shown to replicate actively and when confluent and maintained for 12-20 days, to form in DMEM 10% fetal serum, numerous large and branched myotubes, that are considerably multinucleated (**Fig. 3**). This is reproducible by multiple operators, also in this case, where Hedrick's medium was used. Very surprisingly, this multipotency only was evident after passage 10 and lasted for about 40-45 passages, when myotubes slowly ceased to be formed and only very small MHC polynucleated cells were seen.

This passage-dependence of wt MDSC was only noticed during Year 2, when the time-course records were examined carefully, and therefore the previously reported failure (Year 1) of different



treatments to modulate MDSC myogenesis was assumed to be due to having used late passage cultures. Incubations of the pP6 fraction containing MDSC were then systematically repeated, but at earlier passages (25-35) with recombinant myostatin, antibodies against myostatin, Mst shRNA, Mst cDNA, testosterone, and follistatin, but again none was conclusively demonstrated by quantitative immunohistochemistry and western blot for MHC-II and MyoD, to modulate myotube formation. ASMA as a myofibroblast marker (cell key in fibrosis) was also evaluated and shown to be expressed, but no lipogenic marker was included after showing that these cells do not undergo adipogenesis even in adipogenic medium.

Therefore, other agents unrelated to the TGF $\beta$ /myostatin family, and not initially envisaged, were tested, to try to find positive modulators of wt MDSC conversion into myotubes for their potential application on the mdx and Mstko MDSC. Based on this group previous studies on fibrosis and smooth muscle protective in other organs, molsidomine as a nitric oxide donor, and tadalafil, a long-acting PDE5 inhibitor, both antifibrotic agents, were tested. This was done in comparison with dimethylsulfoxide (**DMSO**), a differentiation agent for embryonic stem cells. Both tadalafil and DMSO considerably increased MHC-II+ myotube formation in vitro after 2 weeks of incubation in DMEM-10% serum and Hedrick's medium.



**Fig. 4 Although wt MDSC form myotubes in vitro at passage 13, mdx MDSC fail to convert into myotubes.** Incubations were carried out for 14 days and stained for MHC-II

On the other hand, we examined also the ability of TGF $\beta$ 1, related to myostatin, and of T3, because of the latter effect on ocular muscle enlargement, on myotube formation by wt MDSC. However, other than occasionally inducing, in the case of T3, unusually large myotubes, no significant modulatory effects could be detected by quantitative image analysis or western blot.

We also tested one by one the myogenic ability of the side fractions pP1 through pP6 in Hedrick's medium that was very effective for pP6, and could not detect any myogenic differentiation of wt MDSC. This suggests that either MDSC or satellite cells were not present in any of these fractions, or were gradually outgrown by other cell types.

Collectively these results indicate that MDSC (pP6 fraction): 1) in vitro have a long-term myogenic capacity, but that starts out after at least 10 passages from the time the culture was established and gradually fades away, for reasons not yet known, after passages 40-45; 2) with the possible exception of tadalafil (increasing cGMP) and DMSO, no other agents could be found to substantially and reproducibly modulate in vitro myogenesis; 3) is the only pre-plating cell population that differentiated into

MHC-II+ myotubes, or into any other form of visually identifiable polynucleated myotubes; 4) in vivo they were shown to repair skeletal muscle, smooth muscle, and epithelial tissues in various organs

### Mdx and Mstko MDSC

The previously reported failure (Year 1) of these MDSC to generate MHC-II+ myotubes or to activate MyoD expression in vitro at early passages (up to 5) was surprising since although dystrophin depletion may be responsible for the inability of mdx MDSC to undergo differentiation and/or subsequent cell fusion, it is counterintuitive that the absence of myostatin in Mstko MDSC would prevent myogenesis. In addition these cultures grow much slower than the wt pP6, again counterintuitive for Mstko MDSC because of the well known role of myostatin in inhibiting myoblast replication.

Therefore, we repeated these experiments assuming that the passage number was a factor and it has to be over 10, but so far even at passage 13 no myotubes were obtained with mdx MDSC. This is shown on **Fig. 4**, where despite wt pP6 formed typical MHC-II+ myotubes, not a single one occurred with mdx MDSC, and only sporadic mononucleated cells showed some MHC-II expression. In addition, the morphology is totally different. Mstko are now being tested at passage 13. None of the mdx or Mstko pP1-5 fractions formed myotubes either.

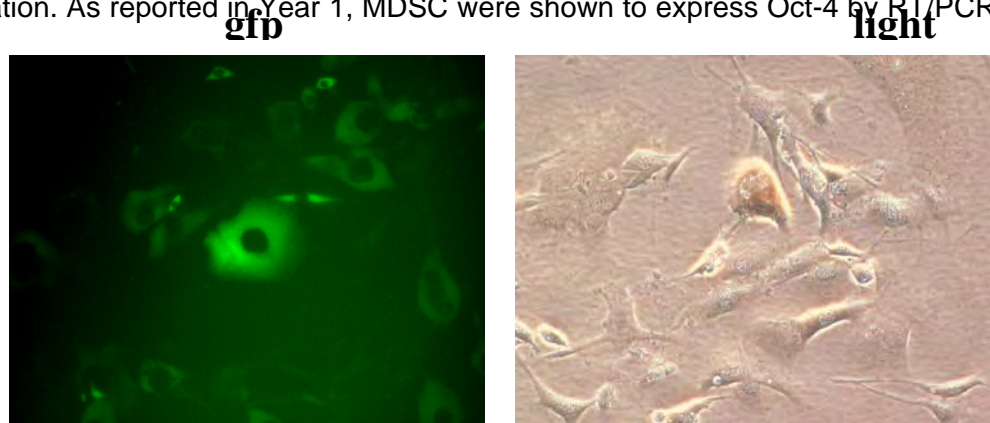
### Alternative in vitro approaches

In order to determine whether the pre-plating procedure of isolation of pP6 may discard a population of pluripotent cells that are much smaller, and therefore detach more easily in the mdx or Mstko muscle cell cultures than in the wt muscle cell cultures, we looked for an alternative procedure of isolation. This is based on the utilization of the Oct-4 Pr-gfp transgenic mouse, that expresses green fluorescent protein under the control of the gene promoter for Oct-4, a key factor in embryonic and adult stem cell activation. As reported in Year 1, MDSC were shown to express Oct-4 by RT/PCR and western blot, so that we started to investigate whether endogenous adult stem cells can be isolated from different organs based on the use of this transgenic mouse and the fact that the Oct-4+ cells should be identified by green fluorescence.

The initial experiments were done in renal and penile tissues, and this

identification was the subject of two related abstracts, **A7 and A8**, that identified Oct-4+ cells in those tissues and demonstrated their multipotent features and the fact that their number is modulated by an underlying histopathology and restored by pharmacological treatment. The endogenous cells resemble the very small embryonic-like stem cells (**VSEL**) described in several adult tissues. In Year 1 we located these cells in vivo in the skeletal muscle around myofibers.

These findings inspired the application of the same methodology to fully characterize Oct-4+ cells along the pre-plating fractions from the wild type mouse skeletal muscle. We observed that these cells were identified better by easy detachment once plated in every pP2-pP6 fraction, size, and morphology, than by their accumulation in the late pP4-pP6 fractions, i.e., they are dispersed all along the different fractions. **Fig. 5** shows that among the muscle pP6 enriched in these easily detachable small cells by a special procedure there are many intensively green fluorescent cells, or Oct-4+ cells. We have tested their myogenic ability, but so far (only passage 6) they have not generated myotubes, what is expected on the basis of what we learned with the standard pre-plating procedures.



**Fig. 5.** Oct-4 expressing cells obtained from the skeletal muscle of the transgenic Oct-4 Pr gfp mouse are detectable by green fluorescence but at early passage they fail to undergo myogenesis.



Another alternative we are exploring, to improve the *in vivo* efficiency of stem cell regeneration of damaged muscle in the mdx mouse, is the use of **iPS** (induced pluripotent stem cells) obtained from keratinocytes, in collaboration with Dr. Iszpirua Belmonte from the Salk Institute. The submitted grants (see list) focus on tissues other than the skeletal muscle, but we intend to apply similar approaches to prevent or repair muscle necrosis in the mdx mouse if the regulat MDSC or the Oct-4+ muscle cells are not effective.

### Objective 3

The experiment to compare the molecular markers of fibro-adipogenic infiltration of the non-injured and regenerating gastrocnemius and diaphragm in the mdx mouse in relation to the myostatin/follistatin ratio is now ongoing, but using younger mice (5 months old), to avoid waiting 5 more months for pursuing the stem cell/pharmacological treatment of the mdx mouse.

### Plan for Year 3

It will focus on all the in vivo experiments planned for tasks 3 (completion) and 4, and part of 5. The host skeletal muscle paracrine and juxtacrine influences affected by the pharmacological and biological modulators, planned in the grant for the treatment of the mdx skeletal muscle, may overcome *in vivo* the relative resistance of wt MDSC to potential upregulators of myogenesis and inhibitors of fibrosis. The latter may include PDE5 inhibitors (not originally envisaged) that have already been tested in the heart in the model of myocardial infarction, replacing those that are less likely to be effective.

We will continue in parallel with the remaining *in vitro* experiments based on dual incubations of wt MDSC with the mdx and Mstko MDSC, to determine whether paracrine and juxtacrine modulation can be detected *in vitro*, and we will compare these three types of MDSC by applying DNA microarrays to try to gain a preliminary insight on whether a differential transcriptional signature may explain the mdx and Mstko resistance to myogenesis. We will also go on with the skeletal muscle Oct-4+ cells characterization from the three skeletal muscles to ascertain whether a selective loss of the small cells similar to VSEL may explain these differences.

A budget carry forward from Year 2 to 3 may allow to increase the % effort of some personnel to accomplish as much as possible these goals.

### Papers (see appendix)

#### **Paper P-4 Summary. Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds**

**Objectives:** Grafts are used for vaginal repair after prolapse, but their use to carry stem cells to regenerate vaginal tissue has not been reported. In this study, we investigated whether: a) muscle derived stem cells (**MDSC**) grown on small intestinal submucosa (**SIS**) generate SMC *in vitro* and upon implantation in a rat model of vaginal defects; b) express markers applicable to the *in vivo* detection of vaginal endogenous stem cells; and c) stimulate the repair of the vagina.

**Methods:** Mouse MDSC grown on monolayer, SIS or polymeric mesh, were tested for cell differentiation by immunocytochemistry (**ICC**), western blot and RT/real time PCR. Stem cell markers were screened by DNA microarrays followed by RT/PCR, ICC, and western blot. Rats that underwent hysterectomy and partial vaginectomy were left as such, or implanted in the vagina with DAPI-labeled MDSC on SIS, or SIS without MDSC, immunosuppressed, and sacrificed at 2-8, weeks. Immunofluorescence, hematoxylin/ eosin, and Masson trichrome were applied to tissue sections.

**Results:** MDSC cultures on monolayer and on scaffolds differentiate into SMC, as shown by  $\alpha$ -smooth muscle actin (**ASMA**), calponin, and smoothelin markers. MDSC express embryonic stem cell markers Oct-4 and nanog. Dual DAPI/ASMA fluorescence indicated MDSC conversion to SMC.

MDSC/SIS stimulated vaginal tissue repair, including keratin-5 positive epithelium formation, and prevented fibrosis, at 4 and 8 weeks. Oct-4+ putative endogenous stem cells were identified.

**Conclusions:** MDSC/SIS implants stimulate vaginal tissue repair in the rat, thus autologous MDSC on scaffolds may be a promissory approach for the treatment of vaginal prolapse.

## Abstracts

### **Abstract A-6. Pharmacological stimulation of NO/cGMP levels as a novel therapeutic approach for myocardial infarction in a rat model.**

**Background.** Inducible nitric oxide synthase (**iNOS**) is expressed during urogenital fibrosis, and iNOS inhibition with L-NIL exacerbates fibrosis. Overexpression of iNOS reduces fibrosis through nitric oxide (**NO**) and cGMP production. Long-term oral PDE5 inhibitors prevent fibrosis via cGMP increase. NO and cGMP protect cardiomyocytes in vitro, and daily injection of sildenafil reduces cardiac fibrosis after myocardial infarction in a mouse model, but functional improvement is marginal and partially inhibited by L-NIL.

**Objectives.** To determine whether the protective iNOS-dependent effects of sildenafil can be conclusively demonstrated in the widely used rat model of myocardial infarction, by defining a larger set of outcomes.

**Methods.** Male rats were subjected to echocardiography to determine the cardiac ejection fraction and to proximal ligation of the left coronary artery, followed by randomization into the following groups (n=6/group): 1) "controls"; 2) "high oral sildenafil": 10 mg/kg/day; 3) "low oral sildenafil", 3 mg/kg/day; 4) "low oral sildenafil-L-NIL", as #3, with L-NIL, 10 mg/kg/day. At 1 and 4 weeks echocardiographies were repeated, and rats were sacrificed. Paraffin embedded sections from the left ventricle were subjected to histo- or immunohisto-chemistry and quantitative image analysis (**QIA**) for: Picrus Sirius red for collagen; TGF $\beta$ 1;  $\alpha$ -smooth muscle actin (myofibroblasts); apoptotic index; and troponin T for cardiomyocytes.

**Results.** Apex ejection fractions (means $\pm$ SEM), with some animals still ongoing, are as follows for groups: **A) Basal:** 1-4): 88.2 $\pm$ 1.8; **1 wk:** 1) 39.6 $\pm$ 2.8; 2) 56.8 $\pm$ 4.2; 3) 39.2 $\pm$ 3.6; 4) 39.9 $\pm$ 7.7; **4 wks:** 1) 49.5 $\pm$ 2.3; 2) 48.8 $\pm$ 4.3; 3) 49.5 $\pm$ 8.9; 4) 40.3 $\pm$ 4.7. Percent increases for 4 wks compared to 1 wk were: 1) 126 $\pm$ 6; 2) \*87 $\pm$ 8; 3) 126 $\pm$ 19; 4) \*86 $\pm$ 17. \*: p<0.05 against 1). Long-term sildenafil treatment reduced infarction size in a process that was not affected by inhibition of iNOS activity. Accumulation of myofibroblasts in the infarction area was considerably reduced by low and high doses of sildenafil, and these effects were iNOS-independent.

**Conclusions.** The postulated beneficial effects of long-term daily administration of sildenafil on cardiac function after AMI in mice were not reproducible in the rat model, despite the considerable iNOS-independent reduction of infarction size and amelioration of fibrosis. This suggests that cGMP may not prevent the loss of cardiomyocytes or restore their number in the rat model, and that this process is more critical than fibrosis for the impairment of cardiac function post AMI (pending tests). In addition, cardiac function in the mouse may be more responsive to sildenafil than in the rat, or lower doses may have to be tested in the rat.

### **Abstract A-7. Activation of the Oct-4 gene identifies stem cells in the kidney that are reduced by type 2 diabetes mellitus in a process counteracted by a PPAR $\gamma$ ligand independently from glycemic control.**

**Objectives.** Endogenous stem cells have been reported in the adult human and rodent kidney, sharing the expression of Oct-4 and other embryonic genes with the "Very Small Embryonic-Like (**VSEL**)" stem cells detected in many adult organs. Diabetic nephropathy is ameliorated in type 2 diabetic rats by pioglitazone, a PPAR $\gamma$  ligand, at doses that do not normalize glycemia. We investigated whether a transgenic mouse expressing green fluorescent protein (**gfp**) under the Oct-4 gene promoter (**Oct-4 Pr-gfp**) may be used to characterize kidney stem cells, and whether the renal effects of diabetes and pioglitazone are partially due to their modulation of these stem cells.

**Methods.** Kidney tissue sections from the Oct-4 Pr-gfp mouse labeled with DAPI-(nuclear blue fluorescence) were examined for green fluorescence. Putative gfp+ stem cells were isolated from fresh tissue by differential attachment on monolayer, and assayed in several cell differentiation media for dual green/red immunofluorescence for  $\alpha$ -smooth muscle actin, calponin, vimentin, nephrin, aquaporin-2, von Wildebrandt factor, and PPAR $\gamma$  (number of positive cells), and by western blot. DAPI-labeled cells were injected into the kidney of intact wild type mice or after ischemia-reperfusion, assaying cell differentiation at 2 weeks. Quantitative immunohistochemistry for Oct-4 was applied to kidney sections from lean Zucker rats (**LZR**), diabetic ZDF fa/fa rats (**ZDFR**), and ZDFR treated with 0.001% pioglitazone in the chow for 4.5 months (n=8 rats/group).

**Results.** Oct-4 gfp+ cells were detected in the Oct-4 Pr-gfp mouse kidneys. In culture, gfp+ cells resembled VSEL stem cells, formed spheroids, and were PPAR $\gamma$ +. They differentiated into renal, endothelial, and smooth muscle cells, and myofibroblasts, by turning off gfp expression, that often coexisted with the differentiated cell marker. Implanted Oct-4+ cells were detected in the mouse kidney, and endogenous Oct-4+ cells were located in the tubules of the LZR. Their number was reduced in the ZDFR and pioglitazone partially restored Oct-4+ cells at doses that ameliorated the nephropathy without normalizing glycemia.

**Conclusions.** This is the first report on: a) the characterization by the activation of the Oct-4 gene promoter of endogenous renal stem cells, b) the down-regulation of renal Oct-4+ cells by type 2 diabetes; and c) the partial preservation of Oct-4+ cells by pioglitazone that may explain its protective effects on the diabetic kidney.

#### **Abstract A-8. Characterization of endogenous stem cells from the mouse penis that express an embryonic stem cell gene and undergo differentiation into several cell lineages**

**Objectives.** Very small embryonic-like (**VSEL**) stem cells expressing embryonic markers, specifically Oct 4, have recently been found in many adult organs. We have previously isolated endogenous multipotent cells from the human penile tunica albuginea, and detected cells in the rat tunica albuginea and corpora cavernosa that express stem cell markers. We and others have demonstrated that stem cells from other organs implanted in the rat corpora cavernosa regenerate smooth muscle cells (**SMC**) and neural cells and correct erectile dysfunction. The current work determined whether VSEL are present in penile tissues, and by using a transgenic mouse that expresses gfp under the control of the Oct 4 promoter (**Oct 4 Pr-gfp mouse**) we isolated and tested these cells for multipotency both in vitro and in vivo.

**Methods.** Shaft penile tissue sections from the rat and wild type (**WT**) mouse were immunostained for Oct 4, and fresh tissues were subjected to RT/PCR and western blot for Oct4 and other embryonic stem cell markers. Penile shaft and crura frozen sections from the Oct 4 Pr-gfp mouse were examined for cellular green fluorescence, and fresh tissues were subjected to a modified stem cell isolation procedure. pP1 to pP6 and related cultures from the "pre-plating" procedure were assayed in several media for multiple cell differentiation by avidin-Texas red immunofluorescence for  $\alpha$ -smooth muscle actin, calponin, vimentin, troponin T, myosin heavy chain-II, and other markers. DAPI-labeled cultures were injected into the corpora cavernosa ( $0.3 \times 10^5$ ) of WT mice and penile tissues were excised at 7 and 14 days to check for cell differentiation.

**Results.** Oct 4 + cells and Oct 4 mRNA and protein were detected in the rat and WT mouse tunical and corporal tissues. Very small, easily detachable green fluorescent cells with a large nuclei, that eventually formed embryonic-like spheroids were shown to differentiate into SMC, myofibroblasts, and cardiomyocytes. Differentiation slowly turned off Oct 4 expression, but it remained active in many cells concurrently with the differentiated marker. Implanted cells also differentiated in vivo in the WT mouse corpora cavernosa.

**Conclusions.** This is the first report on the isolation and characterization of embryonic-like endogenous stem cells from penile tissues. Dormant endogenous stem cells are potential targets for pharmacological activation aimed to non-invasive repair of penile tissue to treat erectile dysfunction and Peyronie's disease.

**Abstract A-9. Modulation of cell lineage commitment by skeletal muscle derived stem cells, MDSC, from mdx and myostatin knockout mice**

**Background.** Muscle lipofibrotic degeneration characterizes Duchenne muscular dystrophy (DMD), hampers cell therapy in the muscle, and is a feasible therapeutic target. Myostatin (**Mst**), a negative regulator of muscle mass, is antimyogenic and stimulates fibrogenic and adipogenic differentiation of stem cells. Inhibiting myostatin in the DMD model, the mdx mouse, improves myogenesis and reduces fibrosis.

**Goals.** To investigate in the mdx mouse a novel therapy for DMD based on the inhibition of myostatin, for the alleviation of muscle lipofibrotic degeneration, and stimulation of myogenesis by implanted muscle-derived stem cells from wild type mice (**Wt MDSC**). This is achieved by: a) comparing the in vitro myogenic and fibrogenic/adipogenic potential of Wt, Mst ko, and mdx MDSC; b) blocking myostatin by follistatin, myostatin short hairpin RNA (**Mst shRNA**), or implantation of MDSC engineered with Mst shRNA, and measuring the myogenic/ fibroadipogenic balance and muscle function.

**Brief description of methodologies.** MDSC isolated from the three mouse strains (pP6 pre-plate fraction) were tested for myogenesis, fibrogenesis, and adipogenesis, in the presence of anti-myostatin antibody, follistatin, or myostatin, or by transfection with AdV-Mst shRNA or AdV-Mst cDNA, comparing with TGF $\beta$ 1, T3, and a nitric oxide donor. Wt MDSC were implanted into various tissues in the rat to assess their tissue repair capacity. MDSC isolated from a transgenic mouse expressing gfp under the embryonic Oct-4 gene promoter were implanted into mdx and Wt mice. Tissue fibrosis and cell death in intact and injured mdx muscles are being studied at several periods. Cell markers were compared by quantitative immunocytochemistry dual fluorescence, and/or quantitative western blot and/or real time RT/PCR.

**Results to date.** Non-confluent Wt pP6 underwent fibrogenic, osteogenic, cardiomyogenic, and SMC differentiation from early passages, but skeletal myotubes were only detected in confluent cultures from passages 10 through 30, when pP6 started to lose this differentiation ability. However, they converted into other lineages for over 50 passages, but did not undergo adipogenesis. In contrast, confluent Mst ko and mdx pP6 were unable to generate myotubes, and the same occurred with pP1-pP5 fractions in all mouse strains. pP6 myogenesis could not be modulated by any of the tested factors, with the exception of testosterone and SNAP, even if TGF $\beta$  and myostatin stimulated fibrogenesis of a multipotent cell line. Wt pP6 implanted in several organs of the rat generated skeletal myofibers, smooth muscle tissue and other cell types, and stimulated tissue repair and recovery of function. A fraction of Wt pP6 from the Oct-4 Pr-gfp express the Oct-4 embryonic stem cell marker, visualized by green fluorescence, and these cells were localized in the muscle.

**Conclusions.** Myogenic differentiation of MDSC in vitro is passage-dependent and refractory to myostatin modulators, despite MDSC undergo myogenesis in vivo, but are moderately responsive to nitric oxide. The resistance of the Mst ko and mdx MDSC to in vitro myogenesis is so far unexplained. Dual cultures with Wt MDSC are ongoing to determine whether myogenesis may be awakened by paracrine or juxtacrine clues. Expression of Oct-4 in MDSC may explain their myogenic activity.

**Impact statement.** Future in vivo experiments should elucidate the role of myostatin and dystrophin in the lineage commitment of MDSC, that remains elusive in vitro.

## **KEY RESEARCH ACCOMPLISHMENTS**

During Year 2, we have:

- confirmed that muscle derived stem cells (**MDSC**) obtained from the wild type (**wt**) mouse skeletal muscle have in vitro a long-term myogenic capacity, but restricted to passages 10 to about 40-45 or earlier;

- The in vitro yield of myotubes from wt MDSC expressing myosin heavy chain II was not modified even at the optimal myotube forming capacity at early passages, by any of the several agents that had previously failed at suboptimal myogenic capacity at late passages, namely myostatin, myostatin antibodies, Mst shRNA, Mst cDNA, T3, follistatin, testosterone or TGF $\beta$ 1;
- However, a PDE5 inhibitor that increases cGMP and dimethylsulfoxide, a modulator of embryonic stem cell differentiation, did upregulate the number of myotubes from wt MDSC;
- These MDSC differentiated in vitro into cells expressing cardiomyocyte markers, and in vivo stimulated the formation of myofibers in the rat skeletal muscle and of smooth muscle and epithelial cells in other organs;
- However, MDSC from the mdx or the Mst ko mouse did not form myotubes or express MyoD in vitro under optimal myogenic conditions for wt MDSC;
- Cultures of multipotent cells have also been obtained from the skeletal muscle and other tissues of the Oct-4 Pr-gfp transgenic mouse, visualized by green fluorescence. The skeletal muscle Oct-4+ cells are currently being characterized to determine whether they are more efficient in myogenesis than the pP6 MDSC. Their selective loss may explain the myogenic deficiency of mdx and Mstko MDSC.

## REPORTABLE OUTCOMES

Numbering is from the start of this grant (P: papers; A: abstracts)

### A. Papers acknowledging this grant in Year 2(see Appendix)

**P-4.** Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini M, Bhatia NN, Gonzalez-Cadavid NF, Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffold. Obst Gynecol, preliminary acceptance

### B. Abstracts and presentations related to results in the current grant in Year 2

**A-6.** Wang, S-C, Nolzco G, Kopchock G, Kovanecz I, White R, Gonzalez-Cadavid NF. Pharmacological stimulation of NO/cGMP levels as a novel therapeutic approach for myocardial infarction in a rat model.

**A-7.** Nolzco G, Toblli J, Kovanecz I, Gelfand R, Lue Y-H, Gonzalez-Cadavid NF (2009) Activation of the Oct-4 gene identifies stem cells in the kidney that are reduced by type 2 diabetes mellitus in a process counteracted by a PPAR $\gamma$  ligand independently from glycemic control. Endocrine Soc Meet, Washington DC

**A-8.** Vernet D, Heydarkhan S, Kovanecz I, Lue Y-H, Rajfer J, Gonzalez-Cadavid NF (2009). Characterization of endogenous stem cells from the mouse penis that express an embryonic stem cell gene and undergo differentiation into several cell lineages. Am Urol Assoc Meet, Chicago, IL, J Urol,

**A-9.** Modulation of cell lineage commitment by skeletal muscle derived stem cells, MDSC, from mdx and myostatin knockout mice Military Health Research Forum 2009, Kansas City, Missouri

### C. New applications for funding in Year 2

The following grant applications, which are still pending have been submitted, by investigators in this DOD grant using in part results obtained during Year 2 of this grant:

1. Pending. PI: Gonzalez-Cadavid NF (2009). Modulation of human iPS differentiation in radical prostatectomy-related erectile dysfunction in rat models. NIH Recovery Challenge Grants. 09/09-08/11. No overlapping



Principal Investigator: Gonzalez-Cadavid, Nestor F.

2. Pending. PI: Gonzalez-Cadavid NF (2009). Erectile Dysfunction and Nitric Oxide Synthase in Aging. RO1 DK53069-07 (resubmission). 11/09-10/14. No overlapping
3. Pending. PI: Gonzalez-Cadavid NF (2009) Nitric oxide and cGMP modulation of Oct-4 renal stem cells in diabetic nephropathy . R21 DK085411-01 09/01/09-08/31/11 No overlapping
4. Pending. PI: Gonzalez-Cadavid NF (2009) Modulation of human iPS differentiation in diabetic nephropathy in rat models NIH Recovery Challenge Grants. 09/09-08/11. No overlapping
5. Pending. PI: Gonzalez-Cadavid NF (2009) PPAR gamma modulation of Oct-4 renal stem cells in diabetic nephropathy R21 DK085413-01 09/01/09-08/31/11 No overlapping

#### D. Appointment and Replacements

None after year 1

### **CONCLUSIONS**

Myogenic differentiation of MDSC from the wild type mouse muscle in vitro is passage-dependent and refractory to myostatin modulators, despite MDSC undergo myogenesis in vivo, but are moderately responsive to nitric oxide and cGMP. The resistance of the Mst ko and mdx MDSC to in vitro myogenesis is so far unexplained, but may be due to a selective loss of other types of stem cells from the pP6 cell fractions containing MDSC. Expression of Oct-4 in MDSC may explain their myogenic activity. Dual cultures with Wt MDSC are ongoing to determine whether myogenesis may be awakened by paracrine or juxtacrine clues.

### **REFERENCES**

They are listed in the papers enclosed in the Appendix, as well below:

### **APPENDICES**

They include:

- 1) The downloaded publications for paper P-4
- 2) The biographical sketches of Gonzalez-Cadavid, Ferrini, Kovanecz, Tsao, and Gelfand

# 1 SKELETAL MUSCLE-DERIVED STEM CELLS SEEDED ON SMALL INTESTINAL 2 SUBMUCOSAL SCAFFOLDS STIMULATE VAGINAL REPAIR IN THE RAT

3  
4 **Ho MH\***§¶, **Heydarkhan S\***§, **Vernet D\***¶, **Kovanecz I\***‡, **Ferrini MG**¶, **Bhatia NN\***§‡,  
5 **Gonzalez-Cadavid NF\***‡¶

6 §Division of Female Pelvic Medicine and Reconstructive Surgery, Department of Obstetrics and  
7 Gynecology, Harbor-UCLA Medical Center, David Geffen School of Medicine at UCLA,  
8 University of California, Los Angeles, CA; \*Los Angeles Biomedical Research Institute at  
9 Harbor-UCLA Medical Center, Urology Research Laboratory, Torrance, CA , ‡Department of  
10 Urology, David Geffen School of Medicine at UCLA, University of California, Los Angeles,  
11 CA, and ¶Division of Endocrinology and Molecular Medicine, Department of Internal Medicine,  
12 College of Medicine, Charles Drew University of Medicine and Science, Los Angeles, CA, USA

13  
14 **Corresponding author:** Nestor F. Gonzalez-Cadavid, Ph.D., LABioMed at Harbor-UCLA  
15 Medical Center, Urology Research Laboratory, Bldg. F-6, 1124 West Carson Street, Torrance,  
16 CA 90502

17  
18 **Telephone:** (310)-222-3824; **Fax:** (310)-222-1914; **e-mail:** ncadavid@ucla.edu

19  
20 **Running Title:** Stem cells in vaginal repair

21  
22 **Key words:** vagina, fibrosis, smooth muscle, stem cells; myofibroblast; Oct-4

23 **Abbreviations:** **FBS:** fetal bovine serum; **ASMA:**  $\alpha$ -smooth muscle actin; **MDSC:** skeletal  
24 muscle derived stem cells; **PCNA:** proliferating cell nuclear antigen; **SIS:** small intestinal  
25 submucosa; **SMC:** smooth muscle cells; **TGF $\beta$ 1:** transforming growth factor  $\beta$ 1.

**ABSTRACT**

**Objectives:** Grafts are used for vaginal repair after prolapse, but their use to carry stem cells to regenerate vaginal tissue has not been reported. In this study, we investigated whether: a) muscle derived stem cells (**MDSC**) grown on small intestinal submucosa (**SIS**) generate SMC *in vitro* and upon implantation in a rat model of apical, anterior and posterior vaginal defects; b) express markers applicable to their *in vivo* detection and to the identification of vaginal endogenous stem cells; and c) stimulate the normal repair of the defected/injured vagina.

**Methods:** Mouse MDSC were grown on monolayer or on porcine SIS scaffolds or polymeric (Vicryl) mesh, under DMEM with 2.5% or 20% fetal serum with or without TGF $\beta$ 1 and tested for cell differentiation by immunocytochemistry (**ICC**), quantitative western blot and RT/real time PCR. Putative stem cell markers were screened by DNA microarrays followed by RT/PCR, ICC, and western blot. Rats that underwent hysterectomy and partial vaginectomy were either implanted in the vagina with: a) MDSC on SIS scaffolds, tagged with DAPI and/or PKH-26, or b) SIS without MDSC, or not implanted. Rats were immunosuppressed and sacrificed at 2, 4, 6 or 8 weeks. Immunohistochemistry, dual immunofluorescence, hematoxylin/eosin, and Masson trichrome were applied to tissue sections.

**Results:** MDSC cultures on monolayer and SIS or polymers differentiate into SMC, as shown by  $\alpha$ -smooth muscle actin (**ASMA**), calponin, and smoothelin. MDSC express embryonic stem cell markers Oct-4 and nanog. Dual tag/ASMA fluorescence indicated MDSC conversion to SMC. Vaginal tissue repair was stimulated by MDSC/SIS, including keratin-5 positive epithelium formation, and prevented fibrosis, at 4 and 8 weeks. Oct-4+ putative endogenous stem cells were also identified.

**Conclusions:** MDSC/SIS implants stimulate vaginal tissue repair in the rat, thus autologous MDSC on scaffolds may be a promissory approach for the treatment of vaginal prolapse.

## INTRODUCTION

Pelvic organ prolapse in general, and specifically vaginal prolapse, is highly prevalent and may occur in up to 50% of parous women (1,2). Surgical cure rates vary and recurrences are common in primary ungrafted methods of anterior vaginal repair, and this has led to the use of synthetic mesh or/and biological grafts to provide support for the existing weakened fascia and musculature (1-6). Short-term data indicates that mesh/graft reduced objective prolapse recurrence rates compared with ungrafted methods, and the use of this procedure is increasing (3,4,7). However, complications are frequent, particularly vaginal fibrosis, inflammation, and epithelial erosion, and the long-term durability and safety of these devices are unknown (8-11).

In other areas of urogenital tissue repair, stem cell therapy is being actively investigated to promote the replacement of lost or damaged tissue, particularly in the bladder and urethra for the treatment of stress urinary incontinence (12-14), and in the penile corpora cavernosa, for erectile dysfunction (15-18). Although embryonic stem cells are promising, their medical use still faces political and ethical hurdles, as well as the still unresolved risks of potential carcinogenesis and immunorejection (19,20). These problems do not present, or are less serious, for stem cells found in accessible adult tissues, such as the bone marrow, skeletal muscle or liver (21,22). Therefore, adult stem cells are starting to be investigated for tissue repair in clinical trials of some non-urogenital conditions (<http://clinicaltrials.gov>), but for vaginal repair not even animal studies have been reported. Differentiated vaginal epithelial and smooth muscle cells have been used to create a neovagina using autologous cells in a rabbit model (23,24).

The skeletal muscle is an adequate source for stem cell isolation for autografts because of the feasibility of biopsies and what is known in rodents in terms of isolation, differentiation ability, and potential of these cells for regenerating tissues (25). Muscle derived stem cells (MDSC) have been also recently isolated from and characterized in human muscle (26,27), and

should not be confused with the myogenically committed satellite cells and/or myoblasts studied for stress urinary incontinence (e.g. **28**). MDSC differentiate in vitro and in vivo into skeletal myotubes, osteoblasts, chondrocytes, and neural cells (**25,29**), and recently we reported that mouse MDSC implanted into the corpora cavernosa smooth muscle of immunosuppressed rats differentiate into smooth muscle cells (SMC) replacing the cells lost during tissue fibrosis induced by aging, thus restoring function, in this case the correction of erectile dysfunction (**18**).

The potential of stem cell therapy for vaginal repair extends to the possible pharmacological activation of "dormant" endogenous stem cells that may be present in this organ. In addition to the "supply" tissues named above, stem cells have been found in most organs, including urogenital organs such as the penis (**18,30**), the ovary (**31**), or the testis (**32**). Some cells in these heterogenous populations may express the embryonic stem cell markers Oct-4 or nanog (**33,34**). However, no information is available on their presence in the vagina.

In the current work we investigated whether MDSC generate in vitro SMC, grow and differentiate into SMC on small intestinal submucosa (SIS), and express embryonic stem cell markers. We have also investigated whether MDSC/SIS implants into the vagina of hysterectomized and partially vaginectomized rats originate SMC and promote tissue repair without inducing fibrosis, and whether potential endogenous stem cells are present in the vagina.

## **MATERIALS AND METHODS**

***Preparation and culture of MDSC.*** Skeletal muscles were obtained from the hind limb of C57BL/6 mice and MDSC were isolated applying the preplating procedure (**18,35**). The mouse skeletal muscle was preferred because these MDSC are the only ones prepared by this method that have been extensively characterized as stem cells (**25**), whereas isolating them from rat skeletal muscle would require to replicate the stem cell identification and characterization. Briefly, tissues were dissociated using sequentially collagenase XI, dispase II and trypsin, and



after filtrations through 60 nylon mesh, and pelleting, the released cells were suspended in GM-20 (Dulbecco's Modified Eagle's Medium (DMEM) with 20% fetal bovine serum (FBS) Cells were then plated onto collagen I-coated flasks for 1 hr (preplate 1 or pP1), and 2 hrs (preplate 2 for pP2), followed by a series of sequential daily transfer of non-adherent cells and re-platings for 2 to 6 days, until preplate 6 (pP6). The latter is the cell population containing MDSC.. In general, cells were maintained in DMEM/20% fetal bovine serum (**FBS**) on regular culture flasks (no coating) and used in the 15th-25<sup>th</sup> passage, since MDSC from mouse muscle have been maintained in our laboratory for at least 30 generations with the same, or even increasing, growth rate, thus confirming their stem cell nature. The absence of **SMC** in these enriched stem cells was verified at the initial passages by immunocytochemistry and western blot for  $\alpha$ -smooth muscle cell actin (**ASMA**) (see below).

MDSC were grown also in two types of scaffolds: 1) porcine small intestinal submucosa (SIS, Cook Biotech Inc., West Lafayette, IN) (**36,37**), and 2) polymeric mesh (Vicryl mesh, Ethicon Inc., Sommerville, NJ) (**38**). Both materials were cut in 0.5 cm<sup>2</sup> fragments, sterilized in alcohol and submerged in DMEM/20% FBS. MDSC were then seeded and allowed to grow until they reached over 60% confluence on the SIS scaffold or they started to cover the small holes in the mesh of the polymeric scaffold.

**Animal experiments.** Retired breeders female Fisher 344 rats (Harlan Sprague-Dawley Inc., San Diego, CA, USA), were used throughout. The animals were treated according to National Institutes of Health regulations with an Institutional Animal Care and Use Committee-approved protocol. Anesthetized rats were divided in the following groups (n=2/group): 1) "intact controls", not subjected to surgery; 2) "defected vagina, untreated", subjected to hysterectomy and a partial vaginectomy to induce apical, anterior and posterior defects. The vagina was closed with absorbable sutures; 3) "defected vagina, treated with SIS implantation",

as # 2, but implanted at the moment of surgery with the SIS scaffold. The SIS scaffold was sutured into the vaginal stump with absorbable sutures. Two nonabsorbable sutures (nylon) were used to mark the ends of the scaffold.; 4) “defected vagina, treated with SIS/MDSC implantation, as # 2, but implanted at the moment of surgery with the SIS scaffold that had been seeded with MDSC. In this case the cells were grown *in vitro* and labelled with either the nuclear blue fluorescent stain DAPI, or with DAPI and the membrane/cytoplasmic red fluorescent stain PKH26, as detailed in each figure. Tacrolimus was given daily to group 4 (1 mg/kg, sc) to avoid immunerejection of the mouse stem cells (18). At 2, 4 or 8 weeks after implantation (and in some cases at 6 weeks), rats were sacrificed under anesthesia, the vagina was excised and divided in approximately 2-3 mm transversal sections numbered from the more distal from 1 to 6 (according to the overall length of the vagina). These sections were cryoprotected in 25% sucrose, immersed in OCT, and subjected to cryosectioning (5 um for regular microscope, or 20-30 um for confocal microscope) without fixation, unless stated.

***Detection of protein expression in cell cultures and tissue sections.*** The general morphology of vaginal frozen tissue sections was evaluated with hematoxylin eosin staining. For immunocytochemistry, cells grown in triplicate on collagen-coated 8 well removable chambers, or in frozen tissue sections, were reacted (18,39) with some of the following primary antibodies against: 1) human ASMA (mouse monoclonal in Sigma kit, 1/2, Sigma Chemical, St Louis, Mo, USA) a marker for both SMC and myofibroblasts, 2) Oct-4 (rabbit polyclonal, 1/500, BioVision, Mountain View, CA, USA), a marker for embryonic stem cells (40); in this case frozen sections were fixed with 2% formaldehyde for 10 min. Immunohistochemical detection was performed by quenching in 0.3% H<sub>2</sub>O<sub>2</sub>-PBS, blocking with goat or corresponding serum, and incubating overnight at 4 °C with the primary antibody. This was followed by biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA), respectively, for 30 min, the ABC complex

containing avidin linked horse radish peroxidase (1:100; Vector), 3,3' diaminobenzidine (DAB), and counterstaining with haematoxylin, or no counterstaining, as indicated.

When DAPI and/or PKH26 fluorescence was detected, immunofluorescence was applied for ASMA (same antibody as above) or keratin 5, a squamous epithelial marker (41) (Monoclonal, 1/200, Vector Laboratories, Burlingame, CA, US) using a secondary anti-mouse IgG antibody that was biotinylated (goat, 1/200, Vector Laboratories) and this complex was detected with streptavidin-Texas Red (red fluorescence), or in the case of Oct-4, also with streptavidin-FITC (green fluorescence). After washing with PBS, the sections were mounted with Prolong antifade (Molecular Probes, Carlsbad, CA, USA). Negative controls for immunohistochemistry or immunofluorescence omitted the first antibodies or they were replaced by IgG isotype. Sections were viewed under an Olympus BH2 fluorescent microscope or in a confocal microscope, using regular light or blue and/or red filters and overlay.

***Histochemical assessment of vaginal fibrosis.*** Masson trichrome staining (39,40) was applied to tissue sections fixed overnight in Bouins fixative (Sigma). For quantitative image analysis, staining intensity was determined by computerized densitometry using the ImagePro Plus 5.1 program (Media Cybernetics, Silver Spring, MD, USA), coupled to the Olympus BH2 microscope with a Spot RT color digital camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA). Results were expressed as the ratio between the areas stained in red comprising the epithelium, lamina propria and muscularis, divided by the area occupied by collagen fibers and extracellular matrix stained in blue. Ten non-overlapping fields were screened per section. Three sections per tissue specimen from the groups of two rats were then used to calculate the mean $\pm$ SEM, based on 30 separate measurements per rat.

***Estimation of protein expression in cell and tissue homogenates by western blots.*** For western blots (18,39), cell homogenates were obtained in boiling lysis buffer (1% SDS, 1mM

sodium orthovanadate, 10 mM Tris pH 7.4 and protease inhibitors: 3  $\mu$ M leupeptin, 1  $\mu$ M pepstatin A, 1mM phenylmethylsulphonyl fluoride), and centrifuging at 16,000 *g* for 5 min. 40  $\mu$ g of protein were run on 7.5% or 10% polyacrylamide gels, and submitted to western blot transfer and immunodetection with the following antibodies against: 1) human  $\alpha$ SMA (monoclonal, 1/1000, Calbiochem, La Jolla, CA, USA); 2) human calponin-1 (basic) (mouse monoclonal, 1/25, Novocastra, Burlingame, CA, USA), as exclusive marker for SMC; 3) proliferating cellular nuclear antigen (**PCNA**) (mouse monoclonal, 1:100, Chemicon, Temecula, CA, USA) a marker for replicating cells; 4) Oct-4 (rabbit polyclonal, 1/500, BioVision, Mountain View, CA, USA), as stem cell marker 5) myoglobin (rabbit polyclonal 1/200, Santa Cruz Biotechnology, Inc., Santa Cruz, CA USA), as muscle origin marker 6) GAPDH (mouse monoclonal, 1/3000, Chemicon, Temecula, CA USA) as a housekeeping gene.

Membranes were incubated with a secondary polyclonal horse anti-mouse IgG linked to horseradish peroxidase (1:2000; BD Transduction Laboratories, Franklin Lakes, NJ, USA, or 1:5000, Amersham GE, Pittsburgh, PA, USA) and bands were visualized with luminol (SuperSignal West Pico, Chemiluminescent, Pierce, Rockford, IL, USA). For the negative controls the primary antibody was omitted.

***Estimation of multipleRNA expression by DNA microarrays.*** Pools of total cellular RNA from three different T75 flasks for MDSC that were incubated with DMEM supplemented with FBS at either 20% (“high serum”) or 2.5% (“low serum”) were isolated with Trizol-Reagent (Invitrogen, Carlsbad, CA). RNA quality was assessed by agarose gel electrophoresis and subjected to cDNA gene microarrays (SuperArray BioScience Corp., Frederick, MD) (**43**), using the following Oligo GEArray microarrays: 1) mouse stem cell (OMM-405), 2) mouse cell surface markers (OMM-055), and 3) mouse cardiovascular disease biomarkers (MM-037), which together covered a series of potential markers for MDSC. Biotin-labeled cDNA probes were

synthesized from total RNA, denatured, and hybridized overnight at 60°C in GEHybridization solution to membranes spotted with the respective pathway-specific genes. Membranes were washed, and chemiluminescent analysis was performed per the manufacturer's instructions. Raw data were analyzed using GEArray Expression Analysis Suite (SuperArray BioScience Corp., Frederick, MD). Expression values for each gene based on spot intensity were subjected to background correction and normalization with housekeeping genes.

***Confirmation of RNA expression by RT/PCR and RT/real time PCR.*** Equal amounts (2ug) of RNA were reverse transcribed in duplicate using a RNA PCR kit (Applied Biosystems, Foster City, CA). The locations of forward/reverse PCR primers for real-time RT-PCR for smoothelin and GAPDH are as follows: 575-597/626-641 on BC074818.2 (67bp) and 606-626/758-738 on BC023196. Mouse gene PCR primer sets (RT2) were purchased from SuperArray Bioscience. The QIAGEN Sybr Green PCR kit with HotStar Taq DNA polymerase (QIAGEN, Valencia, CA) was used with i-Cycler PCR thermocycler and fluorescent detector lid (Bio-Rad, Hercules, CA) (44).

The protocol included melting for 15 min at 95C, 40 cycles of three-step PCR including melting for 15 sec at 95C, annealing for 30 sec at 58C, elongation for 30 sec at 72C with an additional detection step of 15 sec at 81C, followed by a melting curve from 55–95C at the rate of 0.5C per 10 sec (44,45). We confirmed that inverse derivatives of melting curves show sharp peaks for smoothelin and GAPDH 81C and 85C indicating correct products. Samples of 25 ng cDNA were analyzed in quadruplicate in parallel with GAPDH controls; standard curves (threshold cycle vs. log pg cDNA) were generated by log dilutions of from 0.1 pg to 100 ng standard cDNA (reverse-transcribed mRNA from MDSC). Experimental mRNA starting quantities were then calculated from the standard curves and averaged using i-Cycler, iQ



software as described previously (46). The ratios of smoothelin mRNA to GAPDH mRNA were computed.

For regular RT/PCR (45), two ug of total RNA were reverse transcribed, and cDNA was amplified for 38 cycles by PCR at 94°C for 30 seconds, primer annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute. PCR products were analyzed in 2% agarose gels. The locations of the forward/reverse PCR primers in 5'-3' nucleotide positions are as follows: (1) Oct-4 (GenBank Accession No. NM\_013633), forward: nt 830-850; and reverse: nt 1130-1150, 2) nanog (GenBank Accession No. XM\_001471588) forward: nt 732-852; and reverse: nt 1032-1152, 3) CD63 (GenBank Accession No. NM\_001042580) forward: nt 478-898; and reverse: nt 778-798, 4) GAPDH (GenBank Accession No. BC059110) forward: nt 611-631; and reverse: nt 743-763.

**Statistics.** Masson trichrome values (Fig. 10) are expressed as the mean+/-SEM for 30 independent measurements per rat and 60 determinations per group. The normality distribution of the data was established using the Wilk-Shapiro test. Multiple comparisons were analysed by a single factor ANOVA, followed by *post hoc* comparisons with the Newman-Keuls test. Differences among groups were considered statistically significant at  $p<0.05$ .

## RESULTS

In order to show with specific differentiation markers that MDSC are indeed able to originate SMC *in vitro*, they were incubated for various periods of time (7-56 days) in DMEM with 20% FBS. **Fig. 1 top** shows by immunocytochemistry that as early as 9 days some of the cells express ASMA, a marker for both myofibroblasts and SMC, and that TGFβ1 intensifies this expression. Successive periods showed similar results (not shown), and this was confirmed at 22 days by western blot (**Fig. 1 bottom left**). The SMC generation was indicated with an antibody for the SMC marker calponin, not expressed in myofibroblasts, although in this case the increase

of the intensity of the TGF $\beta$ 1 band corrected by GAPDH was only moderate. The cells were actively replicating as shown by proliferating cell nuclear antigen (**PCNA**). The levels of smoothelin mRNA, another specific SMC marker, were elevated by TGF $\beta$ 1, and this effect continued until at least 49 days, as shown by RT/real time PCR (**Fig. 1 bottom right**).

The MDSC may grow on scaffolds and differentiate into the desired lineage, as shown by hematoxylin/eosin staining on an epi-illumination stereomicroscope observation of the monolayer formed on the small intestinal submucosa (SIS) membrane (**Fig. 2 left**). Since it is difficult to perform fluorescence or immunocytochemical observations on the totally opaque SIS scaffold, we opted to grow the MDSC pre-labeled with the nuclear fluorescent stain DAPI onto a translucent polymeric scaffold (Vicryl mesh) and determine whether the cells transform into SMC. The MDSC rapidly proliferated onto the scaffold (**Fig. 2 middle**) and presumably originated SMC, as detected by dual immuno-fluorescence for DAPI and ASMA (**Fig. 2 right**).

DAPI labeling is relatively short-lived, and therefore we searched for potential markers of these cells that would not be expressed in the vagina. First, DNA microarrays representing several stem cell and related genes, as well as tissue markers, were applied to identify RNA transcripts in MDSC that were grown under either high serum (20% FBS) to stimulate cell proliferation or low serum (2.5% FBS) to arrest cell growth and to stimulate cell differentiation. Some embryonic stem cell mRNAs (nanog, Oct-4, CD63, Wnt1) and skeletal muscle or myogenic markers (myoglobin, muscle creatin kinase, notch 3) were identified and the approximate intensity of expression was tabulated (**Fig. 3**).

RT/PCR for two of those genes, nanog and Oct 4, revealed a low expression in MDSC, as well as in the vaginal tissue, whereas CD63 was negligible in MDSC but well expressed in the vagina. Oct 4 and nanog were considerably expressed in the testis, as a source of germinal stem cells, and particularly in the case of nanog in the the penis, as another urogenital organ. In the

skeletal muscle (hind limb), where MDSC originated, Oct 4 expression was also high, whereas nanog was negligible (**Fig. 4 left**). Western blotting with an Oct 4 antibody revealed a strong band in the MDSC that runs as Oct 3B and a very faint one as Oct 3A, whereas both bands were expressed similarly at high levels in the vagina, the skeletal muscle, and the penis. (**Fig. 4 right**). Myoglobin, a skeletal muscle marker, was expressed in MDSC and the vagina, and as expected, very highly in the skeletal muscle, but not in the penis. The myoglobin band in the vaginal tissue possibly results from contamination with adjacent skeletal muscle tissue. Therefore, Oct 4, because of being an embryonic stem cell marker, was used to locate potential endogenous stem cells in the vagina (see below, Fig. 7), whereas due to its low expression in the MDSC it was not useful as a tag to follow MDSC implantation in this tissue.

To study the effects of implanted MDSC on vaginal reconstruction, rats were either left intact and untreated, or were subjected to hysterectomy and partial vaginectomy to create the apical, anterior and posterior vaginal defects. MDSC grown on SIS were labeled with DAPI and implanted on the vagina at the site of vaginal defects, whereas other animal groups were implanted with SIS without cells or left untreated, and sacrificed at 2, 4, 6 and 8 weeks (n=2/group). This time course was selected to follow up qualitatively in a preliminary “proof of concept” cell uptake, survival, differentiation, and effects on histology at several standard periods used in studies of stem cell implantation. The vaginal tissues were divided in regions numbered 1 through 6 from the one most distant to the site of implantation. The DAPI labeled MDSC on the SIS scaffolds were visualized at 4 weeks as numerous blue fluorescent nuclei in frozen unfixed tissue sections of the tissue around the site of implantation (regions 5 and 6), as shown on **Fig. 5 A**. These sections were immunostained with an antibody for ASMA and a red fluorescence Texas red-tagged secondary antibody that shows, in a merge of the blue and red fluorescence, that many of the implanted cells were positive for ASMA expression. Some few

MDSC migrated away from the SIS implants to more distant regions (1 and 3), showing also differentiation into SMC (**Fig. 5 B**).

MDSC growing on SIS were also dually labeled with DAPI and a membrane red fluorescence tag, PKH26, and then implanted in the vagina as above. **Fig. 6 top** shows that at 4 weeks the red fluorescence is somehow diffuse, possibly in regions of the cell membrane and even the cytoplasm, while the blue fluorescence is still visible in discrete nuclei. At 6 weeks (**Fig. 6 bottom**) DAPI labeling has become more diffuse and restricted to a small area of the field where red fluorescence is still apparent and even advanced into the nuclei. This indicates that the MDSC survive for at least 6 weeks.

The other marker identified in the in vitro experiments for MDSC, the Oct 4 gene, detects the endogenous vaginal stem cells and the implanted MDSC. The vaginal tissue from intact rats (no surgery) within a region equivalent to the site of MDSC implantation, displays some discrete staining in isolated regions of the muscularis, apparently in the longitudinal bundles, as well as in the stratified squamous epithelium (not shown). Staining is rather similar in hysterectomized animals with partial vaginectomy after 4 weeks of surgery and no treatment (**Fig. 7 top**). Four weeks after implantation of SIS only without MDSC, there was a more extended staining, with some spreading to both longitudinal and transversal bundles in the muscularis (**Fig. 7 middle**). This is likely due to “activation” of endogenous stem cell replication by the combined SIS implant/tissue repair reaction. The SIS/MDSC intensified these changes with even more dissemination of Oct 4+ cells, particularly in a thicker epithelium, suggesting that many of them are derived from the implanted MDSC and others from endogenous stem cell activation (**Fig. 7 bottom**).

The stimulatory effects of MDSC/SIS on the regeneration of vaginal tissue are seen clearly 4 weeks after surgery on hematoxylin/eosin staining of the vaginal tissue sections, where

there is an apparent increase in the muscularis after receiving the implant (**Fig. 8 bottom**), as compared to the intact vagina (no surgery) (**Fig. 8 top left**) or the untreated hysterectomized/partially vaginectomized tissue (**Fig. 8 top right**). What is remarkable is the considerable stratified squamous epithelium that developed upon treatment with SIS and particularly with the MDSC on the SIS (**Fig. 8 bottom**).

The stimulatory effect of MDSC on the growth of the epithelium was confirmed by immunohistodetection with Texas red fluorescence of keratin 5 a marker of squamous epithelium, compared with the respective hematoxylin/eosin staining at 4 weeks (**Fig. 9**). In this case DAPI was applied after the sections were obtained as a nuclear fluorescent counterstain, not to detect the implanted MDSC. The untreated vagina from hysterectomized rats showed a disorganized and rather thin epithelium with virtually no indentation with connective tissue papillae (**Fig. 9 A,B**). SIS implantation without cells restored some papillae and made the epithelium thicker (**Fig. 9 C,D**), but only the SIS holding the MDSC what normalized the epithelium thickness and appearance (**Fig. 9 E,F**). In order to determine whether the MDSC themselves differentiated into epithelial cells, sections obtained from the vagina of hysterectomized/partially vaginectomized rats where the vagina had been implanted with red fluorescence PKH26-labeled MDSC growing on SIS, were stained for keratin 5 but using FITC green fluorescence, and no MDSC staining was applied. Although red fluorescent implanted cells were visible in the vicinity of the green fluorescent keratin-5 positive area, there was no convincing overlapping in the overlay (not shown) that would suggest a direct conversion, thus implying that the MDSC exert a trophic effect on the epithelium more than a true differentiation.

Masson trichrome staining was applied to determine the extent of fibrosis in the tissue sections upon the different treatments at 8 weeks. **Fig. 10 (panels)** shows in the vagina from intact animals not subjected to surgery the layer of longitudinal smooth muscle bundles in the

muscularis underneath the lamina propria, and the stratified squamous epithelium, stained in red, whereas virtually all the lamina propria and interstitial connective tissue in the smooth muscle is stained in blue indicating collagen fibers. The tissue from hysterectomized and partially vaginectomized animals had a thinner but organized layer of smooth muscle. In contrast, after SIS implantation there was very little smooth muscle and a thin epithelium as if the scaffold would induce mainly connective tissue formation, probably from fibrosis and scar tissue formation. This may also be due, in part, to the wound healing process caused by surgery and regeneration process at longer time points. However, when SIS with MDSC was implanted, there was a remarkable healthy smooth muscle and thicker epithelium. These results suggest that the use of MDSC on SIS scaffold regenerated vaginal smooth muscle and epithelium much faster than SIS alone. Quantitative image analysis (**Fig. 10 bar graph**) of the ratio between the red versus blue areas, as a representation of the cellular/extracellular matrix ratio, was applied to 30 fields per rat. This analysis, limited by a small n imposed by the study design, indicated that MDSC seeded on the SIS generated significantly higher amount of cellular components.

## **DISCUSSION**

To our knowledge, based on an exhaustive PubMed search, this is the first paper on the use of stem cells of any origin to repair defects or injury to the vagina in women or animal models. We have shown that MDSC mounted on a biodegradable SIS scaffold implanted in the defected vagina (apical, anterior and posterior defects) of rats survive for extended periods, differentiate into SMC in the muscularis, and promote epithelium regeneration presumably by a trophic effect. This exploratory “proof of concept” approach was supported by finding a reduction in fibrosis and stimulation of the vaginal repair process, that appears to occur more efficiently than with SIS implants alone without cells or in the spontaneous regeneration occurring in the absence of any treatment. The current study establishes the foundation for future

experimental paradigms to evaluate more precisely with larger number of animals the effects of MDSC/SIS on vaginal tissue fibrosis at even more prolonged periods after implantation. Although we did not perform the functional study of the vaginal defects and this surgically injured vagina model is not the same as pelvic organ prolapse that has developed with time, our results show that implanted MDSC in the surgically injured vagina can survive, differentiate, and regenerate/repair the injured vagina.

In addition, we have defined the expression in MDSC of certain genes, such as Oct-4, that is a well recognized marker of embryonic stem cells. This gene served also to identify potential endogenous stem cells in the tissue that may constitute a target for repair therapy based on their potential activation by pharmacological interventions. MDSC per se, without scaffolds, were not tested because we considered that the physical restriction imposed by the SIS support would on one side facilitate closure of the wound and on the other side help to maintain the implanted cells around the site of repair.

One of the main limitations of the current work is that it was difficult to assess quantitatively the extent of the putative improvement of vaginal regeneration by MDSC/SIS over SIS alone or in the absence of treatment, since with the exception of the effect on fibrosis, we have based our interpretation on qualitative comparisons between vaginal tissues from each experimental group. This was prompted by the difficulty of defining markers that single-handed or in combination represent the complexity of the coordinated repair of the different cellular compartments in the vagina and their histological and anatomical arrangement to restore the normal tissue function. In the case of the correction of the well defined SMC loss in the penile corpora cavernosa occurring during aging we employed ASMA estimated by quantitative western blot (18), but for vaginal repair the possibility that ASMA may also indicate generation of myofibroblasts involved in a fibrotic process (39) may complicate the interpretation. In

addition, the balance between the muscularis and the squamous epithelium and lamina propria may not be well represented by isolated markers for each tissue.

Within these constraints, the in situ estimation in tissue sections of the cellular compartment in relation to collagen and extracellular matrix is a reasonable approximation to evaluate the efficacy of MDSC/SIS, since it measures the functionally desirable restoration of the SMC/keratinocytes content versus the noxious complication of excessive collagen deposition, namely fibrosis. A caveat is that myofibroblasts, the main cell type in fibrosis (47), may also be counted among the red staining area. However, the main reason for our experimental design was that as first stage this work aimed to define the proof of concept of implanted cells surviving in the injured vagina, converting into one of the desired cell types (SMC), and stimulating the formation of the epithelium, while maintaining the proper histological appearance. Further studies will be conducted to identify the most suitable markers for quantitative western blots in order to provide a precise evaluation of the extent of repair. This would facilitate to determine the role of variables such as multiple versus single injection sites, cell loads, pharmacological adjuvants, influence of scaffold types, or ranges of persistence of implanted MDSC, on the relative efficacy of each treatment, and particularly on vaginal function.

Although in the present work we employed immunosuppression with tacrolimus because of the inter-species implantation we used (mouse MDSC into rat vagina), we envisage that this will not be needed in further animal studies or in clinical applications. First, in both cases allografts, e.g, rat MDSC donor in rat recipient, and human MDSC in women, may be used since stem cells are known to be considerably hypoimmunogenic as compared to differentiated cells (21,22). Moreover, in the case of humans, autografts can easily be obtained from biopsies of the skeletal muscle that in a few weeks can generate cell cultures for reimplantation (25). An even less invasive approach would be based on pharmacological awakening of potential dormant



endogenous stem cells from vaginal tissues similar to the Oct-4 cells identified here, which would be the ultimate goal of stem cell therapy (48,49). The endogenous stem cells, that in some cases may be rudimentary embryonic stem cells (32-34), are being increasingly identified in adult tissues as populations different from other stem cells already characterized, and in the vagina they may even be paracrinely stimulated by the implanted MDSC. In conclusion, MDSC seeded onto degradable scaffolds may constitute a promissory approach for the treatment of vaginal prolapse, although much further work is needed to validate and optimize the procedure.

## ACKNOWLEDGEMENTS

This work was supported by an internal grant from the Division of Female Pelvic Medicine and Reconstructive Surgery of Harbor-UCLA Medical Center, University of California at Los Angeles and in part by grants NIH R21DK-070003 and DOD PR 064756.

## REFERENCES

- 1: South M, Amundsen CL. Pelvic organ prolapse: a review of the current literature. *Minerva Ginecol.* 2007 ;59:601-12.
- 2: Maher C, Baessler K, Glazener CM, Adams EJ, Hagen S. Surgical management of pelvic organ prolapse in women: a short version Cochrane review. *Neurourol Urodyn.* 2008;27(1):3-12.
- 3: Nguyen JN. The use of grafts for anterior vaginal prolapse repair: pros and cons. *Curr Opin Obstet Gynecol.* 2008 ;20:501-5.
- 4: Jia X, Glazener C, Mowatt G, MacLennan G, Bain C, Fraser C, et al. Efficacy and safety of using mesh or grafts in surgery for anterior and/or posterior vaginal wall prolapse: systematic review and meta-analysis. *BJOG.* 2008 Aug 18. [Epub ahead of print]
- 5: Mourtzinou A, Raz S. Repair of vaginal vault prolapse and pelvic floor relaxation using polypropylene mesh. *Curr Opin Obstet Gynecol.* 2006 ;18:555-9.

- 432 6: Jakus SM, Shapiro A, Hall CD. Biologic and synthetic graft use in pelvic surgery: a review.  
433 *Obstet Gynecol Surv.* 2008;63:253-66.
- 434 7: Foon R, Tooze-Hobson P, Latthe PM. Adjuvant materials in anterior vaginal wall prolapse  
435 surgery: a systematic review of effectiveness and complications. *Int Urogynecol J Pelvic Floor*  
436 *Dysfunct.* 2008 Jul 8. [Epub ahead of print]
- 437 8: Bako A, Dhar R. Review of synthetic mesh-related complications in pelvic floor  
438 reconstructive surgery. *Int Urogynecol J Pelvic Floor Dysfunct.* 2008 Sep 9. [Epub ahead of print]
- 439 9: Huffaker RK, Muir TW, Rao A, Baumann SS, Kuehl TJ, Pierce LM. Histologic response of  
440 porcine collagen-coated and uncoated polypropylene grafts in a rabbit vagina model. *Am J*  
441 *Obstet Gynecol.* 2008 ;198:582.e1-7.
- 442 10: Tsui KP, Ng SC, Tee YT, Yeh GP, Chen GD. Complications of synthetic graft materials  
443 used in suburethral sling procedures. *Int Urogynecol J Pelvic Floor Dysfunct.* 2005;16:165-7.
- 444 11: Wang AC, Lee LY, Lin CT, Chen JR. A histologic and immunohistochemical analysis of  
445 defective vaginal healing after continence taping procedures: a prospective case-controlled pilot  
446 study. *Am J Obstet Gynecol.* 2004 ;191:1868-74.
- 447 12. Smaldone MC, Chancellor MB. Muscle derived stem cell therapy for stress urinary  
448 incontinence. *World J Urol.* 2008 ;26:327-32.
- 449 13: Furuta A, Carr LK, Yoshimura N, Chancellor MB. Advances in the understanding of stress  
450 urinary incontinence and the promise of stem-cell therapy. *Rev Urol.* 2007 Summer;9:106-12.
- 451 14. Jack GS, Almeida FG, Zhang R, Alfonso ZC, Zuk PA, Rodríguez LV. Processed lipoaspirate  
452 cells for tissue engineering of the lower urinary tract: implications for the treatment of stress  
453 urinary incontinence and bladder reconstruction. *J Urol.* 2005 ;174:2041-5.
- 454 15. Bochinski D, Lin GT, Nunes L, Carrion R, Rahman N, Lin CS, et al. The effect of neural  
455 embryonic stem cell therapy in a rat model of cavernosal nerve injury. *BJU Int* 2004; 94:904-9

- 456 16. Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, et al. Mesenchymal  
457 stem cells alone or ex vivo gene modified with endothelial nitric oxide synthase reverse age-  
458 associated erectile dysfunction. *Am J Physiol Heart Circ Physiol* 2007; 292:H1278-90
- 459 17. Kim Y, de Miguel F, Usiene I, Kwon D, Yoshimura N, Huard J, et al. Injection of skeletal  
460 muscle-derived cells into the penis improves erectile function. *Int J Impot Res* 2006; 18:329-34
- 461 18. Nolzco G, Kovanez I, Vernet D, Gelfand RA, Tsao J, Ferrini MG, et al. Effect of muscle-  
462 derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in  
463 the aged rat. *BJU Int.* 2008 ;101:1156-64.
- 464 19: Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. *Adv Cancer Res.*  
465 2008;100:133-58.
- 466 20: Grinnemo KH, Sylvén C, Hovatta O, Dellgren G, Corbascio M. Immunogenicity of human  
467 embryonic stem cells. *Cell Tissue Res.* 2008 ;331:67-78.
- 468 21: Le Blanc K, Ringdén O. Immunomodulation by mesenchymal stem cells and clinical  
469 experience. *J Intern Med.* 2007;262:509-25.
- 470 22: Jankowski RJ, Huard J. Myogenic cellular transplantation and regeneration: sorting through  
471 progenitor heterogeneity. *Panminerva Med.* 2004 ;46:81-91.
- 472 23: De Filippo RE, Bishop CE, Filho LF, Yoo JJ, Atala A. Tissue engineering a complete  
473 vaginal replacement from a small biopsy of autologous tissue. *Transplantation.* 2008 27;86:208-  
474 14.
- 475 24: De Filippo RE, Yoo JJ, Atala A. Engineering of vaginal tissue in vivo. *Tissue Eng.* 2003  
476 ;9:301-6.
- 477 25: Urish K, Kanda Y, Huard J. Initial failure in myoblast transplantation therapy has led the way  
478 toward the isolation of muscle stem cells: potential for tissue regeneration. *Curr Top Dev Biol.*  
479 2005;68:263-80.

- 480 26: Zheng B, Cao B, Crisan M, Sun B, Li G, Logar A, et al. Prospective identification of  
481 myogenic endothelial cells in human skeletal muscle. *Nat Biotechnol.* 2007 ;25:1025-34.
- 482 27: Alessandri G, Pagano S, Bez A, Benetti A, Pozzi S, Iannolo G, et al. Isolation and culture of  
483 human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell  
484 lineages. *Lancet.* 2004;364:1872-83.
- 485 28. Mitterberger M, Marksteiner R, Margreiter E, Pinggera GM, Frauscher F, Ulmer H, et al.  
486 Myoblast and fibroblast therapy for post-prostatectomy urinary incontinence: 1-year followup of  
487 63 patients. *J Urol.* 2008;179:226-31.
- 488 29. Hwang JH, Yuk SH, Lee JH, Lyoo WS, Ghil SH, Lee SS, et al.. Isolation of muscle derived  
489 stem cells from rat and its smooth muscle differentiation [corrected]. *Mol Cells.* 2004; **17**:57-61.  
490 Erratum in: *Mol Cells* 2004; **17**:381
- 491 30: Vernet D, Qian A, Nolzco G, Cantini L, Magee TR, Ferrini MG, et al (2005) Evidence that  
492 osteogenic progenitor cells in the human tunica albuginea may originate from stem cells.  
493 Implications for Peyronie's disease. *Biol Reprod*, 73:1199-1210.
- 494 31. Virant-Klun I, Zech N, Rozman P, Vogler A, Cvjeticanin B, Klemenc P, et al. Putative stem  
495 cells with an embryonic character isolated from the ovarian surface epithelium of women with no  
496 naturally present follicles and oocytes. *Differentiation.* 2008;76:843-56.
- 497 32: Zovoilis A, Nolte J, Drusenheimer N, Zechner U, Hada H, Guan K, et al. Multipotent adult  
498 germline stem cells and embryonic stem cells have similar microRNA profiles. *Mol Hum*  
499 *Reprod.* 2008 ;14:521-9.
- 500 33: Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, Ratajczak MZ. A  
501 population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells  
502 identified in adult bone marrow. *Leukemia.* 2006 ;20:857-69.

- 503 34: Ratajczak MZ, Zuba-Surma EK, Shin DM, Ratajczak J, Kucia M. Very small embryonic-like  
 504 (VSEL) stem cells in adult organs and their potential role in rejuvenation of tissues and  
 505 longevity. *Exp Gerontol.* 2008 Jun 14. [Epub ahead of print]
- 506 35. Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, et al. Clonal isolation of  
 507 muscle-derived cells capable of enhancing muscle regeneration and bone healing. *J Cell Biol*  
 508 2000; **150**:1085-1100
- 509 36: Chaliha C, Khalid U, Campagna L, Digesu GA, Ajay B, Khullar V. SIS graft for anterior  
 510 vaginal wall prolapse repair--a case-controlled study. *Int Urogynecol J Pelvic Floor Dysfunct.*  
 511 2006 ;17:492-7.
- 512 37: Chaliha C, Khalid U, Campagna L, Digesu GA, Ajay B, Khullar V. SIS graft for anterior  
 513 vaginal wall prolapse repair--a case-controlled study. *Int Urogynecol J Pelvic Floor Dysfunct.*  
 514 2006 ;17:492-7
- 515 38: Allahdin S, Glazener C, Bain C. A randomised controlled trial evaluating the use of  
 516 polyglactin mesh, polydioxanone and polyglactin sutures for pelvic organ prolapse surgery. *J*  
 517 *Obstet Gynaecol.* 2008 ;28:427-31
- 518 39: Cantini LP, Ferrini MG, Vernet D, Magee TR, Qian A, Gelfand RA, et al. Profibrotic role of  
 519 myostatin in Peyronie's disease. *J Sex Med.* 2008 ;5:1607-22.
- 520 40: Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, et al.  
 521 Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs  
 522 after cavernosal nerve resection. *BJU Int.* 2008 ;101:203-10.
- 523 41. Roubelakis MG, Pappa KI, Bitsika V, Zagoura D, Vlahou A, Papadaki HA, et al. Molecular  
 524 and proteomic characterization of human mesenchymal stem cells derived from amniotic fluid:  
 525 comparison to bone marrow mesenchymal stem cells. *Stem Cells Dev.* 2007 ;16:931-52.

42. Lloyd C, Yu QC, Cheng J, Turksen K, Degenstein L, Hutton E, et al. The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. *J Cell Biol.* 1995 ;129:1329-44.
- 43: Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol.* 2008 ;196:235-49.
- 44: Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, Gonzalez-Cadavid NF, et al. Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinology.* 2006 ;147:141-54.
- 45: Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, Vernet D, et al. Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein Inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med.* 2007 ;4:633-43.
46. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology.* 2003 ;144:5081-8
47. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008 ;214:199-210.
48. Goldman SA. Disease targets and strategies for the therapeutic modulation of endogenous neural stem and progenitor cells. *Clin Pharmacol Ther.* 2007 ;82:453-60.
- 49.: Romagnani P, Lasagni L, Mazzinghi B, Lazzeri E, Romagnani S. Pharmacological modulation of stem cell function. *Curr Med Chem.* 2007;14:1129-39.

## LEGENDS TO FIGURES

### **Figure 1. MDSC differentiate in vitro into cells that express smooth muscle cells markers.**

Top: MDSC were incubated in triplicate for up to 56 days on collagen-coated 6-well plates in

DMEM with 2.5 % serum, and immunostained at various periods for ASMA. Panels show an early incubation (9 days) at 200X. Control: no addition; TGF $\beta$ 1: 5 ng/ml. Bottom left: expression of ASMA, specific SMC marker (calponin) and cell proliferation marker (PCNA) at 22 days, by western blot; Bottom right: expression of mRNA for specific SMC marker smoothelin at 21 and 49 days, by RT/real time PCR. Values are expressed as ratios against the respective control.

**Figure 2. MDSC grow in vitro on biological and synthetic scaffolds and undergo differentiation into SMC.** Left: MDSC were seeded on 1 X 1 cm SIS pieces inside 12-well plates in DMEM/2.5% serum (with TGF $\beta$ 1), allowed to grow for 2 weeks, stained with hematoxylin, and examined under a reverse phase microscope (100 X). Middle: MDSC were seeded on Vicryl polymeric mesh fragments as for SIS, stained with DAPI, and examined under a fluorescent microscope (200 X). Right: blue/red fluorescent overlay of cells subjected to immunostaining for ASMA with a Texas red secondary antibody (200 X).

**Figure 3. MDSC express mRNAs for embryonic stem cell markers and for muscle-specific markers, as detected by DNA microarrays.** Top A: RNA was isolated from MDSC cultured in duplicate under low (2.5%) or Top B: high (20%) serum for 3 weeks and hybridized against the stem cell Superarray panel. Bottom: the mRNA levels in this specific experiment were calculated for some embryonic stem cell markers, and also for other selected genes identified with two other panels (OMM-37 and OMM-055), just to confirm a given gene expression.

**Figure 4. Embryonic stem cell and muscle-specific markers are also detected in MDSC by RT/PCR and western blot, although they are expressed at low levels in vaginal tissue.** Left top and middle: RNA from MDSC and from tissues as indicated were subjected to RT/PCR, and the products fractionated by agarose gel electrophoresis, detecting the bands by ethidium bromide staining; Right: MDSC and tissue homogenates as indicated were subjected to western blot and immunodetection as indicated. MGB: myoglobin (17 kDa), GAPDH ( 35 kDa).

**Figure 5. MDSC growing on SIS scaffolds differentiate into smooth muscle cells when implanted into injured vaginal tissue in the rat, and spread away from the site of injection.**

**A:** Blue/red fluorescence overlay of vaginal tissue implanted with DAPI-tagged MDSC/SIS following hysterectomy and partial vaginectomy, and subjected to ASMA red-fluorescence immunodetection, focusing in the muscularis (200X). Frozen tissue sections (10% formalin-fixed) were obtained at 4 weeks after implantation. Region 6 is directly in the site of implantation (suture site around orifice), and region 5 is immediately distal; **B:** As A, but in more distal sections, 3 and 1.

**Figure 6. The implanted MDSC persist in the vagina for at least 6 weeks after implantation and may be traced with PKH26.** **Top:** Separate red and blue filter images of frozen vaginal tissue sections (not fixed) in region 5 at 4 weeks after implantation of SIS with cultures of MDSC tagged with both PKH26 (red fluorescence in membrane and cytoplasm) and DAPI (blue fluorescence in nuclei) (200X). **Bottom:** as top, but at 6 weeks (400X).

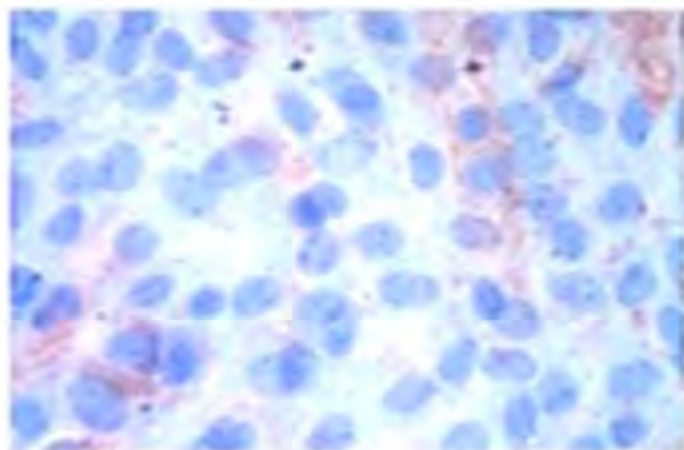
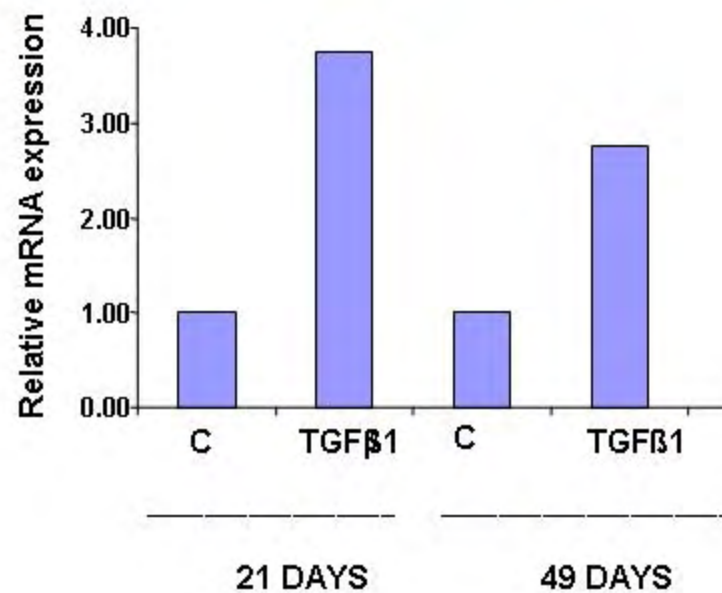
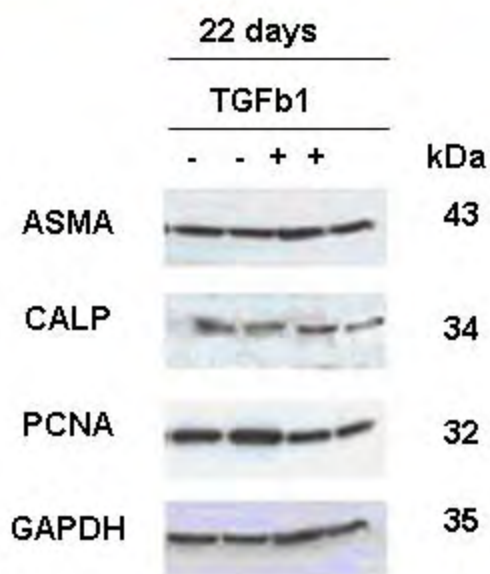
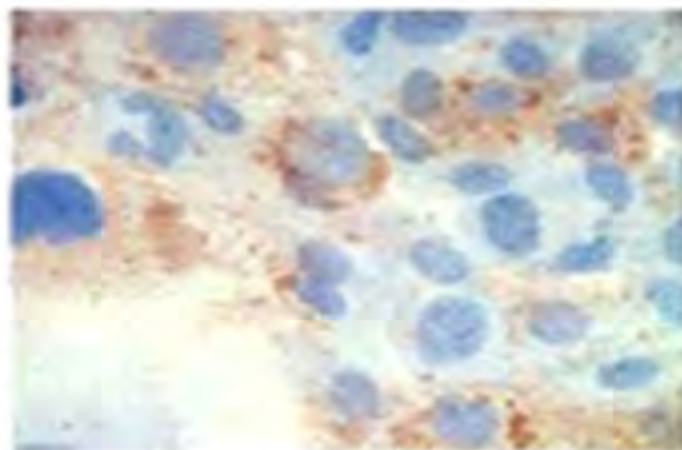
**Figure 7. The embryonic stem cell marker Oct-4 is detectable in the proximal vagina at 4 weeks after surgery and its expression is stimulated in the vaginal tissue that receive the MDSC/SIS implants.** Frozen tissue sections were fixed with 2% formaldehyde for 10 min, and subjected to immunohistochemistry with anti Oct4 antibody followed by immunoperoxidase-AEC staining. **Left row:** 40X; **Mid and right rows:** 200X. Tissues as indicated.

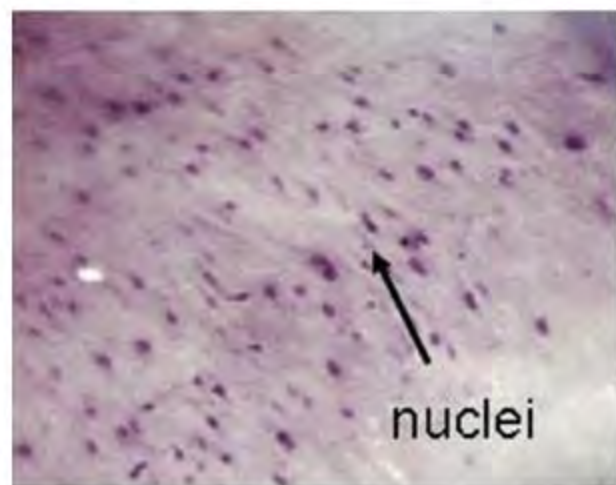
**Figure 8. Implanted MDSC/SIS induce regeneration of the stratified squamous epithelium in the vagina around the site of implantation.** Frozen sections for region 5 (not fixed) were stained with hematoxylin eosin. **Top left:** intact controls, 40X; **Top right:** hysterectomized rats, 40X; proximal; **Bottom:** hysterectomized rats with MDSC/SIS, 200X, at two areas of the tissue section



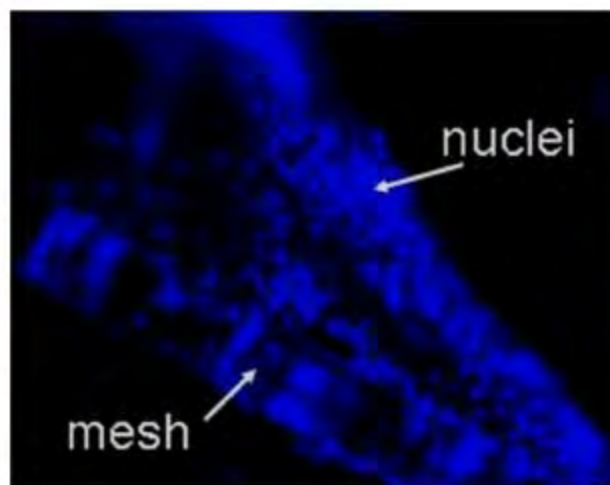
**Figure 9. The effect exerted by MDSC on the stratified squamous epithelium is confirmed by immunohistochemistry for keratin 5.** A,C,E: Frozen sections for region 5 (not fixed) were reacted against an antibody for keratin 5 that was detected by Texas red immunofluorescence, and nuclei were counterstained with DAPI (200X). B,D,F: Adjacent sections were stained with hematoxylin/eosin (200X).

**Figure 10. Implantation of SIS with MDSC increases the cellular/extracellular ratio during vaginal regeneration.** Top and middle: Representative pictures of frozen tissue sections for region 5 (fixed or not) at 8 weeks that were subjected to Masson-trichrome histochemistry (100X). Bottom: quantitative image analysis for 10 fields per specimen, 3 specimens per rat, and 2 rats per group.

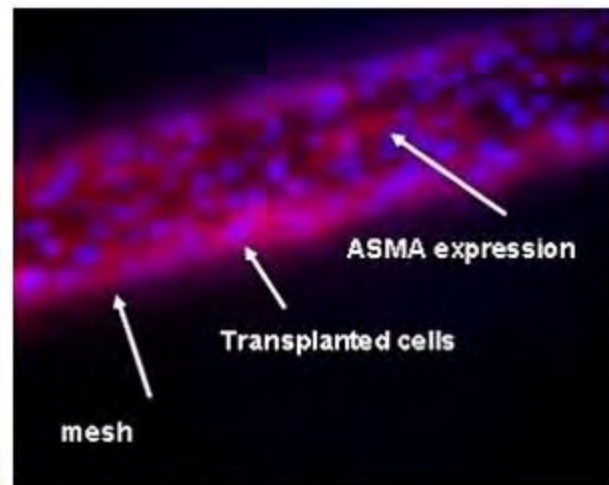
**C****TGFb1**



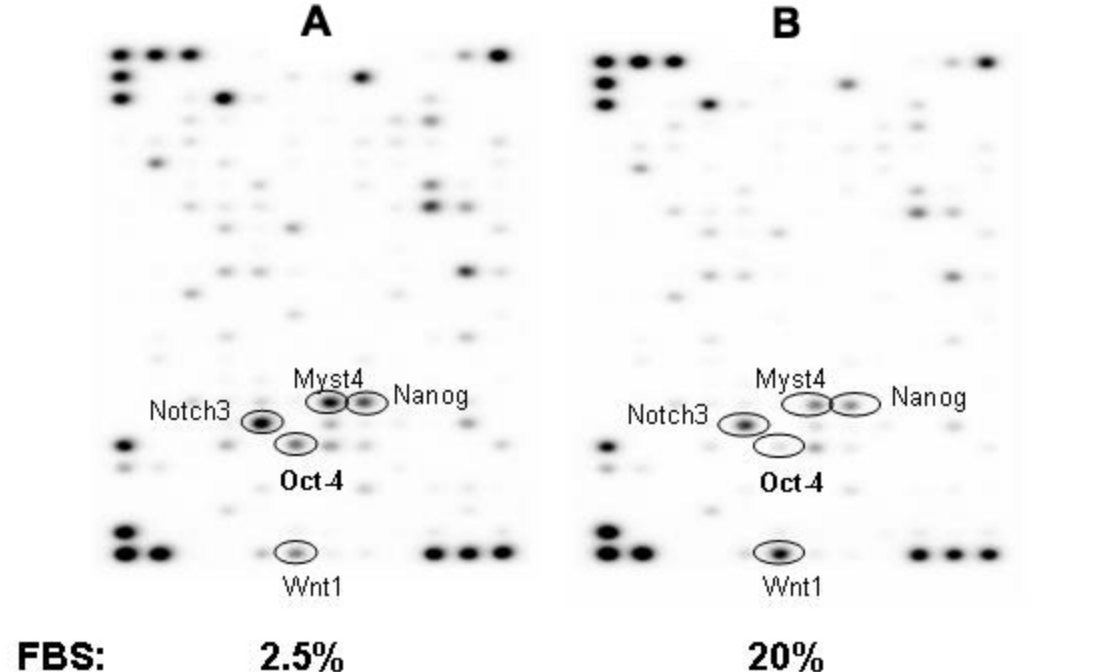
**SIS, hematoxylin**



**Polymer, blue fluorescence**

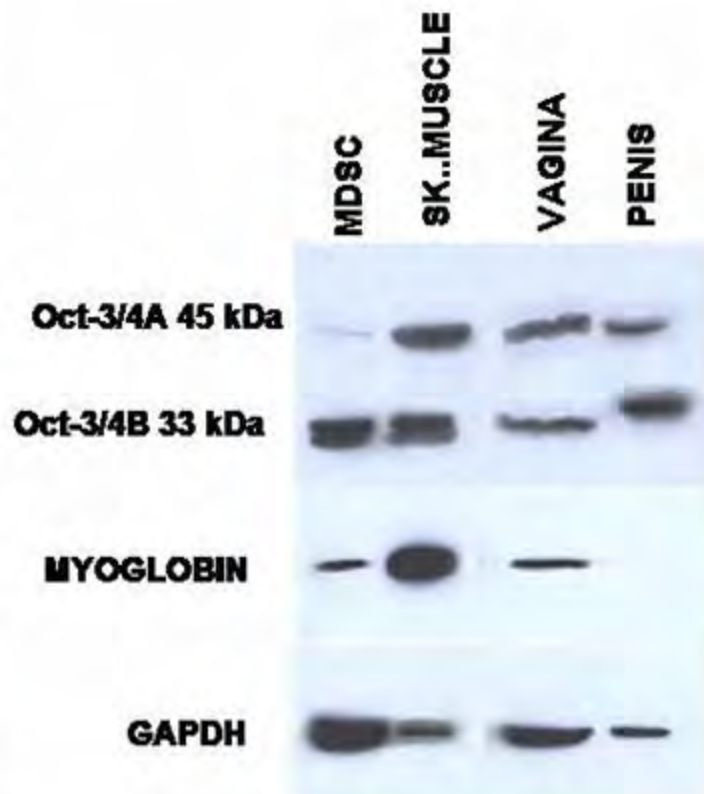
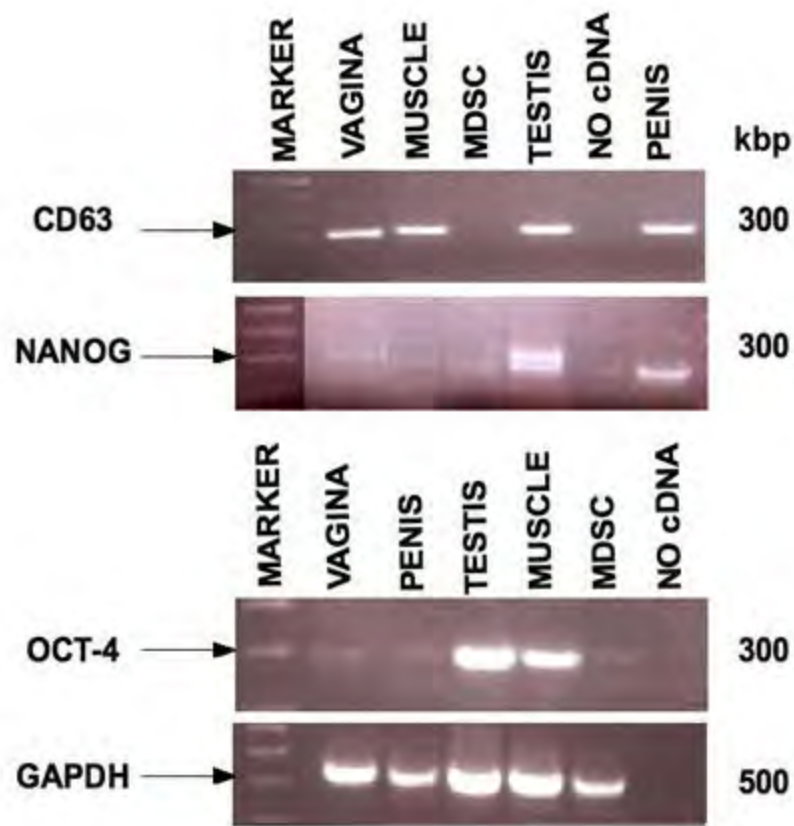


**Polymer, blue/red fluorescence**



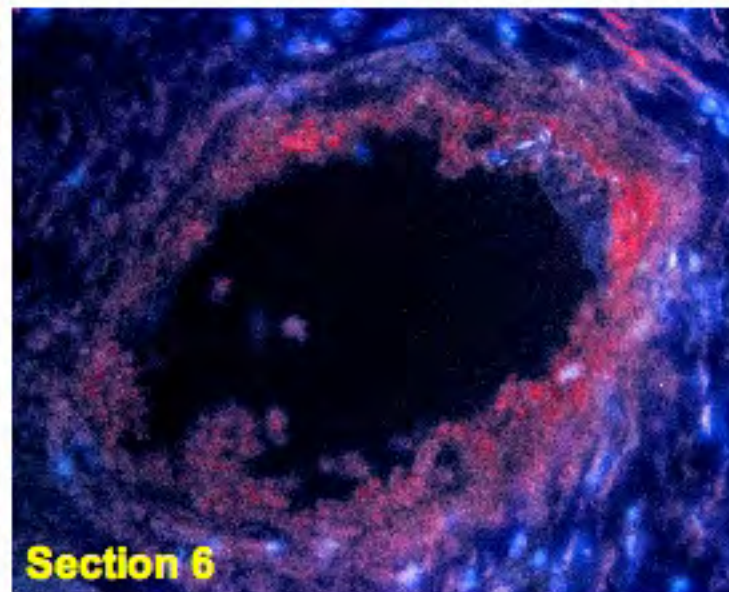
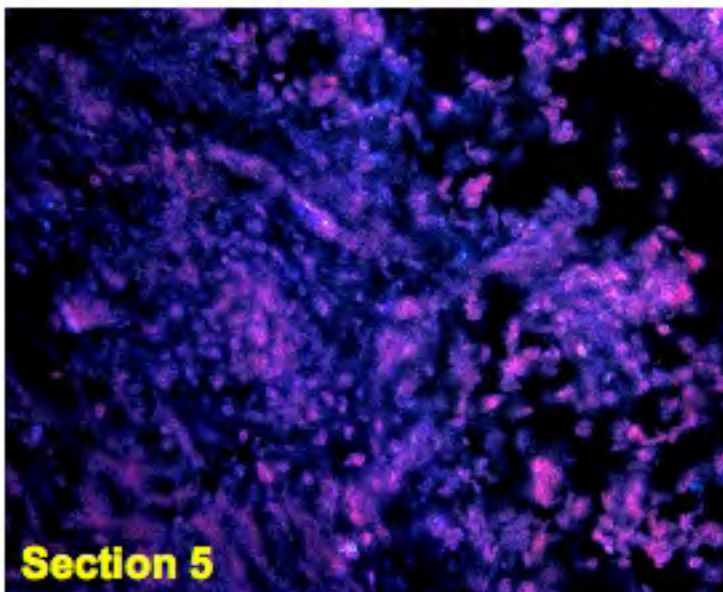
OMM	No.	Abbrev.	Full Name	A	B
405	199	Myst 4	Myst histone acetyltransferase	45	34
	200	Nanog	Nanog homeobox	30	29
	209	Notch 3	Notch3 gene homolog 3	53	56
	222	Pou 5 fi (Oct 4)	Pou domain, class 5	23	8
	282	Wnt 1	Wingless-related MMTV integration site 1	20	72
055	29	CD63	CD63 antigen	259	ND
037	20	Ckm	Muscle creatin kinase	ND	32
	73	Mb	Myoglobin	ND	112

OMM: Array number; Relative intensities: A: 2.5% fetal bovine serum; B: 20% fetal bovine serum; ND: Not Determined

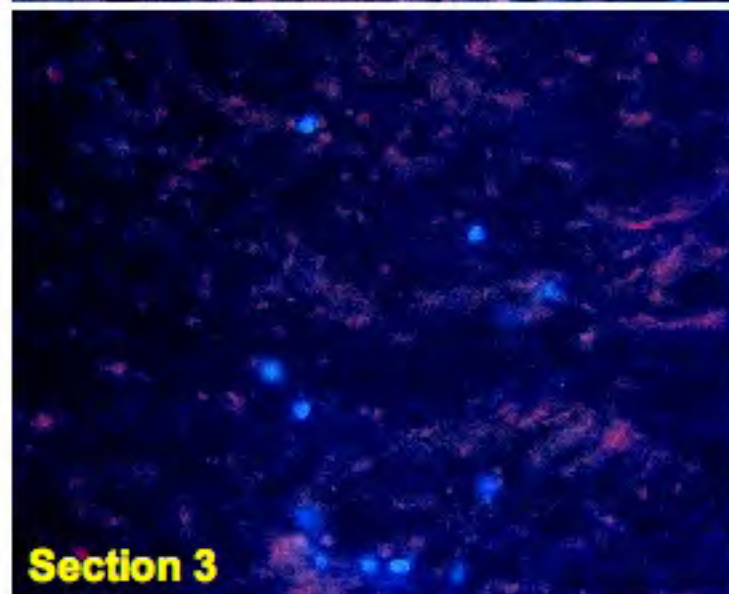
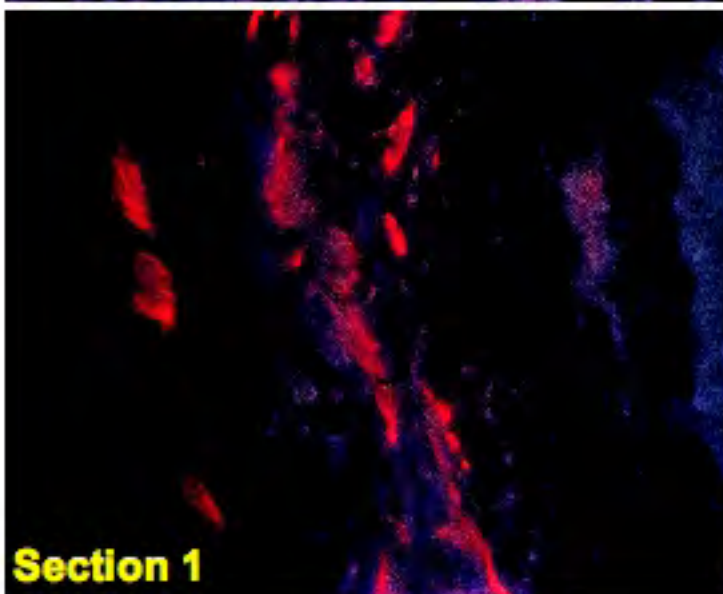




**A**



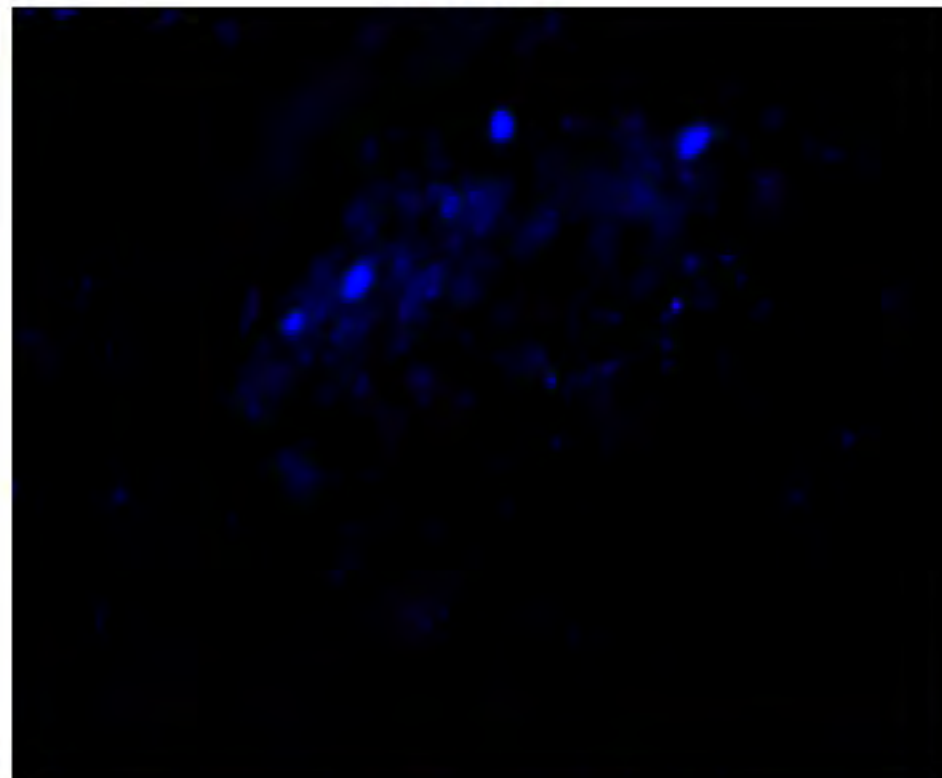
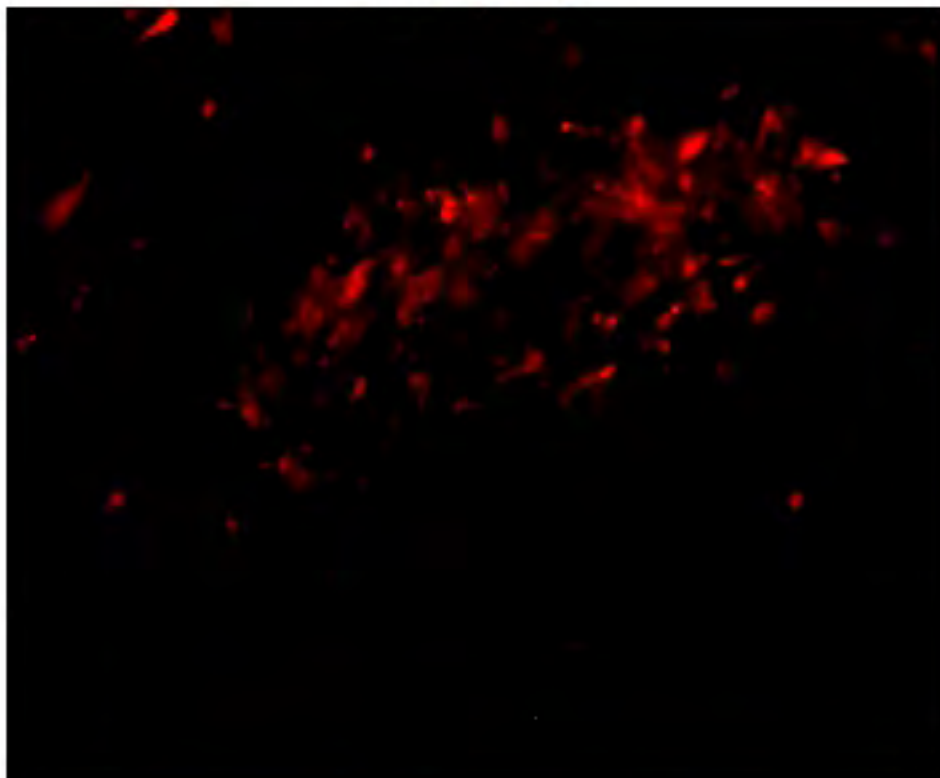
**B**



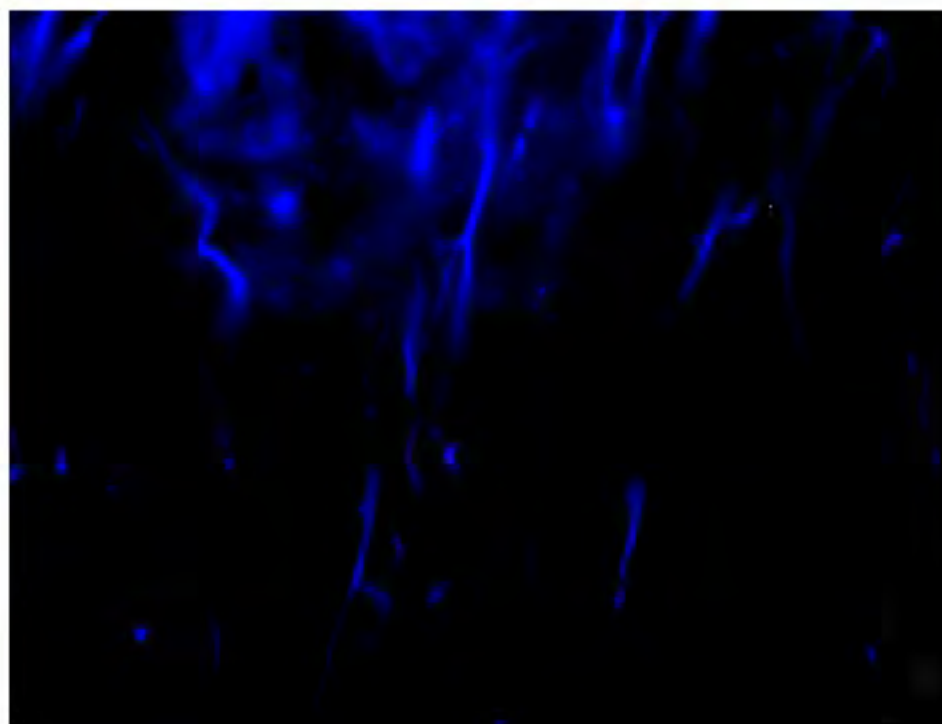
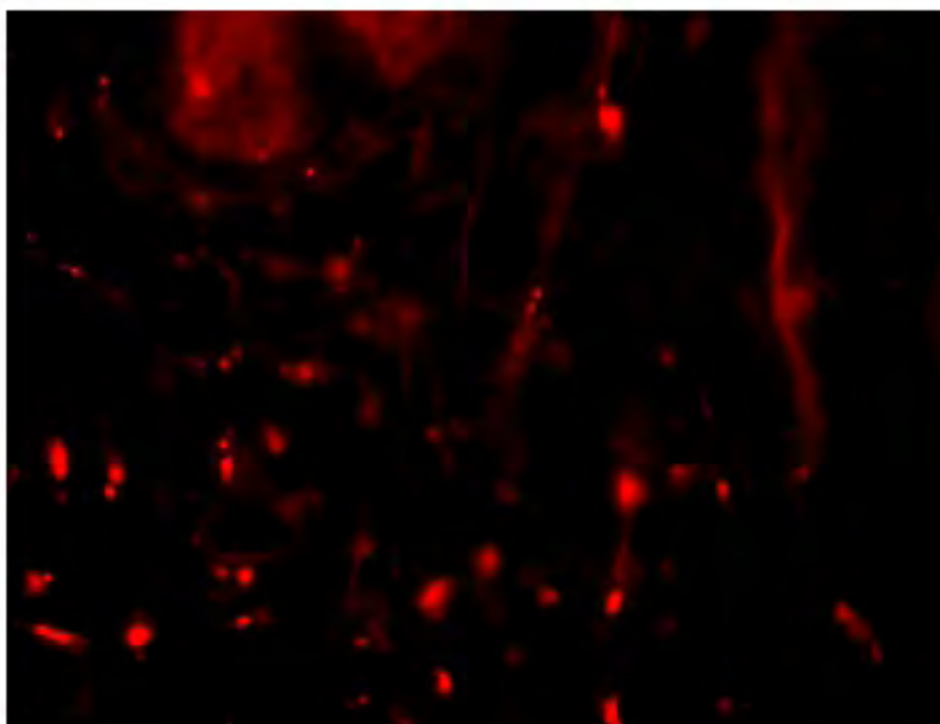
**PKH 26**

**DAPI**

**4 wks**

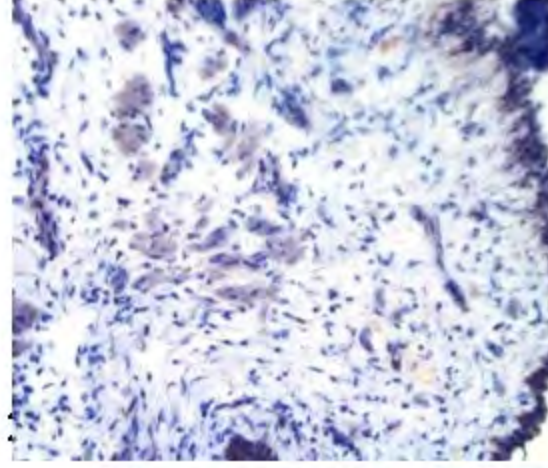
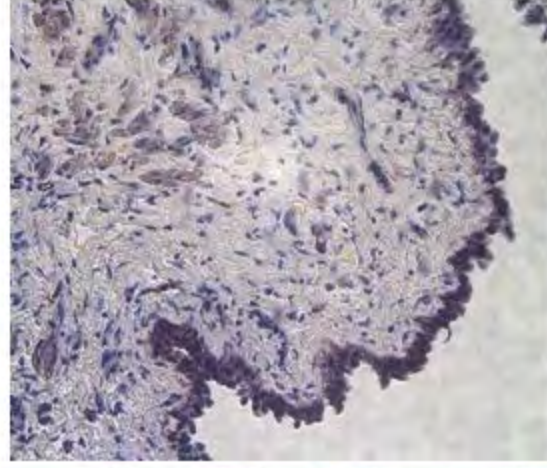
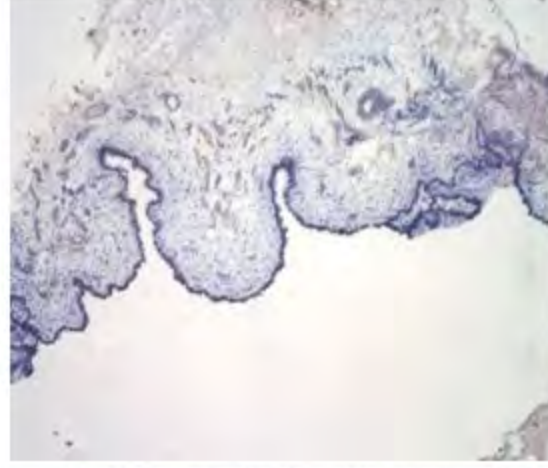


**6 wks**

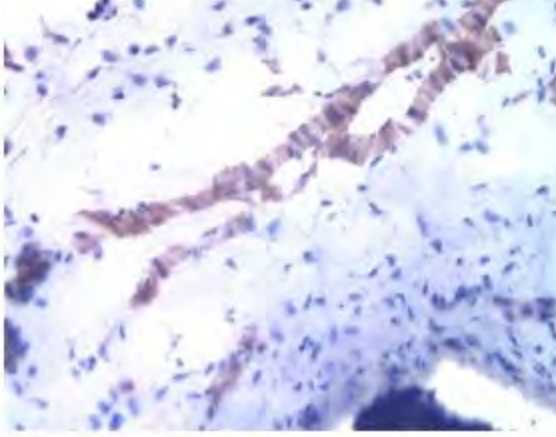
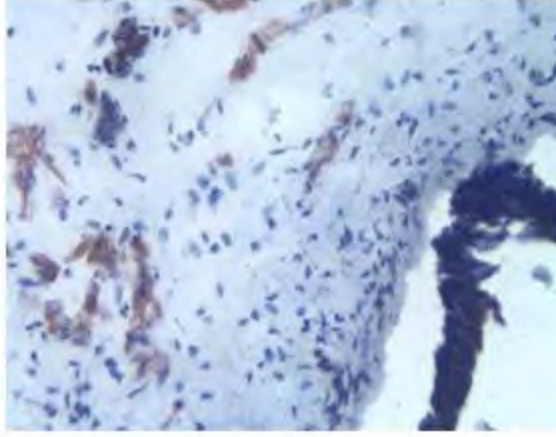
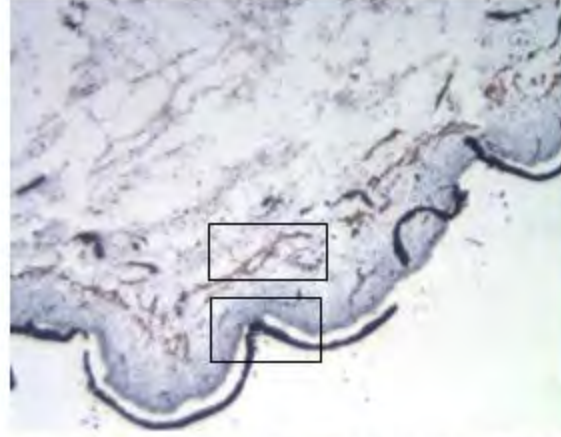




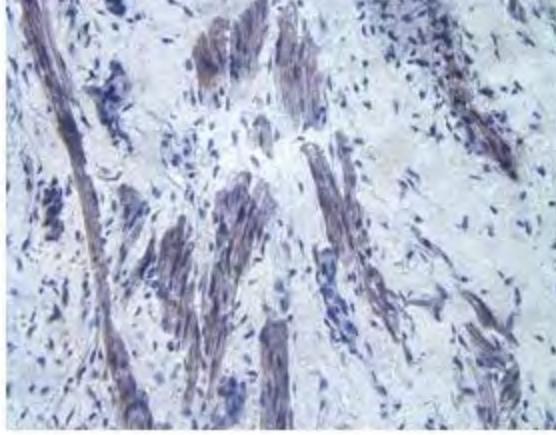
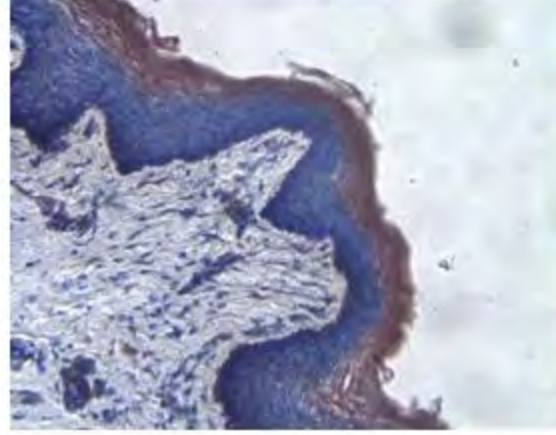
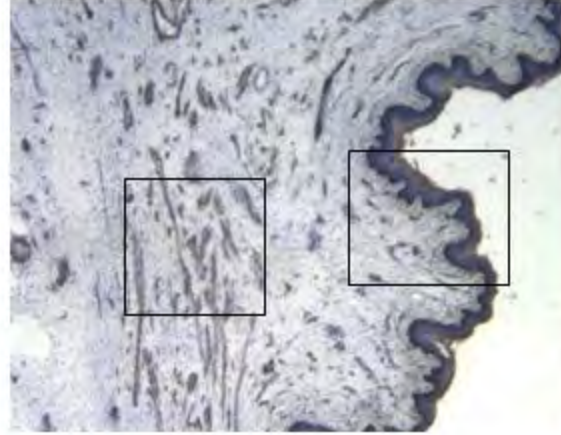
Untreated



SIS

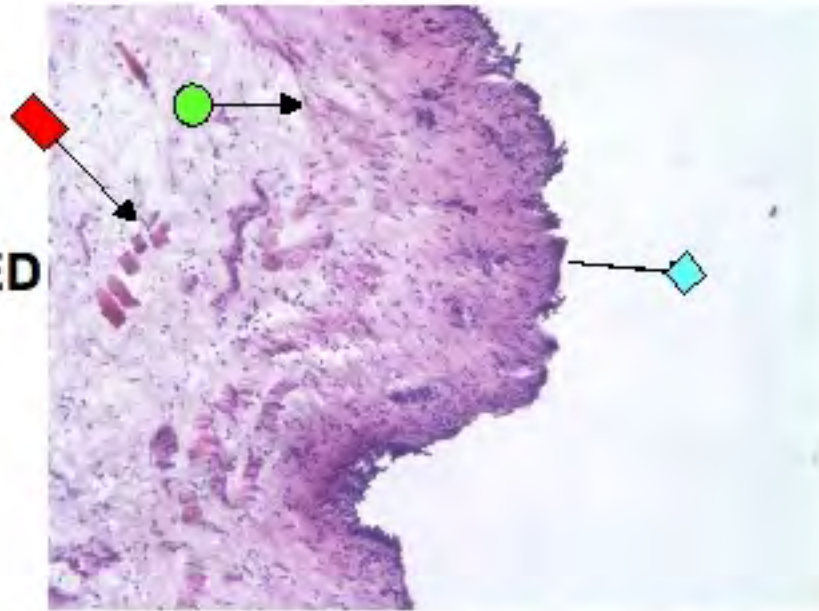


+MDSC/SIS

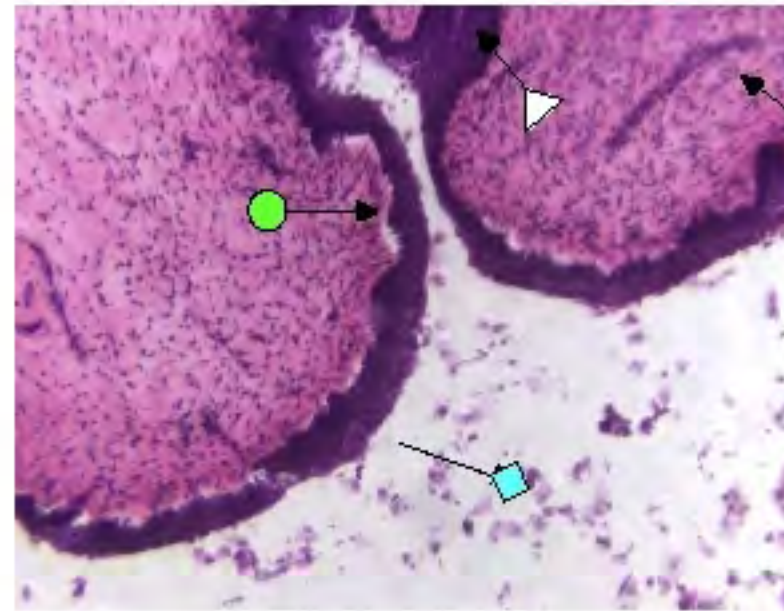




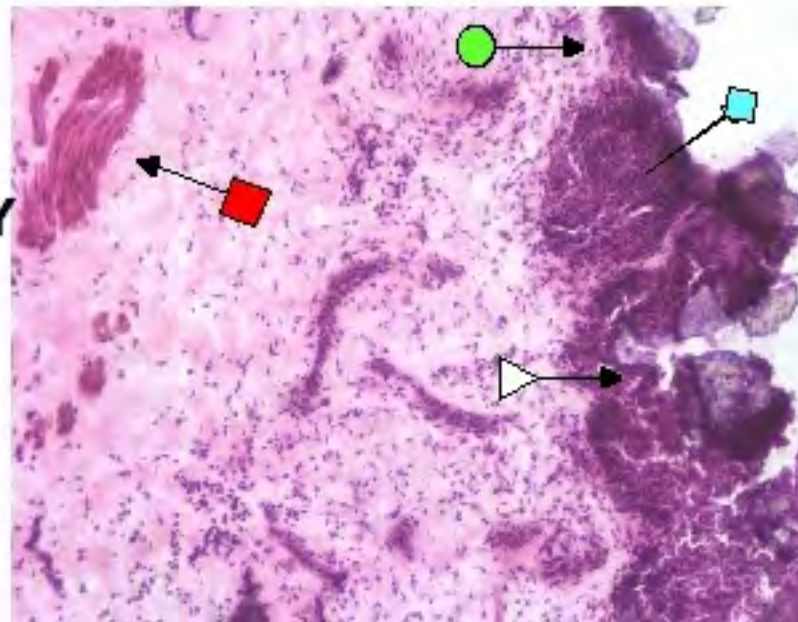
UNTREATED



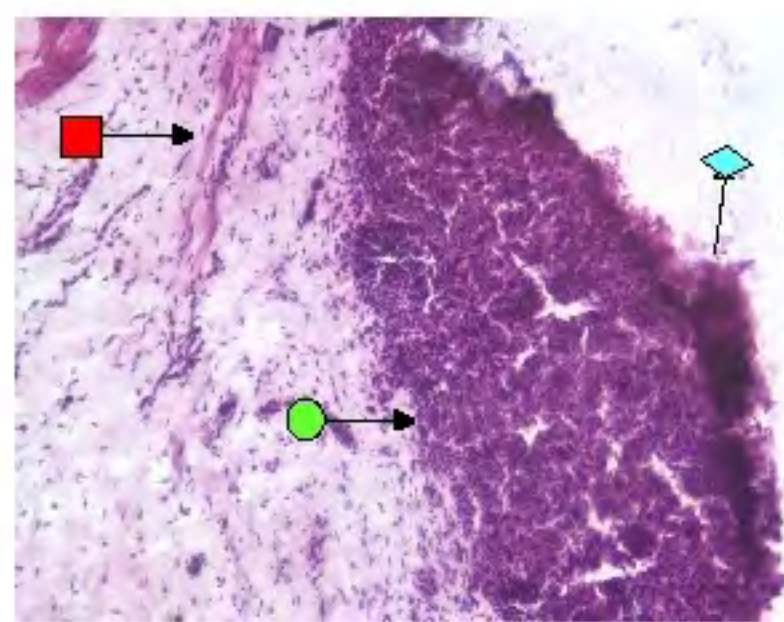
HYSTERECTOMY ONLY





HYSTERECTOMY  
SIS/MDSC



HYSTERECTOMY  
SIS/MDSC



SMOOTH MUSCLE  

LAMINA PROPRIA  

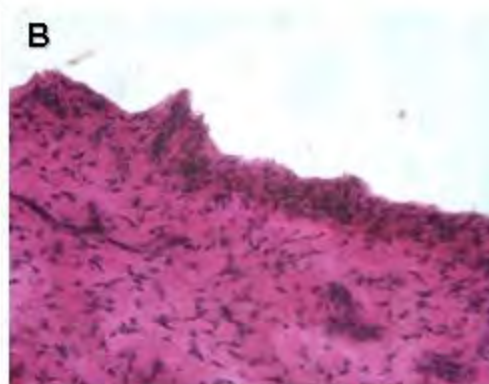
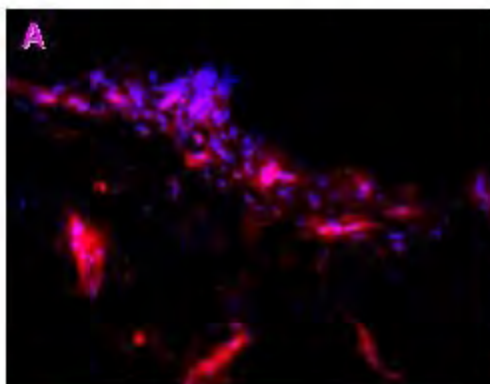
STRATIFIED SQUAMOUS EPITHELIUM  

MUCOSAL FOLD  

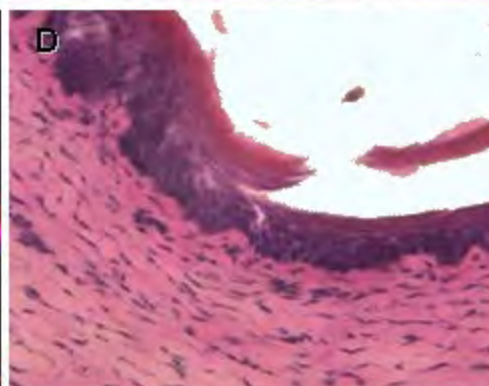
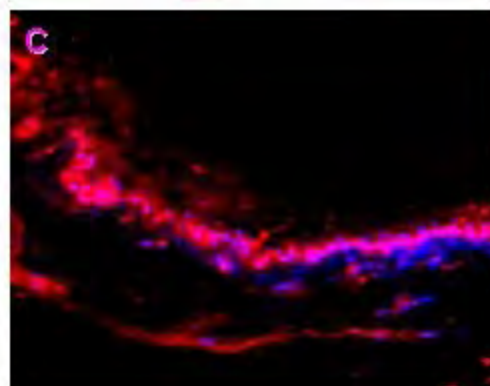
**KERATIN-5  
IMMUNOFLUORESC**

**HEMATOXYLIN/EOSIN**

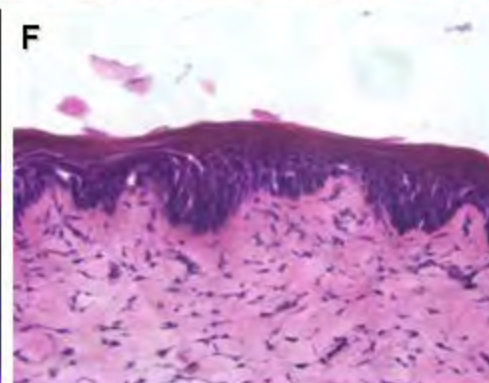
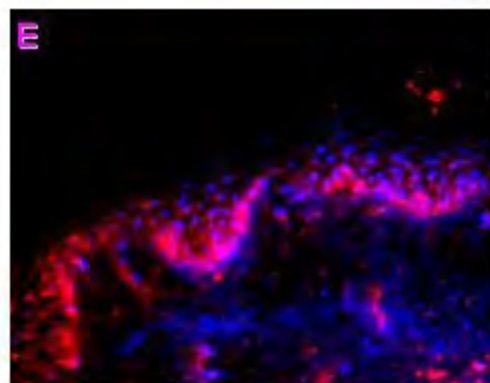
UNTREATED



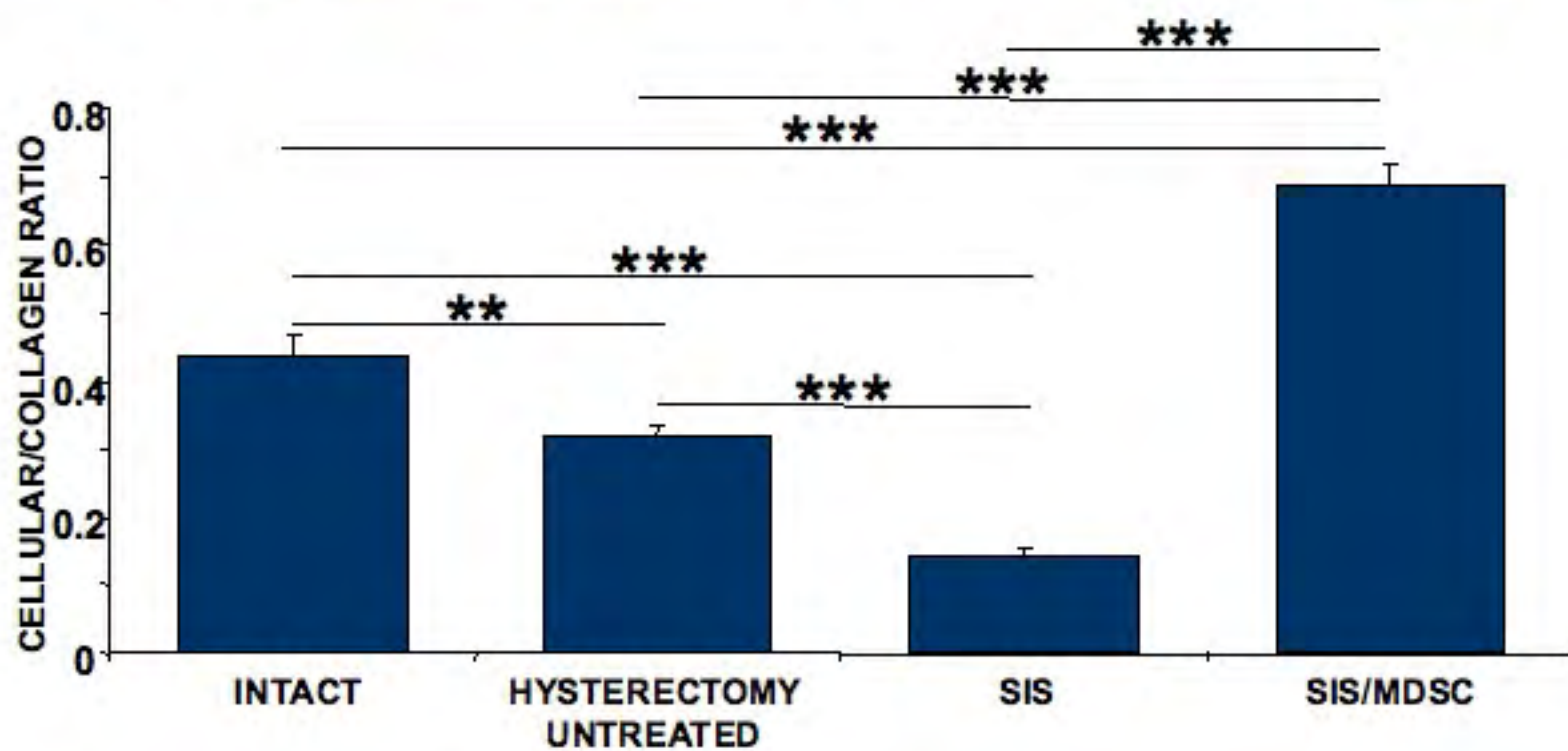
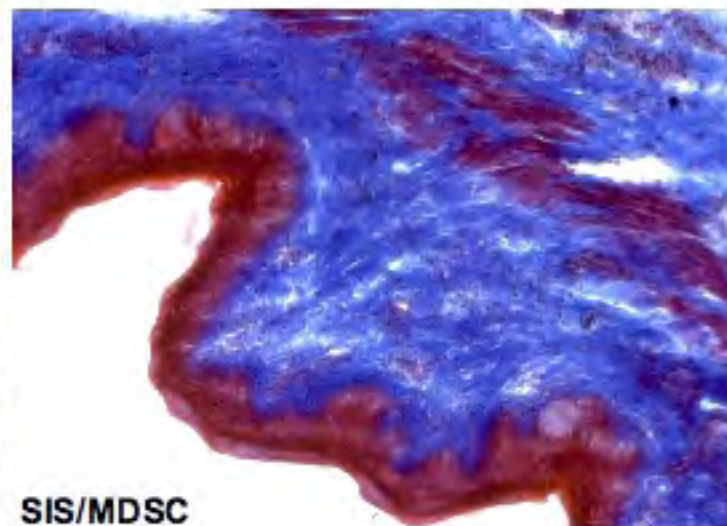
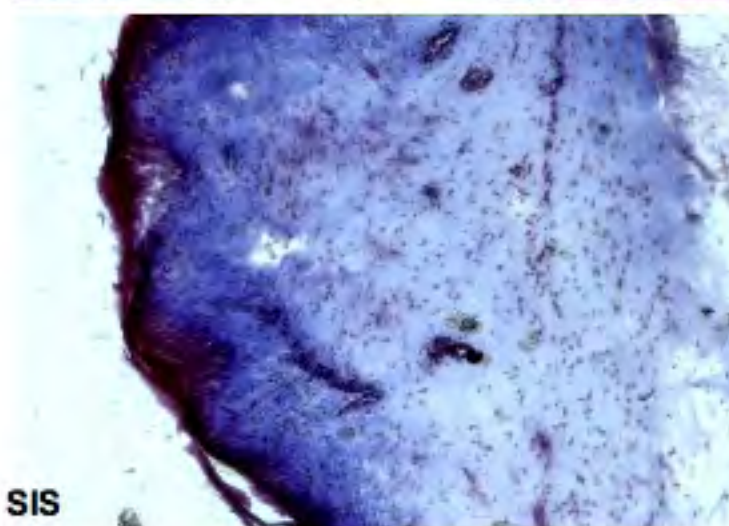
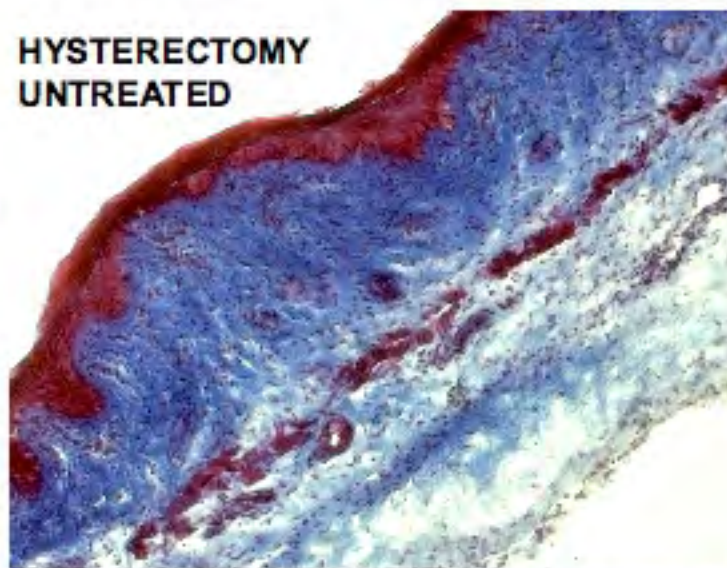
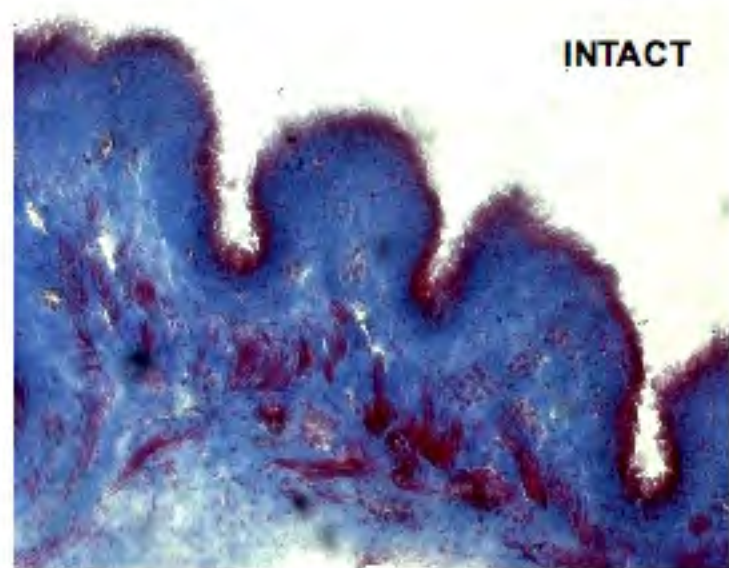
SIS



SIS/MDSC







## BIOGRAPHICAL SKETCH

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

NAME GONZALEZ-CADAVID, NESTOR F	POSITION TITLE Professor		
eRA COMMONS USER NAME (credential, e.g., agency login) NESTORGON			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Buenos Aires, Argentina	M.Sc.	1961	Biochemistry
University of Buenos Aires, Argentina	Ph.D.	1964	Biochemistry
University of London, England	Ph.D.	1967	Biochemistry

### A. Positions and Honors.

1961 Gold medal to the best MSc graduate, University of Buenos Aires  
 1961-62 Fellowship, National Council of Scientific Research (Argentina)  
 1964 Gold Medal to the best doctorate dissertation, University of Buenos Aires  
 1964-66 Fellowship, Natl Council Sci Res (Argentina), work at the Courtauld Inst Biochemistry, London Univ.  
 1967 Fellowship, WellcomeTrust (England), *ibid*  
 1968-71 Assoc. Professor, Dept. Biochemistry., Sch. of Science, Central University, Caracas, Venezuela  
 1971-92 Full Professor, Dept Cell Biology, School of Science, Central University, Caracas, Venezuela  
 1978-79 Gosney Visit. Assoc. in Biology, California Institute of Technology, Biology Division, Pasadena, CA  
 1980 Senior Fellowship, Guggenheim Foundation, Cal. Inst. of Technology, Biology Div., Pasadena, CA  
 1982 Visiting Professor, University of Buenos Aires, School of Biochemistry, Buenos Aires, Argentina  
 1984 Fellowship, Internatl Union Against Cancer, City of Hope Med Center, Div Biology, Duarte (CA).  
 1987-88 Visiting Professor, UCLA School of Medicine, Div of Hematology/Oncology, Los Angeles, CA  
 1987 E. Roosevelt fellowship, Internatl Union Against Cancer, UCLA Med School, Dept Medicine, Los Angeles (CA); Senior Fellowship, United Nations Univ. *ibid*  
 1990-92 National Research Service Award (Senior Fellowship), Popul. Res. Center, Harbor/UCLA Med. Ctr.  
 1990-96 Adj. Associate Professor, Dept of Surgery/Urology, UCLA School of Medicine, Director Urology Research Laboratory, Harbor-UCLA REI  
 1996-on Adjunct Professor, Department of Urology, UCLA School of Medicine, and Director, as above  
 1997-on Professor, Dept of Internal Medicine/Endocrinology, Charles R. Drew University.  
 2001-07 Director, RCMI Molecular Medicine Core, Charles R. Drew University

### B. Selected peer-reviewed publications from 2003-2007 (from a list of 154 on CV)

Ferrini MG, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic and spinal cord regions involved in the control of penile erection. J Compar Neurol 458:46-61  
 Magee T, Zeller CB, Ferrini M, Davila H, Vernet D, Burnett AL, Rajfer J, **González-Cadavid NF** (2003) A protein inhibitor of NOS (PIN) is expressed in the rat and mouse penile nerves and co-localizes with penile neuronal NOS (PnNOS) Biol Reprod 68:478-488.  
 Berman JR, Almeida FG, Jolin J, Raz S, Chaudhuri G, **Gonzalez-Cadavid NF** (2003) Androgen receptors, aromatase, and 5--reductase in human vagina: correlation with menopausal status. Fertil Steril, 79:925-931.  
 Davila H, Ferrini M, Rajfer J, **Gonzalez-Cadavid NF** (2003) Fibrin induction of a Peyronie's-like plaque in the rat penile tunica albuginea. A new model for Peyronie's disease. Br J Urol, 91:830-838.  
 Reisz-Porszasz S, Bhasin S, Artaza JN, Shen R, Sinha-Hikim I, Houge A, **Gonzalez-Cadavid NF** (2003) Reduction of skeletal muscle mass in a transgenic mouse that hyperexpresses myostatin in the muscle. Am J Physiol, Am J Physiol, 2003 Oct;285(4):E876-888.

Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, **Gonzalez-Cadavid N**, Arias J, Salehian B (2003) Glucocorticoid-Induced Skeletal Muscle Atrophy is Associated with Upregulation of Myostatin Gene Expression. Am J Physiol, 285:E363-371.

Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2003) L-arginine and PDE inhibitors counteract fibrosis in Peyronie's plaque and related fibroblast cultures. Nitric Oxide, 9:229-244.

Singh R, Artaza JN, Taylor WE, (**Gonzalez-Cadavid NF\***, Bhasin S\*; \*: equal contributors) (2003) Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent stem cells through an androgen receptor-mediated pathway. Endocrinology, 144:5081-508

Liu W, Thomas SG, Asa SL, **Gonzalez-Cadavid NF**, Bhasin S, Ezzat S (2003) Myostatin is a Skeletal Muscle Target of Growth Hormone Anabolic Action. J Clin Endocrin Metabol, 88:5490-5496

Bhasin S, Taylor W, Singh R, Artaza J, Sinha-hickim I, Jasuja R, Choi H, **Gonzalez-Cadavid NF** (2003) The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action. J Gerontol, 58:M1103-10.

Ferrini MG, Davila H, Valente EG, (**Gonzalez-Cadavid NF\***, Rajfer J\*, \*equal contributors) (2004) Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculo-protective in the arterial media. Cardiovascular Res ;61:796-805.

Qian A, Meals R, Rajfer J, **Gonzalez-Cadavid NF** (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology, 64:399-404.

**Gonzalez-Cadavid NF**, Bhasin S (2004) Role of myostatin in metabolism. Curr Opin Clin Nutr Metabol Care, 7:451-457.

**Gonzalez-Cadavid NF**, Rajfer J (2004). Therapy of erectile function: Potential future treatments. Endocrine, 23:167-176.

**Gonzalez-Cadavid NF**, Rajfer J (2004) Molecular and cellular aspects of the pathophysiology of Peyronie's disease. Drug Discovery Today. Disease mechanisms, 1:99-106

Davila HH, Magee TR, Rajfer J, **Gonzalez-Cadavid NF** (2004) Gene therapy with the inducible nitric oxide synthase (iNOS) cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease. Biol Reprod, 71:1568-1577

Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2004) Corporal veno-occlusive dysfunction in the aging rat. evaluation by cavernosometry and cavernosography. Urology, 64:1261-1266.

**Gonzalez-Cadavid NF**, Rajfer J (2004) Molecular pathophysiology and gene therapy of aging-related erectile dysfunction. Exptl Gerontol, 39:1705-1712

Sinha-Hikim I, Taylor WE, **Gonzalez-Cadavid NF**, Zheng W, Bhasin S (2004) Androgen receptor in human skeletal muscle and cultured muscle satellite cells: Up-regulation by androgen treatment. J Clin Endocr Metabol 89:5245-5255.

Davila H, Magee TR, Rajfer J, **Gonzalez-Cadavid NF** (2005). Peyronie's disease is associated with an increase of plasminogen activator inhibitor-1 (PAI-1) at the RNA and protein levels. Urology, 65:645-648

Hikim AP, Vera Y, Vernet D, Castanares M, Diaz-Romero M, Ferrini M, Swerdloff RS, **Gonzalez-Cadavid NF**, Wang C (2005) Involvement of nitric oxide-mediated intrinsic pathway signaling in age-related increase in germ cell apoptosis in male brown-norway rats. J Gerontol A Biol Sci Med Sci 60:702-708.

Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Fareez MM, **Gonzalez-Cadavid NF** (2005) Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. Endocrinology 146:3547-3557.

Vernet D, Qian A, Nolzco G, Cantini L, Magee TR, Ferrini MG, Rajfer J, **Gonzalez-Cadavid NF** (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod, 73:1199-1210.

Jasuja R, Ramaraj P, Mac RP, Singh AB, Storer TW, Artaza J, Miller A, Singh R, Taylor WE, Lee ML, Davidson T, Sinha-Hikim I, **Gonzalez-Cadavid N**, Bhasin S (2005) Delta-4-androstene-3,17-dione binds androgen receptor, promotes myogenesis in vitro, and increases serum testosterone levels, fat-free mass, and muscle strength in hypogonadal men. J Clin Endocrinol Metab. 90:855-863.

**Gonzalez-Cadavid NF**, Rajfer J (2005) The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. Curr Pharm Des 11:4041-4046

**Gonzalez-Cadavid NF, Rajfer J (2005) Mechanisms of disease: new insights into the cellular and molecular pathology of Peyronie's disease. Nature Clin Pract Urol**, 2:291-298.

Saenz de Tejada I, Angulo J, Celtek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Physiology of erectile function. J Sex Med 1:254-265.

Saenz de Tejada I, Angulo J, Celtek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Pathophysiology of erectile dysfunction. J Sex Med 2:26-39.

Ferrini MG, Nolzco G, Vernet D, **Gonzalez-Cadavid NF\***, Berman J\* (\*: equal contributors) (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. Fertil Steril, 86:1152-1163.

Ferrini MG, Kovanez I, Nolzco G, Rajfer J, **Gonzalez-Cadavid NF** (2006) Effects of long-term treatment with vardenafil on the development of the fibrotic plaque in a rat model of Peyronie's disease BJU Int, 97:625-633

Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, **Gonzalez-Cadavid NF**, Bhasin S (2006) Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell

factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinology*. 2006 Jan;147(1):141-54.

Vernet D, Magee TR, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2006) Long-term continuous incubation with high doses of tadalafil does not up-regulate the levels of phosphodiesterase 5 (pde5) in cultures of human penile smooth muscle cells. *J Sex Med* 3:84-94; discussion 94-95

Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, **Gonzalez-Cadavid NF** (2006). Myostatin shRNA gene therapy increases muscle mass. *J Gene Med* 8:1171-81

Kovanecz I, Ferrini MG, Vernet D, Nolzco G, Rajfer J, **Gonzalez-Cadavid NF** (2006). Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int*, 98:116-124

Ferrini MG, Davila H, Kovanecz I, Sanchez S, **Gonzalez-Cadavid NF\*** Rajfer J\* (\*: equal contributors) (2006) Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. *Urology*, 2006 Aug;68(2):429-35.

Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. *Biol Reprod*, 76:915-923

Artaza JN, Reisz-Porszasz S, Dow JS, Kloner RA, Tsao J, Bhasin S, **Gonzalez-Cadavid NF** (2007) Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. *J Endocrinol*, 194(1):63-76.

**Gonzalez-Cadavid NF**, Rajfer J (2007) Experimental models for the study of the cellular and molecular pathophysiology of Peyronie's disease. In: *Current Clinical Urology: Peyronie's Disease, A Guide to Clinical Management*, ed by L.A. Levine, Humana Press, Totowa, NJ, p 19-39

Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, **Gonzalez-Cadavid NF** (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Medic*, 4(3):633-43.

Kovanecz I, Ferrini MG, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. *BJU Int*. 2007 Oct; 100(4):867-74.

Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res*, 2007, 100(4):867-74.

Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid NF** (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. *BJU Int* 101(2):203-10.

Rambhatla A, Kovanecz I, Ferrini M, **Gonzalez-Cadavid NF**, Rajfer J (2008) Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. *Int J Impot Res*. 20(1):30-4.

Nolzco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, **Gonzalez-Cadavid NF** (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int*, 101(9):1156-64

Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, **Gonzalez-Cadavid NF** (2008) Myostatin promotes a fibrotic phenotypic switch in multipotent C3h 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol* 196:235-49

Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, **Gonzalez-Cadavid NF** (2008) Pro-fibrotic role of myostatin in Peyronie's disease. *J Sex Med*, 5(7):1607-22

**Gonzalez-Cadavid NF**, Rajfer J. Experimental models for Peyronie's disease. Implications for therapy. *J Sex Medic*, 2008, in press

Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, **Gonzalez-Cadavid NF**. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Medic*, 2008, in press

Braga M, Sinha Hikim AP, Datta S, Ferrini MG, Brown D, Kovacheva EL, Gonzalez-Cadavid NF, Sinha-Hikim I (2008) Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. *Apoptosis*. 2008 Jun;13(6):822-32.

Kovanecz I, Nolzco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF** (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. *BJU Int*, 2008, in press.

Gonzalez-Cadavid NF (2008) Mechanisms of penile fibrosis. *J Sex Med*, in press

Toblli JE; Ferrini MG; Cao G; Vernet D; Angerosa M; **Gonzalez-Cadavid NF** (2009) Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrol Dial Transpl*, preliminary acceptance.

Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini MG, Bhatia NN, **Gonzalez-Cadavid NF** (2009) Skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds stimulate vaginal repair in the rat. *Obst Gynecol*, accepted

## C. ACTIVE AND COMPLETED FUNDING

1. PR064756 (PI: Gonzalez-Cadavid) 03/01/07-02/28/10 Department of Defense  
Pharmacological prevention and reversion of erectile dysfunction after radical prostatectomy, by modulation of nitric oxide/cGMP pathways  
The goal is to determine whether long-term treatment with PDE5 inhibitors and nitric oxide donors can prevent corporal veno-occlusive dysfunction in a rat model of erectile dysfunction after radical prostatectomy, and whether this is due to an improvement in the underlying penile corporal fibrosis and loss of smooth muscle  
No overlap
2. PC061300 (PI: Gonzalez-Cadavid) 03/31/07-02/28/11 Department of Defense  
Modulation of stem cell differentiation and myostatin as an approach to counteract fibrosis in dystrophic muscle regeneration after injury.  
The goal is to determine whether skeletal muscle derived stem cells (MDSC) can ameliorate skeletal muscle atrophy and fibrosis in a mouse model of Duchenne's muscular dystrophy, and this is stimulated by ex vivo gene transfer of myostatin shRNA to stem cells, and/or treatment with agents that inhibit myostatin activity  
No overlap
3. NIH R21DK070003 (Gonzalez-Cadavid) 10/01/07-09/30/09 NIH NIDDK  
Cell-selective expression of fibrotic gene pathways  
The goal is to compare the patterns of gene expression related to fibrotic phenotypes in smooth muscle and fibroblasts in the corpora cavernosa in rat models of reproductive aging and Peyronie's disease, and the relationship between stem cells, smooth muscle cells, and fibroblasts, in myofibroblast generation in fibrosis.  
No overlap
4. GCRC Medical Student Program (Gonzalez-Cadavid, PI, Wang J, student) 12/01/07-05/31/09 NIH-GCRC  
Nitric oxide/cGMP modulation of skeletal muscle stem cell differentiation in myocardial infarction in the rat  
The goal is to compare the antifibrotic and tissue repair effects of a long-term continuous treatment with low and high doses of a PDE5 inhibitor with or without concurrent treatment with skeletal muscle derived stem cells and determine whether this pharmacological intervention modulates endogenous and exogenous stem cell differentiation into cardiomyocytes  
No overlap

#### **Research Support (currently submitted)**

1. RO1 DK5306907 (PI: Gonzalez-Cadavid), 05/01/03-04/30/08 NIH/NIDDK. Renewal submitted: 03/05/09  
Erectile Dysfunction and Nitric Oxide Synthase in Aging  
The goal is to apply novel procedures of gene and stem cell therapy for the treatment of aging-related erectile dysfunction, based on the modulation of the nitric oxide/cGMP pathway in the corpora cavernosa in a rat model of reproductive aging, and whether this restores nitrgenic neurotransmission and/or corporal smooth muscle  
No overlap
2. R21 DK (PI: Gonzalez-Cadavid), 12/01/09-11/30/11 NIH/NIDDK. Submitted: 02/17/09  
PPAR gamma modulation of Oct 4 kidney stem cells in diabetic nephropathy  
The goal is to determine whether cells that express the embryonic stem cell gene Oct-4 in the adult kidney and that are visualized by green fluorescence in a transgenic mouse that expresses gfp under the Oct-4 promoter, are true stem cells that intervene in renal repair and that PPAR $\gamma$  agonists at doses that do not exert glycemic control, can still counteract the reduction of their number and differentiation ability caused by diabetes  
No overlap



## BIOGRAPHICAL SKETCH

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

NAME <b>Monica G. Ferrini</b>	POSITION TITLE <b>Assistant Professor</b>
eRA COMMONS USER NAME (credential, e.g., agency login) <b>MFerrini306</b>	

EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Buenos Aires, Argentina School Pharmacy and Biochemistry	M.Sc.	1986	Biochemistry
University of Buenos Aires, Argentina School Pharmacy and Biochemistry	Ph.D.	1995	Physiology

### A. POSITIONS AND HONORS

1992-2001 Assistant Professor. University of Buenos Aires, Argentina

1994-2001 Member of the National Research Council of Argentina (CONICET). Current rank: Adjunct Investigator.

1999-2007 Research Associate, LA BioMed at Harbor UCLA, Torrance, CA

2004-on Assistant Research Professor, David Geffen School Med at UCLA, Department of Urology, Los Angeles, CA

2004-on Assistant Professor, Department Biomedical Sciences, College Health and Science, Charles Drew University, Los Angeles, CA.

2007-on Assistant Professor, Department of Internal Medicine, College of Medicine, Charles Drew University.

### HONORS

1993. "Dr Juan Izquierdo Award" Argentine Society Experimental Pharmacology (SAFE)

1991-1995 "Scientific and Technological Awards University of Buenos Aires"

1996. 3<sup>rd</sup> place in the list of Young Outstanding Researcher, University of Buenos Aires. Argentina.

1997. "Mr Julio Lutfi Award" for the best young endocrinology researcher. Obtained diploma for second place. Argentine Society of Endocrinology and Metabolism.

2000. "AUA: Second place best poster category "Impotence"

2004. Travel Grant Award. Endocrine Society.

### B. PUBLICATIONS selected out of total of 58 papers

3. **Ferrini M**, Magariños A.M, De Nicola AF (1990) Estrogens down-regulate type I but not type II adrenal corticoids receptors in rat anterior pituitary. J Steroid Biochem Mol Biol 35: 671-677.
4. **Ferrini M**, De Nicola AF. (1991) Estrogens up-regulate Type I and Type II glucocorticoid receptors in brain regions from ovariectomized rats. Life Sci 48 (26) 2593-2601.
5. **Ferrini M.**, González S, De Nicola AF. (1993) Estradiol increases glucocorticoid binding and glucocorticoid induction of ornithine decarboxylase in the rat spinal cord. Life Sci, 52: 677-685.
6. **Ferrini M.**, González S, Antakly T, De Nicola AF. (1993) Immunocytochemical localization of glucocorticoid receptors in the spinal cord: Effects of adrenalectomy, glucocorticoid treatment and spinal cord transection. Cell Mol Neurobiol, 13/4: 387-397.
7. **Ferrini, M.**, Lima A, De Nicola AF. (1995) Estradiol abolishes down regulation of glucocorticoid receptors in brain. Life Sci 57: 2403-2412.
8. **Ferrini, M.**, Grillo, C, Piroli, G, De Kloet, ER, De Nicola, AF. (1997) Sex difference in glucocorticoid regulation of vasopressin mRNA in the paraventricular hypothalamic nucleus. Cell Mol Neurobiol, 17: 671-686.
9. De Nicola, AF, **Ferrini, M.**, González, S, González Deniselle, MC, Grillo, C, Piroli, G, Saravia, S, De



- Kloet, ER. (1998) Regulation of gene expression by corticoid hormones in the brain and spinal cord. *J. Steroid Biochem Mol Biol*, 65: 253-272.
10. **Ferrini, M.**, Piroli G, Grillo, C, González-Deniselle, MC, Lima, A, Roig, P, De Nicola, AF. (1998) Effect of estrogen on the immunoreactivity of choline acetyl transferase (CHAT) and mRNA of GAP-43 in aged rats *Act Physiol Latinoam* 78 : 48 abt 23
  11. **Ferrini, M.**, Piroli, G, Frontera M, Falbo, A, Lima, AE. (1999) Estrogen normalize the response to stress and increase glucocorticoid receptors immunoreactivity in aging rats. *Neuroendocrinology* 69 129-137
  12. Gonzalez Deniselle, M.C., Lavista-Llanos, S., **Ferrini, M.**, Lima A.E., Roldan A.G., De Nicola A.F.: (1999) In vitro differences between astrocytes of control and wobbler mice spinal cord. *Neurochem.Res*, 24: 1531-1541
  13. Bisagno, V., **Ferrini, M.**, Rios H., Ziehr, L M and Wikinski S. I: (2000) Chronic corticosterone impairs inhibitory avoidance in rats: possible link with hippocampal CA3 dendritic atrophy. *Pharmacol Biochem Behav.* 66(2):235-40.
  14. **Ferrini M.**, Magee, TR, Vernet D, Rajfer J, Gonzalez-Cadavid, NF (2001) Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. *Biol Reprod* 64: 974-982.
  15. **Ferrini M.**, Wang C, Swerdloff R, Vernet D, Sinha-Hikim A, Gonzalez-Cadavid NF. (2001) Aging related expression of inducible nitric oxide synthase (iNOS) and cytotoxicity markers in the rat hypothalamic regions associated with male reproductive dysfunctions. *Neuroendocrinology* 74: 1-11.
  16. **Ferrini M.**, Vernet D, Magee TR, Shahed A, Qian A, Rajfer J, Gonzalez-Cadavid NF. (2002) Antifibrotic role of inducible nitric oxide synthase. *Nitric Oxide: Biol Chem* 6:283-294
  17. Magee TR, **Ferrini M.**, Garban H, Vernet D, Mitani K, Rajfer J, Gonzalez-Cadavid NF (2002) Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase (PnNOS) cDNA. *Biol Reprod.* 67:20-28
  18. **Ferrini M.**, Bisagno V, Piroli G, Grillo, C, Gonzalez-Deniselle, MC, De Nicola AF. (2002) Effects of estrogens on choline-acetyltransferase immunoreactivity and GAP-43 mRNA in the forebrain of young and aging male rats. *Cell Mol Neurobiol*, 22:289-301.
  19. Gonzalez-Cadavid NF, Magee TR, **Ferrini M.**, Qian A, Vernet D, Rajfer J (2002) Gene expression in Peyronie's disease. In: "Update of Peyronie's disease", ed. by Nera A, Hellstrom W. *Intn J Impot Res*, 14(5): 361-374.
  20. Vernet, D, **Ferrini MG**, Valente EG, Magee TR, Bou Gahrios G, Rajfer, J, Gonzalez-Cadavid, N.F. (2002) Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. *Nitric oxide, Biol Chem* 7:262-276.
  21. **Ferrini M**, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2002) Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. *Int J Impot Res* 14:550 Editorial commentary
  22. Magee, TR, **Ferrini, MG.**, Davila, H, Zeller, CB, Vernet, D, Sun, J, Lalani, R, Burnett, AL, Rajfer, J, Gonzalez-Cadavid, NF. (2003) The protein inhibitor of NOS (PIN) and the NMDAR receptor are expressed in the rat and mouse penile nerves and co-localize with penile neuronal nitric oxide synthase. *Biol Reprod* 68:478-88.
  23. **Ferrini, MG.**, Magee, TR, Vernet, D, Rajfer, J, Gonzalez-Cadavid, NF. (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic regions involved in the control of penile erection. *J Comp Neurol* 458(1): 46-61.
  24. Davila HH, **Ferrini MG.**, Rajfer J, Gonzalez-Cadavid NF (2003) Fibrin as an inducer of fibrosis in the tunica albuginea of the rat: a new animal model of Peyronie's disease. *Brit J Urol Int* 91:830-838.
  25. Valente EG, **Ferrini MG**, Vernet D, Qian A, Rajfer J, Gonzalez-Cadavid NF (2003) L-arginine and PDE inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. *Nitric Oxide*, 9(4): 229-244.
  26. **Ferrini M**, Davila HH, Valente EG, Gonzalez-Cadavid NF, Rajfer J. (2004) Aging-related induction of inducible nitric oxide synthase is vasculo-protective to the arterial media. *Cardiovascular Res.*, 61(4):796-805.
  27. Hikim AP, Vera Y, Vernet D, Castanares M, Diaz-Romero M, **Ferrini M**, Swerdloff RS, Gonzalez-Cadavid NF, Wang C (2005). Involvement of nitric oxide-mediated intrinsic pathway signaling in age-

related increase in germ cell apoptosis in male Brown-Norway rats. *J Gerontol A Biol Sci Med Sci*. 60(6):702-708.

28. **Ferrini MG**, Kovanecz I, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. (2006) Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int*. 97(3):625-33.
29. **Ferrini MG**, Nolzco G, Vernet D, Gonzalez-Cadavid NF, Berman J. Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. *Fertil Steril*. 2006 Oct;86 Suppl 4:1152-63.
30. De Nicola AF, Saravia FE, Beauquis J, Pietranera L, **Ferrini MG**. Estrogens and neuro-endocrine hypothalamic-pituitary-adrenal axis function. *Front Horm Res*. 2006;35:157-68.
31. **Ferrini MG**, Davila HH, Kovanecz I, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. *Urology*. 2006 Aug;68(2):429-35
32. Kovanecz I, **Ferrini MG**, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone prevents corporal veno-occlusive dysfunction in a rat model of type 2 diabetes mellitus. *BJU Int*. 2006 Jul; 98(1):116-24.
33. Magee TR, Artaza JN, Ferrini MG, Vernet D, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, Gonzalez-Cadavid NF. Myostatin short interfering hairpin RNA gene transfer increases skeletal muscle mass. *J Gene Med*. 2006 Sep;8(9):1171-81.
34. Paez Espinosa V, **Ferrini M**, Shen X, Lutfy K, Nillni EA, Friedman TC. Cellular co-localization and co-regulation between hypothalamic pro-TRH and prohormone convertases in hypothyroidism. *Am J Physiol Endocrinol Metab*. 2007 292(1):E175-86
35. **Ferrini MG**, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-Term Continuous Treatment with Sildenafil Ameliorates Aging-Related Erectile Dysfunction and the Underlying Corporal Fibrosis in the Rat. *Biol Reprod*. 2007, 73: 915-923
36. Magee TR, Kovanecz I, Davila HH, **Ferrini MG**, Cantini L, Vernet D, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF. Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein Inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med*. 2007 May;4(3):633-43.
37. Khorram O, Momeni M, **Ferrini M**, Desai M, Ross MG. In utero undernutrition in rats induces increased vascular smooth muscle content in the offspring. *Am J Obstet Gynecol*. 2007 May;196(5):486.e1-8.
38. Kovanecz I, **Ferrini MG**, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Ageing-related corpora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. *BJU Int*. 2007 Oct;100(4):867-74.
39. Rambhatla A, Kovanecz I, **Ferrini MG**, Gonzalez-Cadavid NF, Rajfer J. Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. *Int J Impot Res*. 2008 Jan-Feb;20(1):30-4.
40. Kovanecz I, Rambhatla A, **Ferrini MG**, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid N. Long-term continuous sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res*. 2008 Mar-Apr;20(2):202-12. Epub 2007 Sep 20.
41. Kovanecz I, Rambhatla A, **Ferrini MG**, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid N. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. *BJU Int*. 2008 Jan;101(2):203-10. Epub 2007 Sep 20.
42. Artaza JN, Singh R, **Ferrini MG**, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol*. 2008 Feb;196(2):235-49.
43. Nolzco G, Kovanecz I, Vernet D, Gelfand RA, Tsao J, **Ferrini MG**, Magee T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int*. 2008 May;101(9):1156-64. Epub 2008 Feb 21.
44. Cantini LP\*, **Ferrini MG\***, Vernet D, Magee TR, Qian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF. Profibrotic role of myostatin in Peyronie's disease. *J Sex Med*. 2008 Jul;5(7):1607-22. \* **equal contribution as first author**

45. Braga M, Sinha Hikim AP, Datta S, **Ferrini MG**, Brown D, Kovacheva EL, Gonzalez-Cadavid NF, Sinha-Hikim I. Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. *Apoptosis*. 2008 Jun; 13(6):822-32.
46. Espinosa VP, Liu Y, **Ferrini M**, Anghel A, Nie Y, Tripathi PV, Porche R, Jansen E, Stuart RC, Nillni EA, Lutfy K, Friedman TC. Differential regulation of prohormone convertase 1/3, prohormone convertase 2 and phosphorylated cyclic-AMP-response element binding protein by short-term and long-term morphine treatment: Implications for understanding the "switch" to opiate addiction. *Neuroscience*. 2008 Oct 15;156(3):788-99. Epub 2008 Aug 9.
47. Lakshmanan J, Magee TR, Richard JD, Liu GL, Salido E, Sugano SK, **Ferrini M**, Ross MG. Localization and gestation-dependent pattern of corticotrophin-releasing factor receptor subtypes in ovine fetal distal colon. *Neurogastroenterol Motil*. 2008 Dec;20(12):1328-1339.
48. De Nicola AF, Pietranera L, Beauquis J, **Ferrini MG**, Saravia FE. Steroid protection in aging and age-associated diseases. *Exp Gerontol*. 2009 Jan-Feb;44(1-2):34-40.
49. **Ferrini M.G**, Kovanecz I., Sanchez S., Umeh C., Rajfer J., Gonzalez-Cadavid N. FFibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *Accepted J. Sex Med (in press)* 2008 Dec 2.
50. Kovanecz I, Nolasco G, **Ferrini MG**, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus with erectile dysfunction. *BJU Int*. 2009 Jan 9.

## C. OTHER SUPPORT

### Active

Active/Pending:	Active
Project Number (Principal Investigator):	1SC1NS064611-01 PI: Monica G. Ferrini
Source:	NIH
Title of Project ( <i>and/or Subproject</i> ):	Nitric oxide/cGMP modulation of corporal fibrosis caused by neuropraxia
Dates of Approved/Proposed Project:	08/15/2008 – 07/31/2013
Annual Direct Costs:	
Person months (Calendar months):	

Goals: To define whether PDE5 inhibitors alone or in combination with other drugs that also up-regulate the NO/cGMP pathway, correct not only the underlying histopathology of the corpora but also preserve the normal physiology of the tissue. In addition, to clarify the mechanism of these effects by determining a) to what extent different damaged tissues are affected by these agents; b) what role nitric oxide (NO) and cGMP have on correcting oxidative stress as a factor inducing corporal tissue damage after surgery; and c) what are the downstream targets of NO and cGMP when there is amelioration of corporal tissue damage.

Overlap: None.

## BIOGRAPHICAL SKETCH

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

NAME <b>KOVANECZ, ISTVAN</b>	POSITION TITLE Research Associate Assistant Professor (appointment in progress)		
eRA COMMONS USER NAME (credential, e.g., agency login) <b>IKOVANECZ0308</b>			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Szeged (former Jozsef A. University of Art and Sciences), Szeged, Hungary	M.Sc.	1985	Biochemistry
Budapest University of Technology, Institute of Continuing Engineering Education, Budapest, Hungary	CNRT	1987	Nuclear technology
University of Szeged (former Jozsef A. University of Art and Sciences), Szeged, Hungary	Ph.D	1994	Comparative Physiology
Biological Research Center of The Hungarian Academy of Sciences, Szeged, Hungary		1999-2000	Genomics, IT, Bioinformatics
Research & Education Institute at Harbor-UCLA Medical Center, Torrance, CA, USA		2004	Training course on Protected Health Information
LABioMed Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA		2004	Training course on Protecting Study Volunteers in Research

### A. Positions and Honors

- 1985-1987 Research Fellow, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
- 1987-1991 Research Scientist, Blood Transfusion Center, Szent-Gyorgyi Albert Medical University, Szeged, Hungary
- 1991-1992 Volunteer Researcher, Department of Neurology, Mount Sinai Medical Center, CUNY, New York, NY, USA
- 1993-1999 Senior Research Scientist, Head of the Vivarium, Department of Pharmacology and Pharmacotherapy, Szent-Gyorgyi Albert Medical University, Szeged, Hungary
- 1999-2001 Biologist Chief Counselor, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
- 2000-2001 Member of the Computer Software Council of the Hungarian Academy of Sciences
- 2004–on Research Associate, Urology Research Laboratory, Department of Surgery, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, USA
- 2008-on Assistant Professor, Dept Urology, UCLA David Geffen School Medicine, Los Angeles, CA

### B. Selected publications

#### Original research and theoretical treatises

1. Bodis-Wollner I, Antal A, **Kovanecz I.** (1993) Low-dose scopolamine and acetyl-levo-carnitine dissociate primary from cognitive visual processing in the trained monkey. *Invest Ophth Vis Sci* 34(4): 1174.
2. Antal A., **Kovanecz I**, Bodis-Wollner I. (1994) Visual discrimination and P300 are affected parallel by cholinergic agents in the behaving monkey. *Physiol Behav.* 56(1) 161-66.

3. Tagliati M., Bodis-Wollner I., **Kovanecz I**, Stanzione P. (1994) Spatial frequency tuning in the monkey retina depends on D2 receptor-linked action of dopamine. *Vision Research* 34(16):22051-57.
4. **Kovanecz I**, Csajbok E., Petri I.B. (1994) In vitro steroid sensitivity in chronic uremic and kidney transplant patients: HLA - associated susceptibility to steroid treatment. *Nephrology Dialysis Transplantation* 9(10): 1474-76.
5. **Kovanecz I**, Petri I.B., Kaiser G. (1995) HLA associated lymphocyte panel reactive (cytotoxic) antibody production in dialyzed chronic uremic patients. *Acta Microbiologica Hungarica* 42(1): 81-84.
6. **Kovanecz I**, Papp JG, Szekeres L. (1997) Increased cardiac workload by adrenoreceptor agonists for the estimation of potential antiischemic activity in a conscious rabbit model. *J Pharmacol Toxicol Methods* 37(3): 149-59.
7. Szekeres L, **Kovanecz I**, Papp JG. (1997) Delayed cardiac adaptation to stress moderates response to beta-adrenoceptor agonists. *J Mol Cell Cardiol* 29(5): A134.
8. **Kovanecz I**, Ábrahám A, Makay G, Lukács E, Szekeres L, Papp JGy. (1997) Delayed cardiac adaptation to ischaemic stress - limitation of infarct size in a rabbit model of ischaemia-reperfusion by a single dose of iloprost. 1997 . *J Mol Cell Cardiol* 29(5): A89.
9. Takase H, **Kovanecz I**, Mori T. et al. (2003) Acute and anti-ischemic actions of pranipidine in three animal models. *Asia Pacific Journal of Pharmacology* 16(1): 29-37.
10. Davila HH, Miranda-Sousa AJ, **Kovanecz I**, et al. Effect of bilateral cavernosal nerve resection on the histological alteration in the penile vascular system. *J Urol* 2005; 173(4S): 288.
11. Ferrini MG, **Kovanecz I**, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. (2006) Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int.* 97(3):625-33.
12. **Kovanecz I**, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. (2006) Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int.* 98:116-24
13. Ferrini MG, Davila HH, **Kovanecz I**, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. *Urology* 2006; 68:429-35
14. Ferrini MG, **Kovanecz I**, Sanchez S, Vernet D, Davila HH, Rajfer JA, Gonzalez-Cadavid NF. (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. *Biol Reprod.* 76(5):915-23.
15. Magee TR, **Kovanecz I**, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF. (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med* 4(3):633-43.
16. **Kovanecz I**, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. (2007) Aging-related corpora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. *BJU Int* 100(4):867-74.
17. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. (2007) Long term sildenafil treatment ameliorates corpora veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res* Sep 20; [Epub ahead of print]
18. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. *BJU Int.* 101(2):203-10.
19. Nolzco D, **Kovanecz I**, Vernet D, Ferrini MG, Gelfand B, Tsao J, Mage T, Rajfer J, Gonzalez-Cadavid NF. (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int* Feb 21; [Epub ahead of print]
20. **Kovanecz I**, Nolzco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus with erectile dysfunction. *BJU Int.* 2009 Jan 9. [Epub ahead of print]
21. Ferrini MG, **Kovanecz I**, Sanchez S, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and Loss of Smooth Muscle in the Corpora Cavernosa Precede Corporal Venocclusive Dysfunction (CVOD) Induced by Experimental Cavernosal Nerve Damage in the Rat. *J Sex Med.* 2008 Dec 2. [Epub ahead of print]

### **Non-experimental articles**

1. Rambhatla A, **Kovanecz I**, Ferrini M, Gonzalez-Cadavid NF, Rajfer J. (2008) Rationale For PDE5 Inhibitor Use Post Prostatectomy. *Int J Impot Res* 20(1):30-34. [Epub 2007 Aug 2.]

### **C. Active and Completed Funding.**

None

## BIOGRAPHICAL SKETCH

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

NAME Gelfand, Robert Allen	POSITION TITLE Adjunct Assistant Professor		
eRA COMMONS USER NAME (credential, e.g., agency login)			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Massachusetts Institute of Technology	SB	1970	Mathematics
University of California Santa Barbara	MS	1973	Biological Science
California Institute of Technology	PhD	1980	Biochemistry

### A. Positions and Honors

1973 Graduate Fellowship, California Institute of Technology  
1980-6 Postdoctoral Fellow, Purdue University Department of Biology  
1987-90 Staff Research Associate, University of California Irvine and VA Medical Center Long Beach CA  
1990-94 Biochemist, VA Medical Center, Long Beach CA  
1995-7 Lecturer, Department of Chemistry and Biochemistry, Cal. State Univ Long Beach  
1997-2008 Director, Gelfand Partnership, a management company  
2003-7 Columnist of media, culture, and science, American-Reporter.com  
2004-8 Editor, Coastal Currents, San Pedro CA  
2005-7 Visiting Scientist, LABiomed, Harbor UCLA Medical Center  
2007-8 Staff Research Associate IV, LABiomed Harbor UCLA Medical Center  
2007-8 Adjunct Assistant Professor, Charles R. Drew University of Medicine and Science  
2007-8 Adjunct Faculty, Los Angeles Southwest College

### Professional Membership

1992-2008 Endocrine Society Member

**B. Selected peer-reviewed publications** 1990 – 2008 out of a total of 21 peer reviewed papers and chapters.

Note: Due to the loss of grant funding, I left research in 1994. This was compounded by a malignant melanoma in 2000, which was excised and has not returned. In the interim, I developed a business, taught biochemistry and chemistry in the university setting and published numerous articles in daily newspapers and on the internet, totaling greater than 150 individual pieces on science, culture and the media (not shown). Since late 2005, I have returned to scientific research and teaching and I am committed to continuing as long as I am able.

Levin ER, Frank HJL, Gelfand RA, Loughlin SE, Kaplan G. (1990) Natriuretic peptide receptors in cultured rat diencephalon. J Biol Chem. 265:10019-10024.

Matsuoka LY, Wortsman J, Tang G, Russell RM, Parker L, Gelfand RA, Mehta RG. (1991) Are endogenous retinoids involved in the pathogenesis of acne? Arch. Derm. 127:1072-3.

Gelfand R, Frank HJL, Levin E, Pedram A. (1991) Brain and atrial natriuretic peptides bind to common receptors in brain capillary endothelial cells. Am J. Physiol. 261:E183-E189.

Parker L, Lifrak E, Gelfand R, Shively J, Lee T, Kaplan B, Walker, P, Calaycay J, Florsheim W, Mason I, Soon-Shiong P. (1993) Isolation, purification, synthesis, and binding of human adrenal gland cortical androgen stimulating hormone. *End J* 1:441-445

Frank H, Pedram A, Hu R, Gelfand R, Levin, E. (1994) Regulation of atrial natriuretic peptide receptors in brain capillary endothelial cells. *Endo J.* 2:33-40

Frank H, Pedram A, Hu R, Gelfand R, Levin E. (1994) Regulation of atrial natriuretic peptide receptors in brain capillary endothelial cells. *Endo J.* 2:33-40

Gelfand R, Wepsic HT, Parker L, Jadus, M. (1995) Prostaglandin E<sub>2</sub> induces upregulation of murine macrophage b-endorphin receptors. *Immunol Lett.* 45:143-148

Gelfand R, Bobrow A, Pham L, Young C, Parker, L. (1995) b-endorphin binding in cultured adrenal cortical cells. *Endocrine* 3:201-207

Nolazco G, Kovanecz I, Vernet D, Ferrini M, Gelfand R, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *Br J Urol Int*, 101(9):1156-64. Epub 2008 Feb 21.

Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF (2008) Pro-fibrotic role of myostatin in Peyronie's disease. *J Sex Med.* 2008 Jul;5(7):1607-22. Apr 15. [Epub ahead of print]

### **C. Active and Completed Funding.**

None



## BIOGRAPHICAL SKETCH

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

NAME <b>Tsao, James</b>	POSITION TITLE <b>Principal Research Associate</b>		
eRA COMMONS USER NAME (credential, e.g., agency login)			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of California, Riverside, California	B.S.	1991	Biomedical Science
Ross University, School of Medicine, Dominica	M.D..	1995	Medicine
	.		

### A. Postitions and Honors

1999-2001 Research Associate, Department of ENT, Charles Drew University of Medicine and Science, California, USA  
2001-2003 Senior Research Associate, Department of ENT, Charles Drew University of Medicine and Science, California, USA  
2003-2004 Principal Research Associate, Department of ENT, Charles Drew University of Medicine and Science, California, USA  
2004-2007 Senior Research Associate, Division of Endocrinology, Department of Medicine, Charles Drew University of Medicine and Science, California, USA  
2007-Present Principal Research Associate, Division of Endocrinology, Department of Medicine, Charles Drew University of Medicine and Science, California, USA

### B. Publications

1: Nolzco G, Kovanecz I, Vernet D, Gelfand RA, **Tsao J**, Ferrini MG, Magee T, Rajfer J, Gonzalez-Cadavid NF. (2008) Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int. 101(9):1156-64. Epub 2008 Feb 21.

2: Artaza JN, Singh R, Ferrini MG, Braga M, **Tsao J**, Gonzalez-Cadavid NF. (2008) Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. J Endocrinol. 196(2):235-49.

3: Artaza JN, Reisz-Porszasz S, Dow JS, Kloner RA, **Tsao J**, Bhasin S, Gonzalez-Cadavid NF. (2007) Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. J Endocrinol. 194(1):63-76.

### C. Active and Completed funding

None