W7: Noncellular regenerative therapies for stress urinary incontinence

Workshop Chair: Margot Damaser, United States
28 August 2018 11:00 - 12:30

Start	End	Topic	Speakers
11:00	11:05	Introduction	Margot Damaser
11:05	11:20	Regenerative Medicine in the Context of Stress Urinary Incontinence	Margot Damaser
11:20	11:35	The cell mobilising chemokine CXCL12 as a regenerative pharmacologic treatment for chronic stress urinary incontinence	James Koudy Williams
11:35	11:40	Questions	All
11:40	11:55	Incorporation of oestradiol into a biodegradable mesh as an approach to provide mechanical support and stimulation of tissue regeneration	Sheila MacNeil
11:55	12:00	Questions	All
12:00	12:15	Perigenital transcutaneous electrical stimulation to improve recovery of stress urinary continence in a rat model	Yolanda Cruz
12:15	12:20	Questions	All
12:20	12:30	Discussion	Margot Damaser James Koudy Williams Sheila MacNeil Yolanda Cruz

Aims of Workshop

The Aim of this workshop is to educate attendees on the latest research in regenerative medicine for stress urinary incontinence. Internationally renown researchers from around the world will present their work in the context of current clinical therapies for stress incontinence and current clinical trials. Promising research suggests that noncellular regenerative approaches have great promise for treating and preventing stress incontinence, as well as for reducing complications of surgical treatments such as mesh sling procedures. These promising therapies include regenerative electrical stimulation and regenerative pharmacologic approaches such as treating with estrogen, CXCR12, or the secretions of stem cells.

Learning Objectives

1. Understand the concepts and terms of regenerative medicine in the context of outcomes of recent clinical trials testing regenerative therapies for stress incontinence.
2. Understand the goals and results of recent preclinical research into noncellular regenerative therapies for stress incontinence.
3. Discuss and advance the clinical context for these promising therapeutics and how to translate them into clinical trials.

Learning Outcomes

After the course, the student will be able to:
1. Knowledgeably discuss current research into novel therapies of stress incontinence.
2. Knowledgeably read and critique scientific literature on regenerative medicine.
3. Initiate ideas and design experiments to test novel regenerative therapies for stress incontinence.

Target Audience

Clinicians, researchers, and trainees interested in learning about current state of the art research into the next frontier of therapies for stress urinary incontinence.

Advanced/Basic

Advanced

Conditions for Learning

There should be a lively discussion among the attendees and the presenters.

Suggested Learning before Workshop Attendance

None.

Suggested Reading

Williams JK, Dean A, Badlani G, Andersson KE. Regenerative Medicine Therapies for Stress Urinary Incontinence. J Urol. 2016 Dec;196(6):1619-1626.

Tran C, Damaser MS. Stem cells as drug delivery methods: application of stem cell secretome for regeneration. Adv Drug Deliv Rev. 2015 Mar;82-83:1-11.

Mangir N, Hillary CJ, Chapple CR, MacNeil S. Oestradiol-releasing Biodegradable Mesh Stimulates Collagen Production and Angiogenesis: An Approach to Improving Biomaterial Integration in Pelvic Floor Repair. Eur Urol Focus. 2017 Jun 3. pii: S2405-4569(17)30122-0

Deng K, Lin DL, Hanzlicek B, Balog B, Penn MS, Kiedrowski MJ, Hu Z, Ye Z, Zhu H, Damaser MS. Mesenchymal stem cells and their secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. Am J Physiol Renal Physiol. 2015 Jan 15;308(2):F92-F100.

Williams JK, Dean A, Badra S, Lankford S, Poppante K, Badlani G, Andersson KE. Cell versus Chemokine Therapy in a Nonhuman Primate Model of Chronic Intrinsic Urinary Sphincter Deficiency. J Urol. 2016 Dec;196(6):1809-1815.

Juárez R, Zempoalteca R, Pacheco P, Lucio RA, Medel A, Cruz Y. Activity of the external urethral sphincter evoked by genital stimulation in male rats. Neurourol Urodyn. 2016 Nov;35(8):914-919

Jiang HH, Gill BC, Dissaranan C, Zutshi M, Balog BM, Lin D, Damaser MS. Effects of acute selective pudendal nerve electrical stimulation after simulated childbirth injury. Am J Physiol Renal Physiol. 2013 Feb 1;304(3):F239-47.

Chapple CR, Cruz F, Deffieux X, Milani AL, Arlandis S, Artibani W, Bauer RM, Burkhard F, Cardozo L, Castro-Diaz D, Cornu JN, Deprest J, Gunnemann A, Gyhagen M, Heesakkers J, Koelbl H, MacNeil S, Naumann G, Roovers JWR, Salvatore S, Sievert KD, Tarcan T, Van der Aa F, Montorsi F, Wirth M, Abdel-Fattah M. Consensus Statement of the European Urology Association and the European Urogynaecological Association on the Use of Implanted Materials for Treating Pelvic Organ Prolapse and Stress Urinary Incontinence. Eur Urol. 2017 Sep;72(3):424-431.

Margot Damaser, PhD

Recent FDA warnings and class action lawsuits in the US as well as anti-mesh campaigns and related controversies in UK and Europe and the removal of implantable mesh products from the market have all highlighted the need for improved therapy for stress incontinence. Although it can occur decades later, stress incontinence is strongly associated with the maternal injuries of childbirth, and postpartum incontinence is highly predictive of later development of stress incontinence even if the postpartum incontinence resolves. Thus, there is an opportunity to treat stress incontinence both later in life when it presents in most women and, when possible, earlier in life when it presents as postpartum incontinence. For both treatment paradigms, regenerative medicine holds great potential for regenerating damaged connective tissues, muscles, and nerves, as well as for reducing complications for stress incontinence surgeries.

Recent clinical trials assessing autologous progenitor cells for stress incontinence have demonstrated the safety of regenerative approaches; however, the results are variable and therapeutic efficacy has been difficult to demonstrate in properly controlled clinical trials. The morbidity and complications that did occur were primarily at the biopsy site suggesting that an off the shelf approach would provide improved safety. Noncellular regenerative therapies could be utilized off-the-shelf without the need for biopsy and with reduced risk of oncogenic complications. In addition, if the delivery route is minimally invasive, multiple treatments can easily be provided. Such treatments include regenerative electrical stimulation and regenerative pharmacology including the secretions of stem cells as a whole or individually, as well as regenerative steroids, such as estrogen.

Stem cells secrete a wide variety of molecules, thought to be encoded by approximately 10% of the human genome. These secretions include serum proteins, growth factors, angiogenic factors, hormones, cytokines, chemokines, as well as extracellular matrix proteins and proteases. They have a number of bioactive effects, including antiapoptosis, antiscarring, neovascularization, neuroprotection, neuroregeneration, wound healing, and immune modulation. Several studies, including Dr. Damaser's, have compared treatment with stem cell secretome to treatment with stem cells and have obtained comparable results in several animal models. In those studies, it is the great diversity of secretions, rather than a single element of the secretions, that is thought to have a profound regenerative effect since these many secreted factors can act on multiple pathways at once, multiplying the regenerative effect many times over that possible with a single pharmacologic treatment. Secretome has advantages over cells for manufacturing, storage, handling, product shelf life and their potential for allogenic use as an off-the-shelf regenerative pharmaceutical.

Dr. Damaser will provide an introduction to the state of the art of regenerative medicine research as it applies to stress urinary incontinence. She will provide sufficient background on the field for attendees to understand the subsequent presentations. No prior expertise in regenerative medicine is needed. She will summarize research testing secretome for stress incontinence and the potential it hold for clinical application.

Take Home Message:

- Regenerative medicine approaches show great promise for stress incontinence and noncellular regenerative therapies have advantages over cellular therapies

J. Koudy Williams, PhD

Lower urinary tract disorders remain a major urological problem in both men and women and include several tissue-specific syndromes associated with impaired tissue regeneration. It is more common in women at earlier ages, but increases in frequency in men following radical prostatectomy.

As many as 30% of women older than 20 years have urinary incontinence (UI). It is often the cause of aging and parturition damage to the urinary sphincter and its innervation. Current treatment for SUI in women is largely palliative and often surgically ineffective. Increased interest in permanent cures, but regenerative therapy for patients with chronic SUI (cell therapy) is only modestly effective (around 50% improvement in 50% of patients). Regardless of newer nerve-sparing prostatectomy procedures in men, persistent urinary incontinence occurs in 4-31% of patients, and erectile dysfunction occurs in 54-90% at 12 months following post radical prostatectomy. Similar to women, current treatments are largely palliative and cell therapies provide on modest improvement in symptoms.

As an alternative to cell therapy, this presentation will focus on the use of targeted chemokine CXCL12 (C-X-C motif chemokine 12) treatment for chronic intrinsic urinary sphincter deficiency (ISD) in female nonhuman primates (NHP) and for persistent post prostatectomy urinary and erectile dysfunction in male NHPs. Our results indicate that local (sphincter) injection of CXCL12 provides superior restoration of sphincter function (urodynamic measures of resting and nerve stimulated maximal urethral pressures, muscle content, innervation and vascularization compared to autologous cell therapy. These effects were sustained for at leadt6 12 months most injection. New data indicate that CXCL12, but not cells, stimulated mobilization of labeled bone marrow cells to the urinary sphincters of these female NHPs. In the male NHPs, local injection of CXCL12 at the vesico-urethral anastomosis restored baseline abdominal leak-point pressures and urethral sphincter pressures. Additionally, CXCL12 restored maximal penile pressures in response to papaverine injections and normal sexual function (mating behavior in the male monkeys.

The advantages of non-cellular therapy are that it avoids hesitant FDA approval associated with cell therapy; is a targeted approach; is cheaper; and is potentially more readily available to a wide patient population. However, no molecule acts in a vacuum and requires knowledge of its cross-reactivity with other molecules and pathways and safety issues. Nonetheless, targeted molecular therapy holds the promise of identifying new treatment modalities and pathways that could optimize tissue regeneration.

Take Home Message:

- Targeted molecular therapy holds potential for stress urinary incontinence as a form of noncellular regenerative pharmacotherapy

Sheila MacNeil, PhD

Childbearing and vaginal childbirth often lead to weakened pelvic floors and stress urinary incontinence (SUI) and/or pelvic organ prolapse (POP). 20% of healthy women will require surgery for POP by the age of 80. Unfortunately non-degradable polypropylene (PP) meshes which have been used to support the pelvic organs for the last decade are now known to cause unacceptable side-effects in around 5% of woman when used as small tapes to support the urethra for SUI, and in at least 20% of woman when used as larger areas to support the pelvic organs in POP. Indeed the incidence of severe side-effects continues to rise with time post implantation in these women.

A combination of several factors have contributed to the emergence of the 'vaginal mesh scandal' including problems related to the mesh material, a gap in the regulatory approval process and poor understanding of pelvic floor diseases.

Current surgical meshes evolved over many years from a metal wire to the modern meshes made of polypropylene. Surgical techniques of implantation of the surgical mesh also evolved over the years to overcome complications which were experienced with the early polypropylene mesh materials. These were made in the context of hernia surgeries but there are more lessons yet to be learned when the same materials were implanted into the pelvic floor.

Our understanding of the anatomy of the abdominal hernia is quite mature as is our understanding of the problems of urinary stress incontinence. In contrast our understanding of the disease mechanisms and anatomical problems which lead to pelvic organ prolapse is poor. Materials that were never designed to work in the pelvic floor environment have led to problems of inflammation, pain and erosion through patients tissues. In summary a polypropylene mesh which works well in abdominal hernia repair and reasonably well in supporting the urethra does not work well when introduced through the vagina to support pelvic organs.

Our team of scientists and clinicians are engaged in developing alternative next-generation biomaterials and tissue engineering approaches to provide solutions specifically designed for the dynamic pelvic floor.

For SUI we have developed a fascia mimetic nondegradable mesh of polyurethane which has strength and elasticity much closer to the patient's native tissue than the inflexible PP meshes currently used. This is currently being evaluated in a sheep vagina model developed by Prof Jan Deprest in Leuven. For POP we are developing a slow degrading mesh of polylactic acid (PLA) designed to be introduced with lipoaspirate derived cells which are capable of producing new tissue. The challenge here is to develop a methodology for accessing the cells in a minimally invasive procedure and combining them with the PLA membrane for surgical implantation. Finally in an effort to improve tissue integration we have developed methodologies for releasing oestradiol from both nondegradable (polyurethane) and degradable (polylactic acid) electrospun fibres. Our rationale is that post-menopause women lack oestrogens and surgeons will often use oestrogens to stimulate wound healing prior to operating in the pelvic floor.

Take Home Message:

- Novel synthetic materials with biomechanical properties similar to native tissue have potential to improve treatment of stress incontinence and can be integrated with regenerative therapies

Yolanda Cruz, PhD

Pelvic floor is the anatomical substrate of urinary, sexual and reproductive functions and damage to pelvic organs and/or to its innervation results in pelvic floor dysfunction, including urinary incontinence.

Urinary incontinence is the most significant urinary disorder in women. Although it is well recognized its multifactorial etiology, numerous studies show that vaginal delivery is a common risk factor, due it negatively affects pelvic floor structures and their functions.

The rat vaginal distension model (VD) was created to better understand the injury process during parturition of women. VD in rats induces bladder, urethral, and vaginal hypoxia, as well as urethral obstruction, bladder overdistention and stretch of peri-vaginal nerves, affecting the function of the external urethral sphincter (EUS) and decreasing urethral resistance. VD neuroanatomic injuries correlates with behavioral signs of stress urinary incontinence in unanesthetized rats.

On the other hand, preclinical studies have shown that 1 hour of 20 Hz electrical stimulation of an injured peripheral nerve (femoral or sciatic) promotes acceleration and accuracy of sensory and motor fibers regeneration and reconnection. In VD rats, electrical stimulation of the pudendal nerve motor branch upregulate neurotrophic factors, such as brain-derived neurotrophic factor in the spinal cell body of the external urethral sphincter motoneurons, which in turn promote the synthesis of neural structural proteins important for neuroregeneration. Consequently, electrical stimulation of the injured pudendal nerve has been proposed as a potential treatment of urinary incontinence.

In anesthetized rats we have shown that mechanical stimulation of internal and external genitalia activates the EUS reflexively. At the spinal cord, genital afferents diverge the information to Onuf nucleus motoneurons as well as to other spinal and supraspinal sites, activating several neural pathways, including increase in blood flow. Genital stimulation may facilitate recovery of lower urinary tract polytrauma after VD by reflex activation of the pudendal nerve motor branch to facilitate its regeneration, as well as reducing hypoxia of the lower urinary tract. In addition, the regenerative process may be activated by a non-invasive method, by transcutaneous electrical stimulation.

In this talk I will summarize the work we are doing to investigate in sexually mature female rats the neuroregenerative effects of transcutaneous perigenital stimulation. First, I will describe the electrophysiological studies to determine the parameter of electrical stimulation of the perigenital skin that activates the external urethral sphincter and the effect of estrous cycle on the parameters of stimulation. Then, I will talk about the effect of the transcutaneus perigenital electrical stimulation on the behavior of micturition and the electromyographic activity of the EUS of VD rats. Briefly, we have found that electrical stimulation of the clitoral skin accelerates urinary function recovery of continence of VD rats: reduced the number of animals leaking urine after VD, as well as the time required for continence recovery and for the external urethral sphincter electromographic activity reappearing. Further studies are required to understand the physiological mechanisms involved in perigenital transcutaneous electrical stimulation, information that will enable clinicians to optimize neuromodulation therapy for patients with pelvic floor dysfunction, such as urinary incontinence.

Take Home Message:

Electrical stimulation has regenerative effects and can be done in a minimally invasive manner to facilitate recovery from childbirth injuries and treat and possibly prevent stress incontinence development.

ICS 2018 Workshop 7: Noncellular regenerative therapies for stress urinary incontinence

Chair: Margot S. Damaser, PhD
Speakers: J. Koudy Williams, DVM
Sheila MacNeil, PhD
Yolanda Cruz, PhD

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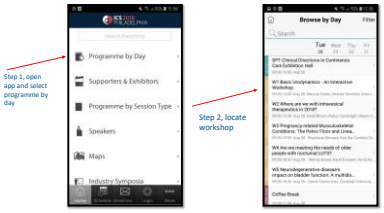

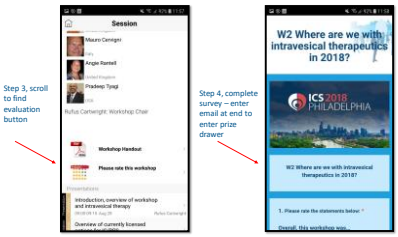

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APT CENTER

Regenerative Medicine in the Context of Stress Urinary Incontinence

Margot S. Damaser, Ph.D.

Professor, Dept of Biomedical Engineering and Glickman Urological and Kidney Institute the Cleveland Clinic, Cleveland, OH

Senior Rehabilitation Research Career Scientist Cleveland VA Medical Center, Cleveland, OH

Margot S. Damaser, PhD

Affiliations to disclose†:

None

† All financial ties (over the last year) that you may have with any business organization with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

- [ ] Self-funded
- [X] Institution (non-industry) funded
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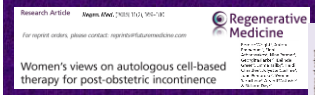
Current Treatments for FPFD

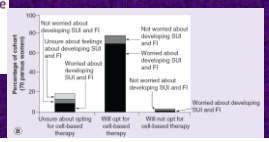
- Stress Incontinence
  - diapers/pads & vaginal pessaries
  - biofeedback & Kegel exercises
  - collagen & bulking agents
  - surgery

The Independent, Monday 9 July 2018
Government agrees to temporarily ban vaginal mesh implants for women with urinary incontinence
Campaigners hail decision as 'vindication' for women 'maimed' by operation

Alternatives are needed

- New prosthetic materials are being investigated in laboratory-based studies
- Regenerative Medicine can provide biologically-driving treatment
- Regenerative therapy with autologous cells currently in clinical trials
- Regenerative Rehabilitation is a promising approach
  - Combining regenerative therapies with exercise





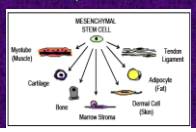
How do stem cells work therapeutically?

- Differentiation
- Paracrine Secretions
- Autocrine Secretions
- Recruitment of other cells
- Exosome Secretions
- Fusion

Classical Approach → Most Speculative

In addition, some stem cells home to injured or hypoxic tissues that secrete homing cytokines

Mechanism is highly dependent on local microenvironment



Secretome of Stem Cells

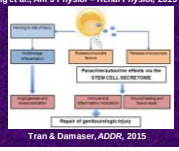
- Trophic factors
- Regulatory chemokines
- Immunomodulatory cytokines
- Signalling cytokines
- extracellular matrix (ECM) proteases
- Hormones
- Lipid mediators

The trophic effects of secretome are observed even in the absence of cell contact and if it is delivered systemically

Uccelli & Prockop, *Curr Op Immunol*, 2010
Makridakis et al., *Biochimica et Biophysica Acta*, 2013
Dissaranan et al., *Cell Transplantation*, 2014
Deng et al., *Am J Physiol – Renal Physiol*, 2015

Preconditioning Methods
- Physiological
- Genetic manipulation
- Molecular
- Cellular
- Pharmacological
- Physical

Test secretome as Concentrated Conditioned Media (CCM)



Tran & Damaser, *ADDR*, 2015

Noncellular Regenerative Therapies

- Treating with secretions of stem cells in the absence of cells
- Regenerative Pharmacology
- Regenerative Electrical Stimulation

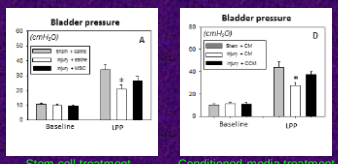
Advantages
- Could be done off-the-shelf or personalized to the patient
- Fewer side effects and complications than cell therapy or mesh implant alone
- Could be given in conjunction with mesh or other prosthetic

Currently in laboratory-based investigations

Noncellular Regenerative Therapies

- Treating with secretions of stem cells in the absence of cells
  - Dr. Damaser will discuss briefly
- Regenerative Pharmacology
  - Dr. Williams will discuss his research with CXCL12 as a regenerative pharmacologic agent for stress incontinence
  - Dr. MacNeil will discuss her research with Estradiol for tissue regeneration in conjunction with a biodegradable mesh
- Regenerative Electrical Stimulation
  - Dr. Cruz will discuss her research using electrical stimulation to improve recovery from stress incontinence

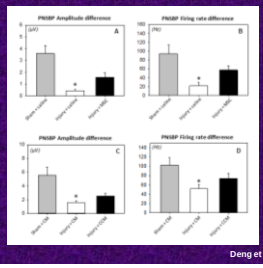
LPP improves with Stem Cell or Secretome treatment in the dual injury rat SUI injury model



Stem cell treatment | Conditioned media treatment

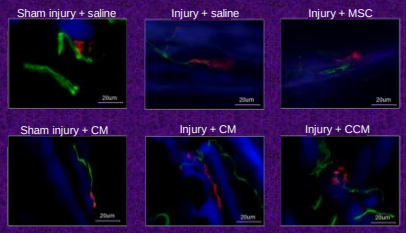
Deng et al., *Am J Physiol – Renal Physiol*, 2015

Pudendal nerve function improves after stem cell or secretome treatment



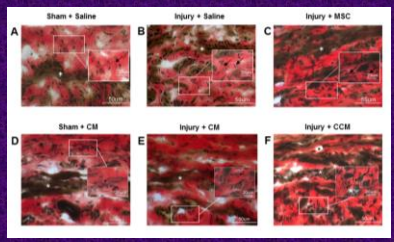
Deng et al., *Am J Physiol – Renal Physiol*, 2015

Innervation of urethral NMJs improves after stem cell or secretome treatment



Deng et al., *Am J Physiol – Renal Physiol*, 2015

Elastin in the urethra changes & increases with stem cells or secretome



Deng et al., *Am J Physiol – Renal Physiol*, 2015

Conclusions

- Noncellular regenerative approaches are feasible and could be used to promote recovery after injury or in a chronic situtation
- The mechanism is likely multifactorial
  via: neuroprotection, neuroregeneration, elastogenesis, and others
  but not likely via differentiation of cells into innervated sphincter muscle
- Repeat treatments could improve outcomes
- Further animal research and controlled clinical trials are needed to test this paradigm-shifting approach

A Non-Cellular Approach to Lower Urinary Tract Regeneration

J. Koudy Williams, Karl-Erik Andersson, Gopal Badlani
Wake Forest Institute for Regenerative Medicine
Winston-Salem, NC

kwilliam@wakehealth.edu

ICS 2018 PHILADELPHIA

Affiliations to disclose†:

National Institutes of Health - NIDDK

† All financial ties (over the last year) that you may have with any business organisation with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

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What are the Different Forms of Regenerative Medicine?

- Cell Therapy **(e.g., Injecting cells in the diseased tissue to restore structure and function.**
- Bioengineered Tissues and Organs **(e.g., some combination of matrix (natural or polymer-based) to implant into diseased or damaged tissues and organs.**
- Endogenous Regeneration **(e.g., using a growth factor, chemokine or genes to stimulate the body to heal itself) - referred to as "Regenerative Pharmacology".**

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A Nonhuman Primate Model of Urinary Sphincter Structural and Functional Deficiency

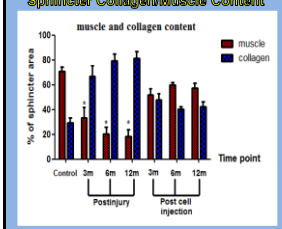
Why Female Nonhuman Primates for Urologic Research?

- 28 day menstrual cycle
- Pelvic bladder and urinary sphincter
- Upright sitting posture
- Age- and hormone-related health problems, including heart disease, osteoporosis, breast/uterine cancer, and cognitive decline.
- Well-defined menarche, pre-menopause peri-menopause, and post-menopause
- Human-like ultrastructure of the sphincter complex and pelvic floor support.

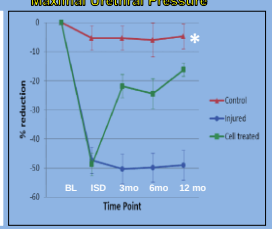
- The pudendal innervation to the urinary sphincter was cut and cauterized.
- Five million autologous skeletal muscle precursor cells (transduced with lenti-M-cherry) injected directly into the urinary sphincter complex post-sphincter injury.
- Partial bone marrow transplantation of lenti-GFP cells 2 weeks prior to cell injection.
- Maximal Urethral pressures (MUP) and sphincter collagen/muscle content

skMPC Effects on Structure and Function

Sphincter Collagen/Muscle Content

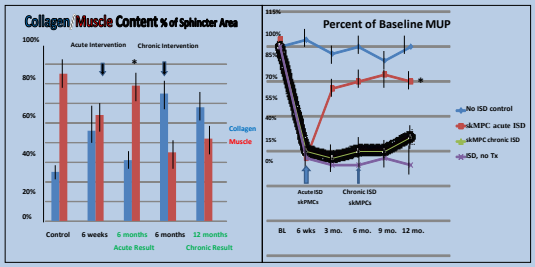


Maximal Urethral Pressure



Chronicity of Disease

Acute (6 weeks) vs. Chronic (6 months) ISD



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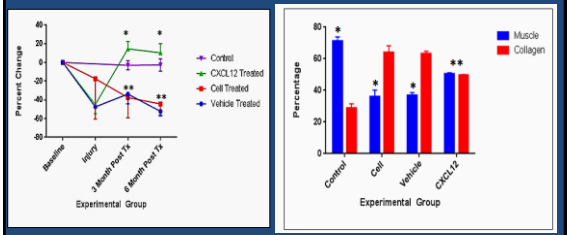
Regenerative Pharmacology and Urologic Diseases.

- **Which molecules are of interest**?
- These cell products include a myriad of molecules including chemokines, growth factors (vascular endothelial growth factor [VEGF], fibroblast growth factor, transforming growth factor-alpha), and interleukins (IL-1, IL8).
- These molecules are involved in important paracrine and receptor-mediated processes associated with tissue regeneration. Identifying the involvement of some of these molecules in disease development and *using them as therapeutic agonists or antagonists* illustrate principles of regenerative pharmacology.

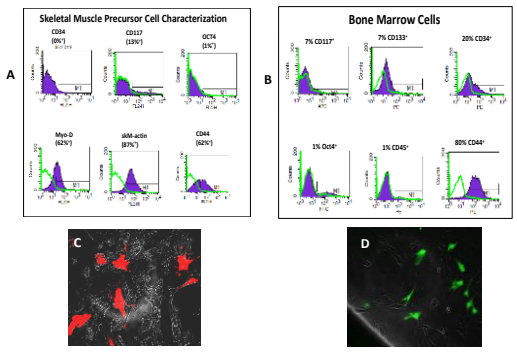
Cell Mobilization Paradigm Using Chemokines: Focus on Stromal Derived Factor (CXCL12)

Stromal derived factor-1α (CXCL-12) plays a major role in cell trafficking and homing of progenitor cells to sites of injury through a receptor [CXCR4, CXCR7] mechanism and enhancing cell survival once at the injury site.

skMPC vs. CXCL-12 Treatment of UI



Tracking Cells *In Vivo*

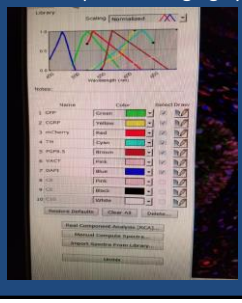


Qantification of Cell Expression Patterns

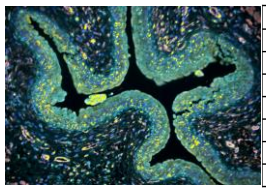
Standard IHC is laborious and has limited ability to quantify expression patterns of injected and mobilized cells

Quantification-Nuance Multispectral Imaging System

- Minimizes autoflourescence by separating cells from background
- Multiple antibodies & flourophores on one slide
- Spectral Library
- Pixel by pixel separation for accurate quantification
- Selective spectral wavelength (nm)



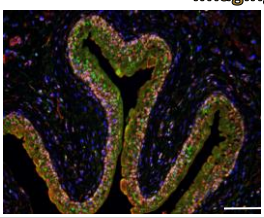
Multiplex/Multispectral Imaging-Innervation



Panel 1		
Antibody (Anti)	Wavelength (nm)	Color
DAPI	450	Blue
GFP	520	Green
mCherry	540	Red
PGP 9.5	570	Orange
Tyrosine Hydroxylase	650	Magenta
vaChT	690	Yellow

Experiment Groups	% GFP+	%M-Cherry+	% positive TH within GFP+	% positive TH within M-Cherry+	% positive PGP9.5 within GFP+	% positive PGP9.5 within M-Cherry+	% positive VaCHT within GFP+	% positive VaCHT within M-Cherry
Untreated	33.3	17.7	65.54	72.48	67.7	82.12	51.24	50.69
Treated	*72.1	16.7	*85.38	75.14	*87.71	77.45	*91.98	45.11

Multiplex/Multispectral Imaging Vascularization



Panel 2		
Antibody (Anti)	Wavelength (nm)	Color
DAPI	450	Blue
GFP	520	Green
mCherry	540	Red
HIF 1 a	570	Brown
αSMA	620	Yellow
Skeletal Muscle Actin	650	Magenta
Von Willebrand Factor	690	Cyan

Experiment Groups	% GFP+	%M - Cherry+	% positive SMA within GFP+	% positive SMA within M - Cherry+	% positive HIF-1 within GFP+	% positive HIF-1 within M - Cherry+	% positive vWF within GFP+	% positive vWF within M - Cherry
Untreated	41.47	13.81	21.68	58.6	66.15	60.39	36.23	60.84
Treated	*78.92	*23.17	*75.38	57.79	*37.86	72.88	*96.23	65.47

Conclusions

- As we proceed through the maturation of regenerative medicine approaches to urological disease, we will need to be mindful of hype vs. hope.
- Regenerative medicine offers the promise of permanent cures for many of these diseases.
- Cell therapy and/or bioengineered tissues have proven to be helpful, but not magic cures.
- A better understanding of the biology of regeneration, and how cells contribute to this regeneration, is essential.
- The answer may lie in the molecules cells produce and how these molecules (alone or in combination) stimulate tissue regeneration.
- Thus, future therapeutic approaches must involve a greater knowledge of these regenerative processes.

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Incorporation of oestradiol into a biodegradable mesh as an approach to provide mechanical support and stimulation of tissue regeneration

Professor Sheila MacNeil
University of Sheffield

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

trust
training urology scientists

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Developing next generation materials for the pelvic floor

- Developed a fascia mimetic nondegradable material for use in SUI
- Protected this with a patent and licenced this to a new company Symimetics to take to the clinic
- Developing other approaches to improve tissue integration looking at both nondegradable and degradable meshes containing agents Oestradiol to drive tissue integration
- Developing degradable meshes of PLA combined with autologous fat for treatment of POP .

Sheila MacNeil

ICS 2018 PHILADELPHIA

Affiliations to disclose†:

Sheila is an Advisor to Symimetics Ltd, a company that is developing a biomimetic material for stress urinary incontinence under licence from Sheffield University .

Sheila was the lead researcher at Sheffield University developing the core licensed technologies

† All financial ties (over the last year) that you may have with any business organisation with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

- ☐ Self-funded
- ☐ Institution (non-industry) funded
- ☒ Sponsored by: *Symimetics*

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THE BURDEN OF SUI AND POP

50% women develop incontinence

50% >50 years have POP

SUI cost NHS £ 536, 000, 000 p/a

SUI cost individuals £ 207, 000, 000 p/a

1 in 5 of all women will require surgery

30 % will need further surgery

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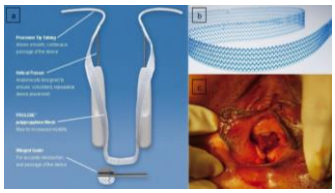

(a) Shows a mid-urethral tape, (b) a close up of this polypropylene mesh and (c) vaginal extrusion of mesh following SUI surgery.

CURRENT HYPOTHESIS OF WHY PPL MESHES DO POORLY, PARTICULARLY IN POP

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Poor tissue integration with host immune attack leading to excessive fibrosis of the implants and contraction.

Biomechanical mismatch between strong rigid PPL mesh and elastic (often damaged) paravaginal tissue, particularly under constant dynamic tension.

A combination of both….

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In designing better materials /product innovation it should be possible to exclude meshes which will not do well in pelvic floor based on

a) Mechanical properties tested in the laboratory under dynamic distension.
b) Sustained inflammatory responses seen in animals,
c) Particularly when associated with extensive contraction
d) Extrusion of materials when implanted in vagina of sheep (or primate model)

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The ideal material should remain relatively elastic to cope with the forces experienced with routine events such as coughing and sneezing but become reversibly stronger at higher strain, similar to native healthy fascia.

Patient's own fascia has good mechanical properties and no bad side effects. The mechanical properties of fascia are known to viscoelastic.

Arguably this is what we need. A biomaterial that mimics the mechanical properties and tissue integration of fascia.

Lets make something like patient's own connective tissue…

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Making sure layers do not delaminate

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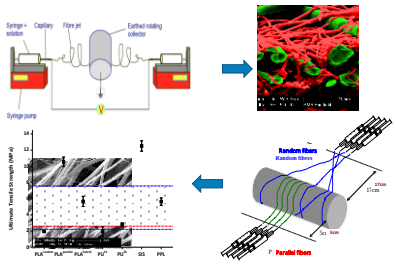

Scanning electron microscope (SEM) of a tri-layer PU scaffold; A-B: bottom sSurface; C-D: top surface; E: cross section.

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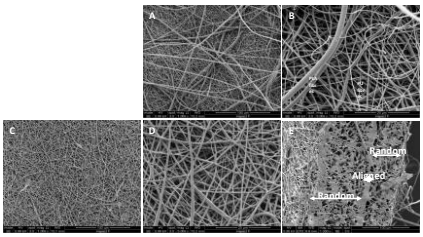

Uniaxial mechanical testing of materials

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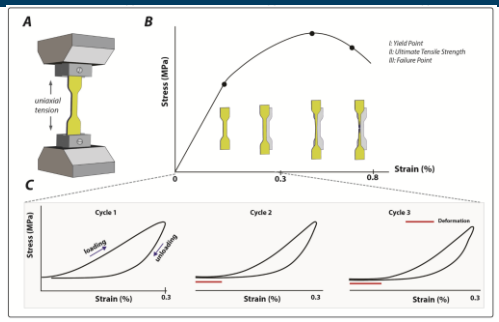

Dynamic testing of materials

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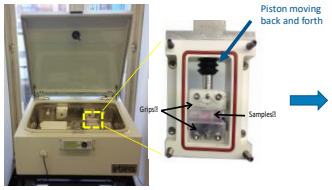
The bioreactor
(37°C, physiologic pH, 5%CO2)



Uniaxial cyclic distention

Effect of 3 days of fatigue testing on materials

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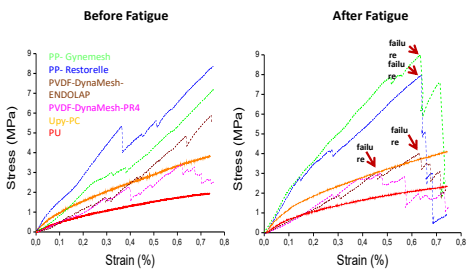
Accepted by Neurourology and Urodynamics 27th August 2017



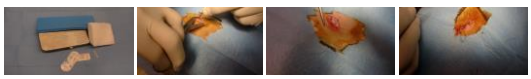
Change before and after EBERS

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 853610, 10 pages
http://dx.doi.org/10.1155/2014/853610

Research Article

Acute *In Vivo* Response to an Alternative Implant for Urogynecology

Sabiniano Roman Regueros,[1] Maarten Albersen,[2] Stefano Manodoro,[3] Silvia Zia,[3] Nadir I. Osman,[1,4] Anthony J. Bullock,[1] Christopher R. Chapple,[4] Jan Deprest,[2,5] and Sheila MacNeil[1]



Animals were sacrificed after 3 and 7 days and samples were fixed and paraffin embedded to analyze by histology the host response against our engineered tissues, such as their integration into native tissues, cell infiltration, neovascularization and new collagen ingrowth.

RESULTS

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Biodegradable poly-L-lactic acid scaffold implanted in the abdomen of rats with or without cells (human and rat adipose derived stem cells) showed extensive host cell penetration, new blood vessel formation and new collagen deposition without any obvious differences between cell containing and cell-free scaffolds.

Acute (7 day) response in rats shows good acute integration of PLLA mesh into the host.

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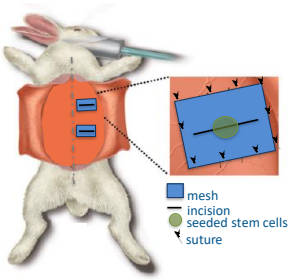
Evaluating Alternative Materials for the Treatment of Stress Urinary Incontinence and Pelvic Organ Prolapse: A Comparison of the In Vivo Response to Meshes Implanted in Rabbits

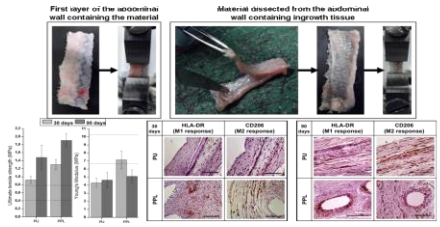
Sabiniano Roman, Iva Urbánková, Geertje Callewaert, Flore Lesage, Christopher Hillary, Nadir I. Osman, Christopher R. Chapple, Jan Deprest and Sheila MacNeil*

From the Kroto Research Institute, University of Sheffield (SR, CH, NIO, SM) and Royal Hallamshire Hospital (CH, NIO, CRC) Sheffield, United Kingdom, and Organ Systems, Department of Development and Regeneration, Katholieke Universiteit Leuven-University of Leuven and Department of Obstetrics and Gynaecology, University Hospitals Leuven, Leuven (IU, GC, JD), Belgium

IN VIVO STUDY – ABDOMINAL WALL DEFECT RABBIT MODEL



- 3 month implantation in rabbit model
- Leuven, Belgium
- Cell-free or cell seeded PLA or PU scaffolds
- Sham controls and polypropylene reference material
- 1 month and 3 month explants:
  - Histology
  - Biomechanical properties



This shows material dissected from the abdominal wall of rabbits subject to mechanical testing. Mechanical properties of PP and PU were essentially similar after 1 and 3 months of implantation. However the M1 response to PP remained very evident after 3 months with little M2 response, whereas the M1 response to PU was not very evident by 3 months and there was a clear M2 response indicative of immune cells "calming down".

RESULTS

Two materials in clinical use – polypropylene and polyvinylidene were compared with two experimental materials – poly-L-lactic acid and polyurethane meshes in rabbits.

Materials were implanted in the abdomen for 1 or 3 months.

All materials showed similar biomechanical properties without significant differences.

However polypropylene and polyvinylidene fluoride demonstrated sustained chronic inflammatory response by 90 days. In contrast, poly-L-lactic acid and polyurethane meshes showed a decreased inflammatory response by 90 days indicative of constructive remodelling.

EVIDENCE OF PP MESH FAILURE IN SHEEP

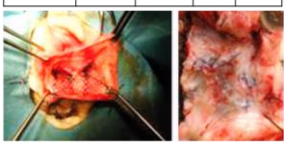
Manodoro et al, BJOG, 2013 120:244-250.

Gynacare PP mesh 50x50mm was implanted in the abdomen or vagina or sheep.

Results indicate both contraction and exposure are site specific for the PP mesh.

Mesh in the vagina of sheep was exposed in 3 out of 10 sheep by 90 days.

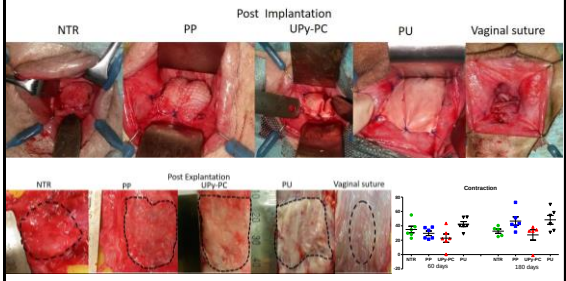
Implantation	Contraction (%)		Exposure	
	60 days	90 days	60 days	90 days
Abdominal	9.35±14.56 (n=10)	7.9±10.1 (n=9+1)	0	0
Vaginal	51.0±11.2 (n=7)	55.0±17.0 (n=5)	2/5 (n=5)	1/5 (n=5)



Assessment of Electrospun and Ultra-lightweight Polypropylene Meshes in the Sheep Model for Vaginal Surgery

Lucie Lymphanova , Rita Rynkevic· Sabiniano Román#Marina G.M.C. Mori da Cunha· Edoardo Mazza· Manuel Zündel. Iva Urbánková· ,Monica R. Gallego· ,Jakob Vange Geertje Callewaert· Christopher Chapple· Sheila MacNeil and Jan Deprest[a, b, d, *]

European Urology Focus Accepted 19th July 2018



There were two exposed PP stitches (n=1 NTR, n=1 UPy-PC) and there were 7 ewes with limited adhesions that were not dividable by blunt dissection (n=2 in NTR, n=3 in PP, n=2 in UPy-PC).

* No significant differences between groups.

Immunohistochemistry of vaginal explants at 60 days

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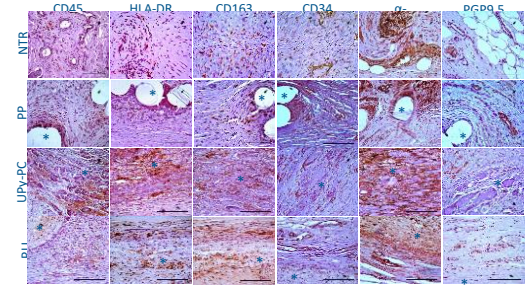

Immunohistochemistry of vaginal explants at 180 days

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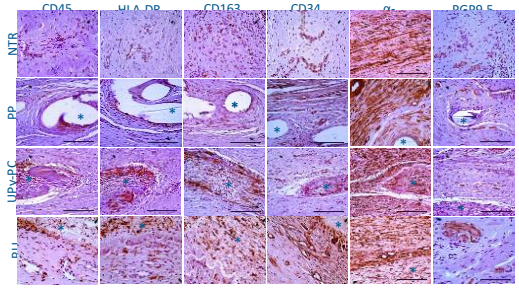

Summary of evaluation of trilayer PU mesh in sheep vagina

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- The fascia mimetic material of 3 layers of PU of different orientation demonstrated a functional repair of sheep vaginal tissues comparable to NTR.
- It integrated well into native tissues with good cell infiltration and formation of blood vessels within the material
- The inflammatory response against the material suggests a constructive remodelling process

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Oestradiol-releasing Biodegradable Mesh Stimulates Collagen Production and Angiogenesis: An Approach to Improving Biomaterial Integration in Pelvic Floor Repair

Mangir, N.; Hillary, C. J.; Chapple, C.; MacNeil, S.

European Urology Focus

2017

Demonstration of improved tissue integration and angiogenesis with an elastic, estradiol releasing polyurethane material designed for use in pelvic floor repair

Shafaat, S.; Mangir, N.; Regueros, S. R.; Chapple, C. R.; MacNeil, S.

Journal Neurourology and Urodynamics, 37, 716-725, 2018

Cumulative release of oestradiol from PLA and PU meshes .

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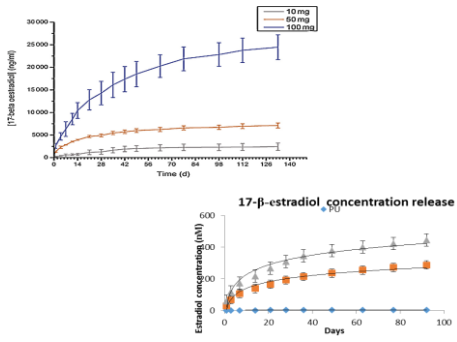

Oestradiol does not affect properties of PLA mesh

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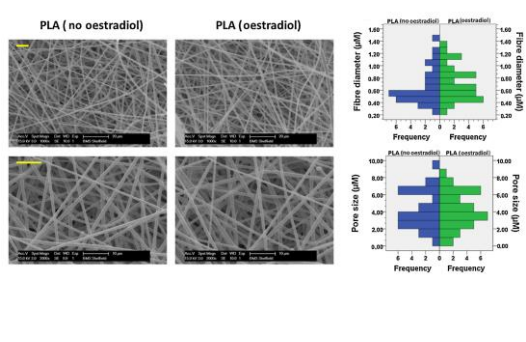
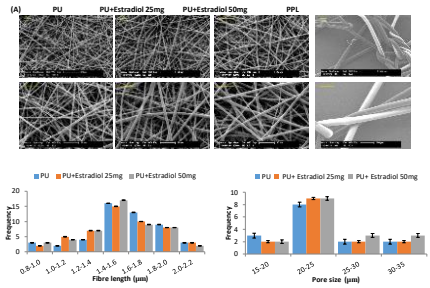
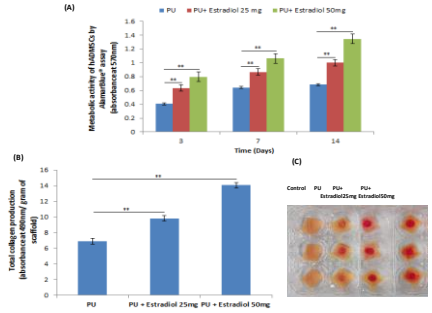


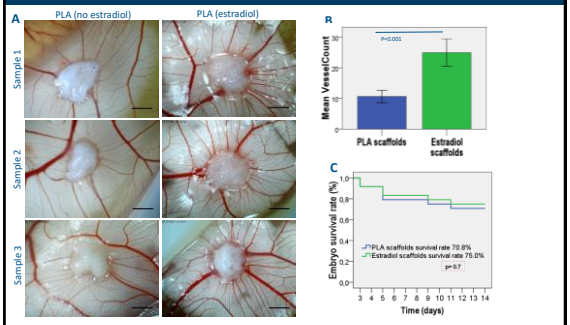
Oestradiol does not affect properties of PU mesh



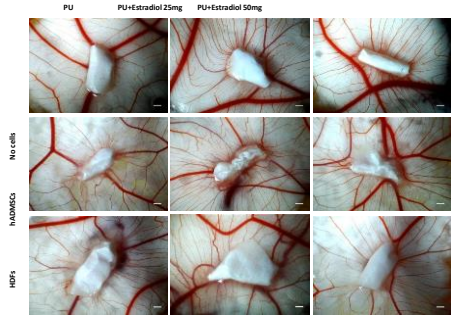
Inclusion of oestradiol improves metabolic activity and collagen production of hADMSCs seeded on scaffolds for 14 days



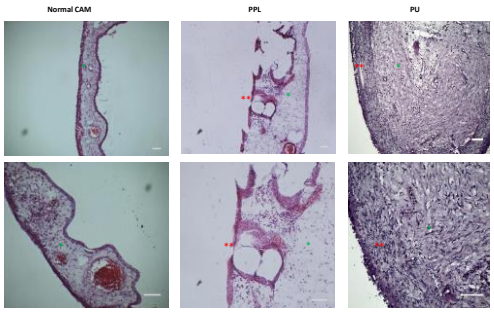
Use of CAM assay to assess angiogenesis of PLA



Use of CAM assay to assess angiogenesis of PU



PU mesh well tolerated on CAM - PPL mesh poorly tolerated after only 7days.



Conclusions

- Simple mechanical testing in vitro can discriminate between materials which have already caused problems clinically and new as yet untested materials
- Both electrospun PLA and PU integrate well into native tissues in animals with good cell infiltration and formation of blood vessels within the material
- The addition of oestradiol can improve pro-angiogenic response to both materials

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Professor Christopher Chapple

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Julio Bissoli 2012-2013
Chris Hilary 2013-2016
Naside Mangir 2014-current
Emma Mironska 2017-current

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Anthony Bullock
Sabi Roman

PhD students:
Sabi Roman
Frazer Bye
Giulia Gigliobianco

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Professor Jan Deprest

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Perigenital transcutaneous electrical stimulation to improve recovery of urinary continence in a rat childbirth injury model

Yolanda Cruz Ph. D.
Autonomous University of Tlaxcala

August 28th, 2018

Yolanda Cruz

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Affiliations to disclose+:

Professor of the Autonomous University of Tlaxcala

+ All financial ties (over the last year) that you may have with any business organization with respect to the subjects mentioned during your presentation

Funding for speaker to attend:

- [X] Self-funded
- [X] Institution (non-industry) funded
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Urinary incontinence

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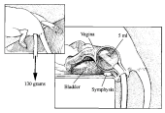
Urinary incontinence is the most significant urinary disorder in women. Although it is well recognized its multifactorial etiology, numerous studies have shown that vaginal delivery is a common risk factor, due it negatively affects pelvic floor structures and their functions.

A rat vaginal distension model (VD) was created to better understand the injury process during parturition of women.



Sievert et al., 2001

LUT injury VD induced (acute studies)

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- **Hypoxia of the bladder, urethra and vagina.**
- **Decreased smooth and striated urethral musculature.**
- **Stretching of pelvic and perineal nerves, including the pudendal nerve.**
- **Abolish activity of EUS.**
- **Decreases urethral resistance denoted by lower LPP, which suggest stress urinary incontinence.**

Does VD induced anatomical and physiological damage is enough to reproduce clinical signs of stress urinary incontinence?

In the clinic, stress urinary incontinence is recognized as a complaint of involuntary loss of urine during effort (Haylen et al., 2010).

Lin et al., 1998; Sievert et al., 2001; Damaser et al., 2004; Jiang et al., 2009, 2011; Pull et al., 2011; Palacios et al., 2016 .

Behavior or micturition

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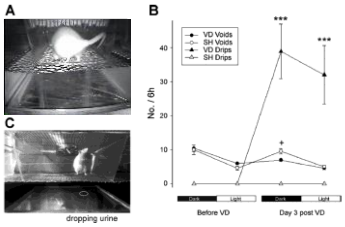

VD during 4h with a Foley catheter inflated with 3 ml of water.

Dropping urine during behaviors implying effort was considered as a sign of stress urinary continence.

Stereotyped behavior of micturition indicates the animals feel bladder fullness and maintain urinary continence until reach the corner.

Palacios et al. 2016

How could urinary continence be restored?

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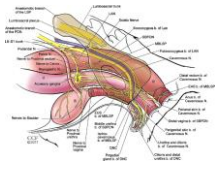
The contribution of urethral striated muscles to urethral resistance is 30% to 40% (Jiang et al. 2011).
The EUS and its innervation is damaged during VD.



The aim was to find a way to facilitate recovery of the urethral somatic innervation.

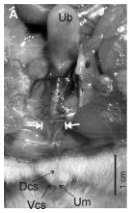
Peripheral nerve regeneration is improved with electrical stimulation (ES) applied to the injured nerve (Geremia et al. 2007).

Hypothesis. ES of the pudendal nerve of VD animals facilitates recovery of urinary continence.

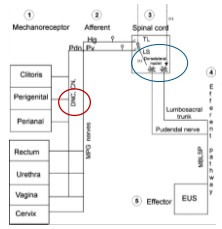
1 hour of electrical stimulation (20 Hz, 0.2 ms, 0.3 mA) of the PdN of childbirth injured rats upregulates BDNF in the spinal cell body of the EUS motoneurons, which in turn promoted the synthesis of BII tubulin, neural structural protein important for nerve regeneration (Jiang et al. 2013).

Electrical stimulation of the childbirth injured pudendal nerve is a potentials treatment of stress urinary incontinence.

May the regenerative effect of ES of pudendal nerve be activated with a minimal invasive method?



Mechanostimulation of perigenital skin discharge EUS activity.



Pudendal nerve regenerative process may be triggered transinaptically through a reflex activity of the EUS neuromuscular pathway.

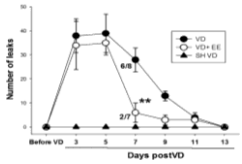
Pastelin et al., 2012, Cruz et al 2016

Transcutaneous electrical stimulation of the DNC

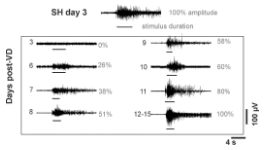
VD rats → Electrical stimulation (20 Hz, 0.2 ms, 1 mA) was applied to clitoral skin at 0, 2 and 3 days after VD.

Outcome measures: Behavior of micturition
EUS EMG

Time course recovery of urinary continence



EUS EMG activity after VD



Conclusions

Electrical stimulation has regenerative effects and can be done in a minimally invasive manner to facilitate recovery from childbirth injuries and treat and possibly prevent stress incontinence development.

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