**W7: Noncellular regenerative therapies for stress urinary incontinence**
 Workshop Chair: Margot Damaser, United States 
 28 August 2018 11:00 - 12:30

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<td>Yolanda Cruz</td>
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**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**
An off-the-shelf approach would provide improved safety. Noncellular regenerative therapies could be utilized properly controlled clinical trials. The morbidity and complications that did occur were primarily at the biopsy site suggesting that secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model.

**Take Home Message:**

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

**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 urinary 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 proctectomy 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 least 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 mimic 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 transcutaneous 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 electromyographic 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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• PDF versions of the slides (where approved) will be made available after the meeting via the ICS website so please keep taking photos and video to a minimum.

Margot S. Damaser, PhD
Affiliations to disclose:\nNone

Funding for speaker to attend:
[ ] Self-funded
[ ] Institution (non-industry) funded
[ ] Sponsored by:
Current Treatments for FPFD

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

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

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
- Signalizing cytokines
- Extracellular matrix (ECM) proteases
- Hormones
- Lipid mediators

Preconditioning Methods

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

Secretome of Stem Cells

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

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


Pudendal nerve function improves after stem cell or secretome treatment


Innervation of urethral NMJs improves after stem cell or secretome treatment

Sham injury + saline
Injury + saline
Injury + MSC
Sham injury + CM
Injury + CM
Injury + CCM


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


Conclusions

• Noncellular regenerative approaches are feasible and could be used to promote recovery after injury or in a chronic situation
• 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

L. Kouly Williams, Karl-Erik Andersson, Gopal Badlani
Wake Forest Institute for Regenerative Medicine
Winston-Salem, NC
kwilliams@wakehealth.edu

Affiliations to disclose†:

National Institutes of Health - NIDDK

Funding for speaker to attend:

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

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

• The pudendal innervation to the urinary sphincter was cut and cauterized.

• Five million autologous skeletal muscle precursor cells (transduced with lent-i-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

Why Female Nonhuman Primates for Urologic Research?

Female bladder and urinary sphincter larger, stronger
High sex hormone-related health benefits, including lower risk of urinary tract and gastrointestinal problems, sexual maturity, and regenerative ability.
Well-defined organ, no spontaneous pre-menopause, and post-partum

skMPC Effects on Structure and Function

Sphincter Collagen/Muscle Content

Maximal Urethral Pressure

% of control area

-30 0 +30

0 2 4 6 8 10 12 14 16 Time point

BL 1w 3m 6m 12m

0 15 30 45 60 75 90 105 mL

0 10 20 30 mL

0 2 4 6 8 10 12 14 16 Time point

Control

Lent-i-M-cherry

Lent-i-GFP

Lent-i-GFP+Lent-i-M-cherry
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.
Quantification 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 autofluorescence by separating cells from background
- Multiple antibodies & fluorophores on one slide
- Spectral Library
- Pixel by pixel separation for accurate quantification
- Selective spectral wavelength (nm)

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Panel 1

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<th>Antibody (Anti)</th>
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<tr>
<td>mCherry</td>
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Experiment Groups

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Panel 2

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<th>% positive vWF within GFP+</th>
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<td>77.45</td>
<td>*51.96</td>
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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.
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

Developing next generation materials for the pelvic floor

- Developed a fascia mimetic nondegradable material for use in SUI
- Protected this with a patent and licensed 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.

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

The Sheffield Urology Team

Chris Chapple
Saurabh Bhargava
Jake Patterson
Altaf Mangera
Sabiniano Roman
Frazer Bye
Giulia Gigliobianco
Nadir Osman
Julio Bissoli
Anthony Bullock

Funding for speaker to attend:
- Self-funded
- Institution (non-industry) funded
- Sponsored by: Symimetics

Sheila MacNeil

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

50% women develop incontinence
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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
CURRENT HYPOTHESIS OF WHY PPL MESHES DO POORLY, PARTICULARLY IN POP

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....

INTERIM CONCLUSIONS

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)

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.

Making sure layers do not delaminate

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

What is needed?

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.
Uniaxial mechanical testing of materials

Dynamic testing of materials

Effect of 3 days of fatigue testing on materials

RESULTS

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.

Research Article

Acute In Vivo Response to an Alternative Implant for Urogynecology

Accepted by Neurourology and Urodynamics 27th August 2017

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.

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

Sabrinano Roman, Iva Urbánková, Geerjte Callewaert, Flore Lenoig, Christopher Hillary, Nadir L Omana, Christopher R. Chapple, Jan Deprest and Sheila MacNeil®

From the Institute of Research Institute, University of Sheffield (SR, CH, NO, BI) and Royal Infirmary of Hospital, University of Sheffield, United Kingdom, and Imperial, Department of Surgery and Radiology, Katholieke Universiteit Leuven, University of Leuven and Department of Obstetrics and Gynecology, University Hospitals Leuven, Leuven, NO, IC, JH, 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 fluoride 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.

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

Lucie Lymperousova, Rita Rynkovic, Sabineev Rohmel, Marina G.M.C. Mori de Carvalho, Edoardo Mazze, Manuel Zündel, Manuel Zündel, Iva Urbánková, Sheila MacNeil and Jan Deprest

European Urology Focus Accepted 19th July 2018

**Comparison of Meshes**

<table>
<thead>
<tr>
<th>Mesh Type</th>
<th>Contraction (%) 60 days</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal</td>
<td>9±0 (0NTR), 15±0 (UPy)</td>
<td>0±0</td>
</tr>
<tr>
<td>Vaginal</td>
<td>15±0 (PP), 16±0 (UPy)</td>
<td>0±0, 33±5 (m/s)</td>
</tr>
</tbody>
</table>

*No significant differences between groups.*
Immunohistochemistry of vaginal explants at 60 days

Immunohistochemistry of vaginal explants at 180 days

Summary of evaluation of trilayer PU mesh in sheep vagina

- 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.

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.; Regureos, S. R.; Chapple, C. R.; MacNeil, S.
Journal Neurourology and Urodynamics, 37, 716-725, 2018

Cumulative release of oestradiol from PLA and PU meshes.

Oestradiol does not affect properties of PLA mesh.
**Oestradiol does not affect properties of PU mesh**

(A) PU  | PU+Estradiol 25mg | PU+Estradiol 50mg | PPL

(B) Frequency

(C) Fibre length (µm)

(D) Pore size (µm)

**Use of CAM assay to assess angiogenesis of PLA**

(A) PLA (no estradiol)  | PLA (estradiol)

(B) Use of CAM assay to assess angiogenesis of PU

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

**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
Acknowledgements

Professor Sheila MacNeil
Clinical Lead:
Professor Christopher Chapple
Clinical Fellows:
Alif Mangera 2009-2011
Nadir Osman 2011-2013
Julio Bissoli 2012-2013
Chris Hilary 2013-2014
Naside Mangir 2014-current
Emma Miranda 2017-current
Postdoctoral Scientists:
Anthony Bullock
Sabi Roman
PhD students:
Sabi Roman
Frazer Bye
Gilda Eglistobrano
European Collaborator:
Professor Jan Deprest

Funding:
• Robert Luff Foundation
• The Urology Foundation
• TRUST European Marie Curie Network
• EPSRC DTC PhD
• BBSRC PhD funding
Urinary incontinence

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.

LUT injury VD induced (acute studies)

- Hyposia 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.

Urinary incontinence is recognized as a complaint of involuntary loss of urine during effort (Haylen et al., 2010).

Behavior or micturition

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

How could urinary continence be restored?

The contribution of urethral striated muscles to urethral resistance is 30% to 40% (Jiang et al., 2011).

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 βIII tubulin, neural structural protein important for nerve regeneration (Jiang et al. 2013).

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

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.