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Bioelectrical Stimulation for the Treatment of Lower Urinary Symptoms:

A Randomized Controlled Trial.

Cristiane Carboni, MSC, PhD

Porto Alegre, Brazil

Introduction:

Dysfunction of the lower urinary tract, including incontinence is a very common clinical problem effecting millions of men and women of all ages. Regenerative medicine has a potential to provide a hope for the recovery of lost tissue and organ functions. In urology, regenerative medicine approaches include for the treatment of lower urinary tract or bladder dysfunctions (1), which can be caused by several diseases altering the normal cellular and extracellular matrix (ECM) compartments which accomplish the role of storage and voiding. The urothelium is necessary to prevent the passage of hypertonic urine to the blood and the exchange of toxic metabolites. In a state of homeostasis, adult urothelial cells are quiescent, however, if the urothelium is damaged by an acute lesion or a exposure to toxins, a rapid process of exfoliation and regeneration is induced (2).

The normal functioning urinary bladder is composed of two main parts: a compliant muscular wall and a highly specialized urothelium. It is well know that the pelvic floor muscles (PFM) play an important roll in the management of lower urinary tract symptoms as well (LUTS) (3). When the bladder is damaged, all these properties must be taken into account for a global functional recovery (4).

The mechanism of action of bioelectrical stimulation (BES) for lower urinary tract dysfunction was initially investigated in animal models, where it caused bladder relaxation by inhibiting the parasympathetic motor neurons. In animal model BES revealed that stress urinary incontinence (SUI) mice exhibit disordered collagen metabolism and corresponding changes in the TGF- β 1-Smad2/3 pathway. After BES therapy, collagen levels increased and the TGF- β 1-Smad2/3 pathway was activated in response to mechanical strain. These results indicate that collagen metabolism and the TGF- β 1-Smad2/3 pathway are potentially involved in the pathogenesis of SUI as well as in the response to BES therapy (5).

Other studies showed that BES causes contractions of the pelvic floor, increasing the number of muscle fibers with rapid contraction (6). The BES also increases the blood flow to the urethra and PFM, improving the neuromuscular connections, muscular fiber function, genital atrophy improving the mechanism of urethral closure (7),(8) and also induces epithelial cell migration, macrophages, mast inhibition, fibroblasts proliferation and motor axonal regeneration (9).

BES is widely used for overactive bladder syndrome (OAB). The effect of BES in afferent pathways provides a preganglionic central inhibition of bladder's motor neuron influencing the detrusor activity, but the effects of this therapy in the sacral region are better known. In animal models it has been observed bladder relaxation by the inhibition of parasympathetic motor neurons (10). Remembering that OAB usually occurs with weakness of the pelvic floor muscles too (11).

Considering that BES might be an opportunity to treat bladder dysfunction by the tissue regeneration engineered by significant upregulation of several stem cell homing and regenerative proteins to enhance bladder muscle tone. We propose this study using three different routes of micro-current bioelectric stimulation on bladder function and LTUS.

Study Name: Bioelectric Stimulation for treatment of Bladder Incontinence

Study Sponsor: BladderCell, a subsidiary of Leonhardt Ventures, LLC

Principle Investigator: Cristiane Carboni, MSC, PhD, Director Pelvic Floor World Clinic

Number of Study Sites: 1

Study Location: Pelvic Floor Dysfunction Clinic, Porte Allegre, Brazil

Study Design: Prospective, Randomized, 3 Arm, Open Label, Pilot Trial

Number of Subjects: 30

Number of Treatment Arms: 3

Duration of Each Treatment: 30 minutes

Frequency of Treatments: 2 x's/week

Number of Treatments: 16

General objectives

The present study aims to evaluate three different sites of BES on the improving bladder function and LTUS using questionnaires and clinical patient assessment.

Protocol:

This will be a prospective, randomized, 3 arm, open label clinical trial that will enroll 30 patients selected from a private physician's office in the city of Porto Alegre / RS with

chronic complaints of urinary incontinence . The study will randomize patients equally into one of three arms comparing three locations and two methods (intravaginal probe and nerve acupuncture) for delivery of micro-current bioelectric stimulation of compare the change in symptoms of incontinence of women. The intervention period will last 8 weeks with two months follow up after the end of treatment to evaluate the durability of benefit without ongoing treatment. All subjects will complete self-assessment of the severity of their symptoms, both pre and post treatment using validated questionnaires for severity of incontinence including: PRAFAB-score, OAB-V8, PGI-I, Pelvic Floor Impact Questionnaire-short form 7 and King's Health Questionnaire.

Randomization will be carried out in two steps: generation of random numbers in each group, using the RANDOM subroutine of the PEPI software suit (computer programs for urologist); and allocation concealment, placing numbers in letter-sized manila envelopes. Participants will be randomly assigned to three groups based on site of delivery of the BES including: Gp I: intravaginal probe; Gp II: sacral nerve using acupuncture needles; and Gp III: Tibial nerve stimulation also via acupuncture needles. Each treatment for all subjects be for 30 minutes with intensity increased to the motor threshold, twice a week for 8 weeks. All treatments will take place under the supervision of the PI in her Pelvic Floor World Clinic.

Patients assigned to the Intravaginal Probe will self-insert the probe to a depth required for proximity to the bladder and comfort. The cord from the probe will be connected to the Mettler stimulator and current increased to a comfort predetermined by the patient that does not cause muscle contraction. Those assigned to delivery via acupuncture needles, will have the needles inserted along the course of the nerve, with the patient prone for the sacral nerve, with the needle inserted just above the buttock, and the tibial nerve as it courses along the lower extremity near the ankle. The needles will be connected to alligator clips which will be connected to the Mettler stimulator and turned on for the 30 minute periods of BES.

Inclusion criteria:

1. Women, 18 - 80 age
2. Symptoms of stress, urge or mixed urinary incontinence of more than 3 month duration.
3. Willing and able to sign the Informed Consent

4. Able to attend all clinic visits defined in the protocol

Exclusion criteria:

1. Current urinary tract infection (positive urine culture after antibiotic treatment)
2. History of recurrent urinary tract infection (> 3 within the previous year)
3. Bladder pathology or dysfunction because of fistula, tumor, pelvic irradiation, neurological or other
4. Any previous surgical or device treatment of incontinence during the previous 12 months
5. Genital prolapse to, or beyond, the introitus,
6. Cardiac pacemaker or internal defibrillator
7. Cognitive impairment or insufficient mental condition/cognition.

Primary End Point: Change in questionnaires and self-assessment of severity of urinary incontinence.

Second End Points:

1. Any device-related or BES related adverse events including local pain, skin irritation, urinary track infection,
2. painful urination,
3. vaginal irritation/ burning or discharge in patients randomized to intravaginal probe for delivery of BES.

Statistical Analysis: All data will be collated and analyzed by a statistician not involved in the study and unaware of treatment assignment. Simple ANOVA of the difference between and within groups will be performed. Final analysis will be given to the Principal Investigator and study sponsor.

Annexes

Annexes 1 - PRAFAB score

PRAFAB score: The patient was asked to give the most appropriate answer; only one answer per section was possible

Protection

- 1 I never use pads for urinary incontinence
- 2 I occasionally use pads or have to change underwear
- 3 As a rule I use pads or have to change underwear more than once a day
- 4 I always use pads for urinary incontinence

Amount

- 1 My loss of urine is limited to an occasional drop
- 2 My loss of urine can sometimes be as much as a teaspoon
- 3 My loss of urine is enough to really wet my pads and/or clothing
- 4 My loss of urine usually completely soaks my pads and/or clothing

Frequency: Involuntary loss of urine occurs

- 1 Once a week or less
- 2 More than once but less than three times a week
- 3 More than three times a week but not every day
- 4 Every day

Adjustment: My urinary incontinence

- 1 Does not inhibit me in my daily activities
- 2 Made me quit some activities like sports or other strenuous exertion
- 3 Made me quit most activities that provoke my urinary incontinence
- 4 Prevents me from leaving the house altogether

Body image

- 1 I am not really bothered by my urinary incontinence
- 2 My urinary incontinence is an inconvenience but not a real problem
- 3 My urinary incontinence makes me feel unclean
- 4 I am disgusted with myself because of my urinary incontinence

Annexes 2 - OAB-V8

OAB-V8

Overactive Bladder-Validated 8-question Screener¹

The questions below ask about how bothered you may be by some bladder symptoms. Some people are bothered by bladder symptoms and may not realize that there are treatments available for their symptoms. Please circle the number that best describes how much you have been bothered by each symptom. Add the numbers together for a total score and record the score in the box provided at the bottom.

How bothered have you been by...	Not at all	A little bit	Some-what	Quite a bit	A great deal	A very great deal
1. Frequent urination during the daytime hours?	0	1	2	3	4	5
2. An uncomfortable urge to urinate?	0	1	2	3	4	5
3. A sudden urge to urinate with little or no warning?	0	1	2	3	4	5
4. Accidental loss of small amounts of urine?	0	1	2	3	4	5
5. Nighttime urination?	0	1	2	3	4	5
6. Waking up at night because you had to urinate?	0	1	2	3	4	5
7. An uncontrollable urge to urinate?	0	1	2	3	4	5
8. Urine loss associated with a strong desire to urinate?	0	1	2	3	4	5
Are you male?	If male <input type="checkbox"/> add 2 points to your score					

Please add up your responses to the questions above

Please hand this page to your doctor when you see him/her for your visit.

If your score is 8 or greater, you may have overactive bladder. There are effective treatments for this condition. You may want to talk with a healthcare professional about your symptoms.

Note: You may be asked to give a urine sample. Please ask before going to the bathroom.

Reference: 1. Data on File. Pfizer Inc, New York, NY.

Annexes 3 – Patient Global Impression of Improvement

Patient Global Impression of Improvement (PGI-I)

Check the number that best describes how your post-treatment condition is now, compared with how it was before you had the treatment	
Very much better	1
Much better	2
A little better	3
No change	4
A little worse	5
Much worse	6
Very much worse	7

Annexes 4 - Pelvic Floor Impact Questionnaire—short form 7 (PFIQ-7)

Pelvic Floor Impact Questionnaire—short form 7 (PFIQ-7)

Name _____ DATE _____

DOB _____

Instructions: Some women find that bladder, bowel, or vaginal symptoms affect their activities, relationships, and feelings. For each question, check the response that best describes how much your activities, relationships, or feelings have been affected by your bladder, bowel, or vaginal symptoms or conditions **over the last 3 months**. Please make sure you mark an answer in **all 3 columns** for each question.

How do symptoms or conditions in the following usually affect your	<i>Bladder or urine</i>	<i>Bowel or rectum</i>	<i>Vagina or pelvis</i>
1. Ability to do household chores (cooking, laundry housecleaning)?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit
2. Ability to do physical activities such as walking, swimming, or other exercise?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit
3. Entertainment activities such as going to a movie or concert?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit
4. Ability to travel by car or bus for a distance greater than 30 minutes away from home?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit
5. Participating in social activities outside your	home?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat	<input type="checkbox"/> Moderately <input type="checkbox"/> Quite a bit

- Not at all
- Somewhat
- Moderately
- Quite a bit

- Not at all
- Somewhat
- Moderately
- Quite a bit

6. Emotional health (nervousness, depression, etc)?

- | | | |
|--------------------------------------|--------------------------------------|--------------------------------------|
| <input type="checkbox"/> Not at all | <input type="checkbox"/> Not at all | <input type="checkbox"/> Not at all |
| <input type="checkbox"/> Somewhat | <input type="checkbox"/> Somewhat | <input type="checkbox"/> Somewhat |
| <input type="checkbox"/> Moderately | <input type="checkbox"/> Moderately | <input type="checkbox"/> Moderately |
| <input type="checkbox"/> Quite a bit | <input type="checkbox"/> Quite a bit | <input type="checkbox"/> Quite a bit |

7. Feeling frustrated?

- | | | |
|--------------------------------------|--------------------------------------|--------------------------------------|
| <input type="checkbox"/> Not at all | <input type="checkbox"/> Not at all | <input type="checkbox"/> Not at all |
| <input type="checkbox"/> Somewhat | <input type="checkbox"/> Somewhat | <input type="checkbox"/> Somewhat |
| <input type="checkbox"/> Moderately | <input type="checkbox"/> Moderately | <input type="checkbox"/> Moderately |
| <input type="checkbox"/> Quite a bit | <input type="checkbox"/> Quite a bit | <input type="checkbox"/> Quite a bit |

Total x 100 x 100 x 100 Scoring the PFIQ-7: =

All of the items use the following response scale:

0, Not at all; 1, somewhat; 2, moderately; 3, quite a bit **PFIQ-7 Score Scales:**

Urinary Impact Questionnaire (UIQ-7): 7 items under column heading “Bladder or urine”

Colorectal-Anal Impact questionnaire (CRAIQ-7): 7 items under column heading “Bowel / rectum”

Pelvic Organ Prolapse Impact Questionnaire (POPIQ-7): Items under column “Pelvis / Vagina”

Scale Scores: Obtain the mean value for all of the answered items within the corresponding scale (possible value 0 – 3) and then multiply by (100/3) to obtain the scale score (range 0-100).

Missing items are dealt with by using the mean from answered items only. **PFIQ-7 Summary Score:** Add the scores from the 3 scales together to obtain the summary score (range 0-300).

THE KING’S HEALTH QUESTIONNAIRE

1. How would you describe your health at the present?

Please tick one answer

Very good

Good Fair

Poor Very

poor

2. How much do you think your bladder problem affects your life?

Please tick one answer

Not at all

A little

Moderately A

lot

Below are some daily activities that can be affected by bladder problems. How much does your bladder problem affect you?

We would like you to answer every question. Simply tick the box that applies to you

3. ROLE LIMITATIONS	1 Not at all	2 Slightly	3 Moderately	4 A lot
A. Does your bladder problem affect your household tasks? (cleaning, shopping etc)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Does your bladder problem affect your job, or your normal daily activities outside the home?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

4. PHYSICAL/SOCIAL LIMITATION	1 Not at all	2 Slightly	3 Moderately	4 A lot
A Does your bladder problem affect your physical activities (e.g. going for a walk, running, sport, gym etc)?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Does your bladder problem affect your ability to travel?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
C. Does your bladder problem limit your social life?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D. Does your bladder problem limit your ability to see and visit friends?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

5. PERSONAL RELATIONSHIPS	0 Not at all	1 Not at all	2 Slightly	3 Moderately	4 A lot
A. Does your bladder problem affect your relationship with your partner?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Does your bladder problem affect your sex life?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

6. EMOTIONS

	1 Not at all	2 Slightly	3 Moderately	4 Very much
A. Does your bladder problem make you feel depressed?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Does your bladder problem make you feel anxious or nervous?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
C. Does your bladder problem make you feel bad about yourself?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

7.SLEEP/ENERGY

	1 Never	2 Sometimes	3 Often	4 All the time
A. Does your bladder problem affect your sleep?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Does your bladder problem make you feel worn out and tired ?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

8.Do you do any of the following?

	If so how much?			
	1 Never	2 Sometimes	3 Often	4 All the time
A. Wear pads to keep dry?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B. Be careful how much fluid you drink ?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
C. Change your underclothes because they get wet?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D. Worry in case you smell?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

We would like to know what your bladder problems are and how much they affect you ? From the list below choose only those problems that you have at present. Leave out those that don't apply to you.

How much do they affect you? FREQUENCY: going to the toilet very often

1. A little

2. Moderately

3. A lot

NOCTURIA: getting up at night to pass urine

1. A little

2. Moderately

3. A lot

URGENCY: a strong and difficult to control desire to pass urine

1. A little

2. Moderately

3. A lot

URGE INCONTINENCE: urinary leakage associated with a strong desire to pass urine

1. A little

2. Moderately

3. A lot

STRESS INCONTINENCE: urinary leakage with physical activity eg. coughing, running

1. A little

2. Moderately

3. A lot

NOCTURNAL ENURESIS: wetting the bed at night

1. A little

2. Moderately

3. A lot

INTERCOURSE INCONTINENCE: urinary leakage with sexual intercourse

1. A little

2. Moderately

3. A lot

WATERWORKS INFECTIONS

1. A little

2. Moderately

3. A lot

BLADDER PAIN

1. A little

2. Moderately

3. A lot

Thank You For Your Time

To Calculate Scores

PART 1

1) General Health Perceptions

Very good	1
Good	2
Fair	3
Poor	4
Very poor	5

$$\text{Score} = ((\text{Score to Q1} - 1)/4) \times 100$$

2) Incontinence Impact

Not at all	1
A little	2
Moderately	3
A lot	4

$$\text{Score} = ((\text{Score to Q2} - 1)/3) \times 100$$

PART 2

Individual scores as recorded at the top of each column of possible responses

3) Role limitations

$$\text{Score} = (((\text{Scores to Q 3A} + 3\text{B}) - 2)/6) \times 100$$

4) Physical limitations

$$\text{Score} = (((\text{Scores to Q 4A} + 4\text{B}) - 2)/6) \times 100$$

5) Social limitations

$$\text{[If 5C} \geq 1\text{]} \text{ Score} = (((\text{Score to Q 4C} + 4\text{D} + 5\text{C}) - 3)/9) \times 100$$

$$\text{[If 5C} = 0\text{]} \text{ Score} = (((\text{Score to Q 4C} + 4\text{D}) - 2)/6) \times 100$$

6) *Personal relationships*

[If 5A+5B >=2] Score =(((Scores to Q 5A + 5B) – 2)/6) x 100
[If 5A+5B =1] Score =(((Scores to Q 5A + 5B) – 1)/3) x 100
[If 5A+5B =0] Treat as missing value

7) *Emotions*

Score =(((Score to Q 6A + 6B + 6C) – 3)/9) X 100

8) *Sleep / energy*

Score =(((Scores to Q 7A + 7B) – 2)/6) x 100

9) *Severity measures*

Score =(((Scores to Q 8A + 8B + 8C + 8D) – 4)/12) x 100

PART 3

<i>Scale</i>	<i>score</i>
Omitted	0
A little	1
Moderately	2
A lot	3

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