# CENTER FOR RECONSTRUCTIVE URETHRAL SURGERY


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# Complejo Hospitalario Universitario - Granada

## April 28 – 29

# 1° Meeting on Reconstructive Urethral Surgery

## 2015

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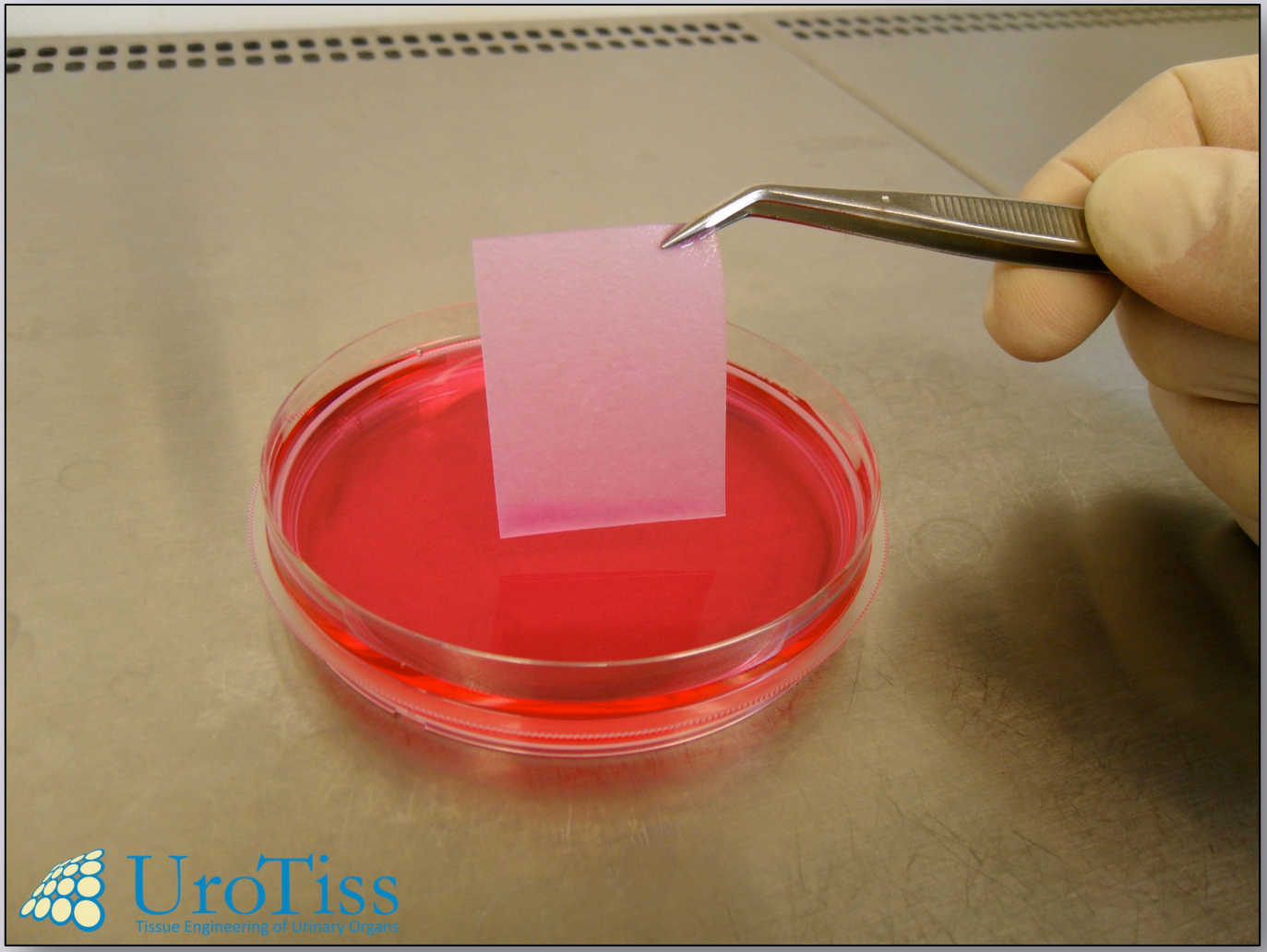
CENTER FOR RECONSTRUCTIVE URETHRAL SURGERY

# Tissue engineering urethroplasty

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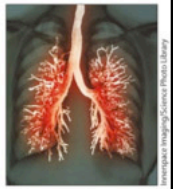
# The Lancet

## The end of the beginning for tissue engineering

For three decades we have heard about the hope of tissue engineering. Hyperbole has become routine, but amidst unreasonable expectations are serious scientists, like Paulo Macchiarini, who believe that by combining cells and degradable materials ex vivo they can develop organs to replace or repair diseased tissues. After many years of trying to build engineered tissues on a backbone of synthetic degradable polymers,[1] a growing body of evidence suggests that decellularised whole organs and tissues are clinically effective degradable scaffolds.[2] Until recently, decellularised tissues were used clinically without the addition of cells, and in many cases—eg, the decellularised porcine small intestine submucosa family of surgical implants—this approach was sufficient to generate a healing response. The idea of whole organ engineering—whereby organs are decellularised and then repopulated with desired mixtures of cells—seems to be a realistic path towards complex three-dimensional tissue engineering.

In 2008, Macchiarini's team announced that they had successfully grown a neo-trachea from a decellularised evidence that the tracheal graft is now naturalised. More importantly, given the data for extracellular-matrix-derived restorative degradable materials and their use in airway and bladder neo-organ development[4,5] we can celebrate the end of the beginning for tissue engineering; the groundwork has been laid for clinical implementation in other specialties.

Excitement about tracheal regenerative therapy might be muted by realisation that the patient in this study was not restored to full health. Although heroic in complying with the needs of a research study, the patient is suffering from ongoing complications from scarring at the proximal anastomotic site. There is nothing unusual about a tracheal stricture forming at a surgical site and, in fact, this patient had already had such a post-surgical stricture. Rather, the formation of a stricture shows that the remaining challenges for tissue engineering of thin hollow organs such as trachea, oesophagus, intestine, blood vessels, and bladder relate to how neo-tissues are incorporated into existing structures. Research is needed to understand how to



Published Online
October 23, 2013
http://dx.doi.org/10.1016/S0140-6736(13)62110-8
See Online/Articles
http://dx.doi.org/10.1016/S0140-6736(13)62033-4

**The Lancet, October 2013**

Comment

## Tissue engineering's green shoots of disruptive innovation

The ready availability of tissues or organs to replace or repair those diseased or damaged is a ubiquitous clinical need, and the rapidly developing field of tissue engineering might offer innovative solutions. Two Articles[1,2] in *The Lancet* show the incremental expansion of the applications of tissue-engineering technology to reconstructive surgery.

Application of cells to a scaffold, with or without added chemical or mechanical stimuli, followed by their use for repairing congenital or acquired defects was well established in the 1990s[3] and has led notably to the treatment for congenital bladder defects clinically available in the USA. Following on well documented successes in the clinical application of tissue-engineered skin,[4] blood vessels,[5] urethra,[6] and bladder wall,[7] investigators used similar principles of seeding autologous differentiated cells onto collagen scaffolds to treat patients with nasal defects[2] after cancer resection, or seeding onto extracellular-matrix-derived scaffolds to treat patients with vaginal aplasia.[3]

In four girls aged 13–18 years, with a rare form of vaginal aplasia, Atlántida Raya-Rivera's team restoration of contour and nasal airflow to the noses of two women and three men, aged 76–88 years, undergoing substantial resections of external nasal tissue as treatment for skin cancer. After flap refinement at 6 months, Fulco and colleagues[2] took biopsy samples of repair tissues and histologically analysed them. Safety and feasibility of the procedure 12 months after reconstruction were the primary outcomes. Importantly, the staged reconstruction in the patients permitted histological assessment of the implanted tissue and confirmed sustained restoration of all three layers of the nose that had been reconstructed. At 12 months, no adverse events had been recorded and patients were satisfied with the aesthetic and functional outcomes.

Together, these two studies[1,2] begin to answer three of the key scientific questions posed to, and by, the translational tissue-engineering community.[8] First, are biological scaffolds, with or without cells, replaced by scar tissue or native quality tissue over time? In both studies, findings show excellent evidence of multilayer remodelling in a manner consistent with the normal tissues restored. Second, can tissue-engineered

Published Online
April 11, 2014
http://dx.doi.org/10.1016/S0140-6736(14)60533-X
See Online/Articles
http://dx.doi.org/10.1016/S0140-6736(14)60544-4 and
http://dx.doi.org/10.1016/S0140-6736(14)60542-0

**The Lancet, April 2014**

**Repair of the anterior urethra is one of the most demanding surgical problems in urology. In recent years, regenerative medicine and tissue engineering studies have led to the development of novel biomaterials for urethral repair.**

# Tissue-engineered repair of urethral stricture:
## When we will get there?

The number of publications referring to stem cells has increased from 4.402 publications in 1996, to 21.193 publications in 2012 with a compound annual growth rate of 7.0%.

In the urological literature, there is a myriad of reports about experimental different tissue-engineered products.

Only three reports on the use of these materials in patients with urethral strictures are available.

# Tissue-engineered repair of urethral stricture:
## When we will get there?

**How tissue engineered material for urethral reconstruction should be used in a large scale in different countries?**

**How tissue engineered material for urethral reconstruction should be used in a large scale in different urethral conditions (congenital or acquired, simple vs. complex)?**

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*Here we describe the first clinical report of a large series of patients who underwent tissue-engineered oral mucosal graft urethroplasty for anterior urethral stricture.*

**Dr. Gouya Ram-Liebig and Dr. Soeren Liebig**
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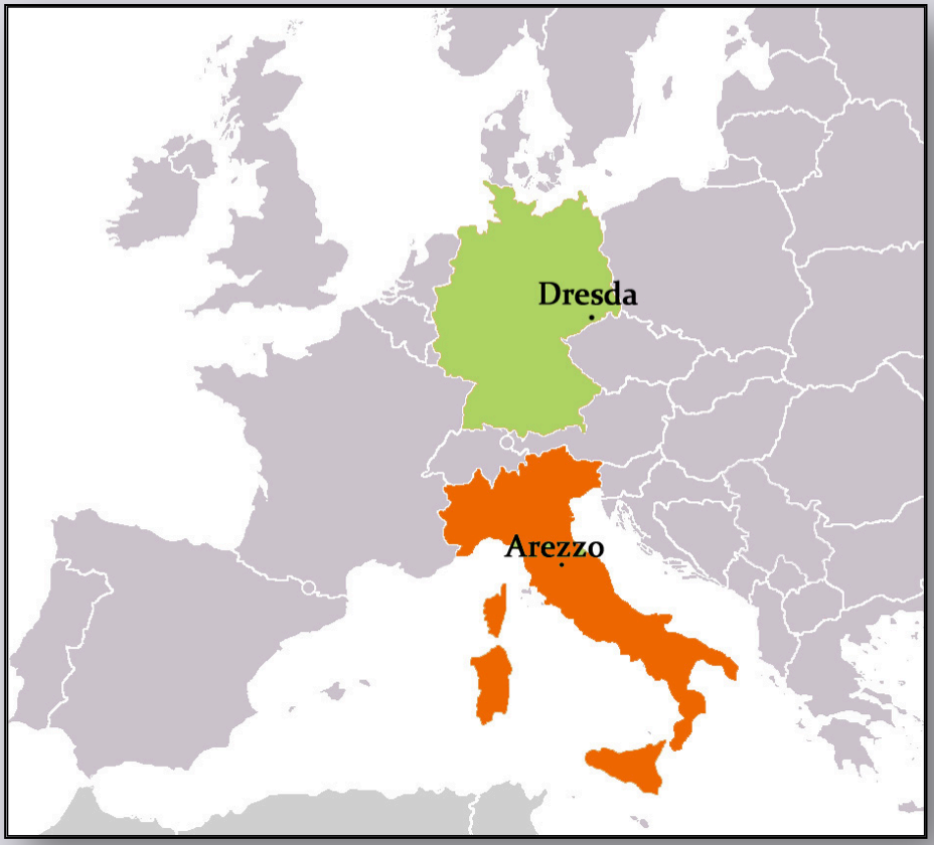


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**UroTiss GmbH is a pharmaceutical company, founded in Germany in 2005 by Dr. Gouya Ram-Liebig and Dr. Soeren Liebig. UroTiss provides products with highest safety and quality, in accordance to current Good Manufacturing Practices (GMP).**

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AUA 2014

# Preclinical and clinical examination of tissue-engineered graft for urethral reconstruction (MukoCell®) with regard to its safety

*Massimo Lazzeri[1], Guido Barbagli[1], Dirk Fahlenkamp[2], Giuseppe Romano[3], Ulf Balsmeyer[2], Helmut Knispel[4], Maria-Elsa Spiegeler[4], Burkard Stuerzebecher[4], and Gouya Ram-Liebig[5]*

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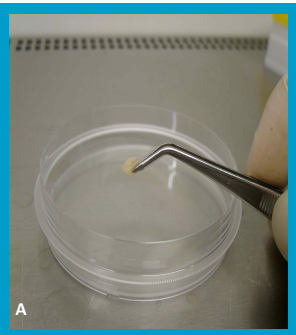
## I. Introduction

MukoCell® is a national authorized, autologous tissue-engineered oral mucosa graft. The present report sums up some of MukoCell®' s preclinical safety data. Additional reported data of 70 patients, treated with MukoCell®, are also considered with regards to safety analysis.



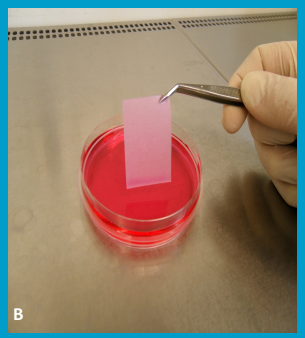




Fig. 1: Patient's oral mucosa cells are generated from a small oral mucosa biopsy (A) and cultured on the surface of a biocompatible scaffold (B).

Source of Funding: UroTiss GmbH, Germany

## II. Methods

For MukoCell® production, patient's oral mucosa cells were generated from a small oral mucosa biopsy and cultured on the surface of a biocompatible scaffold (Fig. 1).

The tumorigenic potential of MukoCell® was examined in vivo. For this purpose, human cultured cells of 4 different runs were injected by intraperitoneal and subcutaneous route into each of ten immunodeficient athymic nude mice. $4x10^7$ cells ± 2 × $10^6$ cells were injected into each animal on Days 1, 18, 25 and 46 of the study. An additional group consisting of ten animals each received cell culture medium as vehicle control (Table 1).

To examine the potential migration of cells into distant organs, murine MukoCell® constructs from eGFP-transgenic mice were implanted into peritoneal cavity of histocompatible non-transgenic mice and vice versa. The 24 test animals were sacrificed either at weeks 1, 2, 4 or 12 for histological analysis (Table 2).

To investigate the degradation of implanted MukoCell® with time, scaffolds with the size of 0.5 x 1.5 cm were implanted into the peritoneal cavity of 20 BALBc/C57BL6J mices.

Additionally, reported clinical safety data from 70 MukoCell®-treated patients with urethral stricture, which have been recruited in an ongoing observational study with up to 2 year follow-up period, were evaluated on the basis of a pharmacivigilance system. Ethical committee votum was available for the study.

## III. Tables

Table 1. Experimental groups allocated during the tumourigenicity study

| Group | No. of animals | Item | Injection on days[a)] | Injection volume (i.p. + s.c.) [µL] | Total no. of cells at each day |
|---|---|---|---|---|---|
| 1a | 5 | Test items (n=4) | 1, 18, 25, 46 | 200 + 200 | $10^7$ ± 2 x $10^6$ |
| 1b | 5 | Test items (n=4) | 1, 18, 25, 46 | 200 +200 | $10^7$ ± 2 x $10^6$ |
| 2a | 5 | Control item | 1, 18, 25, 46 | 200 + 200 | - |
| 2b | 5 | Control item | 1, 18, 25, 46 | 200 + 200 | - |

Notice [a)] Cell preparations generated independently from four different runs were used. A separate cell preparation was used on each day.

Table 2. Allocation and treatment of animals in the biodistribution study

| Group (Cage) | No. of animals | Donor for engineered tissue graft | Recipient of engineered tissue graft | Sacrifice after implantation week |
|---|---|---|---|---|
| A-1 (11/5/0) | 3 | EGFP-tg | ntg | after 1 week |
| A-2 (11/6/0) | 3 | EGFP-tg | ntg | after 2 weeks |
| A-3 (11/7/0) | 3 | EGFP-tg | ntg | after 4 weeks |
| A-4 (11/8/0) | 3 | EGFP-tg | ntg | after 3 months |
| reserve animal (11/H3/0) | 1 | EGFP-tg | ntg | |
| B-1 (11/1/0) | 3 | ntg | EGFP-tg | after 1 week |
| B-2 (11/2/0) | 3 | ntg | EGFP-tg | after 2 weeks |
| B-3 (11/3/0) | 3 | ntg | EGFP-tg | after 4 weeks |
| B-4 (11/4/0) | 3 | ntg | EGFP-tg | after 3 months |
| reserve animal (11/H1/9) | 1 | ntg | EGFP-tg | |

Notice: Material used for histology after sacrifice: Brain (cerebrum, cerebellum, brain stem, paraventricular parts); heart; kidneys;

large intestine (caecum, colon, rectum); liver; lung; lymph nodes (mesenteric)

intestine; (duodenum, jejunum, ileum) / peyer plaques; spleen; thymus; transplants (including surrounding tissue)

## IV. Results

Evaluation of tumorigenicity study in nude mice did not reveal macroscopic and microscopic malignancies attributable to MukoCell® in 60 different examined tissues and organs. Additionally, migration of the transplanted cells into distant organs was excluded at all examined time intervals after implantation of murine homologue of MukoCell®. While the grafts were still present in all 10 animals 9 days after implantation, 6 of 10 grafts were degraded 40 days after implantation in the remaining 10 animals. Clinical data of 70 with MukoCell® treated patients demonstrated no peri- or post-operative adverse events related to MukoCell®.

## V. Conclusion

MukoCell® seems to be a safe graft for urethroplasty for patients with urethral stricture. The graft is degrading within a few weeks and hence avoids complication associated with persistent implants.

# MukoCell® is an autologous tissue-engineered oral mucosa graft

**AUA 2014**
MP9 – Abstract ID: 14-578

## Tumorigenic study:

- cultured cells of human donors were injected by intraperitoneal and subcutaneous route into each of ten immunodeficient athymic nude mice.
- 4x107 cells ± 2 × 106 cells were injected into each animal on Days 1, 18, 25 and 46 of the study.
- An additional group consisting of ten animals each received cell culture medium as vehicle control

**Results: No macroscopic and microscopic malignancies attributable to MukoCell® in 60 different examined tissues and organs.**

## Biodistribution study:

- Murine MukoCell® constructs from eGFP-transgenic mice were implanted into peritoneal cavity of histocompatible non-transgenic mice and vice versa.
- The 24 test animals were sacrificed either at weeks 1, 2, 4 or 12 for histological analysis

**Results: No migration of the transplanted cells into distant organs.**

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# MukoCell® is an autologous tissue-engineered oral mucosa graft

**AUA 2014**
**MP9 – Abstract ID: 14-578**

## Degradation study:

- 0.5 x 1.5 cm MukoCell® scaffolds were implanted into the peritoneal cavity of 20 female BALBc/C57BL6J mices

**Results: 60% of the grafts were degraded 40 days after implantation.**

## Clinical observational study:

- data from 70 MukoCell®-treated patients with urethral stricture, with up to 2 year follow-up period, were evaluated

**Results: No peri- or post-operative adverse events related to MukoCell®.**

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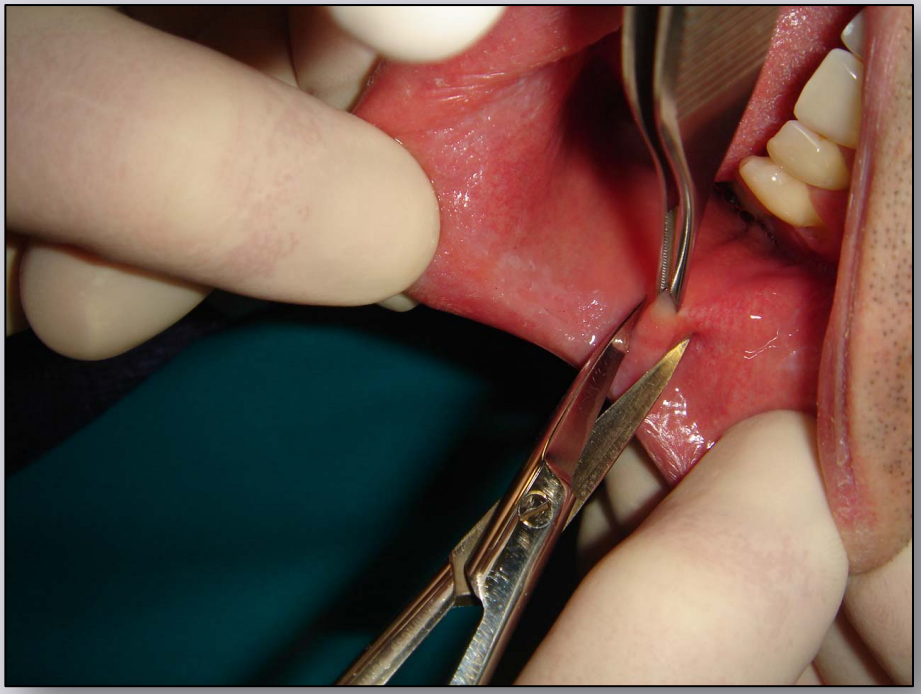
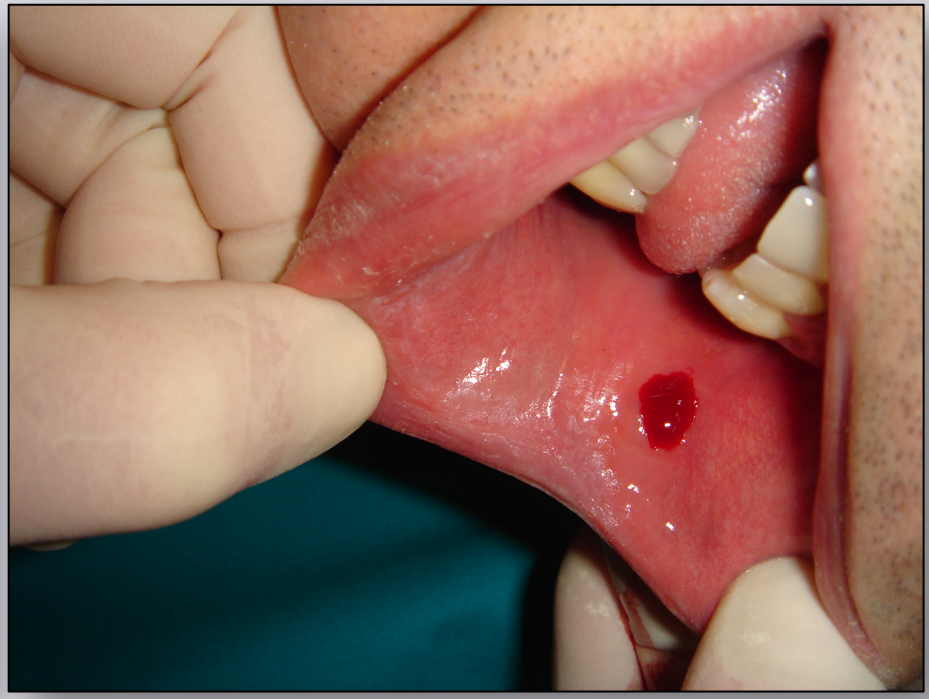
# Step 1

# Harvesting sample from the cheek

# Local anaesthesia





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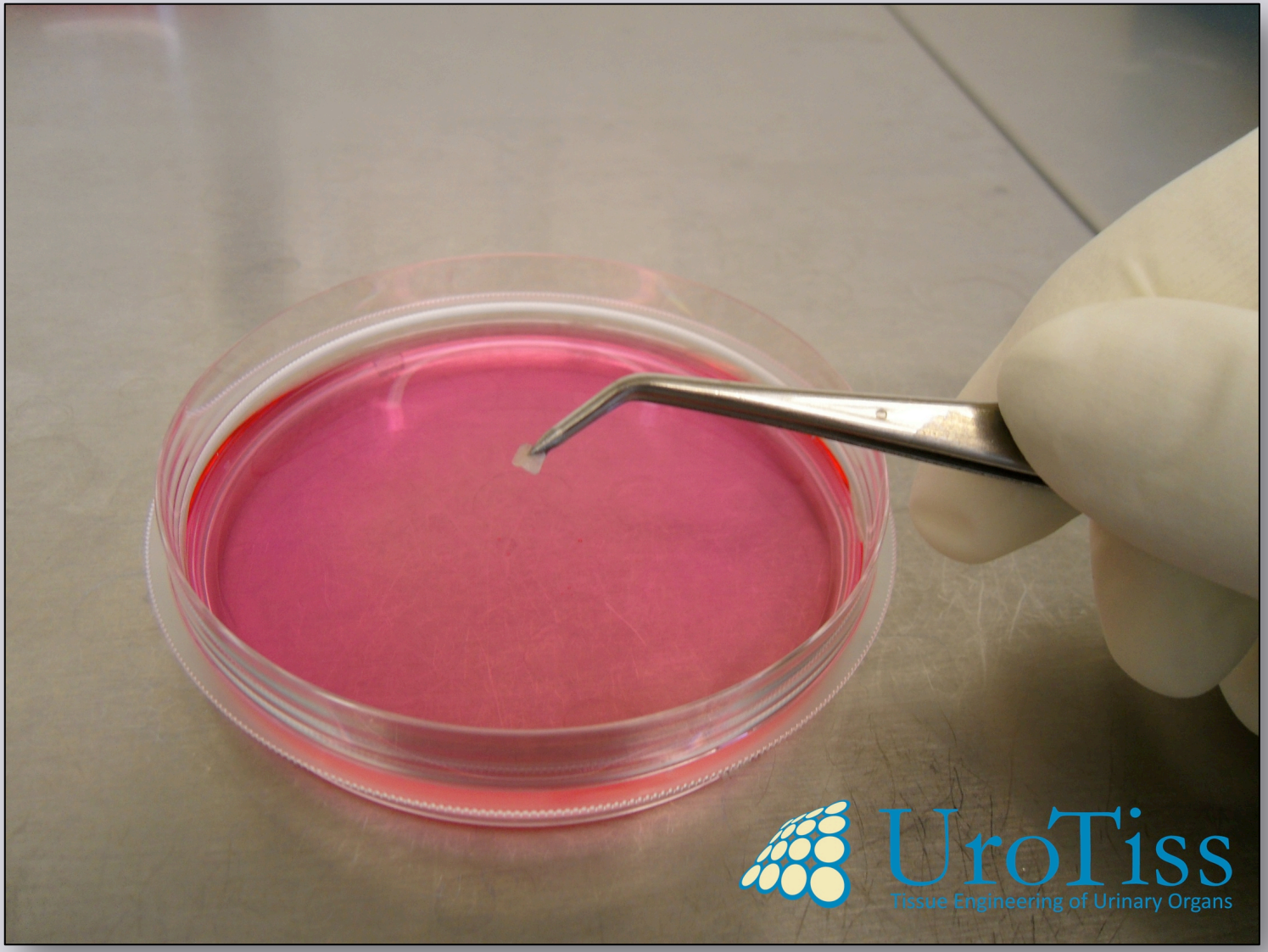


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# Step 2

# GMP Laboratory

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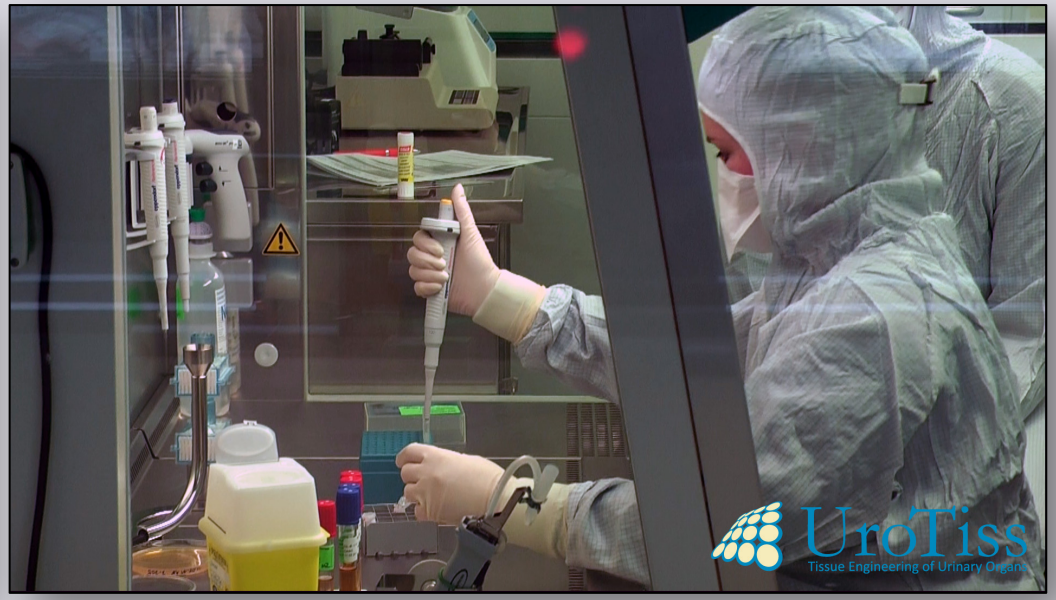
UroTiss
Tissue Engineering of Urinary Organs




**Clean roon Laboratory in accordance to current Good Manufacturing Practices (GMP).**

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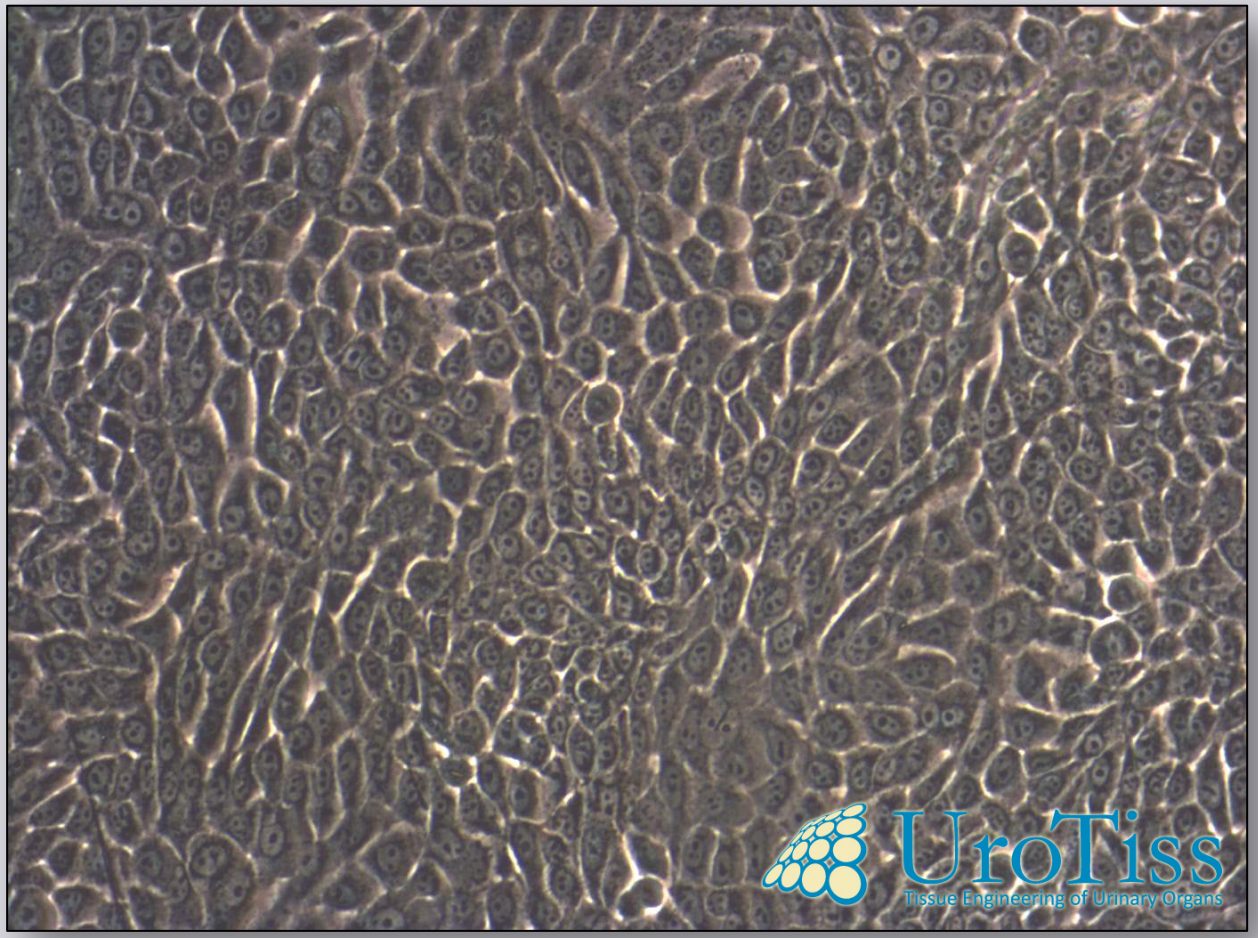


**Cells were expanded**
**and cultured on the surface of a biocompatible scaffold.**

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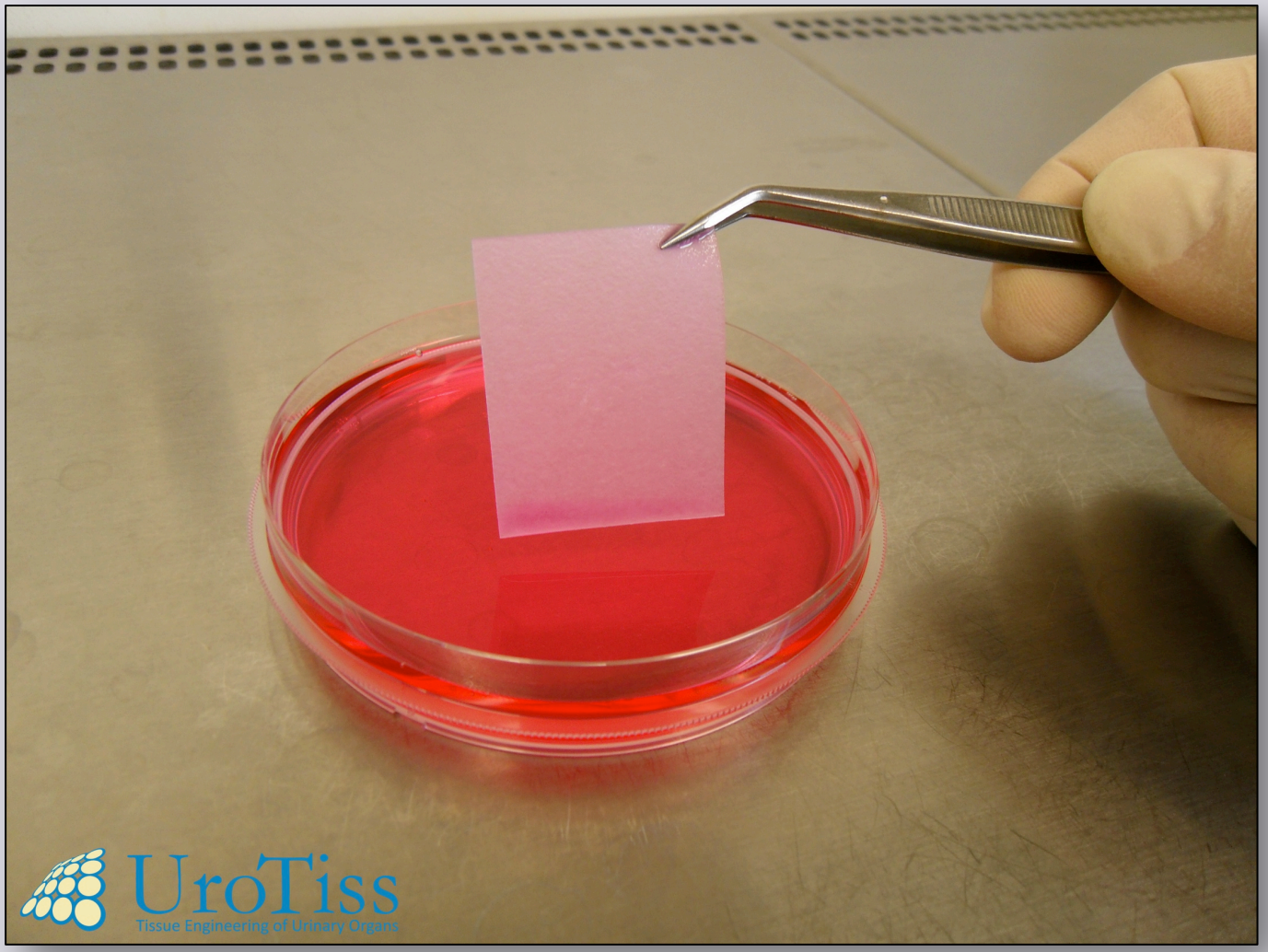
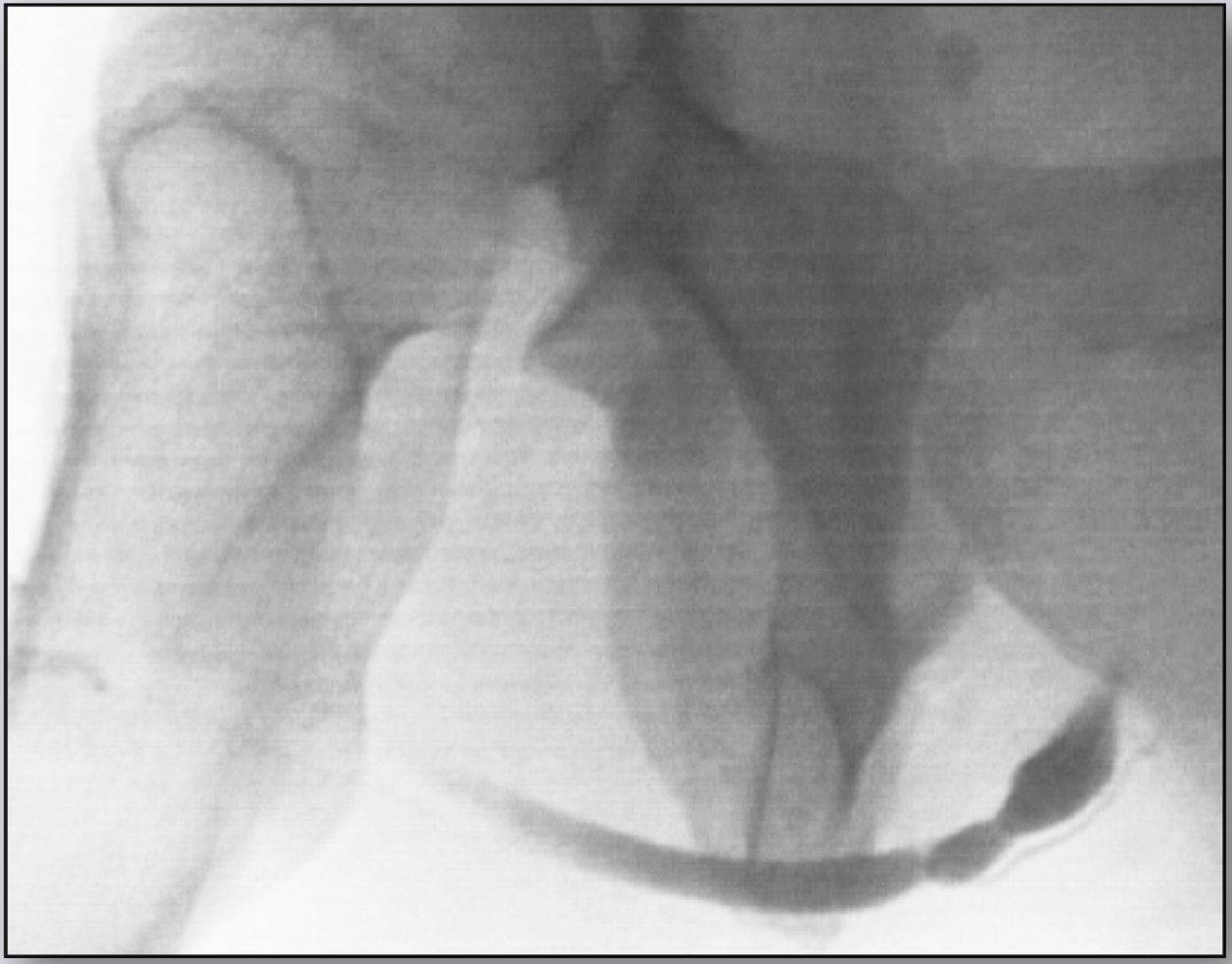



**3 weeks later**

**48 hours for transplant**

# Pre-operative retrograde urethrography



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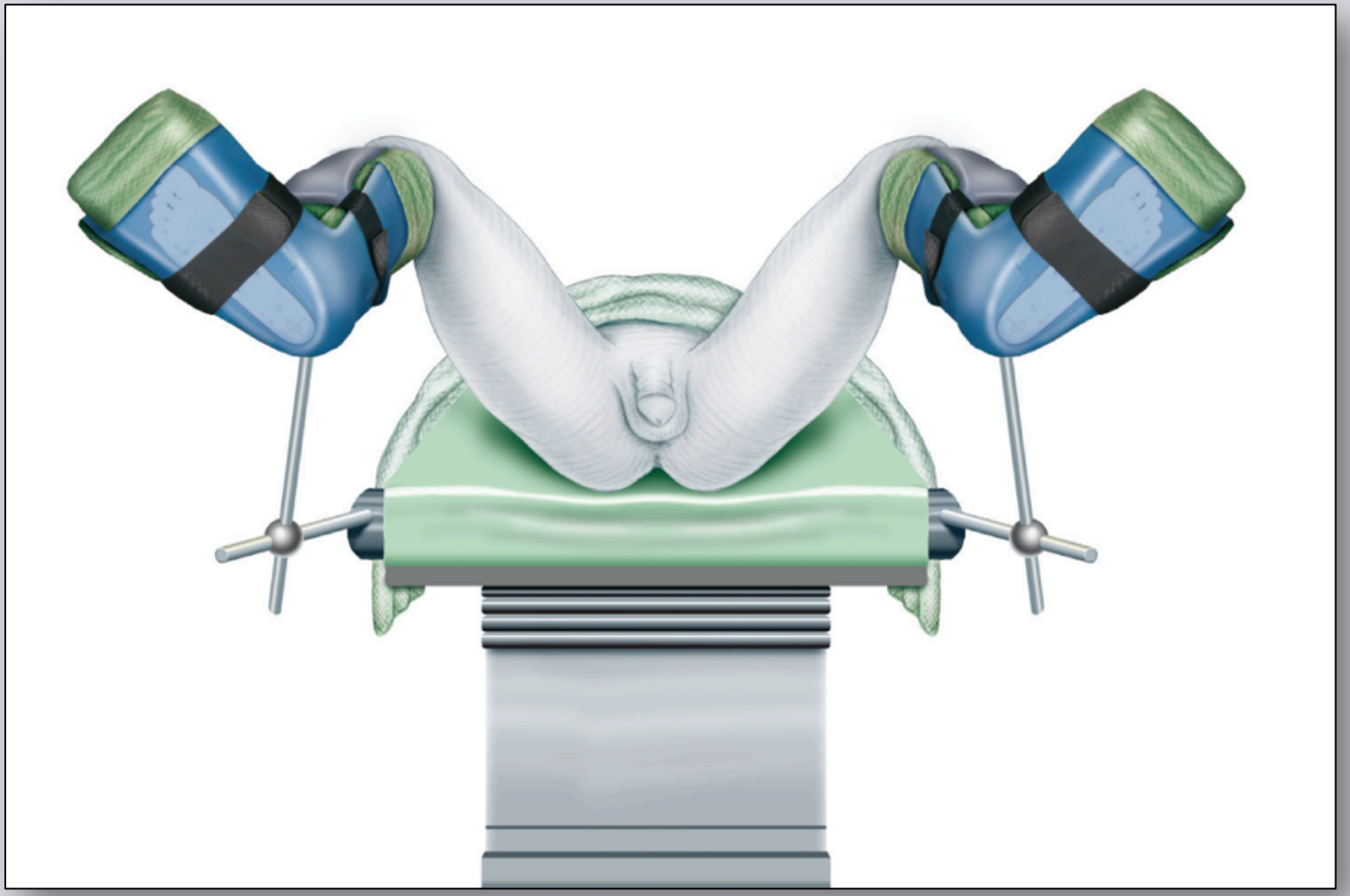
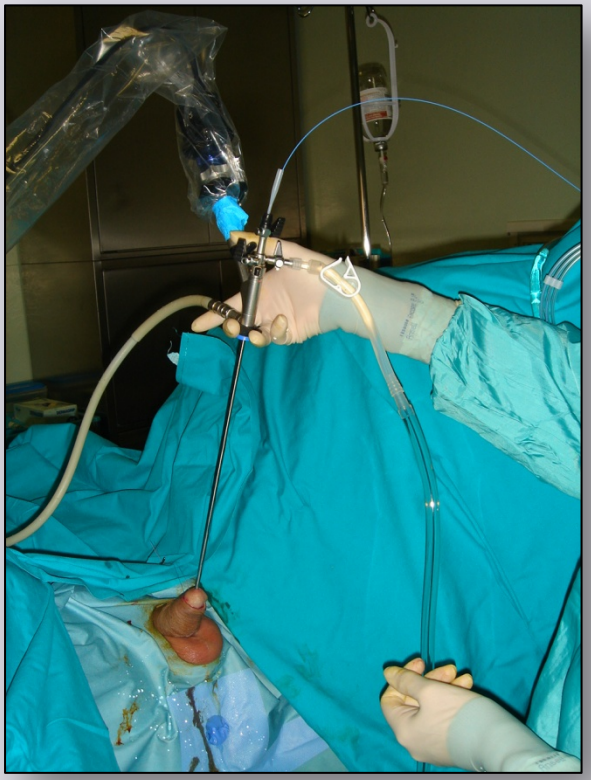
## Step 3

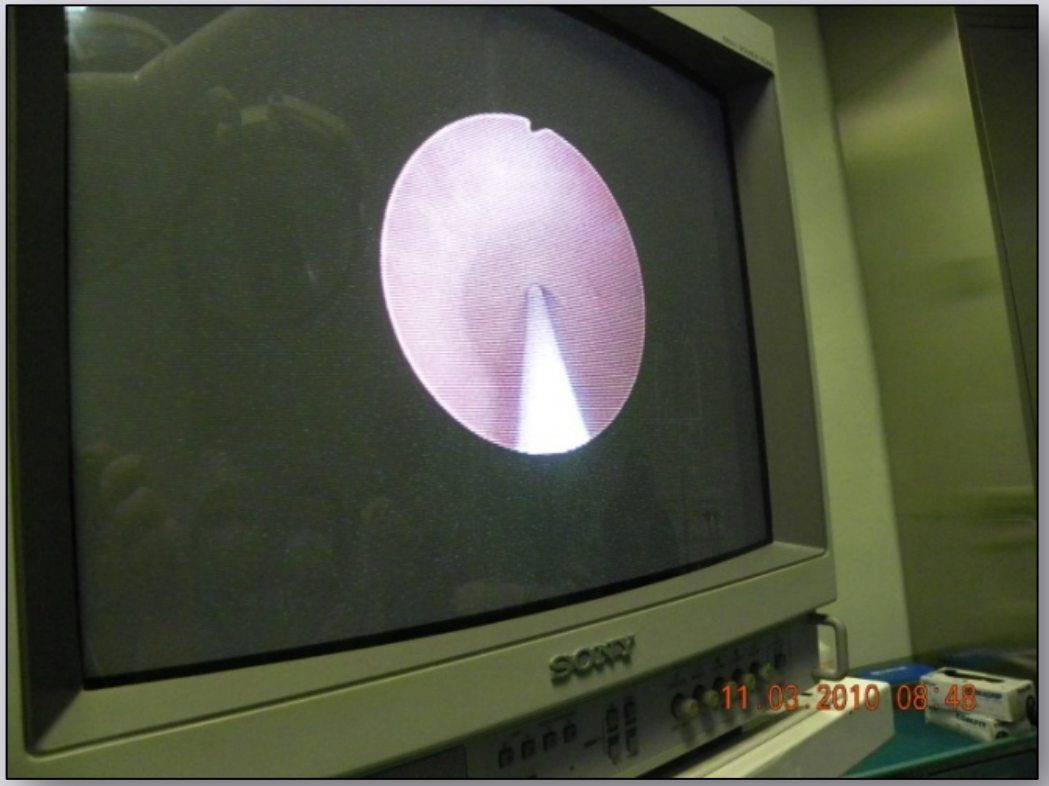
# Surgical transplant using dorsal inlay technique



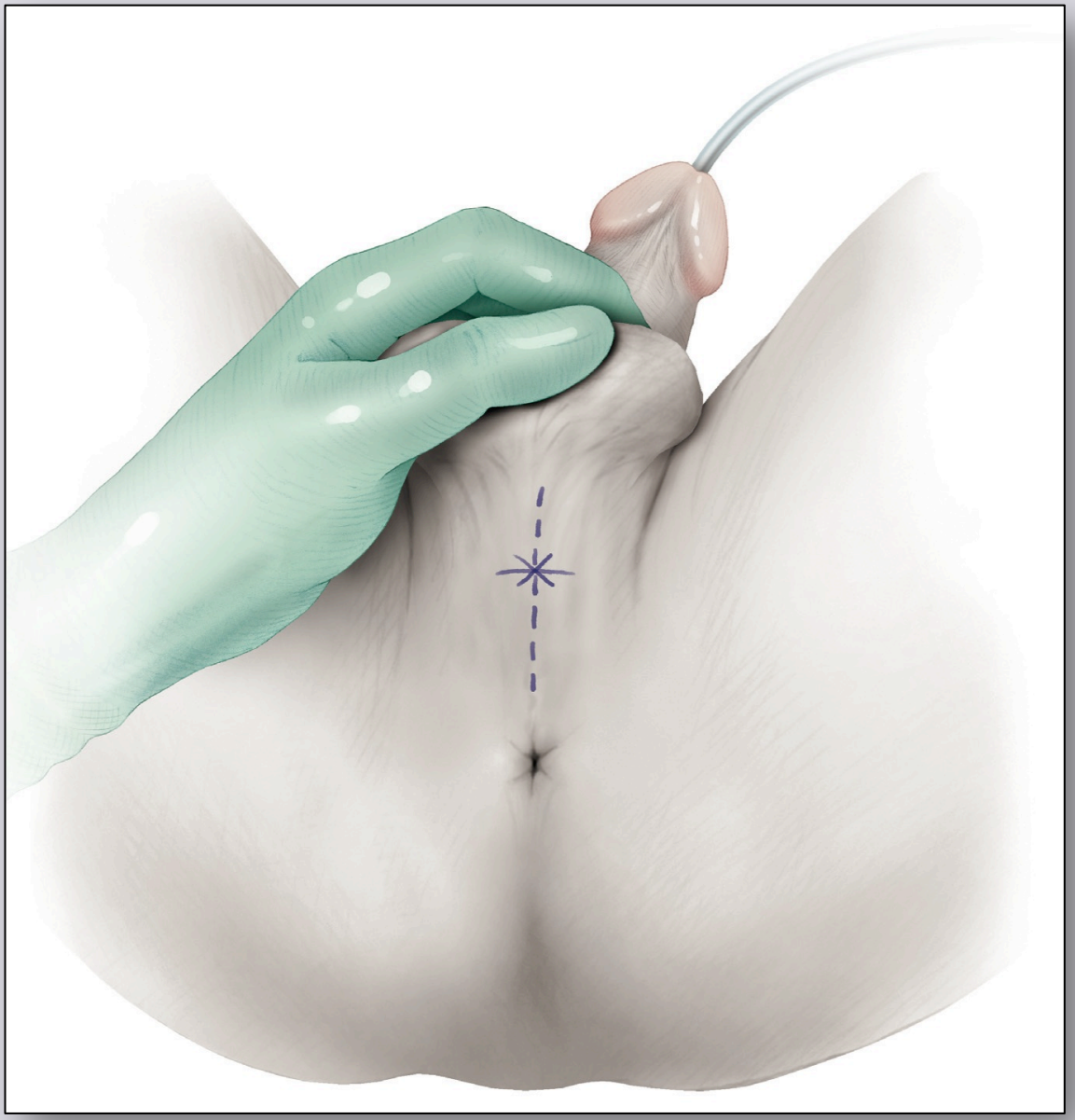
**Simple lithotomy position**



**Pre-operative urethroscopy**




**Insert Sensor 3 Fr. guidewire**



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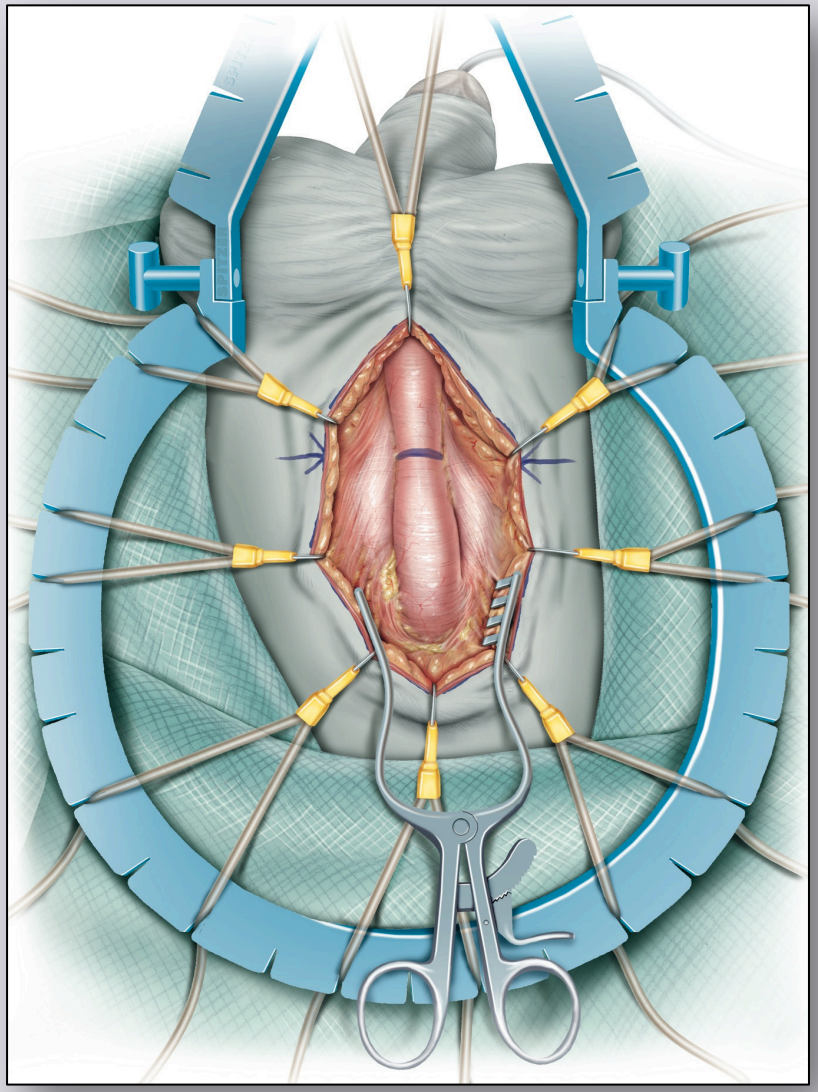

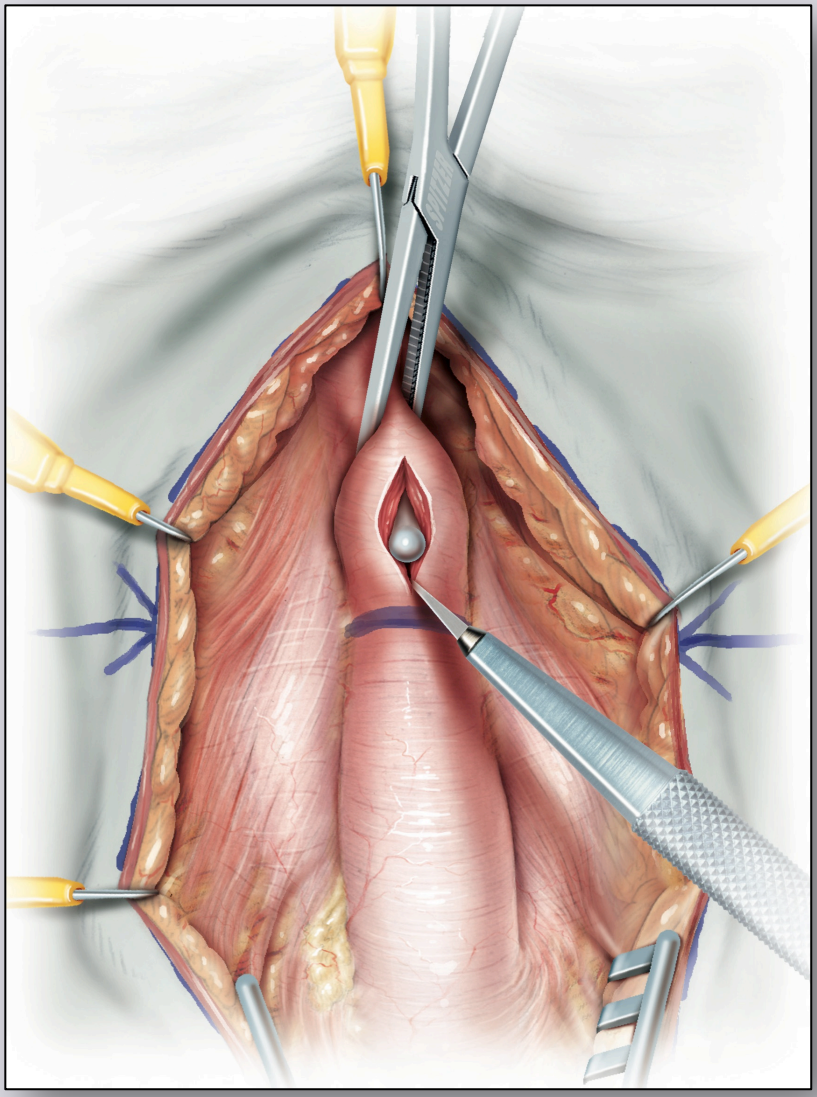

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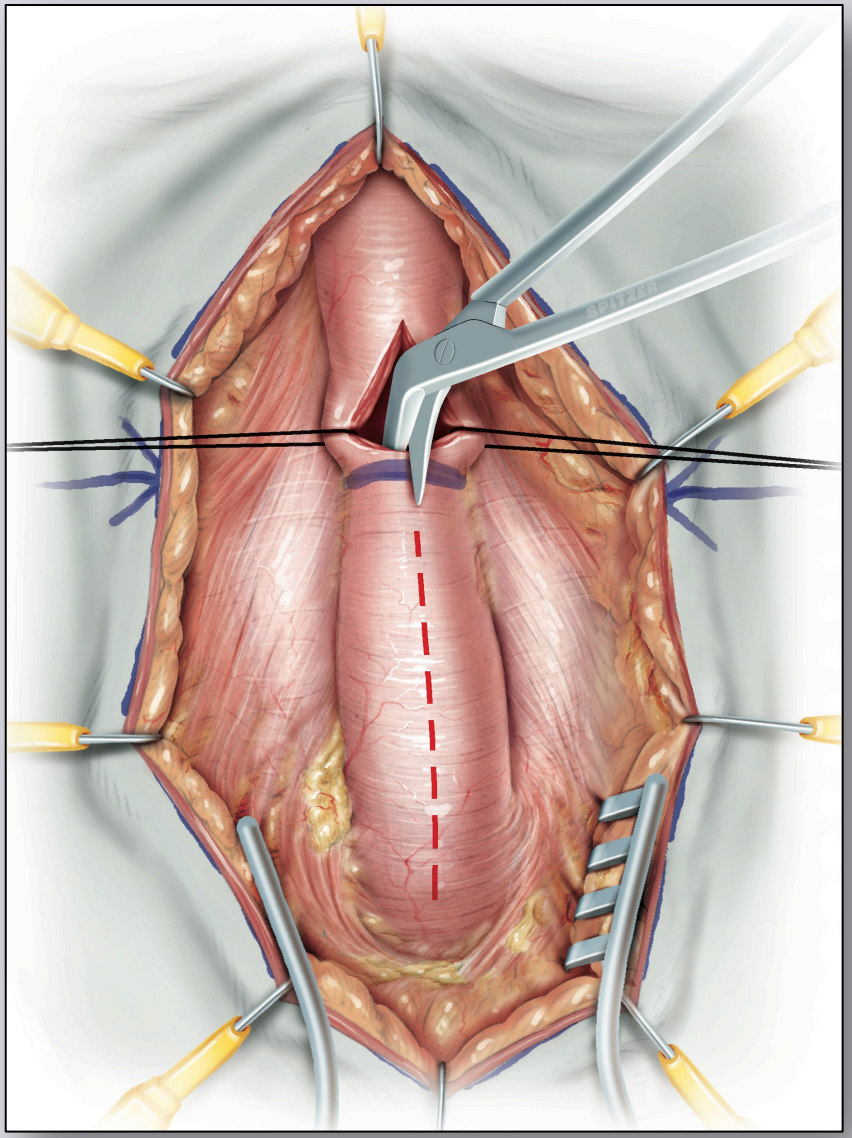

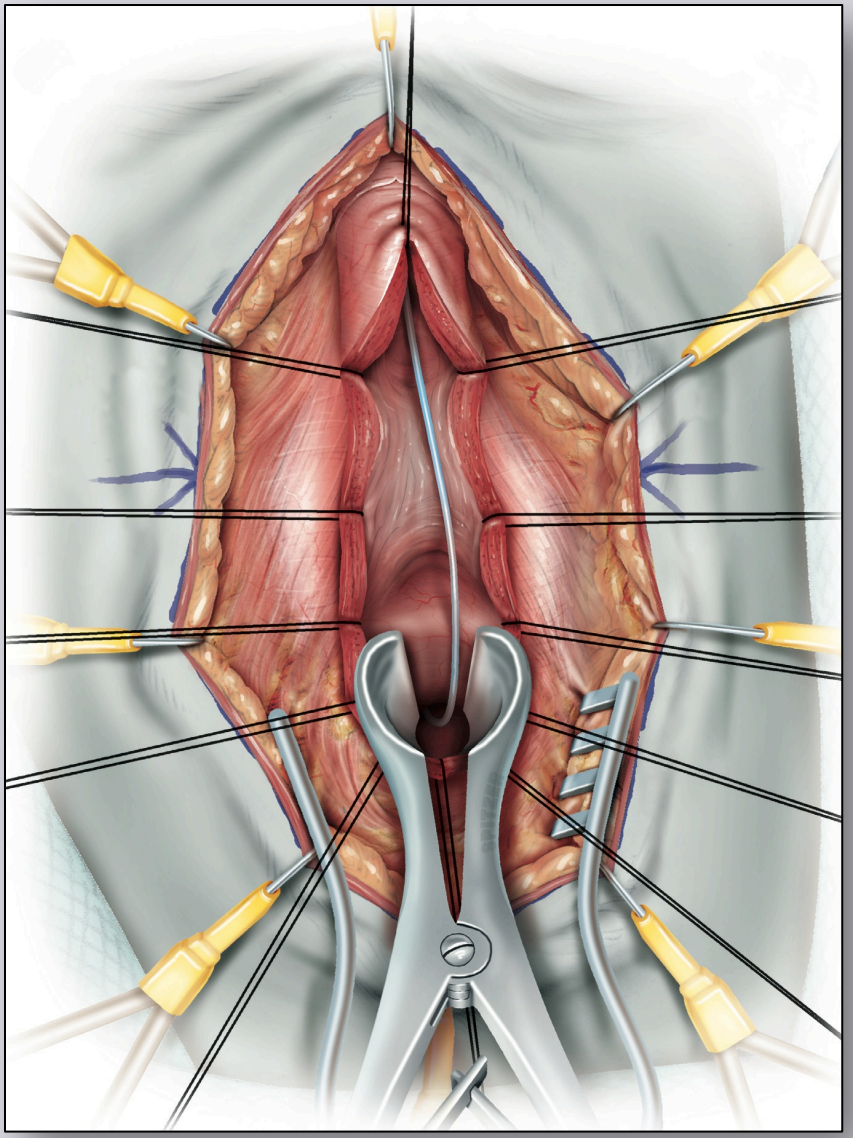

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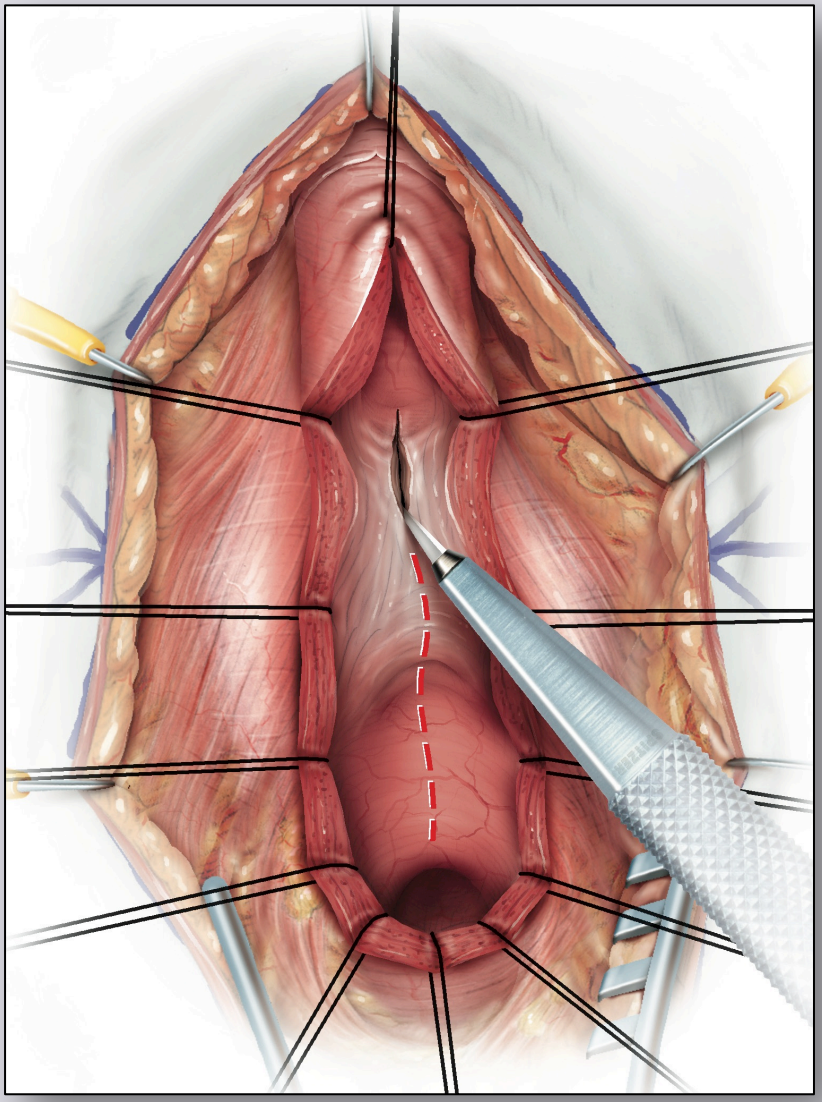

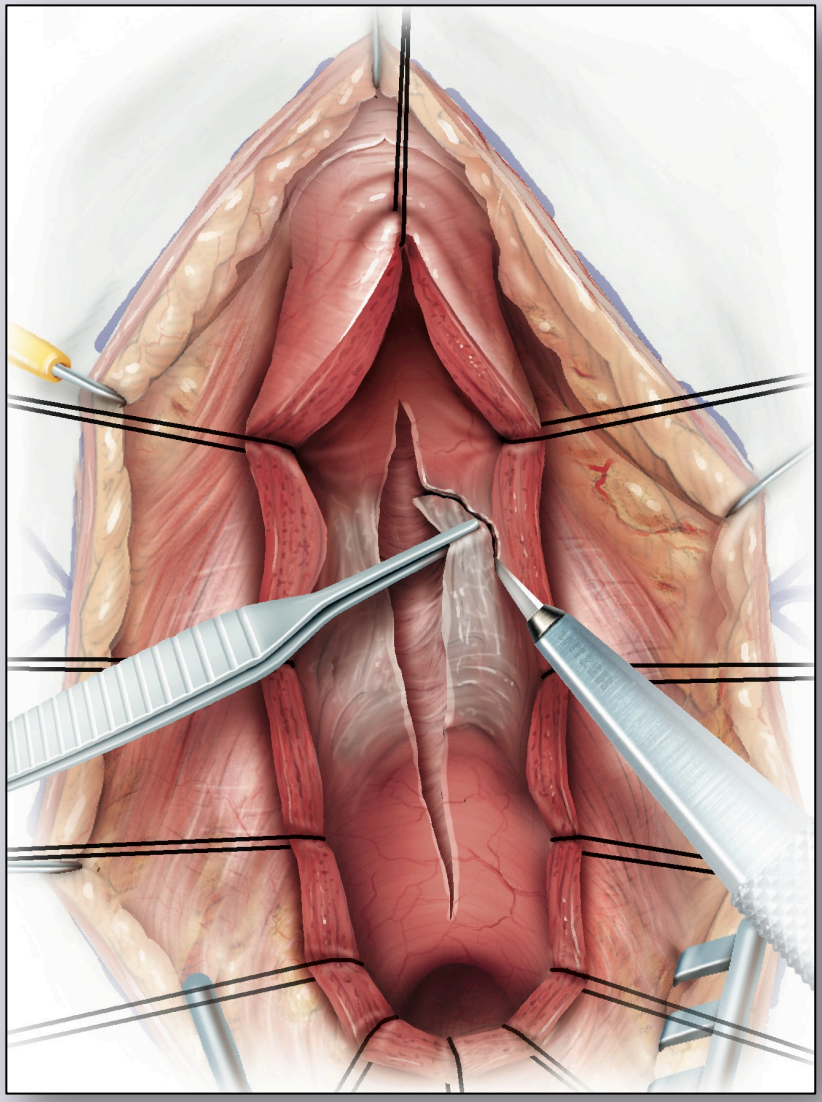

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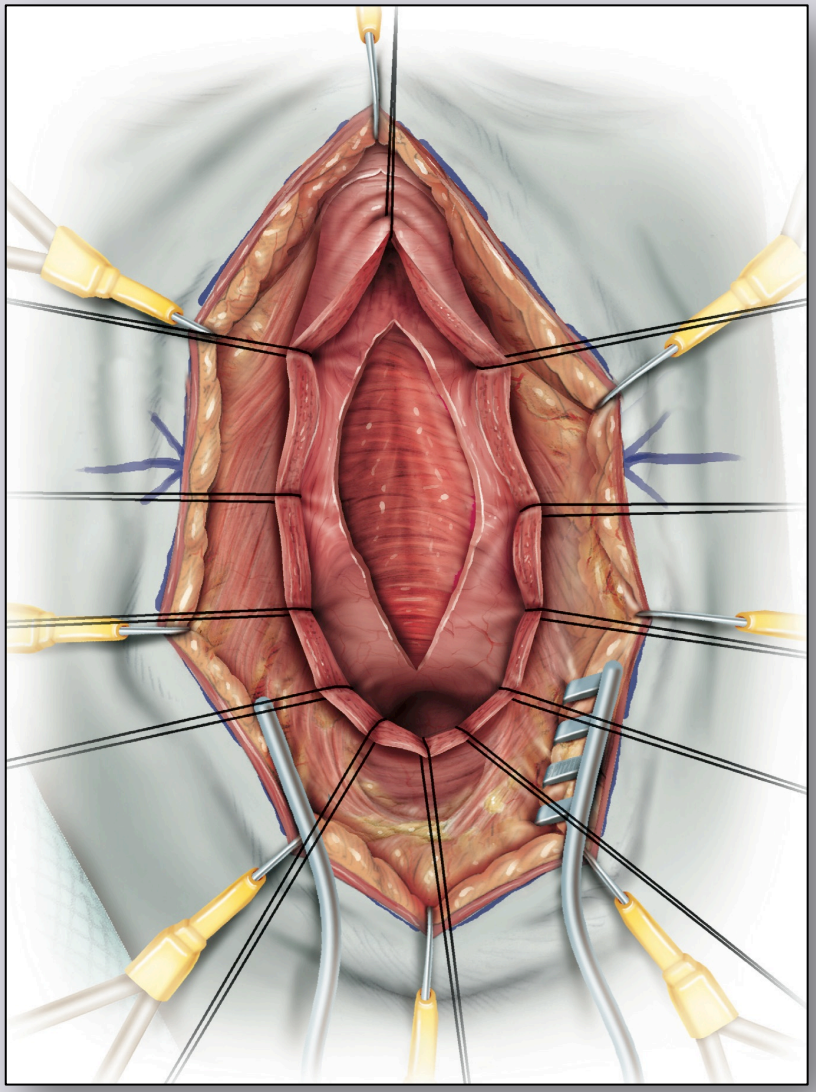

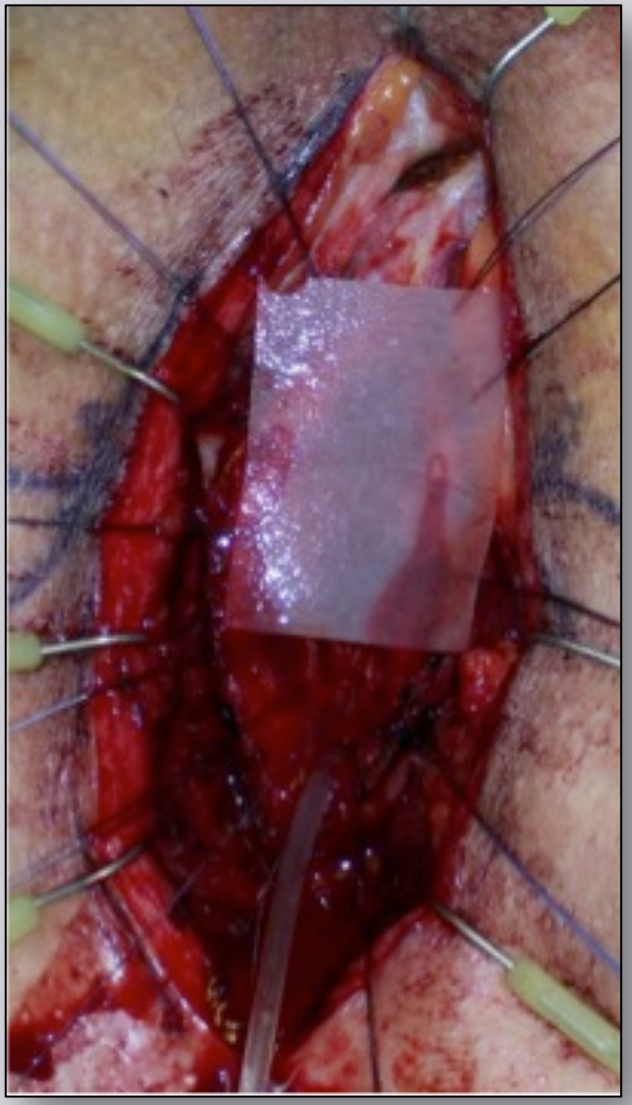

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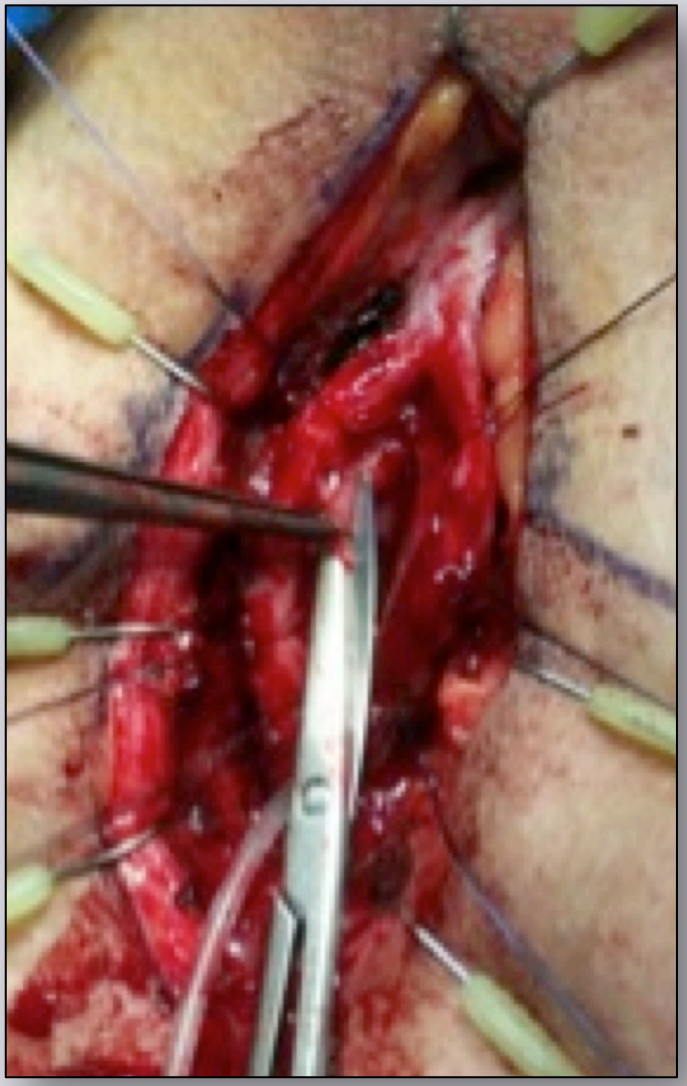

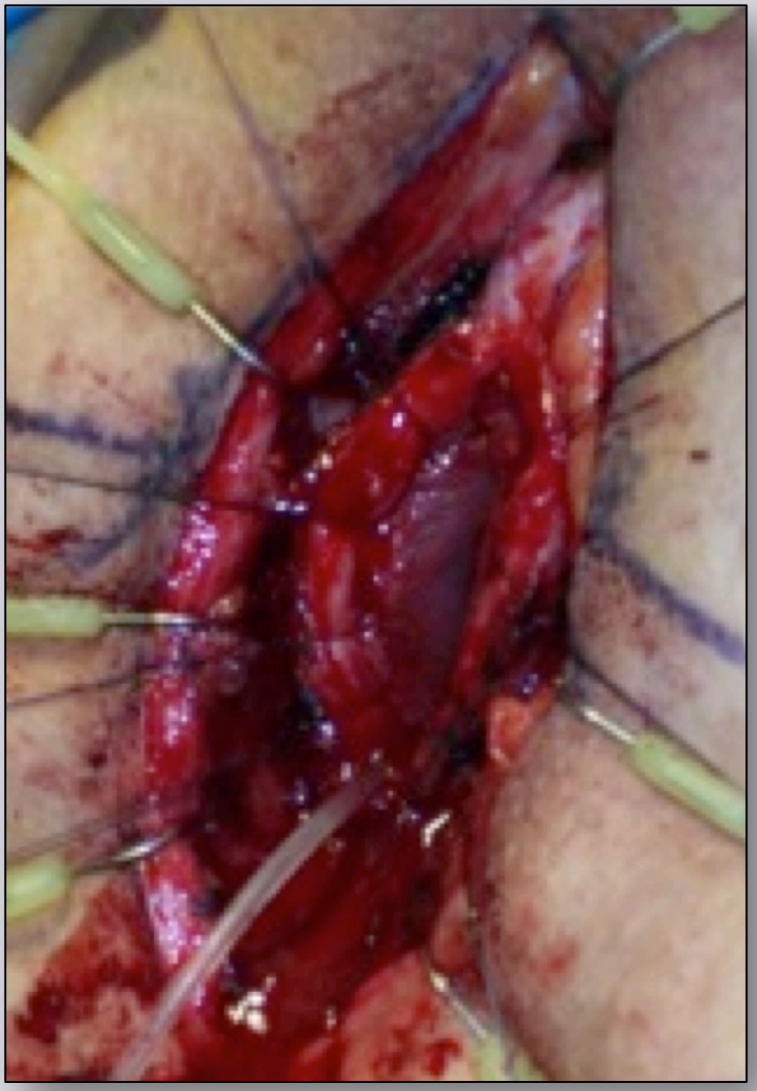

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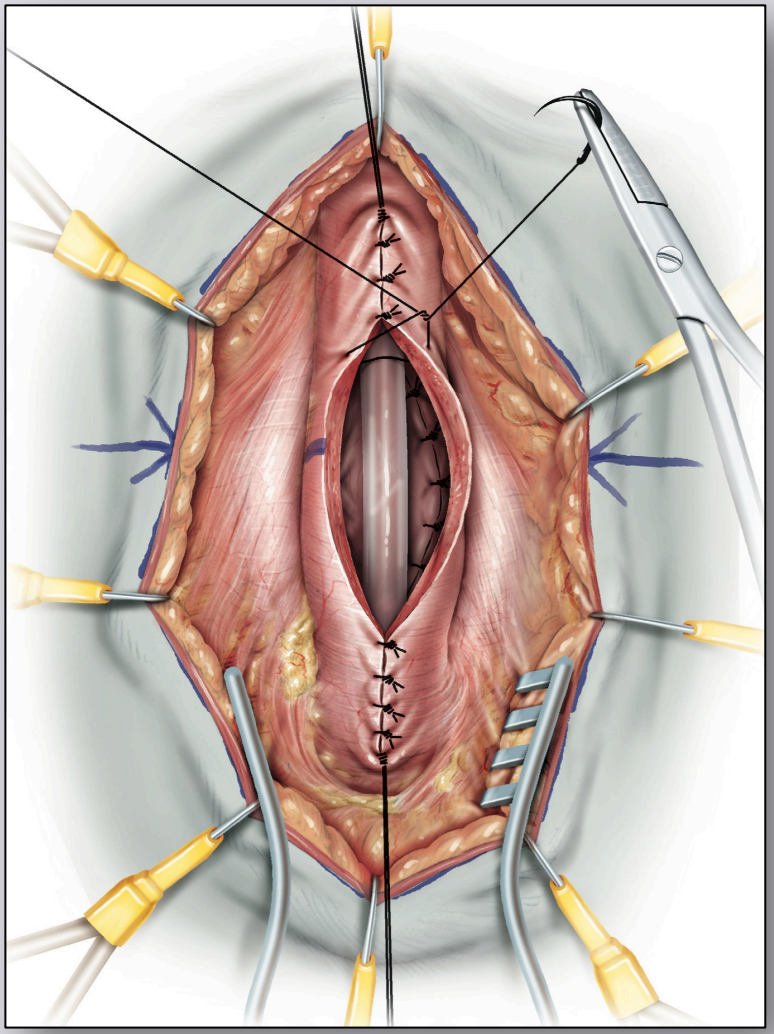

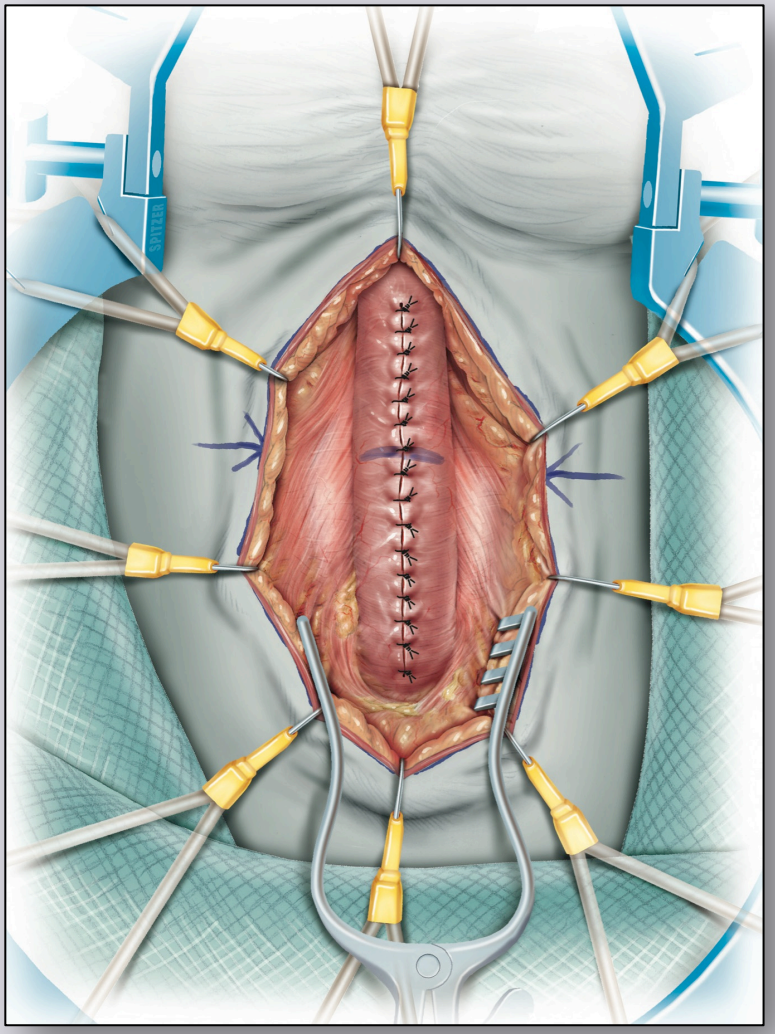

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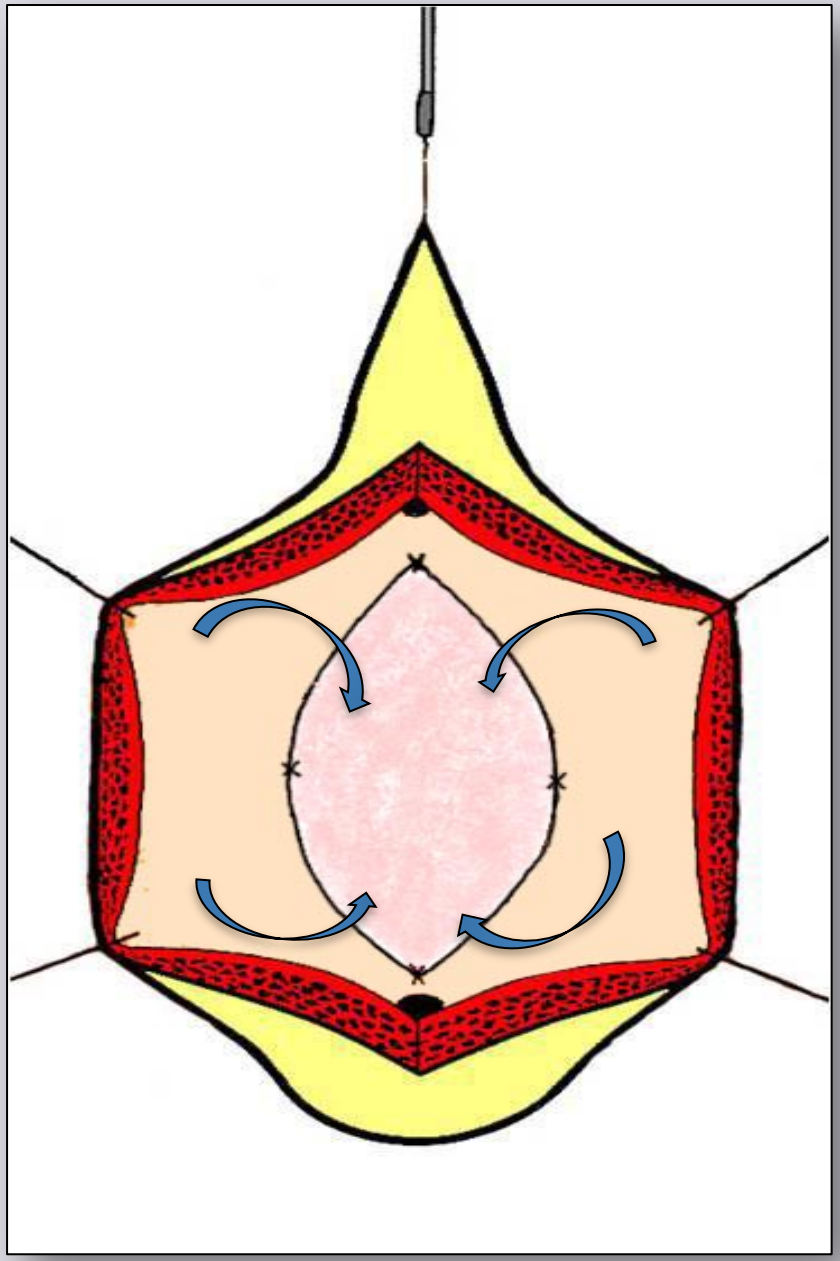

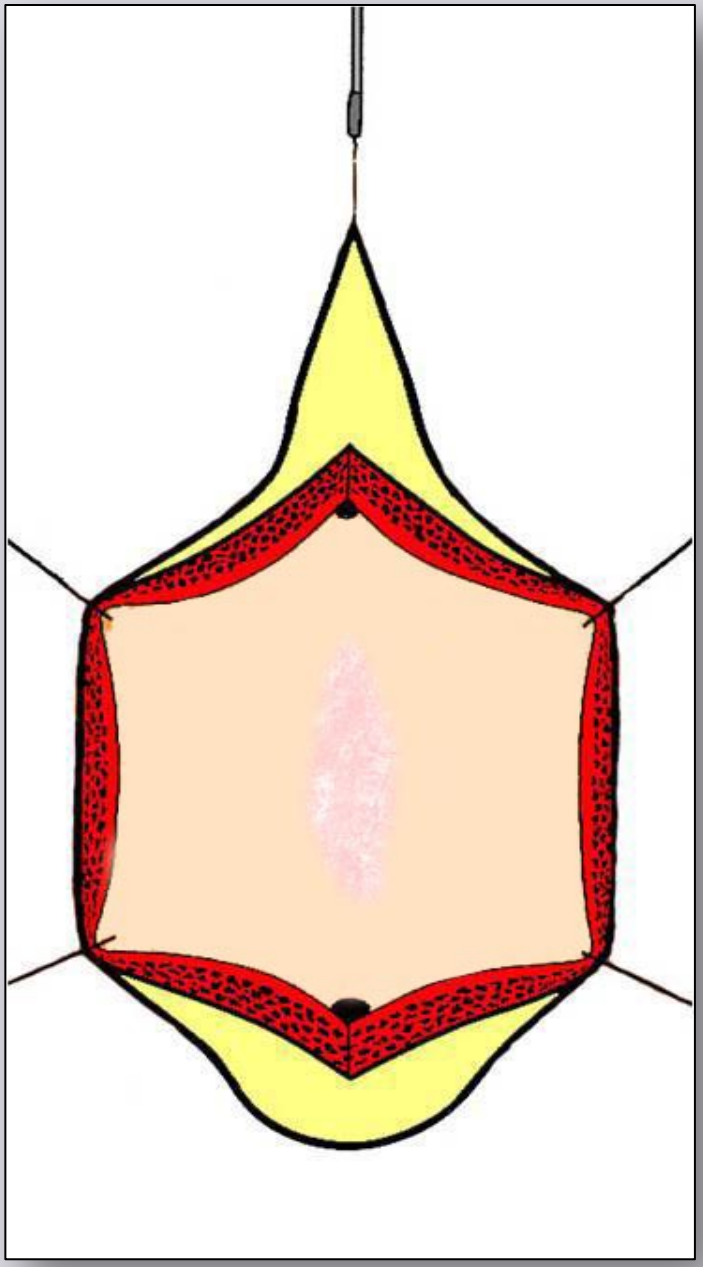

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Websites: www.uretra.it
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# Surgical transplant using ventral onlay technique

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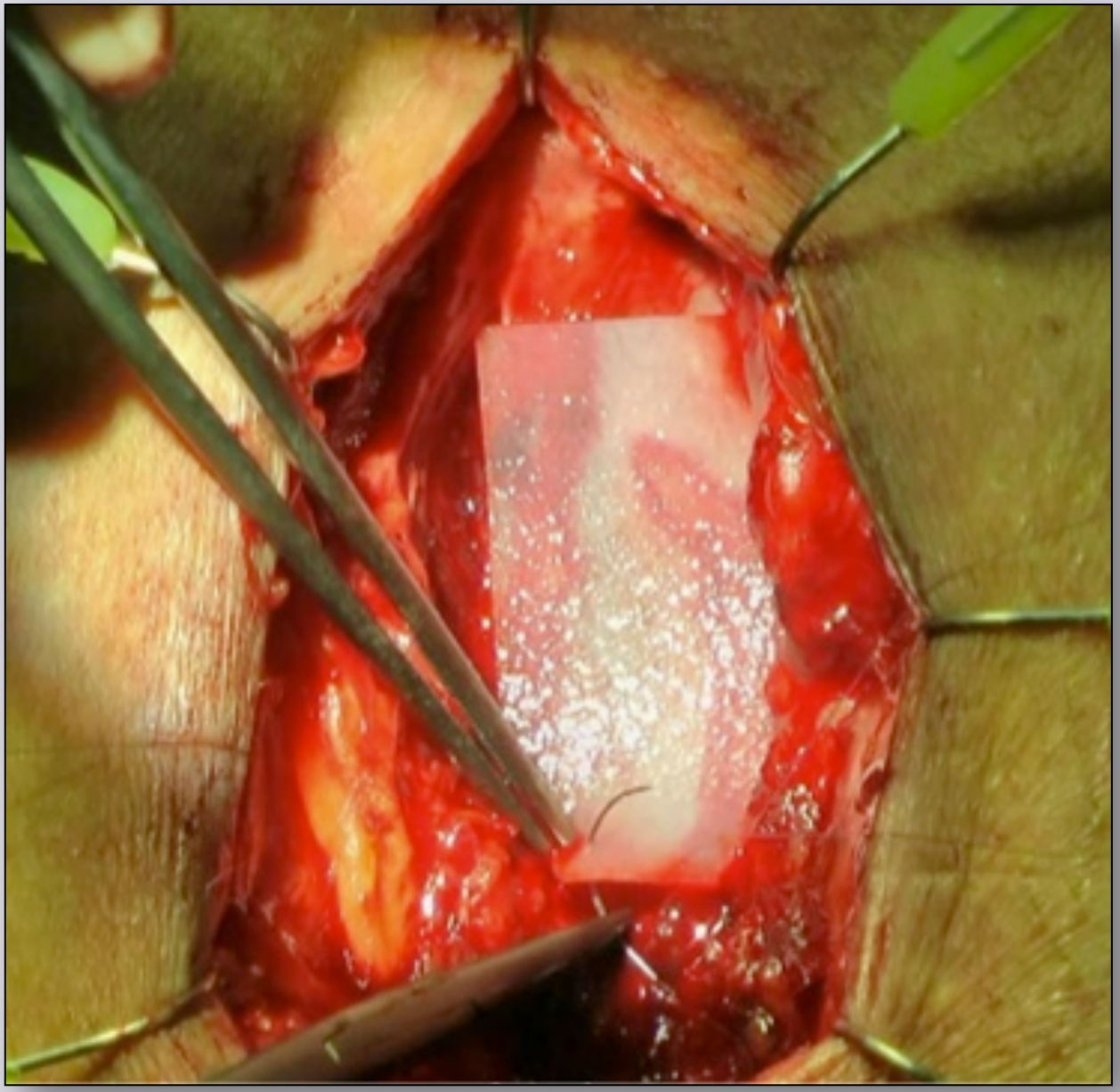

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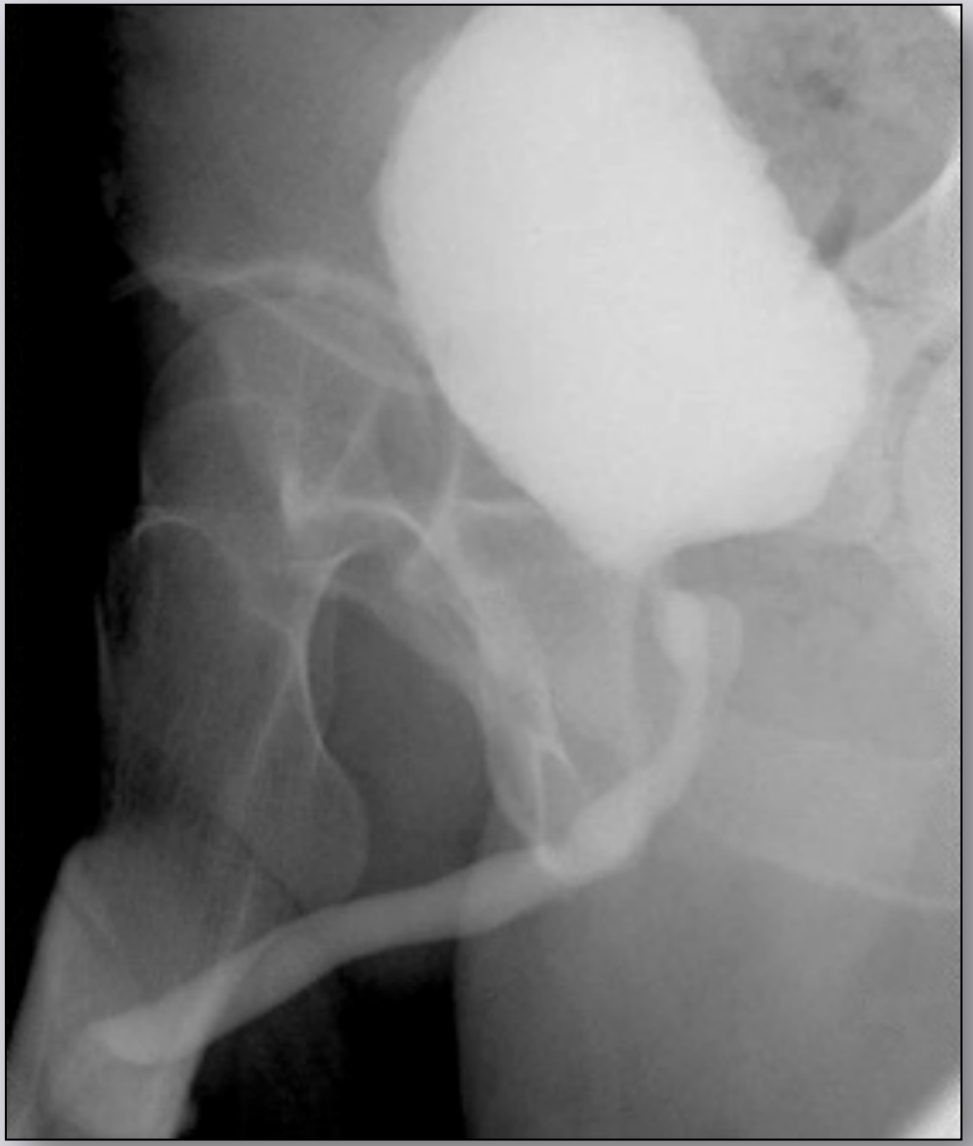
# Post-operative voiding cysto-urethrography



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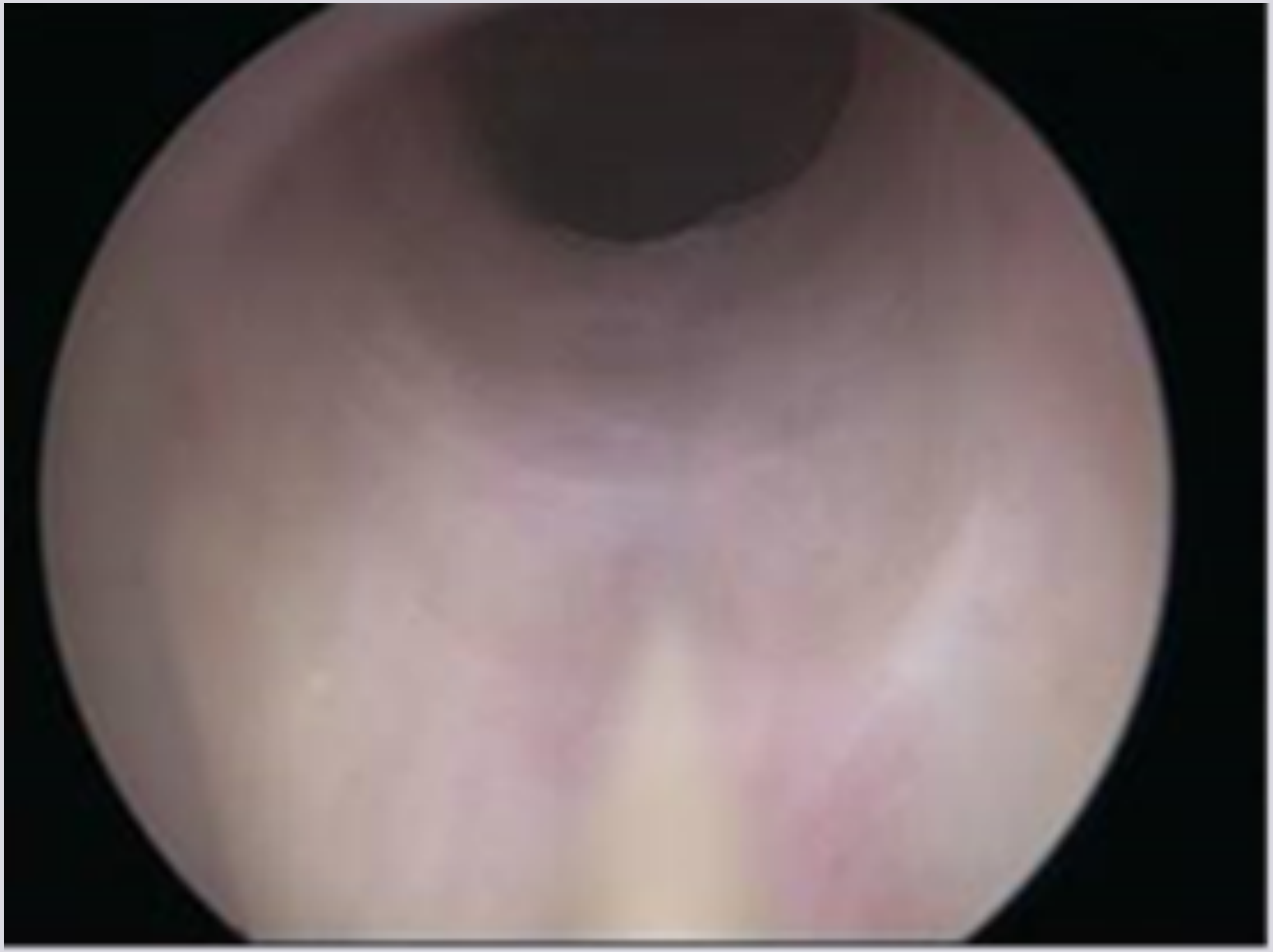

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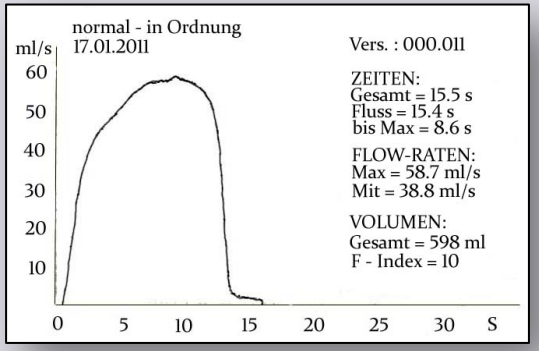
# Clinical experience with MuKocell implant in Germany

**Osnabruck**
**Chemnitz**
**Berlin**
**Hamburg**
**Luneburg**
**Lipsia**

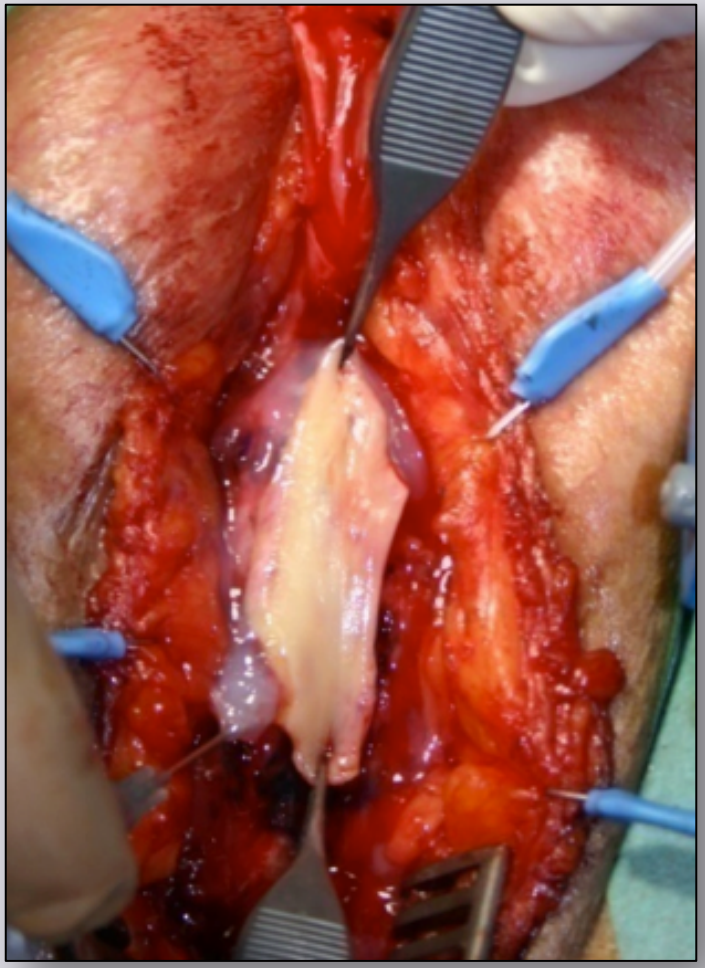
**103 patients**

**Period: from 2010 to 2013**

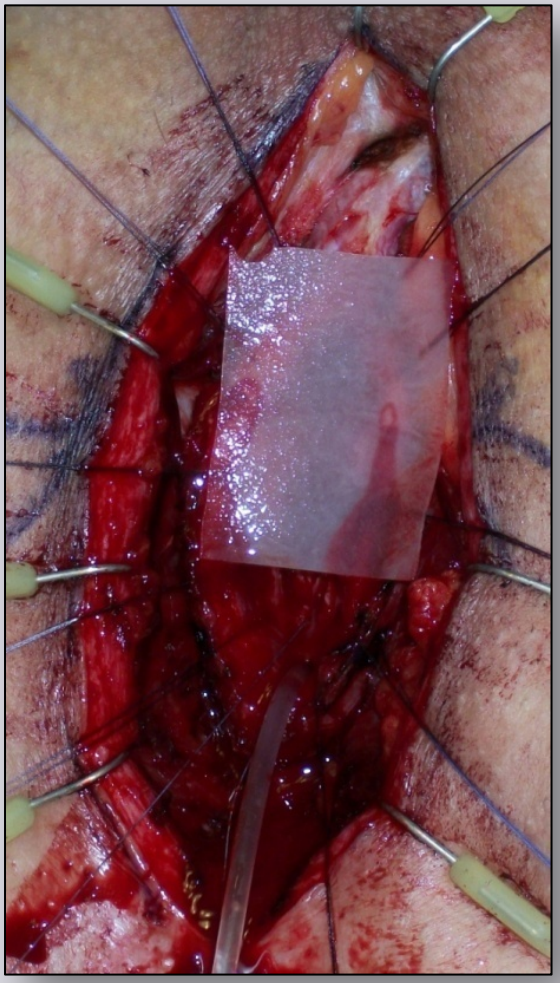



**Overall success rate: from 80% to 85%**

# Our preliminary experience



Oral mucosa



Tissue engineered oral mucosa

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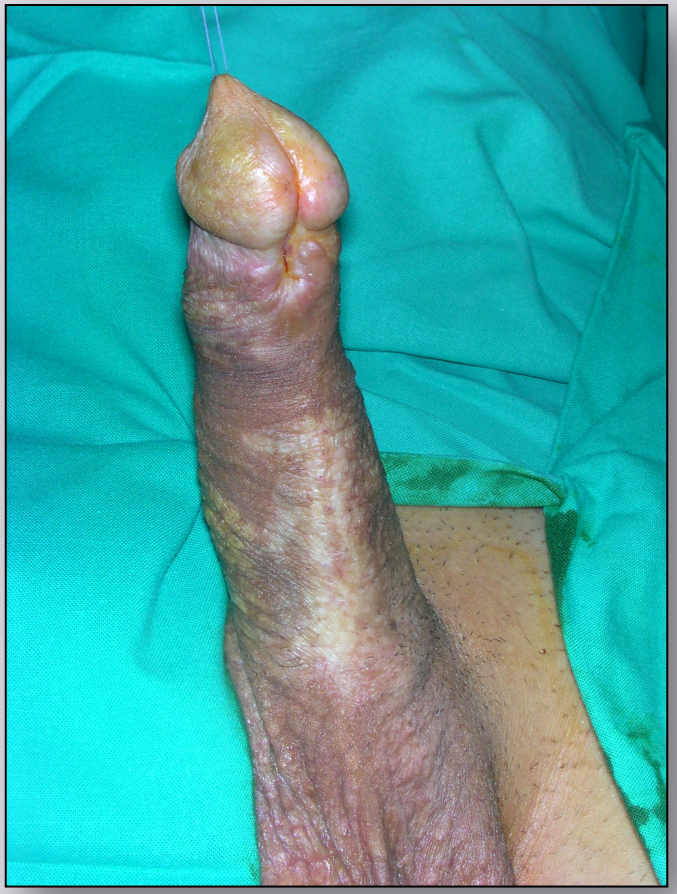
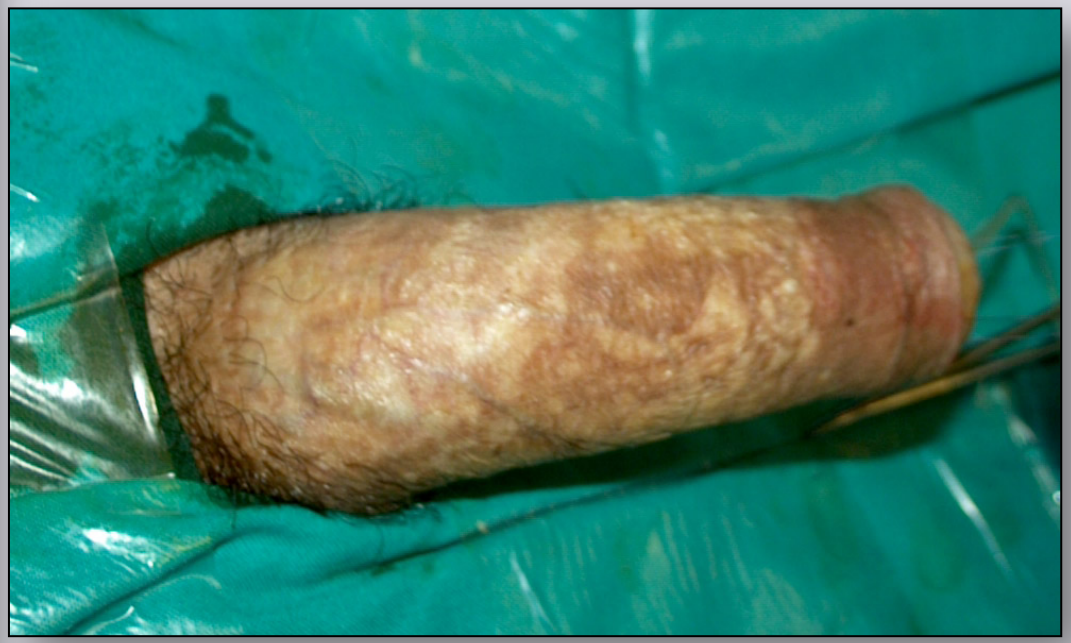
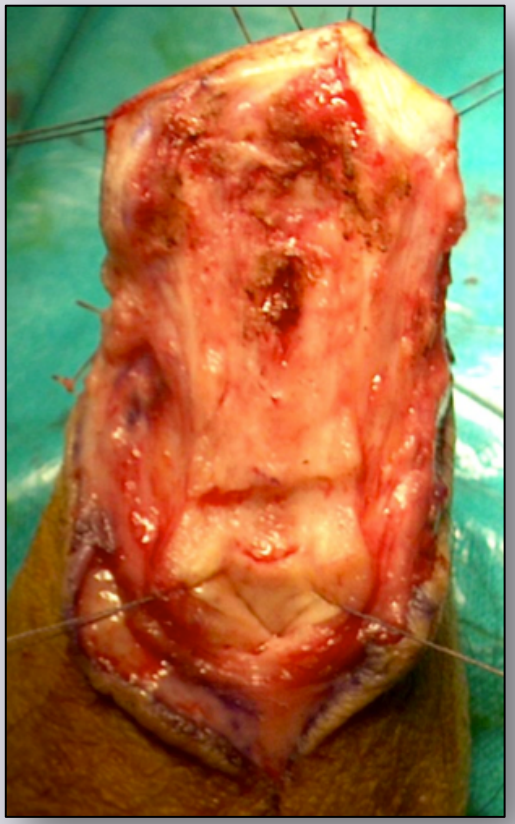
# Our preliminary experience

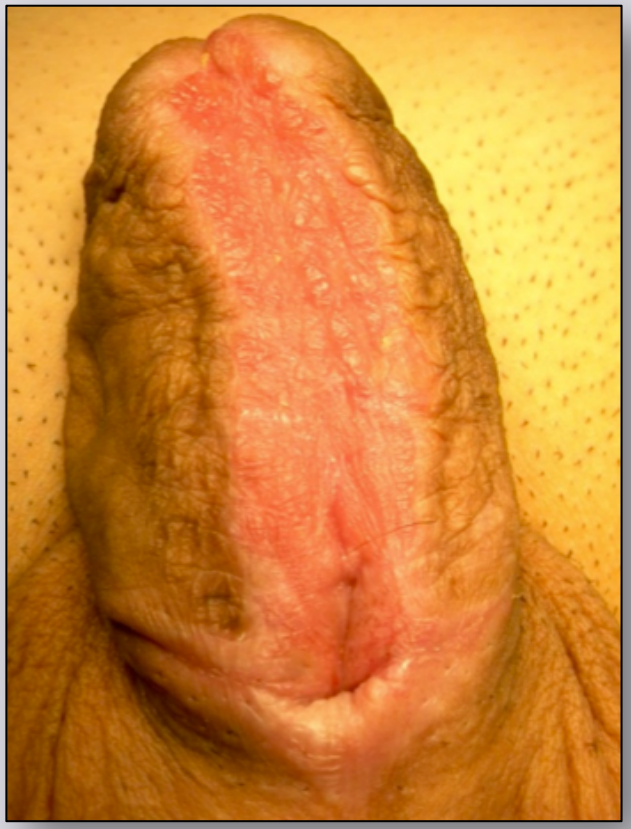




**Is tissue engineered oral material fit for use in patient with immunogical disorder like Lichen Sclerosus?**

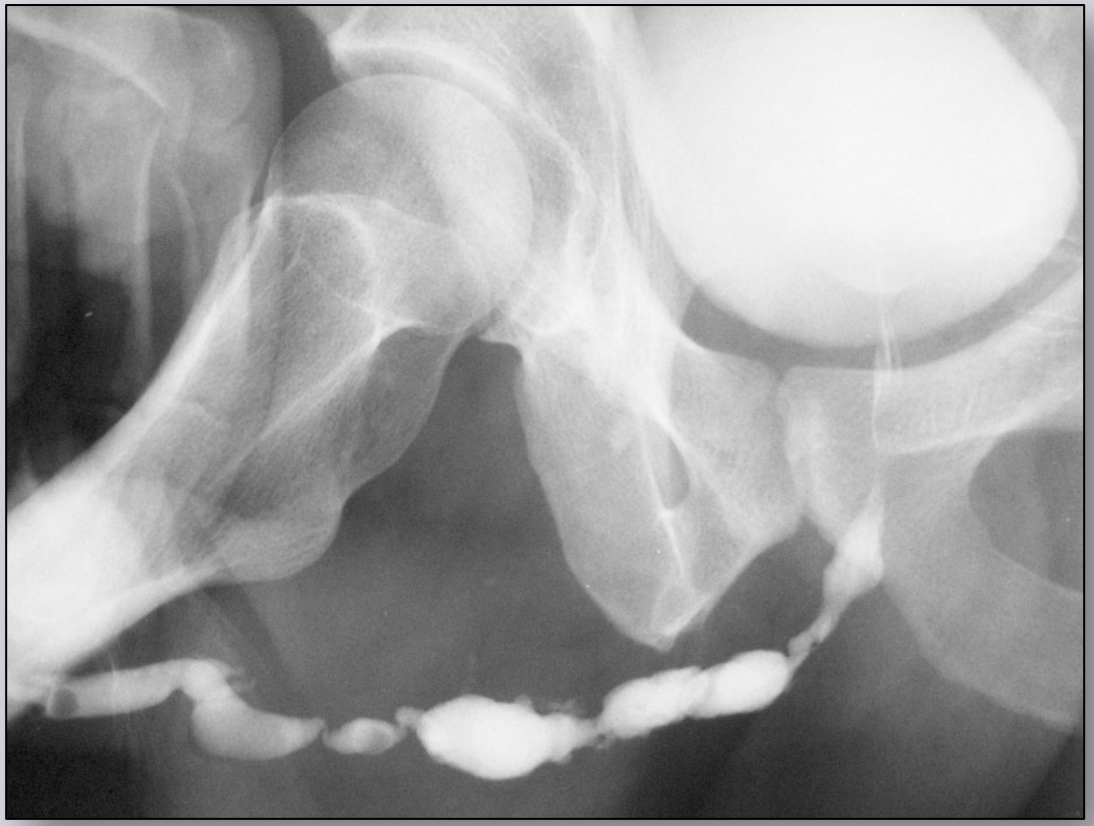
# Our preliminary experience





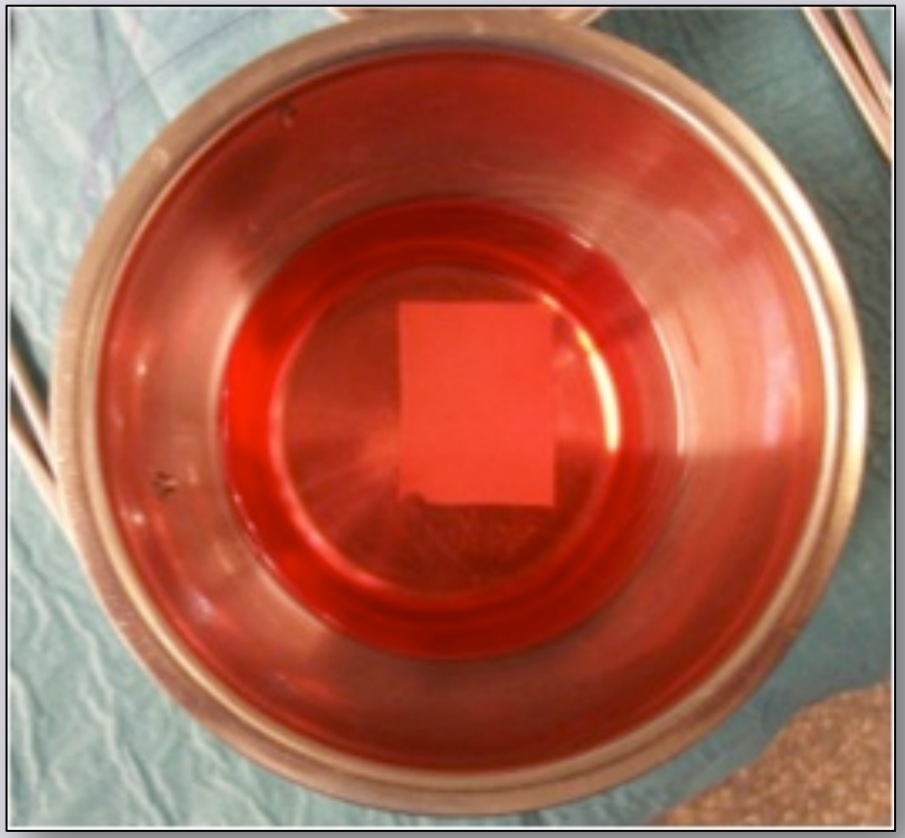
**Is tissue engineered oral material fit for use in patient requiring two-stage urethral reconstruction?**

# Our preliminary experience



**Is tissue engineered oral material fit for use in patient requiring pan-urethral reconstruction?**

# Our preliminary experience



**The use of tssue engineered oral mucosa is not a simple surgical procedure and should be performed only in a Centre of excellence for urethral surgery**

# Limitations of this study

- **Short follow-up**

✓ **This material should be used only in Germany**

✓ **The cost is about 4.000,00 to 5.000,00 Euro**

✓ **This material should be used in 48 hours**

**Take home message:**

**It is not the end line of the long history of urethral reconstruction, but the first step for a new future of urethral surgery.**

1987


2014

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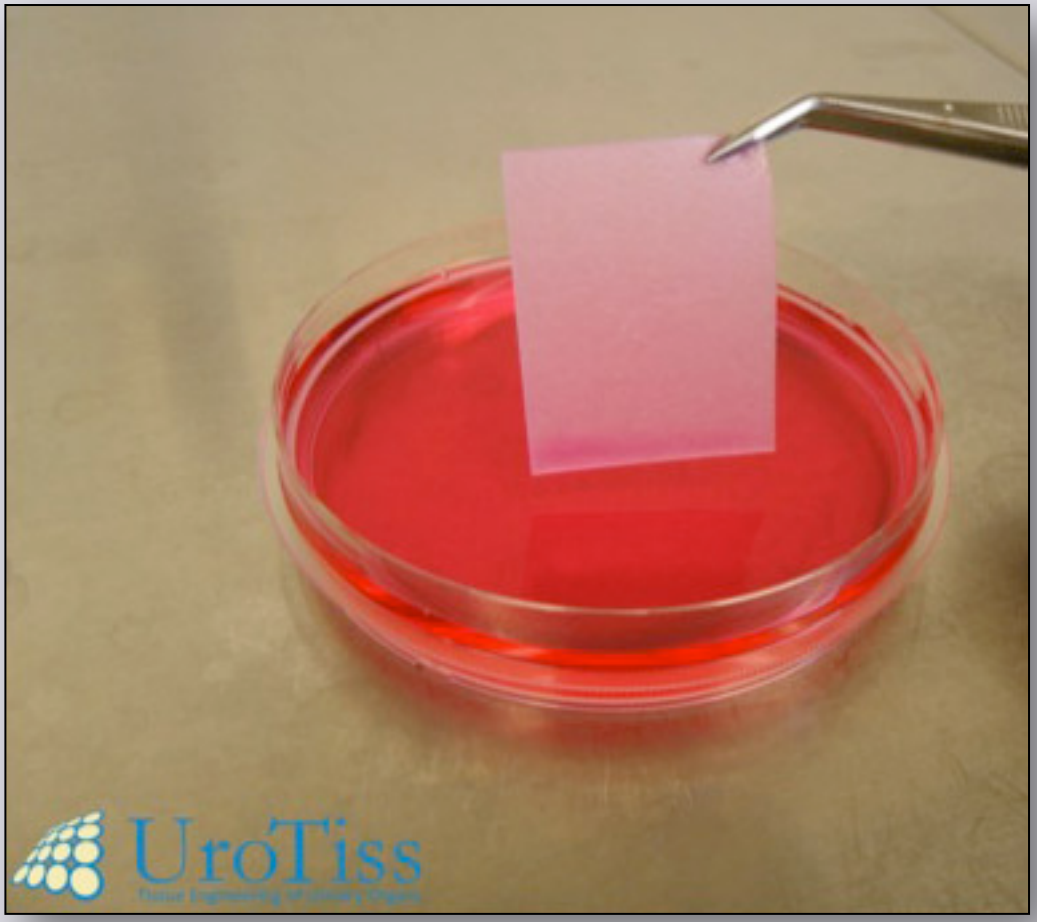


**2015**

**Next future**

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# Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe ☆

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ARTICLE INFO

Available online 14 November 2014

*Keywords:*
Tissue-engineering
Urethral stricture
ATMP
Regulation
Oral mucosa graft

ABSTRACT

Since the late eighties of last century the high potential of tissue engineered products (TEP)s has been shown for the treatment of various diseases and many scientific publications appeared in this field. However, only few products reached the market since. Development of TEPs is a promising but owing to its novelty a very challenging task that requires experts in this still developing field as well as ample financial resources. This paper summarises relevant regulatory challenges during quality, preclinical and clinical development of autologous TEPs in Europe. Selected strategies on how to manage major issues are presented, together with some examples from the development of an autologous TEP for urethroplasty. Considering these aspects may help other investigators with potential strategies during the development of novel TEPs.



**Advanced Drug Delivery Reviews 2015; 82-83: 181-191**

**For more information about MukoCell manifacturing or if you are interested in developing this technology in your country please contact Dr. Gouya Ram-liebig at:**

**Email: g.ram-liebig@urotiss.com**

**www.urotiss.com**

UroTiss
Tissue Engineering of Urinary Organs

**Dresden - Germany**

# Tissue-engineered repair of urethral stricture:
## When we will get there?

**To realize this project represents a very difficult development and we need pay attention to not deceive our patients that this "quiet revolution" in urethral reconstruction will be available soon for all urethral conditions (congenital or acquired, simple or complex) requiring surgery.**

**This step require also a large series of patients involved in a prospective, multi-centre, randomised, double blinded and placebo controlled/comparative is the standard design for the following phase III study.**

**We are still far-off from this step.**


# Register now !

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