



Universitätsklinikum
Tübingen

Master Medical Technology

Modul Implantology

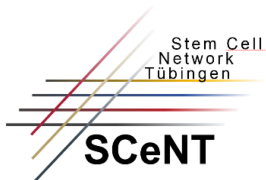


Minimal Invasive Transport of Cells to Remote Sites of Patients by a Novel Waterjet Technology

Lecture 25-04-2022

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EBERHARD KARLS
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TÜBINGEN**



Disclosure:

Data are in part the results of a collaboration with Erbe Elektromedizin GmbH Tübingen. The projects have been and/or are supported in part by grants from the BMBF, DFG, EU, and in part by institutional funds.

Prof. Aicher has no other conflict of interests to report

The data presented are in part original work of the above mentioned projects and therefore must not be communicated or disclosed to any third party. They are included in this lecture for teaching purposes of MedTech students at EKUT/MFT only.

Overview

Urinary incontinence. The clinical situation and challenges

Lessons from pre-clinical studies

Principles of the waterjet technology

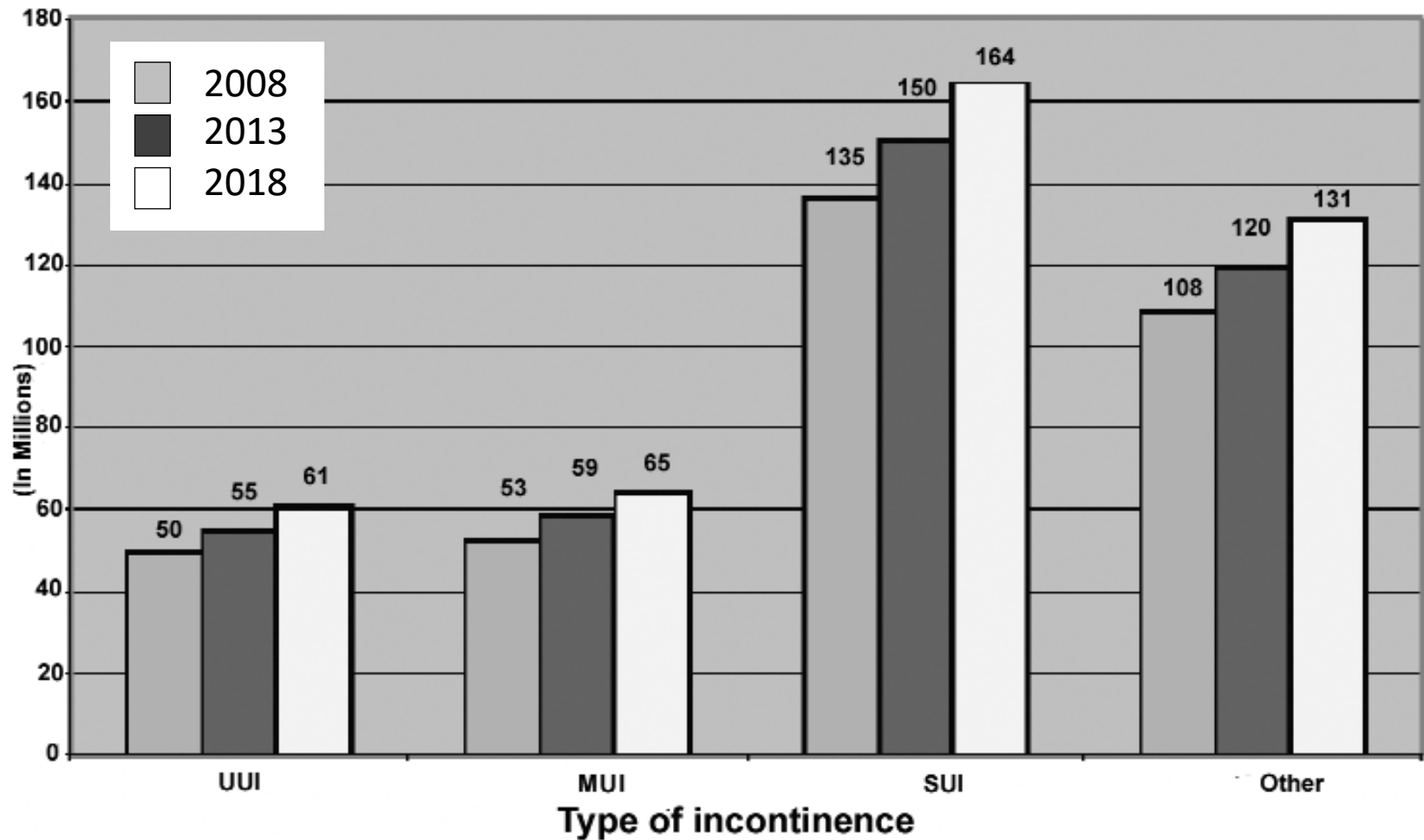
Application of the waterjet for GI applications. Spray of cells on surfaces

Application of the waterjet for urological applications: Injection of cells into media and cadaveric tissues

Cell injection of regeneration-competent cells in a SUI animal model

Future perspectives

The Clinical Situation: Prevalence of Urinary Incontinence in Western Countries



approx. 12% of people in Europe present with urinary incontinence

Risk Factors to Develop Stress Urinary Incontinence

Men:

Prostate surgery

Women:

Pregnancy

Vaginal childbirth

Both Gender:

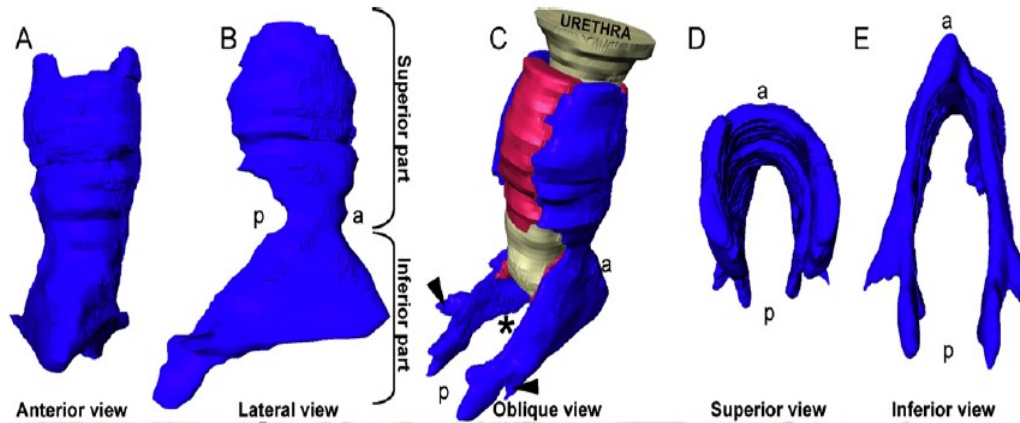
Adipositas per magna

Diabetes

Aging

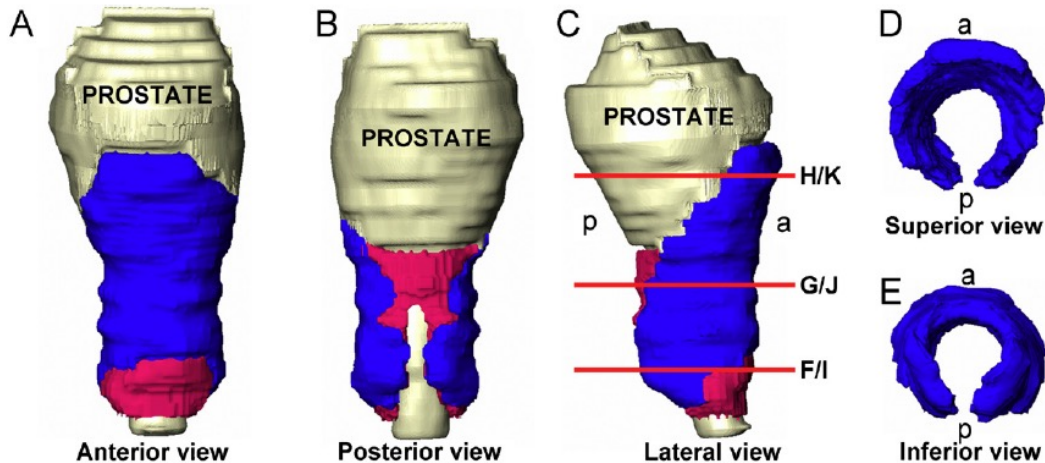
Mental Illness

The Anatomical Situation of the Urethral Sphincter Complex

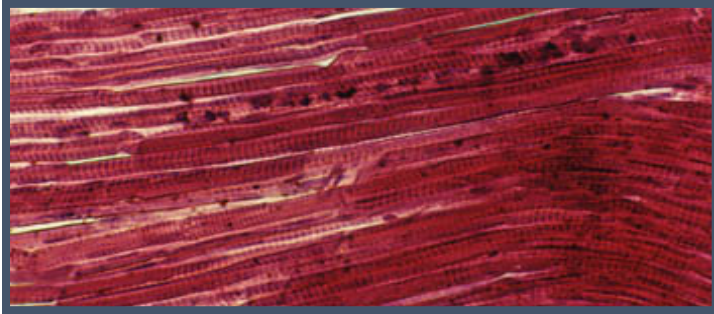


The urethral sphincter consists of:

- smooth muscle
- striated muscle

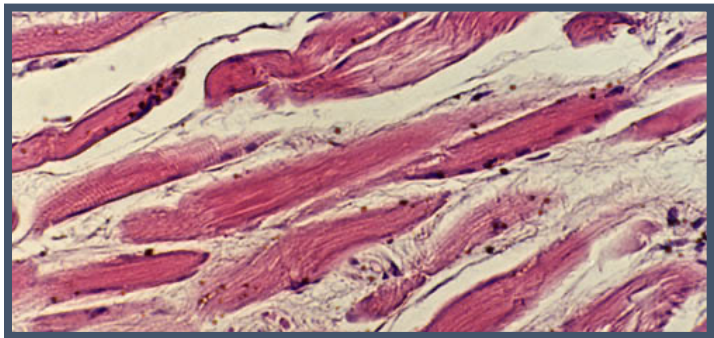


The Histological Situation of the Sphincter Tissue As Function of Aging



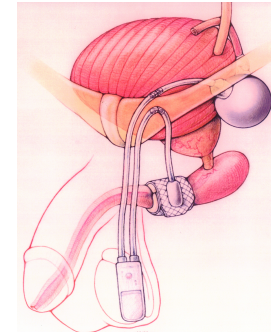
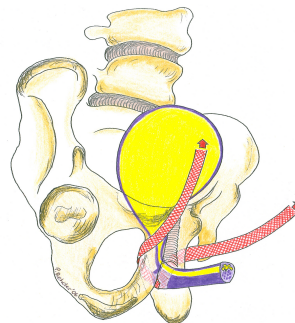
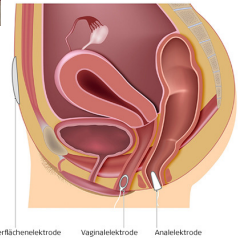
insufficient contractile function of the muscle

- apoptosis of muscle cells
- lack of innervation
- ageing
- injury / inflammation / fibrosis



Overview on Current Treatment Options

- reduction of bodyweight to reduce the load on the lower pelvic floor and behavioral changes of daily routines
- exercise to strengthen the sphincter muscle
- bio feed back exercise, possibly in combination with electro stimulation therapy
- pharmacological therapy
- surgical intervention



Most current therapies applied for significant SUI
ameliorate the sequela

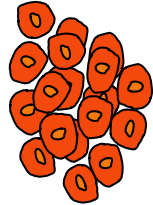
... but do not cure the conditions

Hyopthesis:

Loss of innervation and / or loss functional muscle fibres contributes to further **degeneration** of the sphincter muscle and consequently contributes to stress urinary incontinence.

Can injection of muscular progenitor cells facilitate a functional **regeneration** of this muscle?

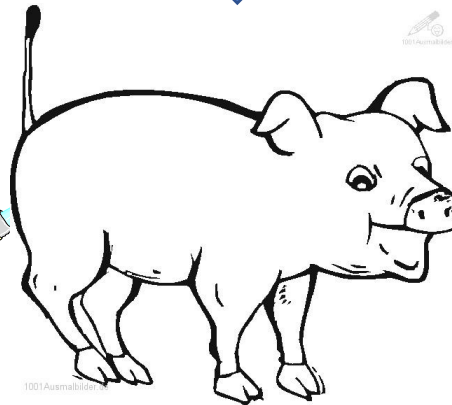
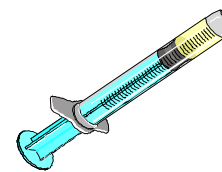
Design of the Current Pre-clinical Animal Study*



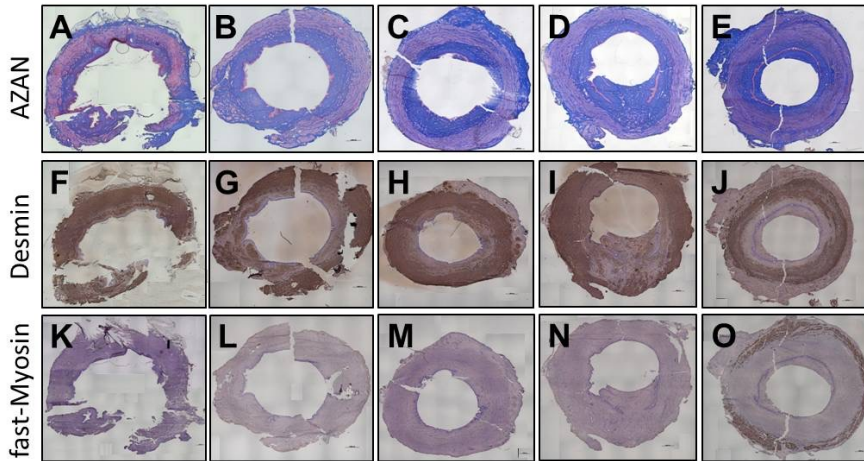
Collect homologous
cells from male littermates



Expand *in vitro*
Quality management
Fluorescent label



Inject in
incontinent femal animals



Analysis

* EU project MUS.I.C. / DFG projects Ai16/28-1 PoTuS & Ai16/29-1 MyoRepair

Results from a Pre-clinical Model of Cell-based Therapies of Experimental SUI*

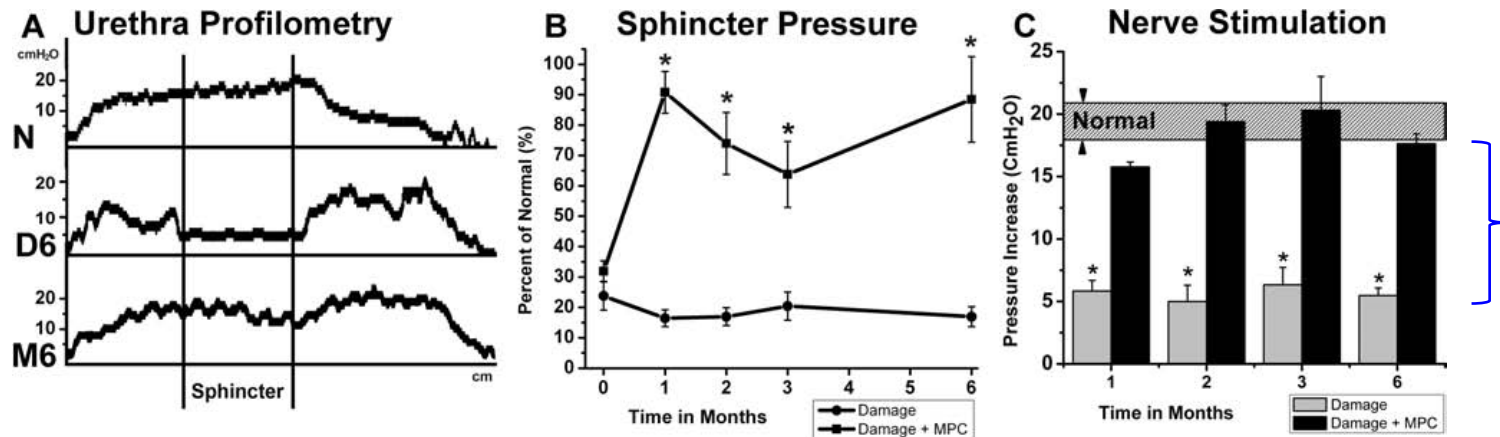


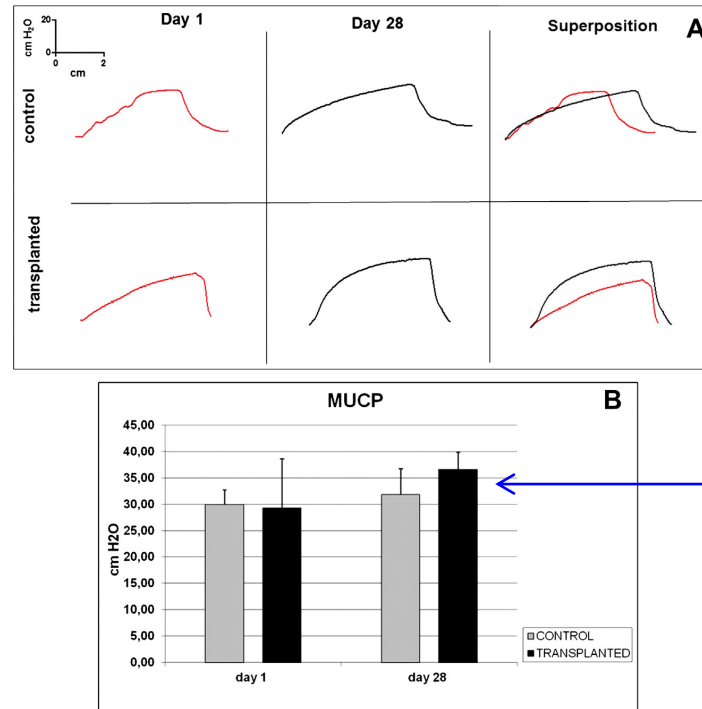
Figure 2. Functional assessment of sphincter function. (A) Representative urethra profiles showing the increase of sphincter pressure in the sphincter area after cell treatment. N, normal control; D6, damage-only control at 6 months; M6, MPC-treated animal at 6 months. (B) Graph showing sphincter pressures over time. Animals treated with MPC injection showed a significant functional recovery with pressures of approximately 80% of normal, while the damage-only group remained low at 20% ($p < 0.025$). (C) Pressure increase during electrical stimulation of the pudendal nerve showed a significant improvement for the cell-treated group, when compared to the damage-only control ($p < 0.003$).

significant improvement of sphincter function

> very good recovery, promising pre-clinical data

The clinical feasibility study closed in 2022. Data are under evaluation

Results from another Pre-clinical Model of Cell-based Therapies of Experimental SUI*



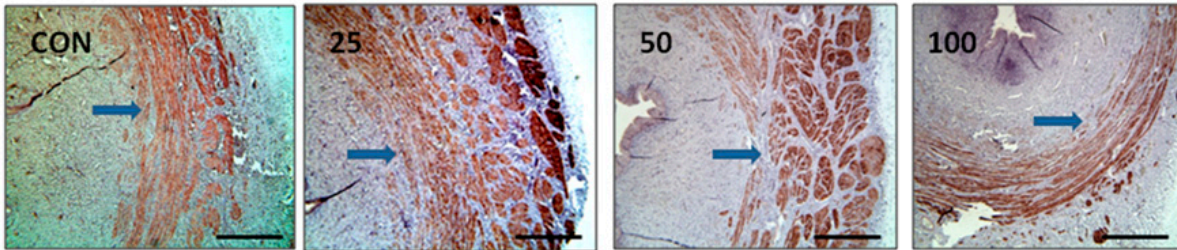
no significant change in sphincter function

Figure 3. The functional evaluation of the porcine muscle-derived cells (pMDCs) transplantation effect. **(A)** The urethral profiles recorded during urodynamic evaluation. The profiles recorded at day 1 and day 28 of the same pig from the representative control animal and experimental animal. All profiles refer to the presented scale. **(B)** The graph represents the mean maximal urethral closure pressure (MUCP) values of control and transplanted animals at day 1 and day 28. The differences between groups were not statistically significant ($P = .2$).

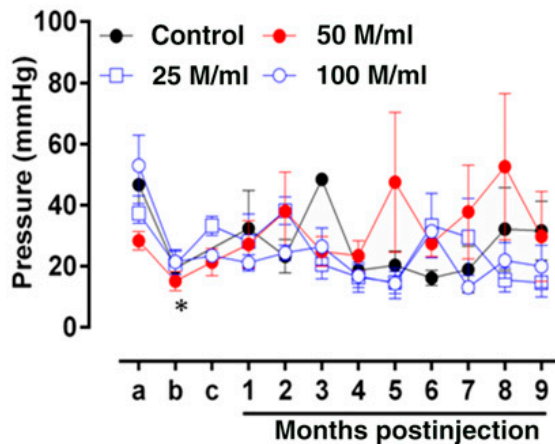
> results depend very much on the model used

Results from yet Another Pre-clinical Model of Cell-based Therapies of Experimental SUI*

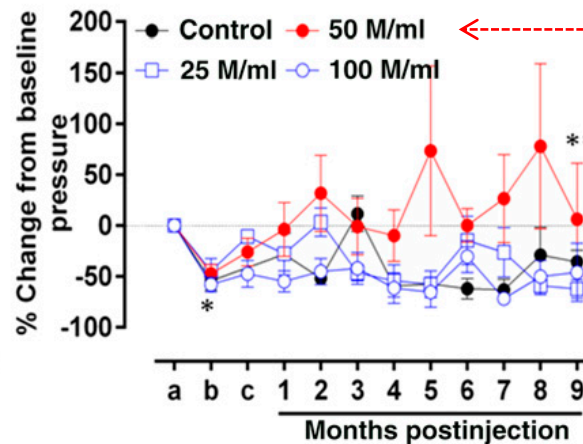
A



B Maximal Urethral Pressure (MUP)



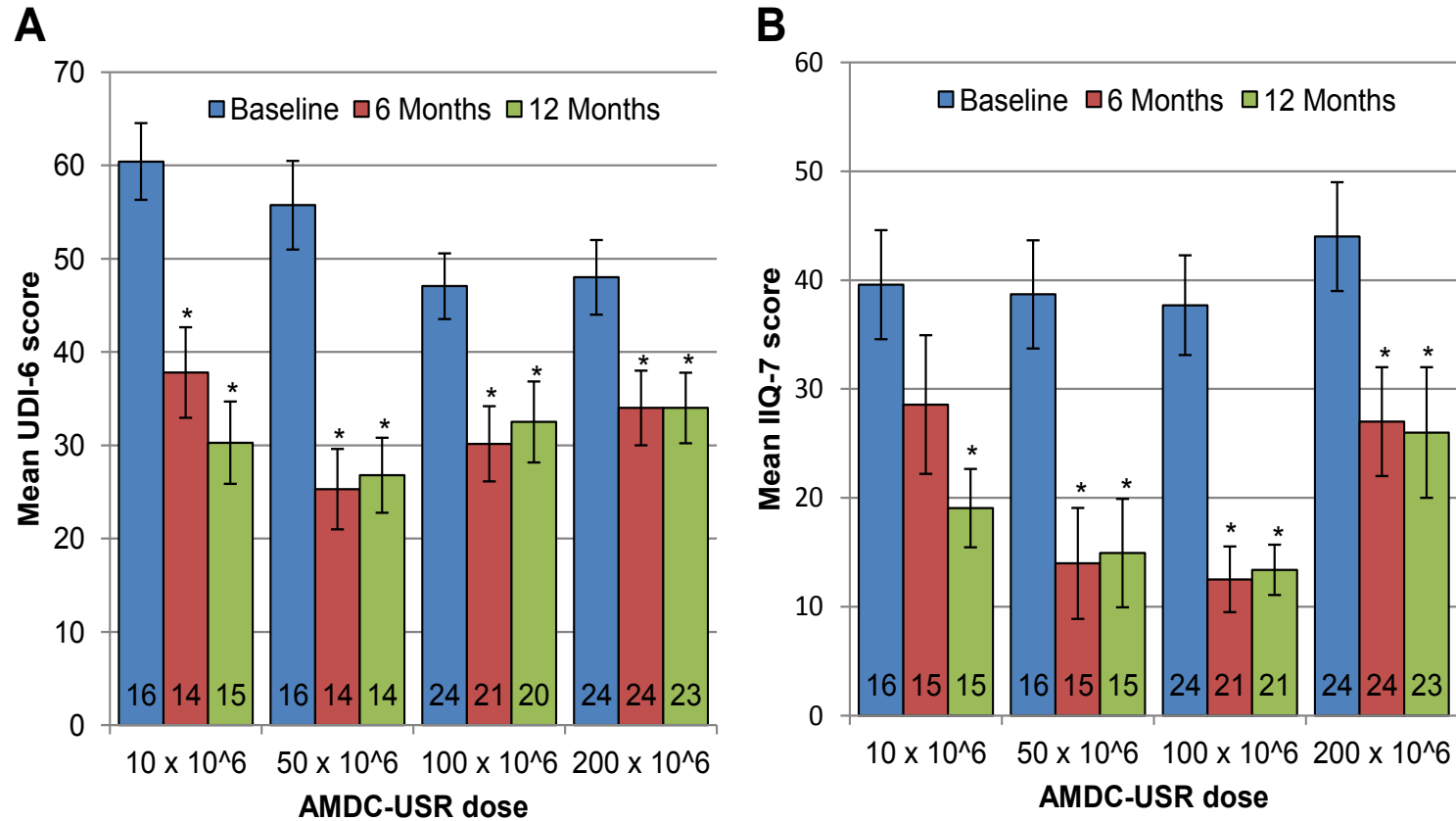
C MUP: % Change from baseline pressure



minor but significant improvement only with 5×10^7 cells / animal

> outcome is very variable and depends very much on the cell dosage used
> during follow-up only in 1 cohort significant improvement was observed

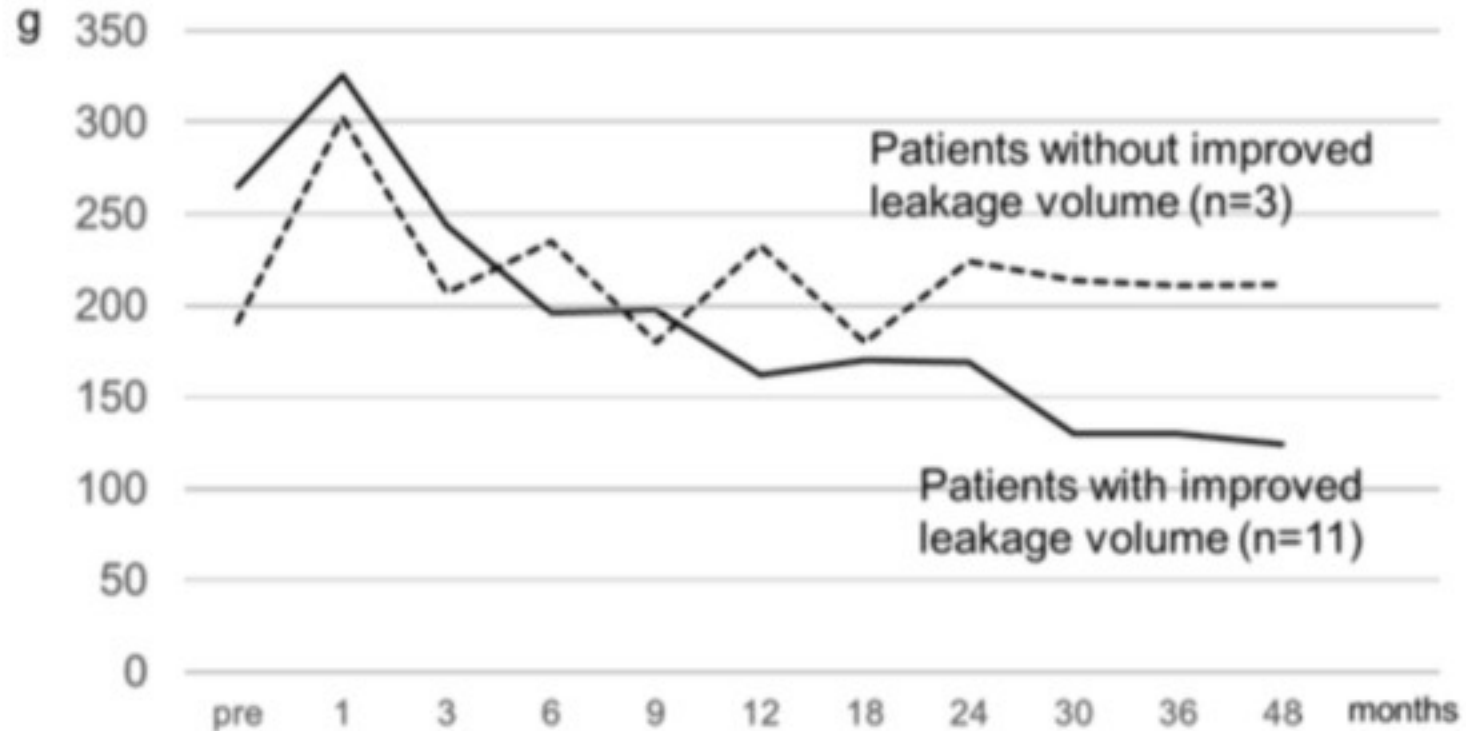
Clinical Application of Muscle-derived Cells



Result:

- 72/80 patients completed the study after 1 year
- no adverse effects
- significant improvement independent of cell dose

Clinical Application of Autologous Adipose Tissue-derived Cells



14 patients after radical prostatectomy
f/u ø 60 months
11/14 improved

Ambivalent Situation in the Current Literature on Cellular Therapies

Many pre-clinical studies utilizing animal models of SUI reported on success of cell based therapies, but others reported no improvement*

Some clinical studies reported on success of SUI treatment of patients....

ported on success but were retracted thereafter,

Int Urogynecol J (2008) 19:881–883
DOI 10.1007/s00192-007-0553-z

CASE REPORT

1-year follow-up of autologous muscle-derived stem cell injection pilot study to treat stress urinary incontinence

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R. Pruchnic · R. Jankowski · J. Erickson · J. Huard ·
M. B. Chancellor

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Abstract We hereby report a 1-year follow-up on eight women in the first North America trial in which stress urinary incontinence (SUI) was treated with muscle-derived stem cell injections. Mean and median follow-up in this group was 16.5 and 17 months (range 3–24 months). Improvement in SUI was seen in five of eight women, with one achieving total continence. Onset of improvement was between 3 and 8 months after injection. Cure or improvement continued at a median of 10 months. No serious adverse events were reported.

Keywords Stress urinary incontinence · Stem cell · Muscle · Urethra

Introduction There are many causes of stress urinary incontinence (SUI), and the most common is a deficiency of the urethral sphincter. The urethral sphincter is a complex of muscle and connective tissue. It is located at the junction of the bladder and the urethra. The urethral sphincter is responsible for maintaining continence. In women, the urethral sphincter is often weakened by factors such as aging, childbirth, and menopause. This can lead to SUI, which is a common condition that affects many women. The goal of this study was to evaluate the safety and efficacy of a new treatment for SUI, which involves the injection of muscle-derived stem cells into the urethral sphincter. This treatment is based on the idea that stem cells can differentiate into muscle cells and help to strengthen the sphincter. The study involved eight women who were treated with this therapy. The results showed that five of the women experienced improvement in their SUI, with one woman achieving total continence. The improvement was seen between 3 and 8 months after the injection. The improvement continued at a median of 10 months. No serious adverse events were reported. These findings suggest that the injection of muscle-derived stem cells may be a safe and effective treatment for SUI. Further studies are needed to confirm these results and to determine the long-term effects of this treatment.

ea
European Association of Urology

Female Urology – Incontinence

Adult Stem Cell Therapy of Female Stress Urinary Incontinence

J Korean Med Sci 2010; 25: 813–6

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Myoblasts
Rhabdosphincter
Stem cells
Transurethral ultrasound
Urethra

Abstract

Objectives:
To evaluate the safety and efficacy of transurethral injection of autologous muscle-derived stem cells (MDSCs) for the treatment of stress urinary incontinence (SUI) in women. Methods:
A pilot study was conducted in which eight women with SUI were treated with MDSCs. The women were followed up for 12 months. Results:
Five of the eight women (62.5%) showed improvement in their SUI. The improvement was seen between 3 and 8 months after the injection. The improvement continued at a median of 10 months. No serious adverse events were reported. Conclusion:
The injection of MDSCs may be a safe and effective treatment for SUI. Further studies are needed to confirm these results and to determine the long-term effects of this treatment.

Human Cord Blood Stem Cell Therapy for Urinary Incontinence

Our objective in this study was to evaluate the safety and efficacy of trans cord blood stem cell injection for treatment of stress urinary incontinence. Between July 2005 and July 2006, 39 women underwent transurethral u cord blood stem cell injection performed by one operator at a single hospital. All patients had stress urinary incontinence. The patients were evaluated 1, 3, 6, 12, and 24 months postoperatively. No postoperative complications were observed. 28 (71.8%) were more than 50% satisfied according to the Patient's Satisfaction Scale after 1 month, 29 patients (83%) were more than 50% satisfied according to Patient's Satisfaction results after 3 months, and 26 (72.2%) continuously more than 50% improvement after 12 months. Intrinsic sphincter deficiency mixed stress incontinence improved in the ten patients evaluated by urodynamic study. Our results suggest that transurethral umbilical cord blood stem cell injection is an effective treatment for women with all types of stress urinary incontinence.

Key Words : Urinary Incontinence, Stress, Intrinsic Sphincter Deficiency, Umbilical Cord Blood Stem Cell Therapy

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World J Urol (2008) 26:327–332
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TOPIC PAPER

Muscle derived stem cell therapy for stress urinary incontinence

Marc C. Smaldone · Michael B. Chancellor

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Abstract

Aim The aim of this article is to discuss the potential of muscle-derived stem cells (MDSCs) for rhabdosphincter regeneration and to review the early clinical experiences with its application in patients with stress urinary incontinence.

Results In anatomical and functional studies of the human and animal urethra, the middle urethral contained rhabdosphincter is critical for maintaining continence. Transplanted stem cells have the ability to undergo self-renewal and multipotent differentiation, leading to sphincter regeneration. In addition, such cells may release, or be engineered to release, neurotrophins with subsequent paracrine recruitment of endogenous host cells to concomitantly promote a regenerative response of nerve-integrated muscle. **Conclusion** Cell-based therapies are most often associated with the use of autologous multipotent stem cells, such as bone marrow stromal cells. However, harvesting bone marrow stromal stem cells requires a general anesthetic, can be painful, and has variable yield of stem cells upon processing. In contrast, with appropriate experience, alter-

native autologous adult stem cells such as muscle-derived stem cells and adipose-derived stem cells can be obtained in large quantities and with minimal discomfort.

Keywords Stem cells · Muscle · Urinary incontinence · Urethral sphincter

Introduction

There are over 200 million people worldwide with incontinence, a condition that is associated with social impact and a reduced quality of life. Stress urinary incontinence (SUI) has been reported as the most common type of urinary incontinence [1]. Risk factors for developing SUI include increasing parity, age, and obesity. Injury during childbirth to pelvic floor musculature, connective tissue, and nervous structures appears to be the most important risk factor for urinary incontinence in later life [2].

SUI can be grouped into two major categories: urethral hypermobility and intrinsic sphincter deficiency (ISD). Most research has focused on the SUI caused by urethral

Animal studies applied variable regimen to induce incontinence and therefore can't be compared head to head

In small animals precise cell injections are a challenge

Many pre-clinical and clinical studies have not (yet) published their results

Some clinical feasibility studies with selected cohorts reported initial success

Some clinical studies with larger cohorts suffered from significant drop-out

Overall cohorts investigated so far have been small often due to regulatory affairs

Individual treated in feasibility studies so far differ significantly in their clinical situation

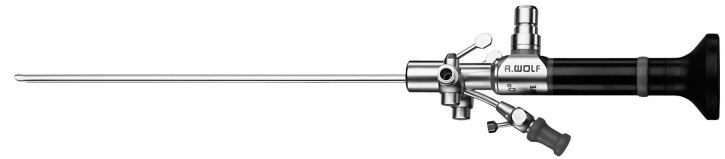
Additional Reasons for Variability of the Results

- Localisation of cells injected can't be investigated in patients
- Viability of cells injected can't be monitored during follow-up in patients

The Surgical Method

Navigation by Ultrasound

Sphincter



Bladder

Urethra



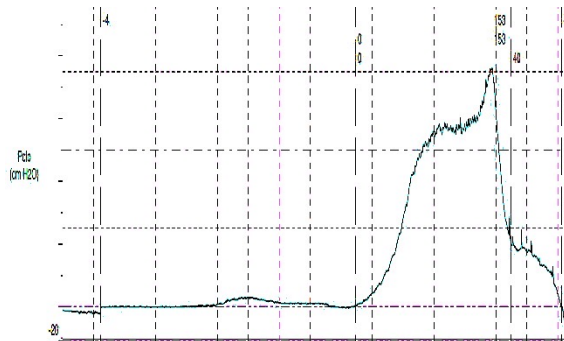
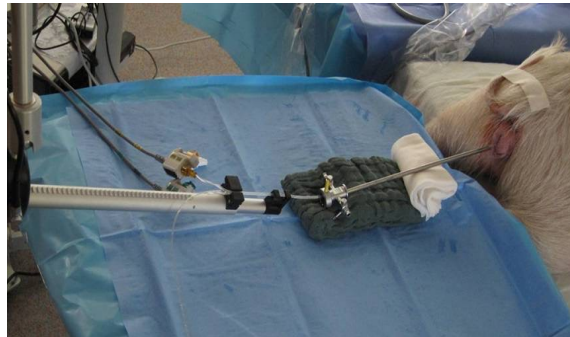
Vagina/Anus

Functional Mapping of the Urethra by Urodynamics to Inject Cells Precisely in the Sphincter

Application *in vivo*

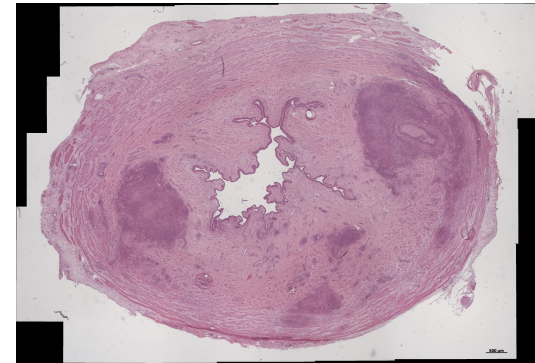


Urodynamical evaluation

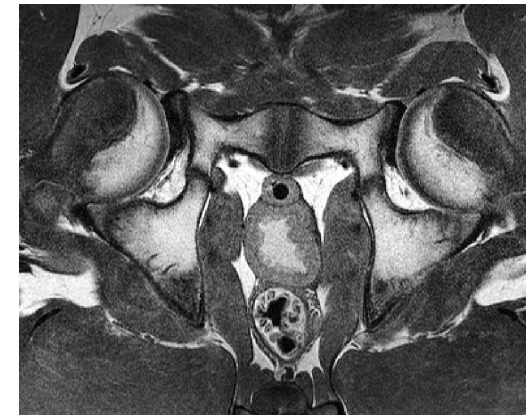


Incubation 3 wks - 12 months

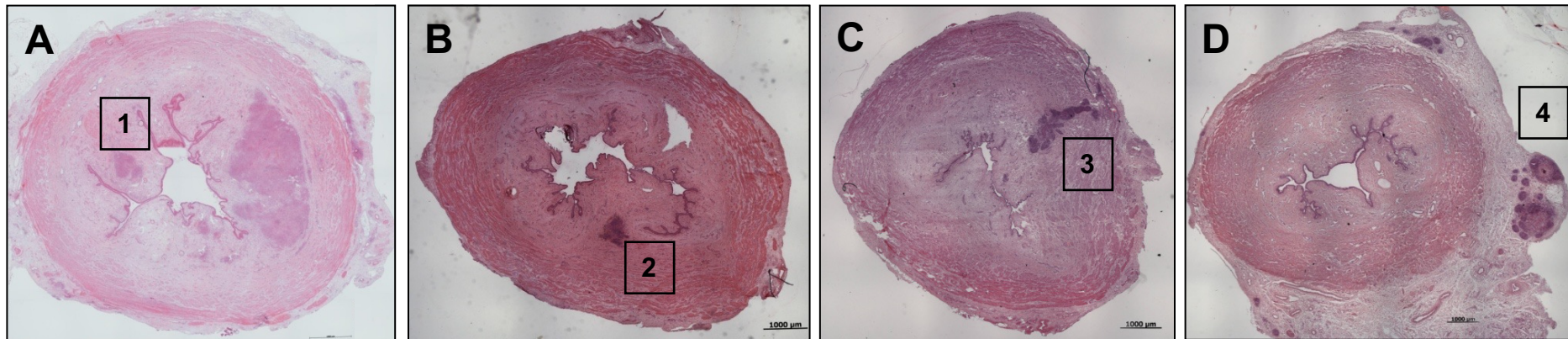
Follow-up by histology



Follow-up by MRI



Technical challenges for transurethral injection of cells by cystoscope under visual control

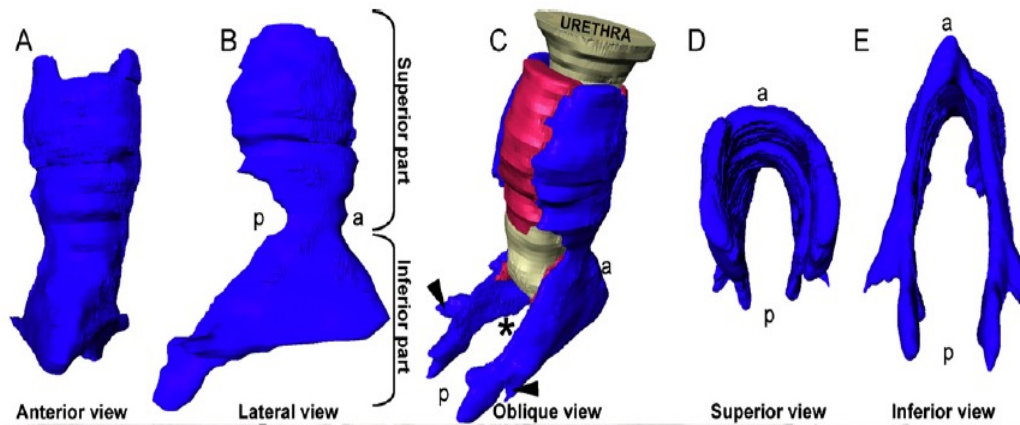


localisation	close to lumen / urothelial cells	connective tissue / intermediate	muscular layer	outside urethra
8 mm	8%	20%	13%	59%
4 mm	12%	45%	16%	27%

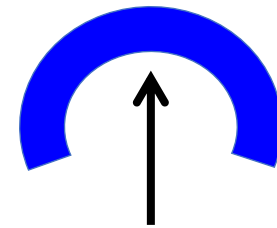
Frequent Loss of Cells Due to Improper Needle Injection



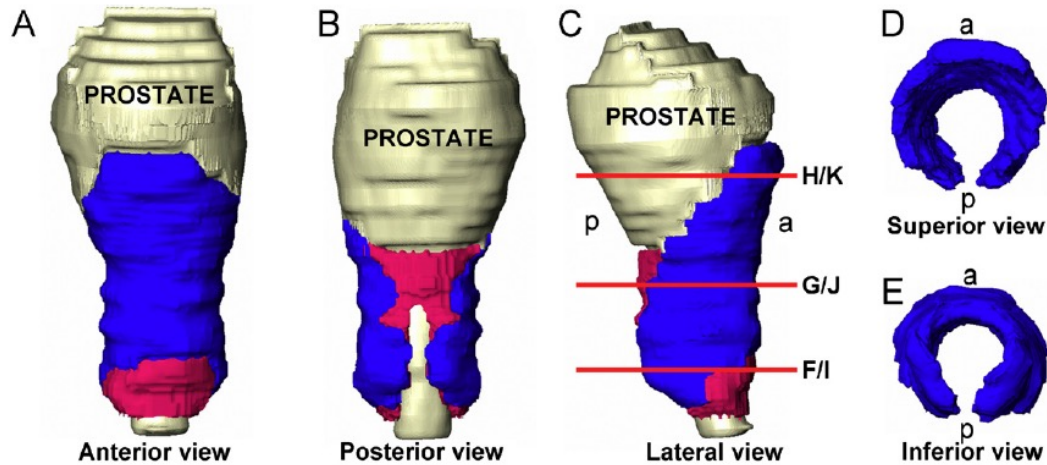
Cell Injection Strategy: Single Shot vs. Multiple Sides



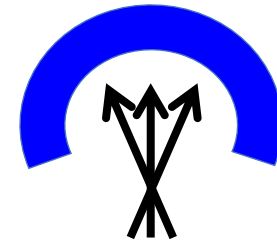
female embryo, 18wks p.g.



less invasive, less distribution

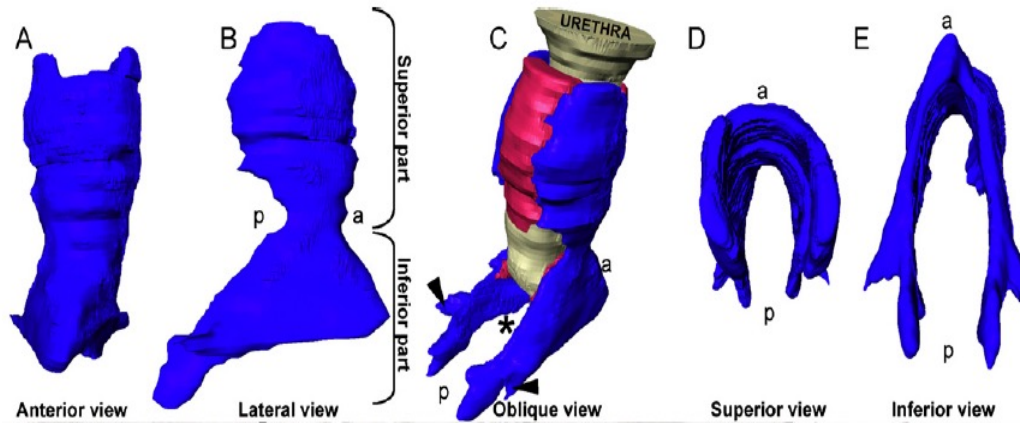


male embryo, 12wks p.g.



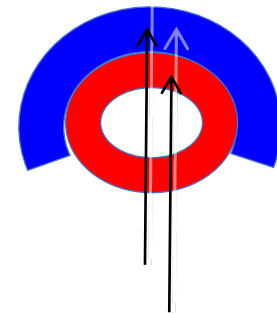
more invasive, better distribution

Cell Injection Strategy: Targeting the Rhabdosphincter or the Lissosphincter

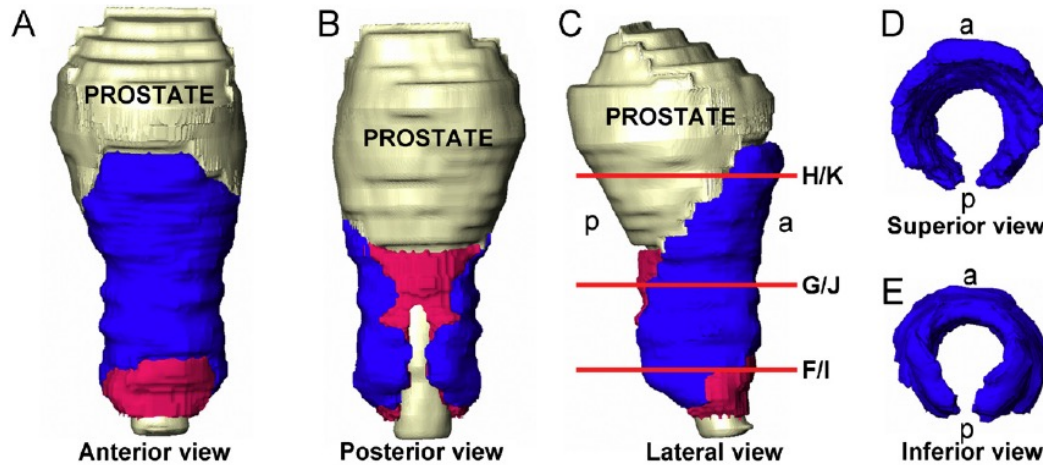


female embryo, 18wks p.g.

target the rhabdosphincter



target the lissosphincter



male embryo, 12wks p.g.

target the connective tissue
improve vascularization

How to Resolve these Issues?

Replace the Injection Needle by a WaterJet Device

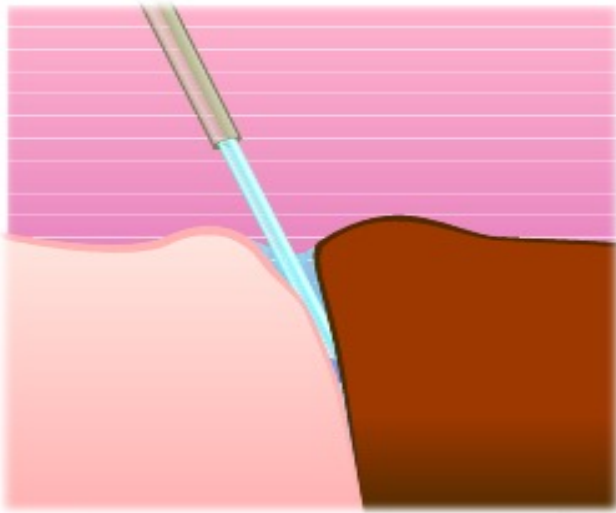
- Define the Energy of the Jet (sc. pressure)
- Define the Form of the Jet (sc. geometrie)
- Define Optimal Transportation Media

Pressure and Geometry of the Jet will Determine the Depth and Distribution of the Cells in the Region of Interest

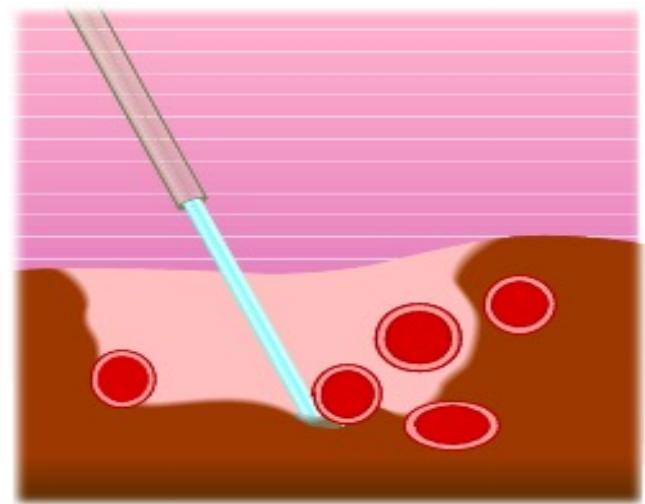
WaterJets in our Everyday Life



Basics of the WaterJet Technology in Medicine

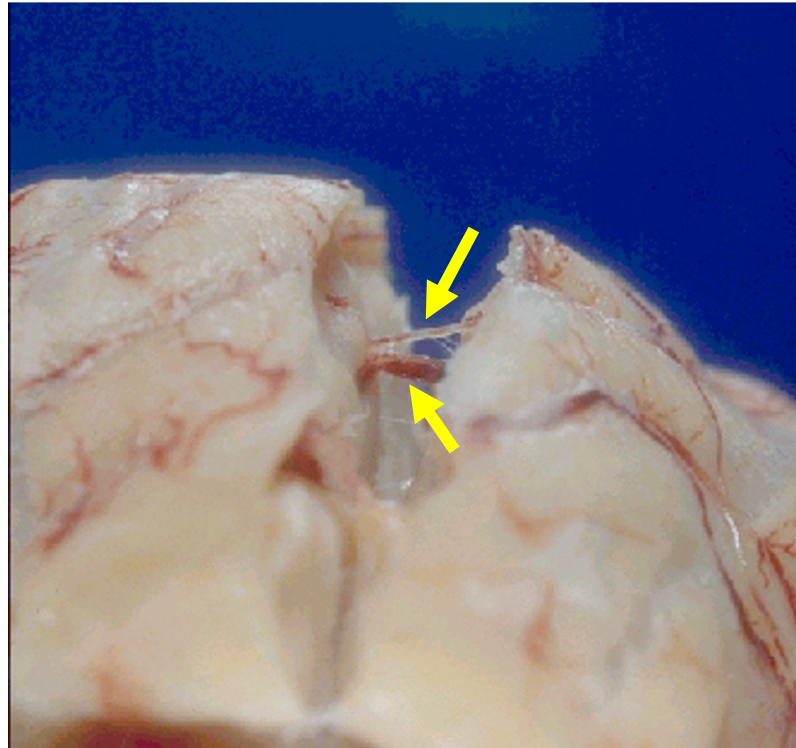


Separate layers of tissue along
density / elasticity borders



Remove soft tissue, spare
blood vessels / nerves

Experimental Cut in Rodent Brain Tissue by the WaterJet Technology

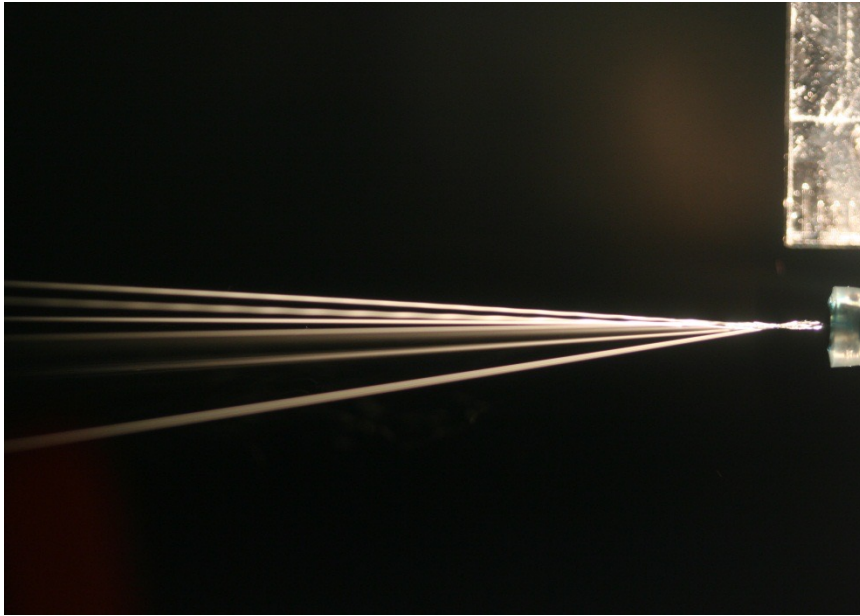


Blood vessel and nerve remain intact

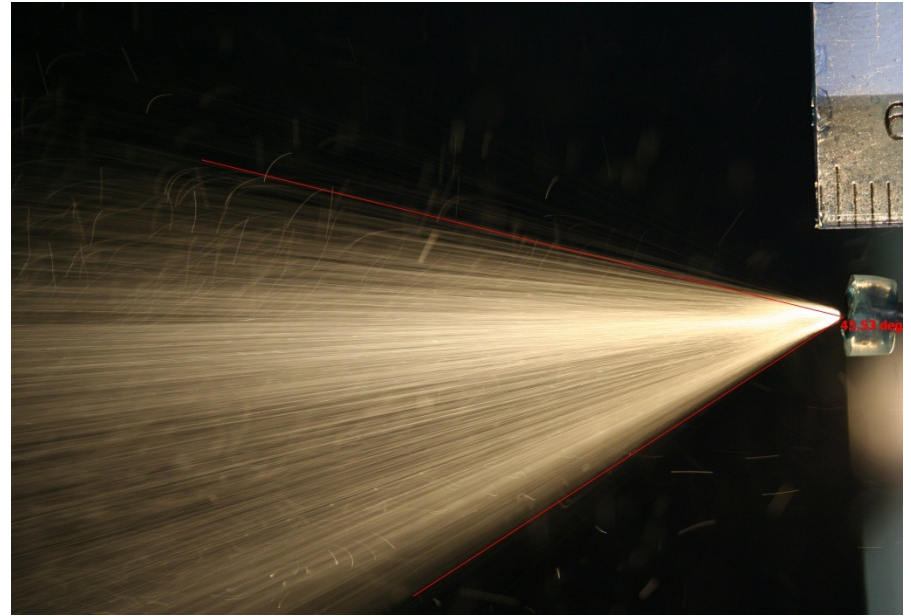
Developing a Multi-Purpose Waterjet Device for both,
Precise Tissue Penetration plus Gentle Cell Transport

a challenge for applied medical technology

Modification of the Jet from Cutting to Application of Biomaterials

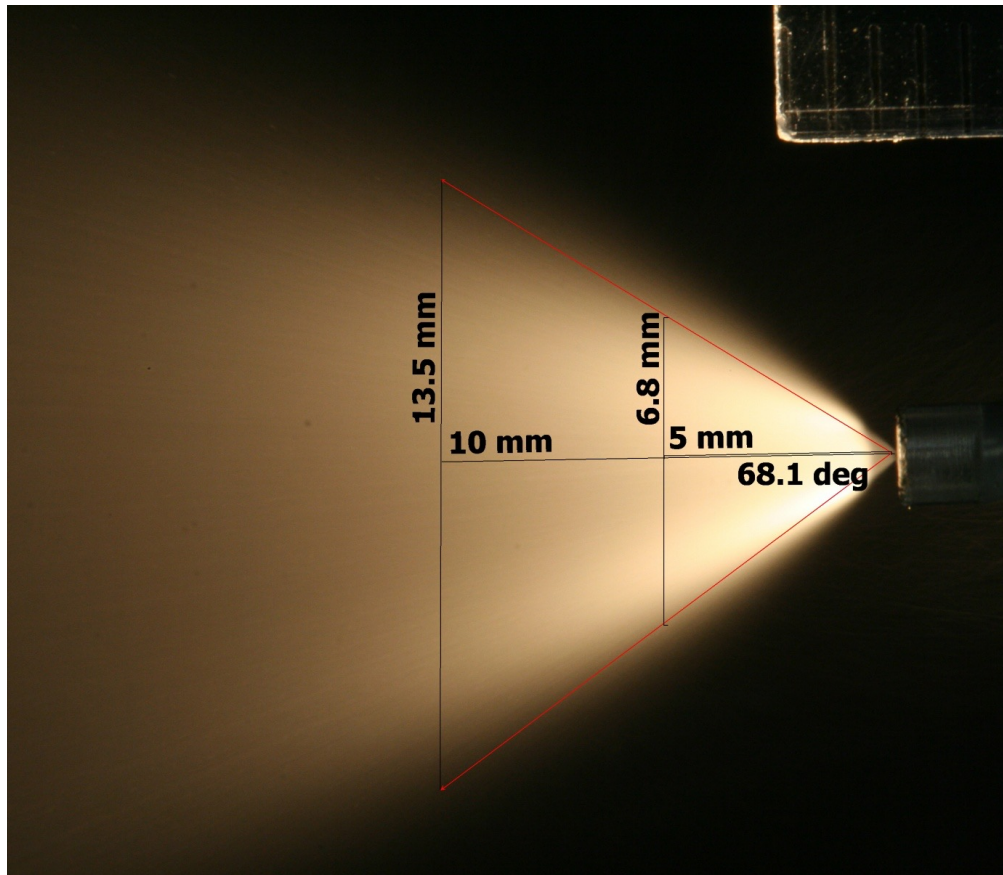


Narrow jet:
for a precise tissue cut



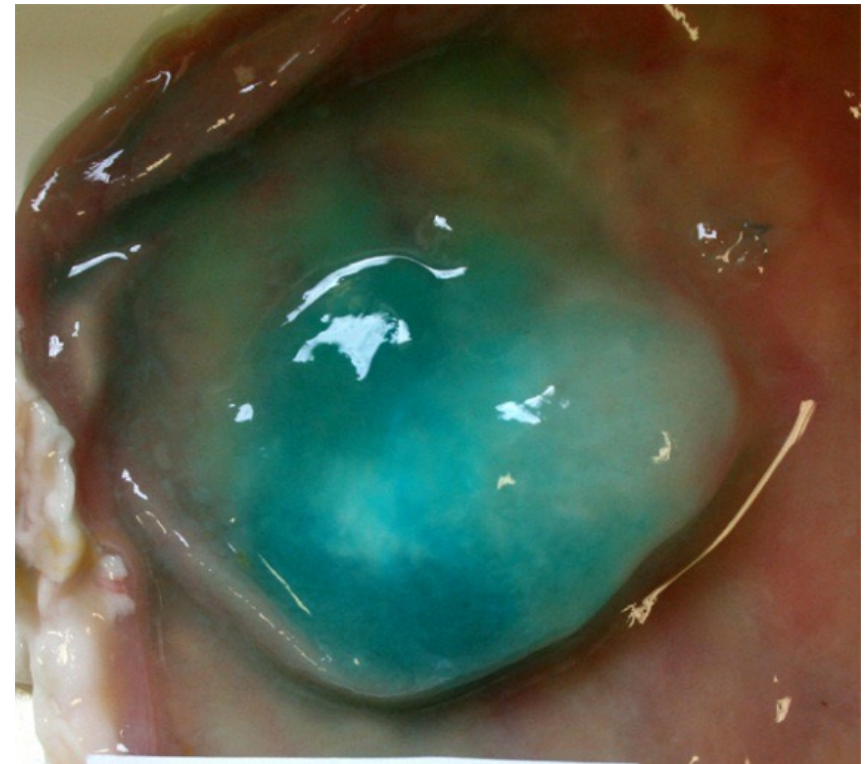
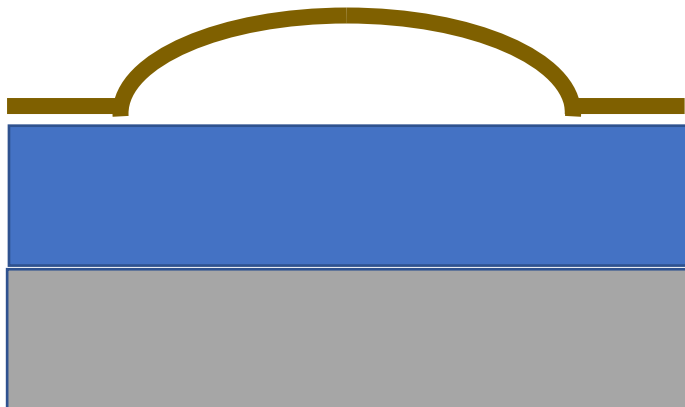
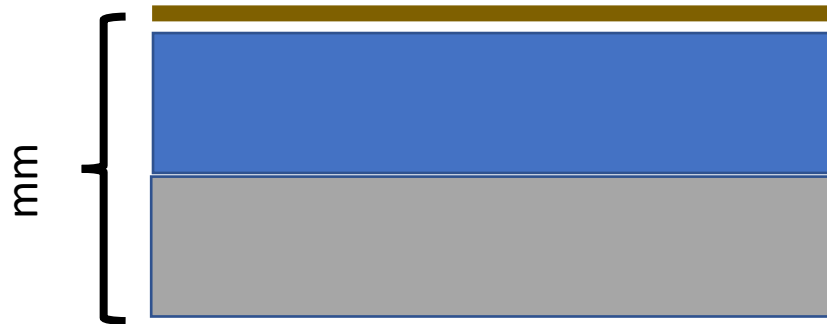
Wide but flat jet:
for application / distribution of active
components on tissue surfaces

Modification of the Jet from Delivery of Biomaterials to Delivery of Viable Cells



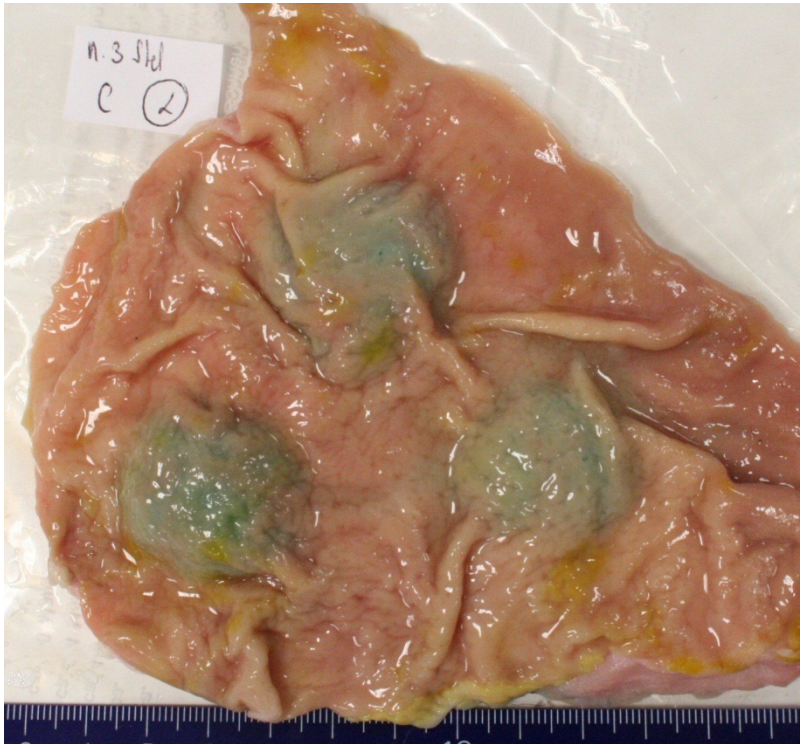
Modification of the Spray Fluid
Breeze by Biomaterial

Injection of Colored Buffer in the Cadaveric GI
Epithelium to Elevate the Area of Surgery and
to Protect the Tissue Underneath



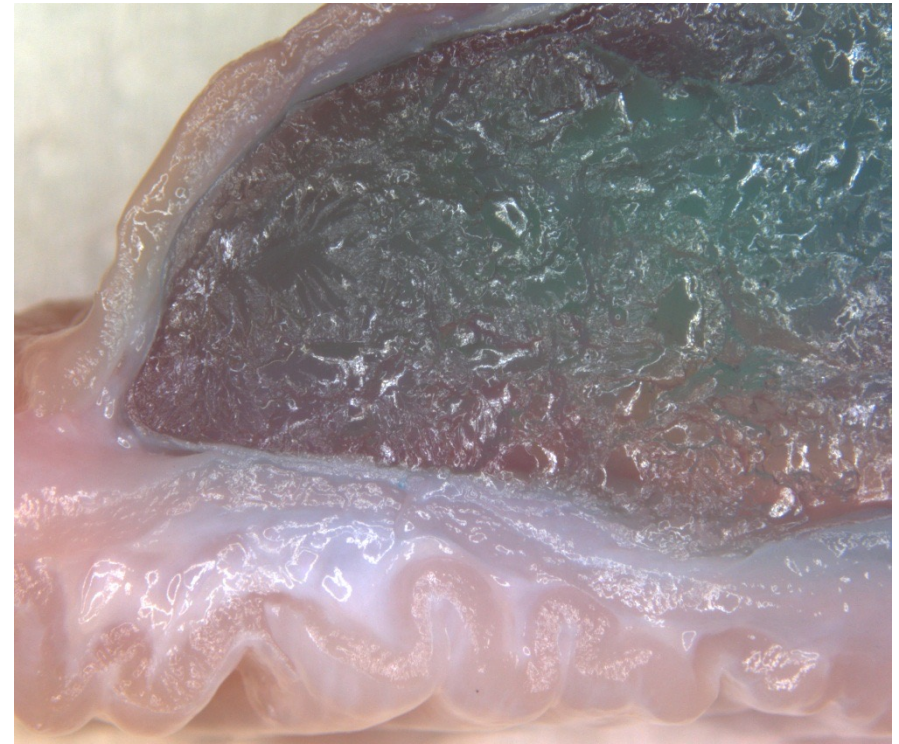
Injection of Active Components under Visual Control on Gastrointestinal Surfaces by an Endoscope-based Minimal Invasive Technique

Investing Injection of Different Self-Hardening Biomaterials for Application in the GI Tract



Top view

Injection of a green colored material in the submucosa (stomach, swine)



Cross-section

Selective enrichment of the injected material in the submucosa (stomach, swine)

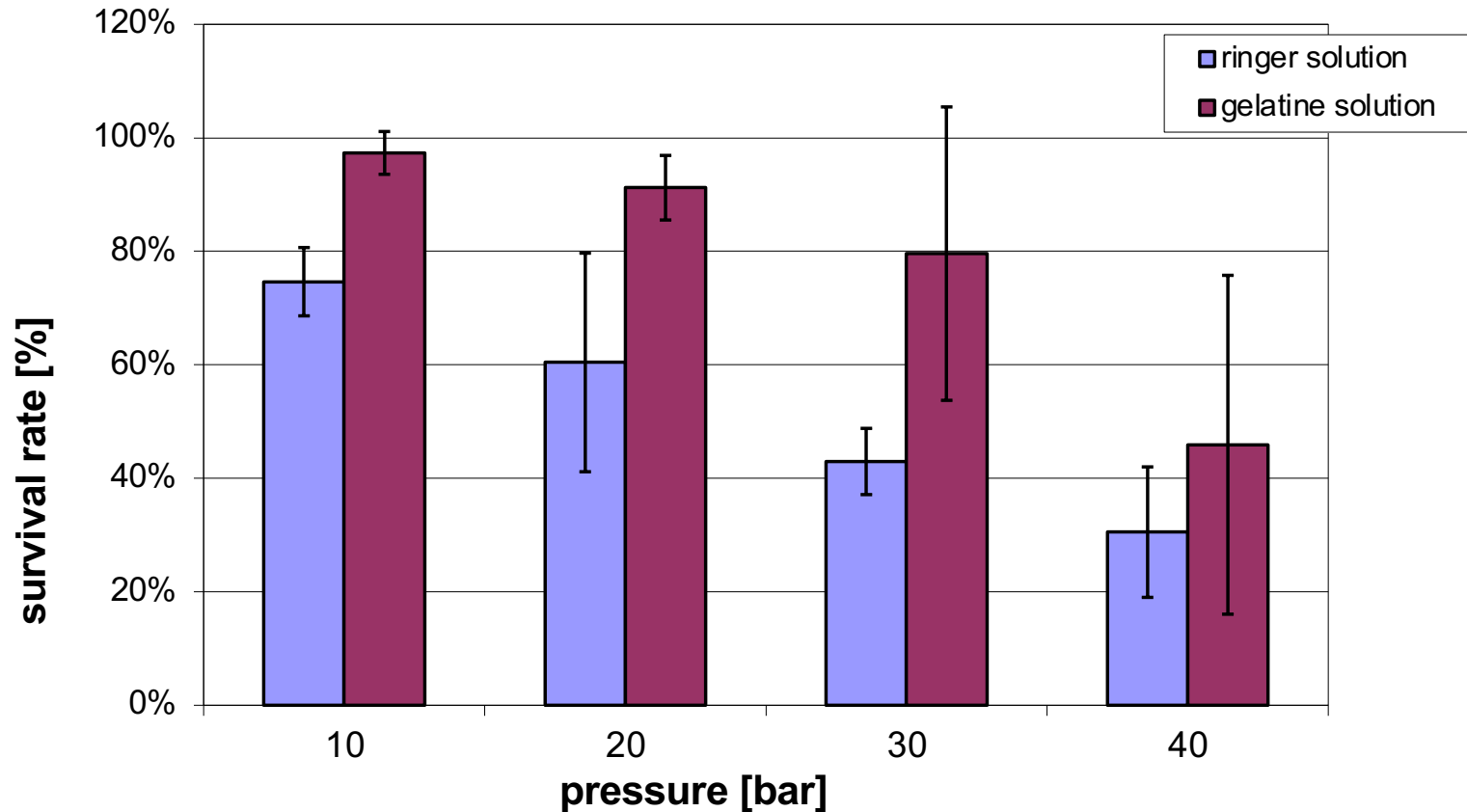
Hypotheses:

We can spray buffers and biomaterials on surfaces and beneath tissue layers

- i) Can we spray cells as well on surfaces by waterjet?
- ii) Can we spray/inject cells between layers of tissues or in a given tissue?

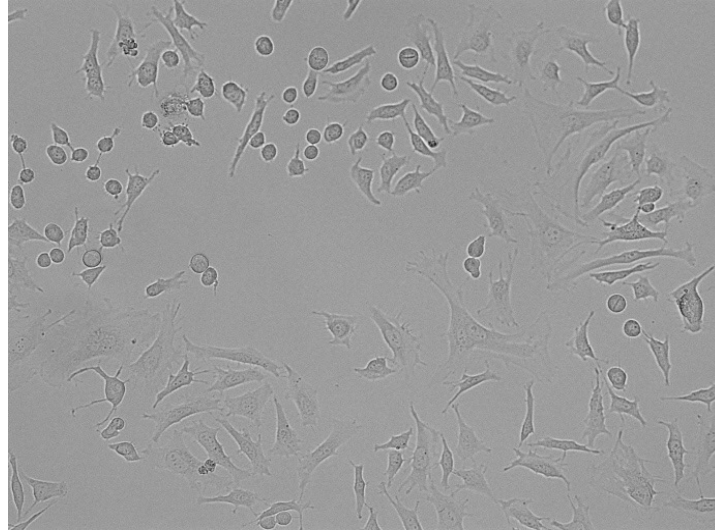
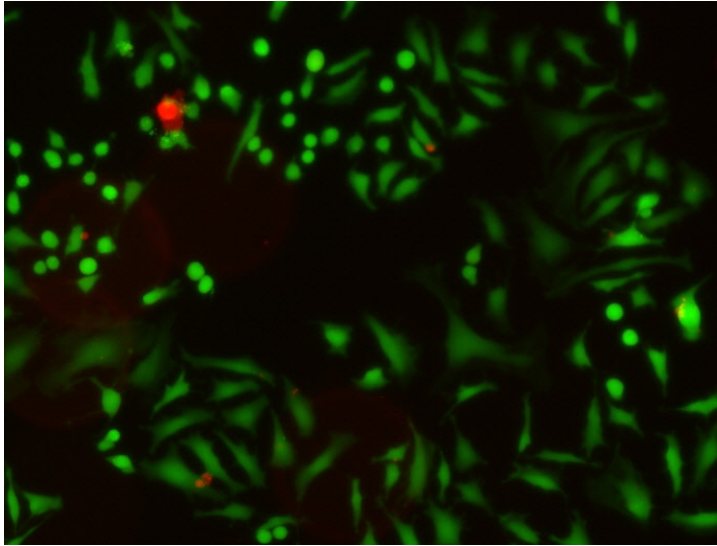
Gelatin – a Clinically Approved Biomaterial Improves Viability of Cells after WJ Injection

Injection of HeLa cells with a **straight GI** WaterJet device

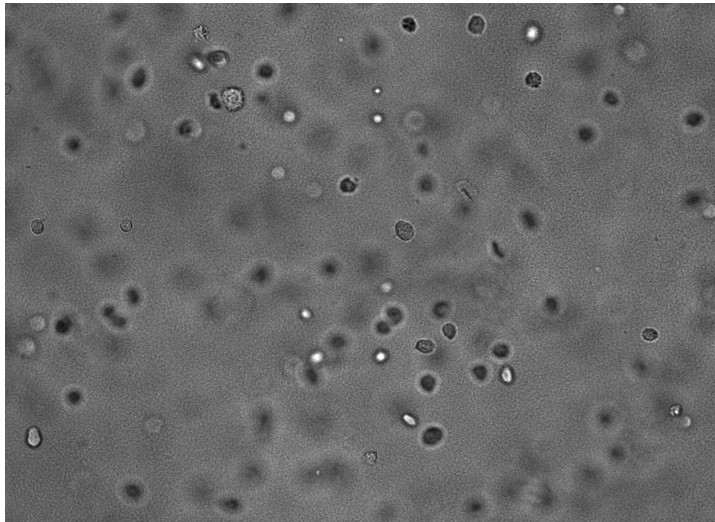
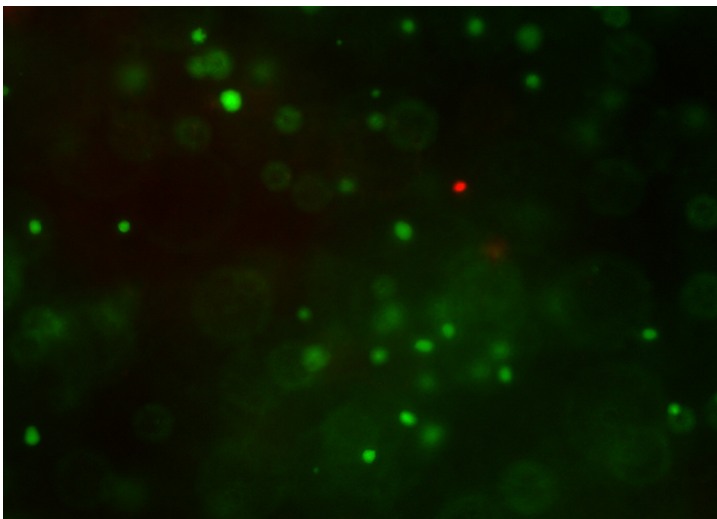


Investigating Fibrin Glue as Carrier for WaterJet Applications

Viability test of HeLa cells with fibrin glue

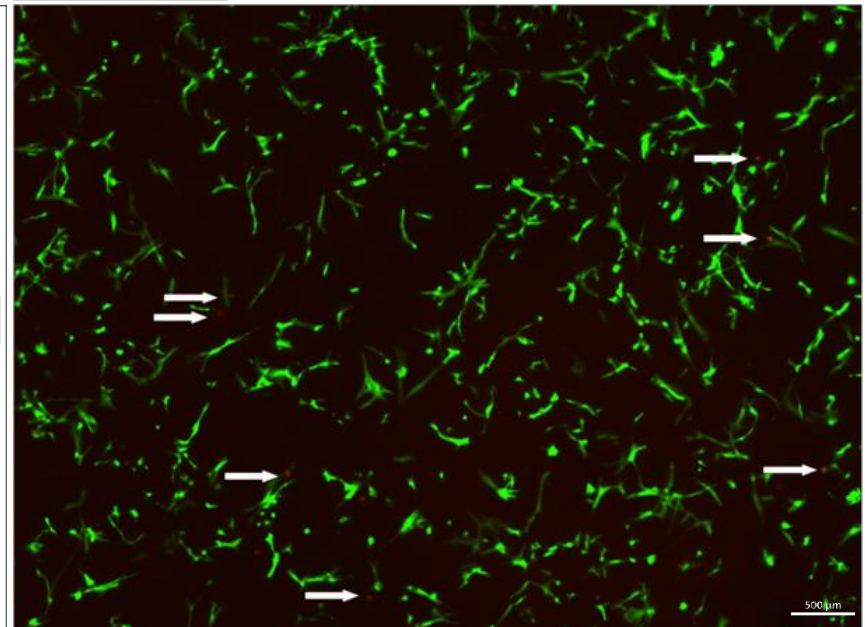
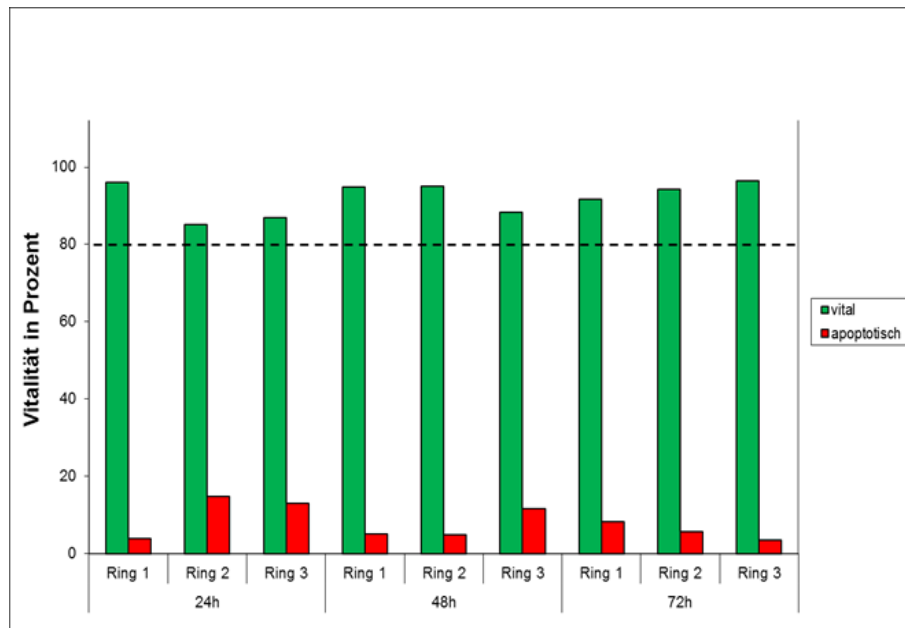


HeLa on fibrin glue



HeLa in fibrin glue

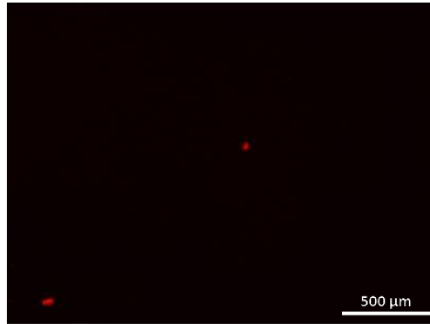
Long-term Survival and Attachment of Human Mesenchymal Stromal Cells (MSCs) upon Injection with Fibrin Glue



Survival of MSCs resuspended in Fibrin Glue and Injected in High Layers of Biomaterial

A

380 μ l (2mm)
Thrombin/Fibrin 1/20

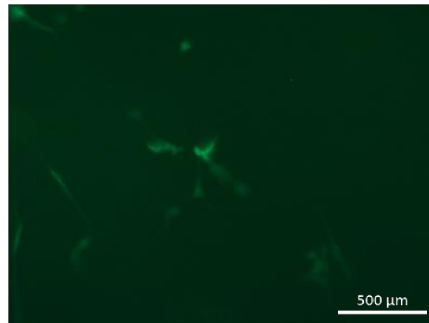
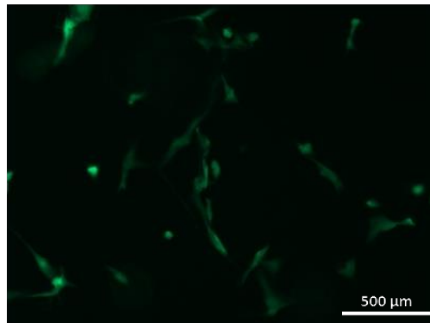
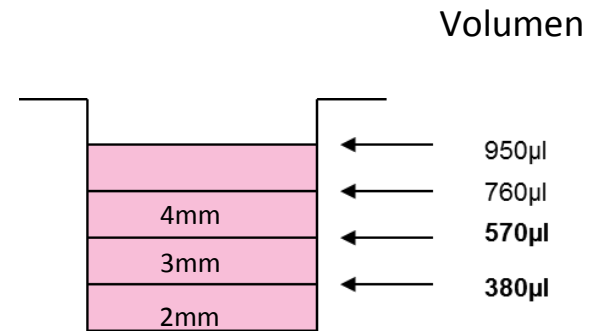


B

570 μ l (3mm)
Thrombin/Fibrin 1/20

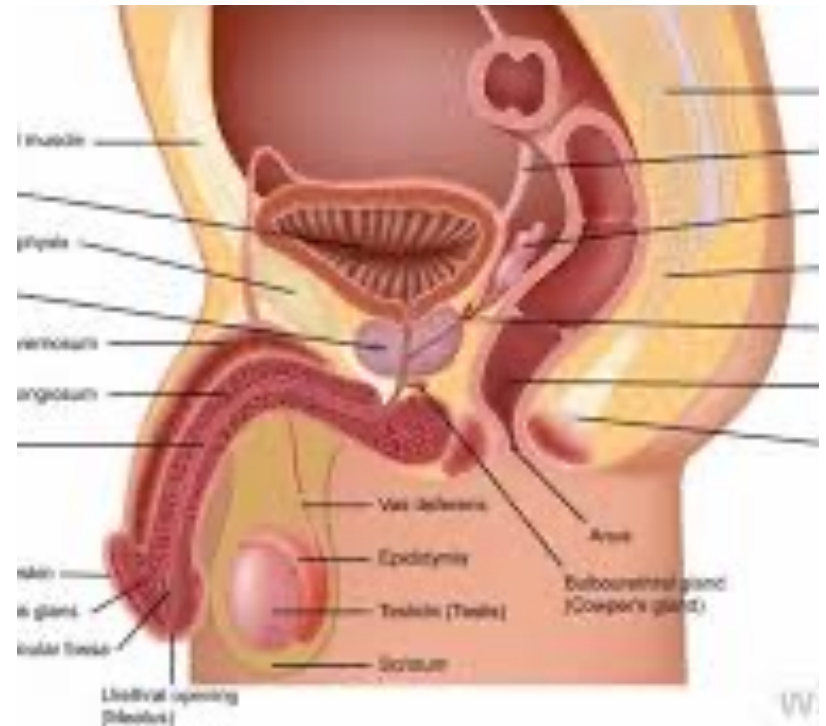
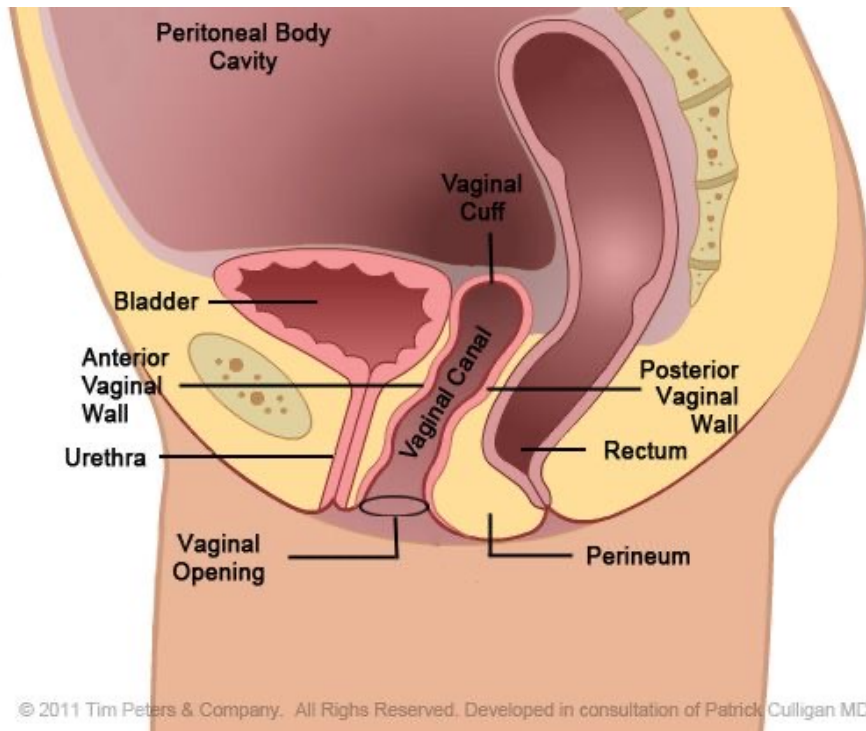


C



Injection of Cells in the Submucosal Tissue of the Female or Male Urethra

The Anatomy of the Human Urethra



Redesign of the Injection Device to Fit an Urological Endoscope

GI Application:

Wide tube

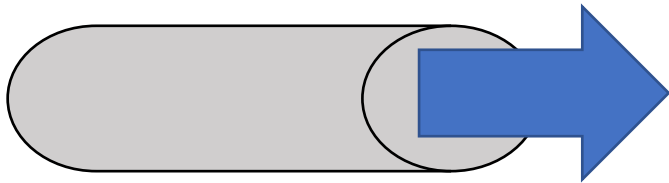
Straight Nozzle

Spray Cells **ON** Surface:

-> Low Pressure

Spray materials **IN** Tissue:

-> Elevated Pressure



Urological Application

Narrow tube

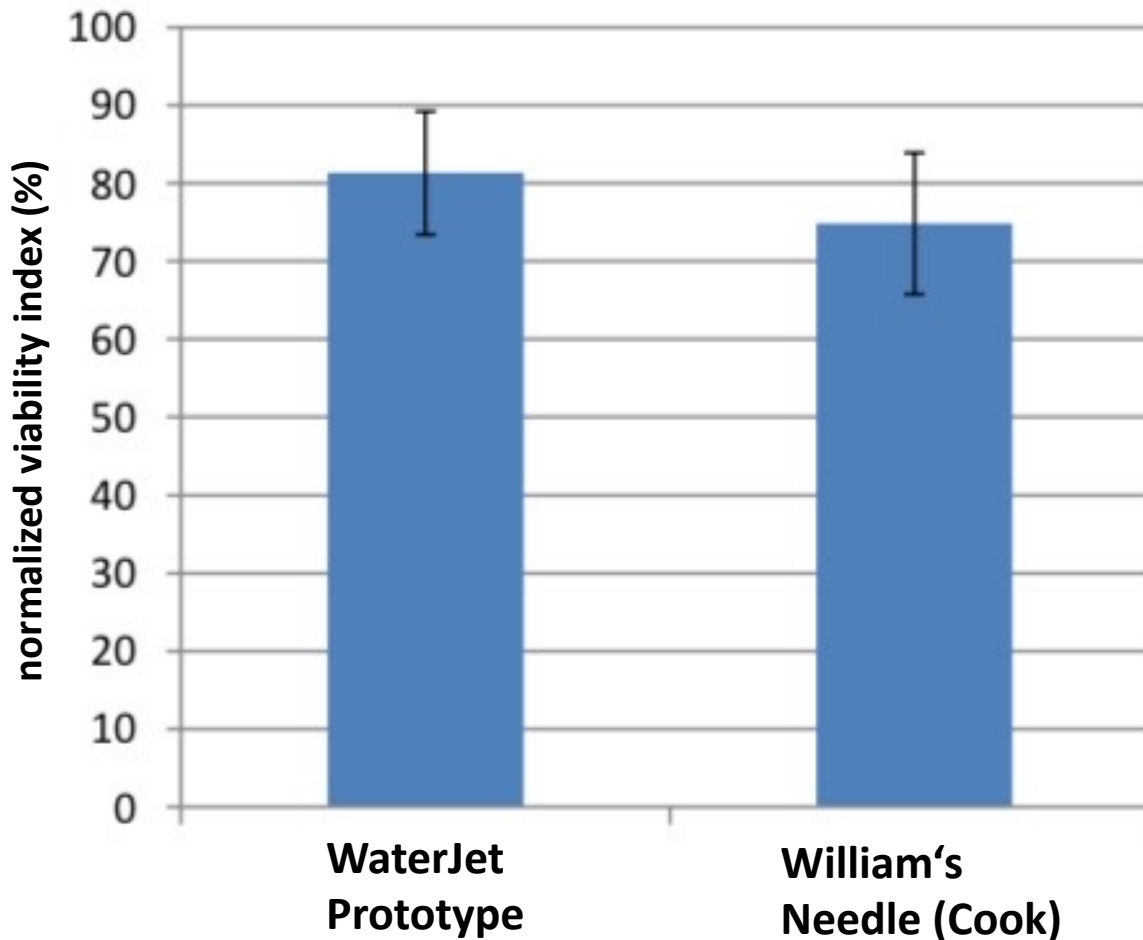
Angular Nozzle

Inject Cells **IN** Tissue

-> Moderately Elevated Pressure

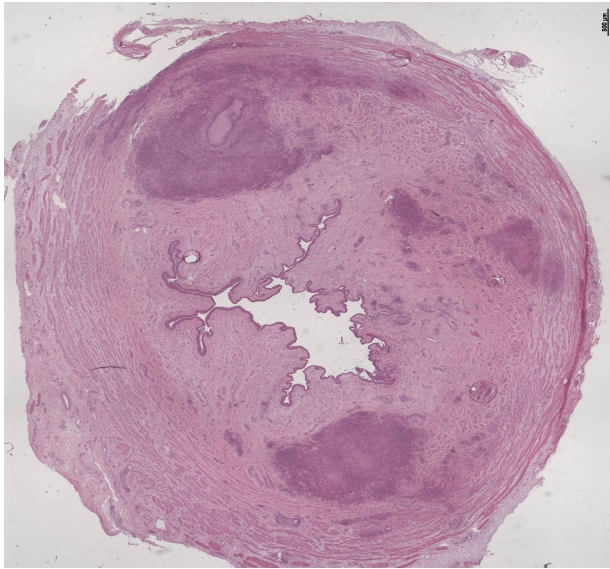


Current Injection Device Delivers MSCs with a Higher Yield of Viable Cells Compared to the Standard William's Needle

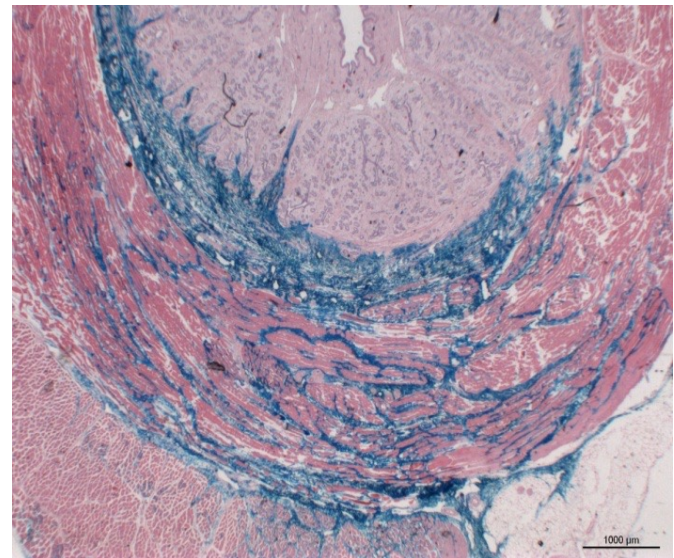


Exploring the WJ injection conditions in cadaveric tissue samples

Adaptation of the *in vitro* Systems to Pre-clinical Animal Models of Incontinence

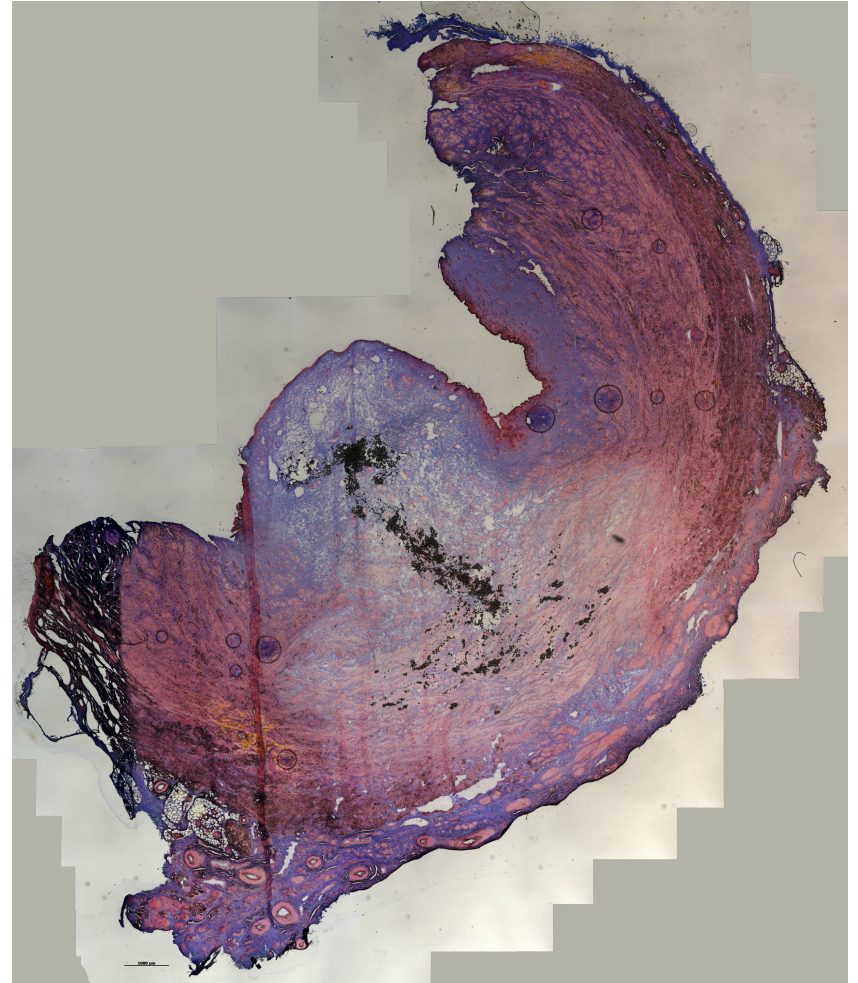
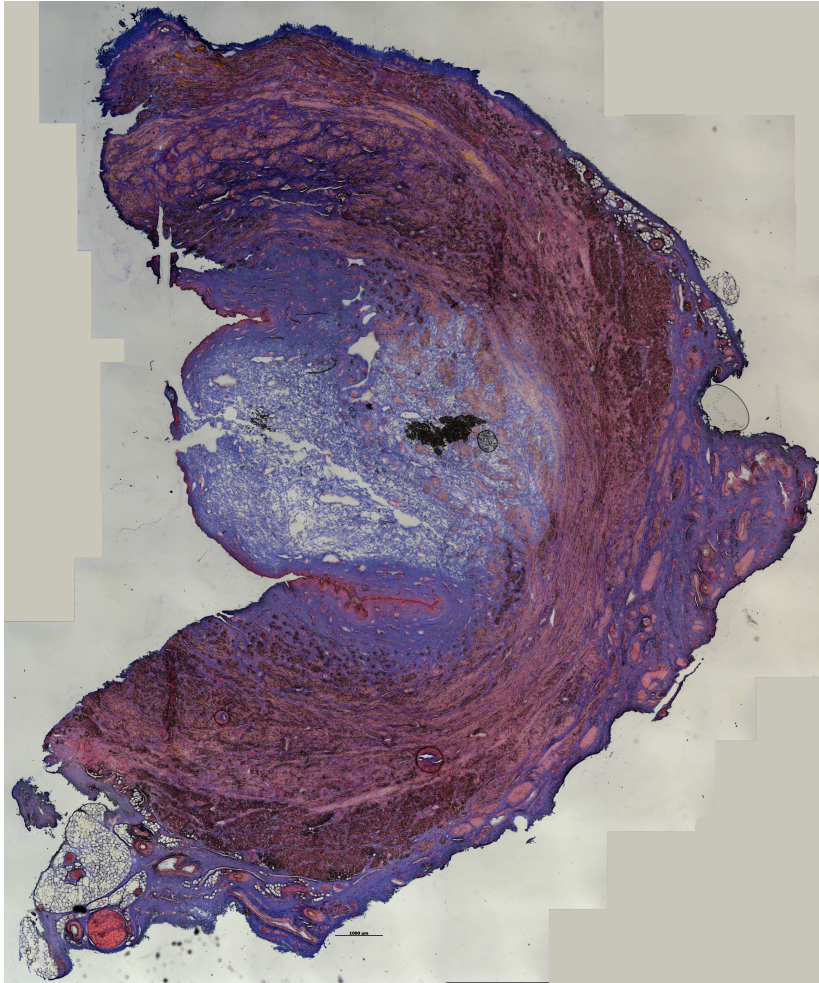


Injection of MSCs
by Needle

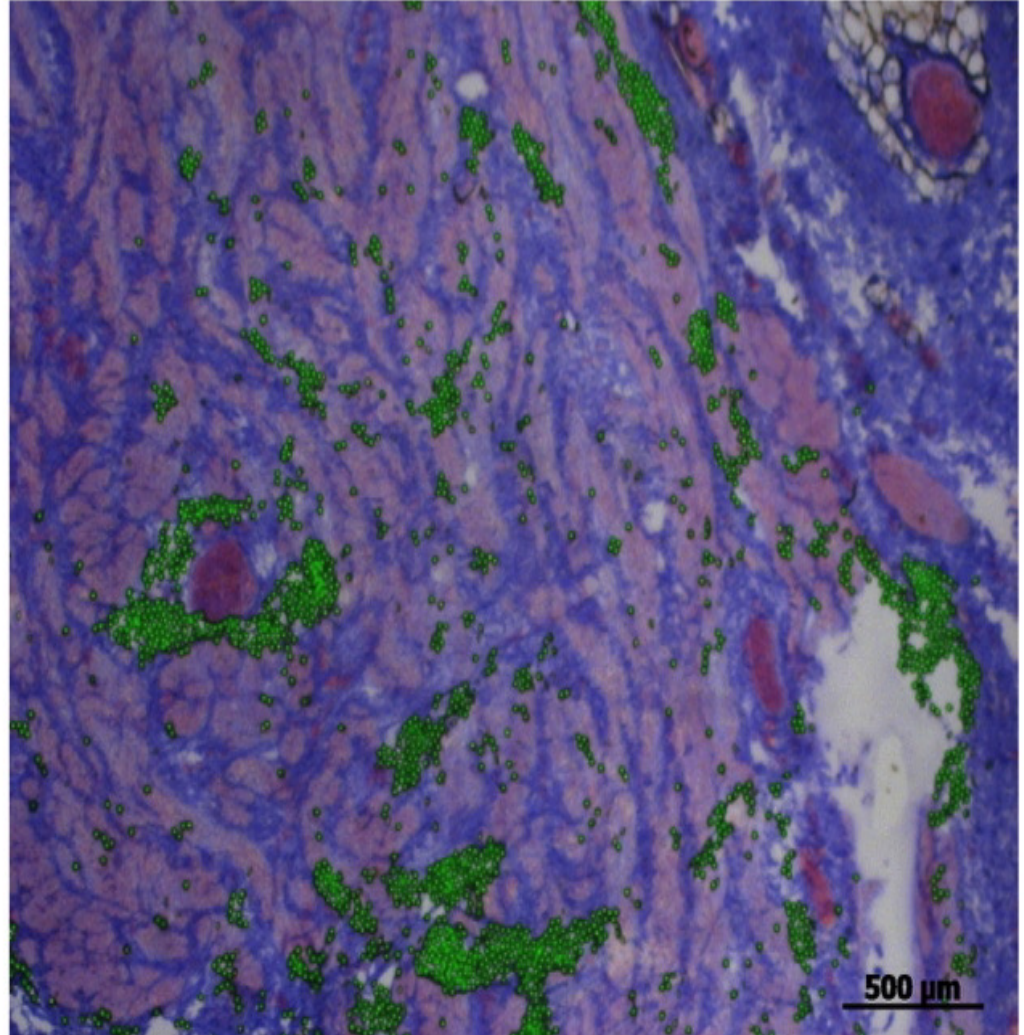
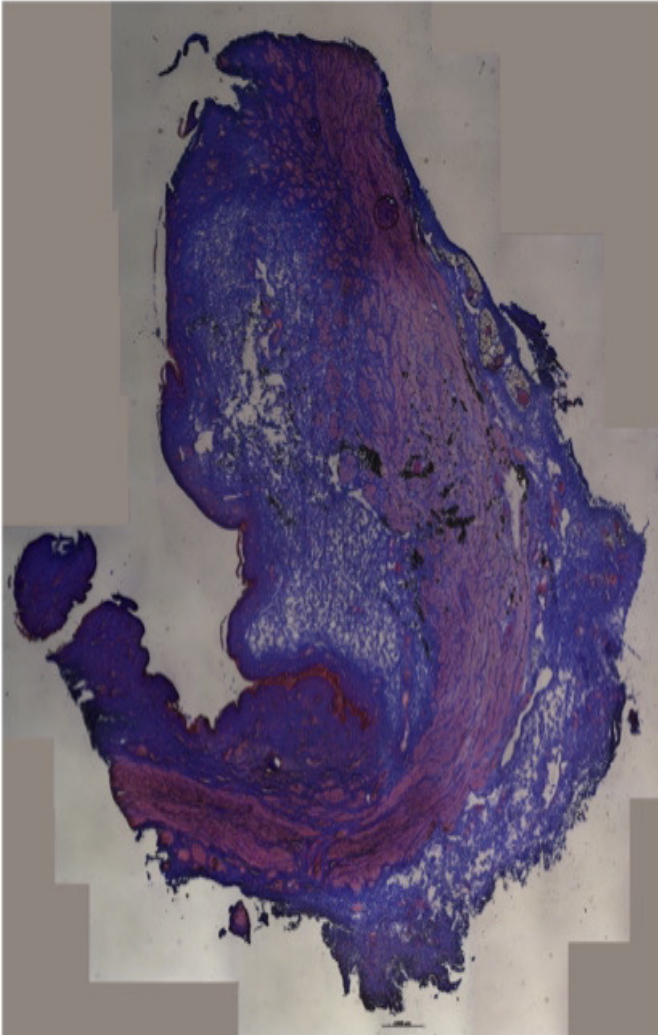


Injection of ink
by WaterJet

Injection of Nanoparticles by Novel WaterJet in Cadaveric Samples of Porcine Urethra



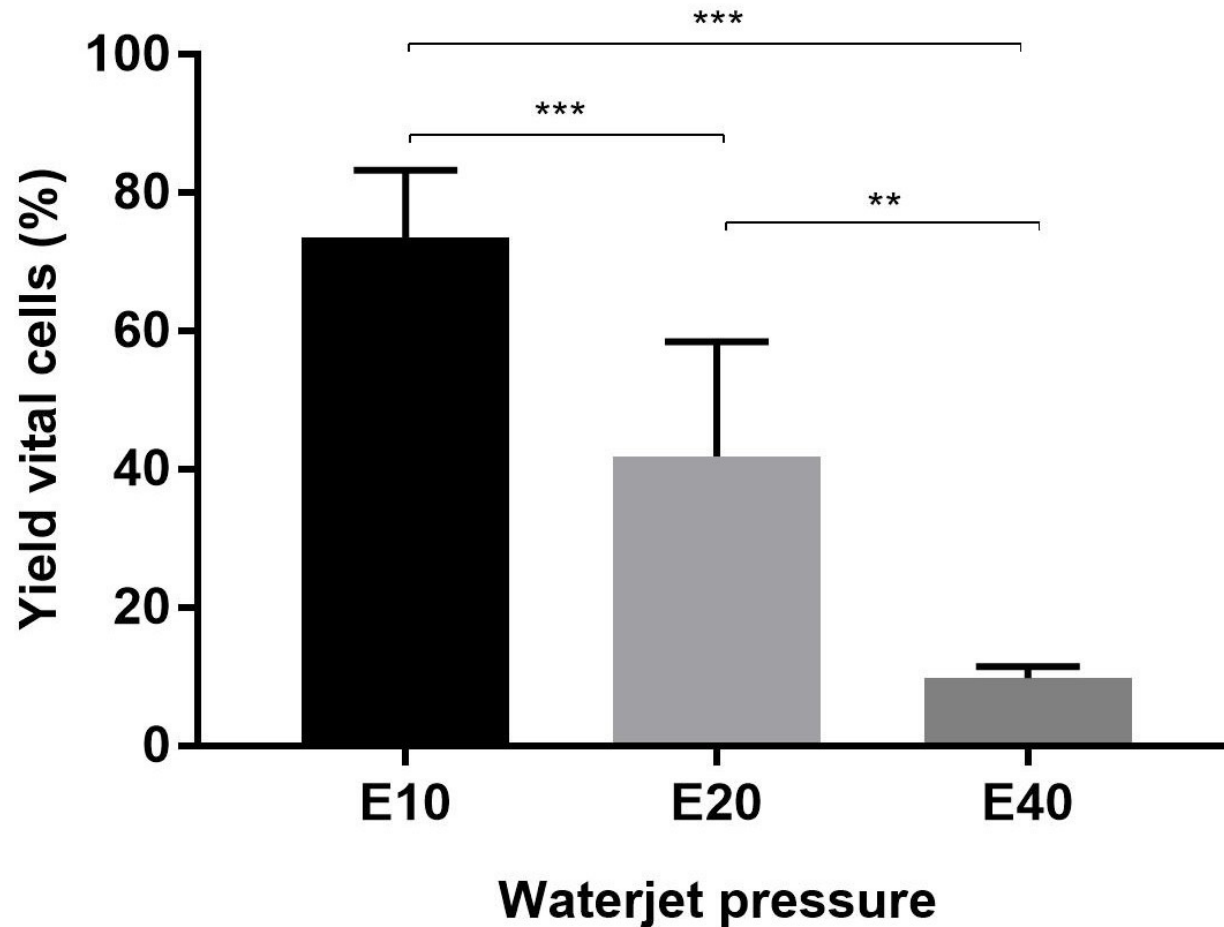
Depth of Injection Depends on the Pressure Applied During WaterJet Application



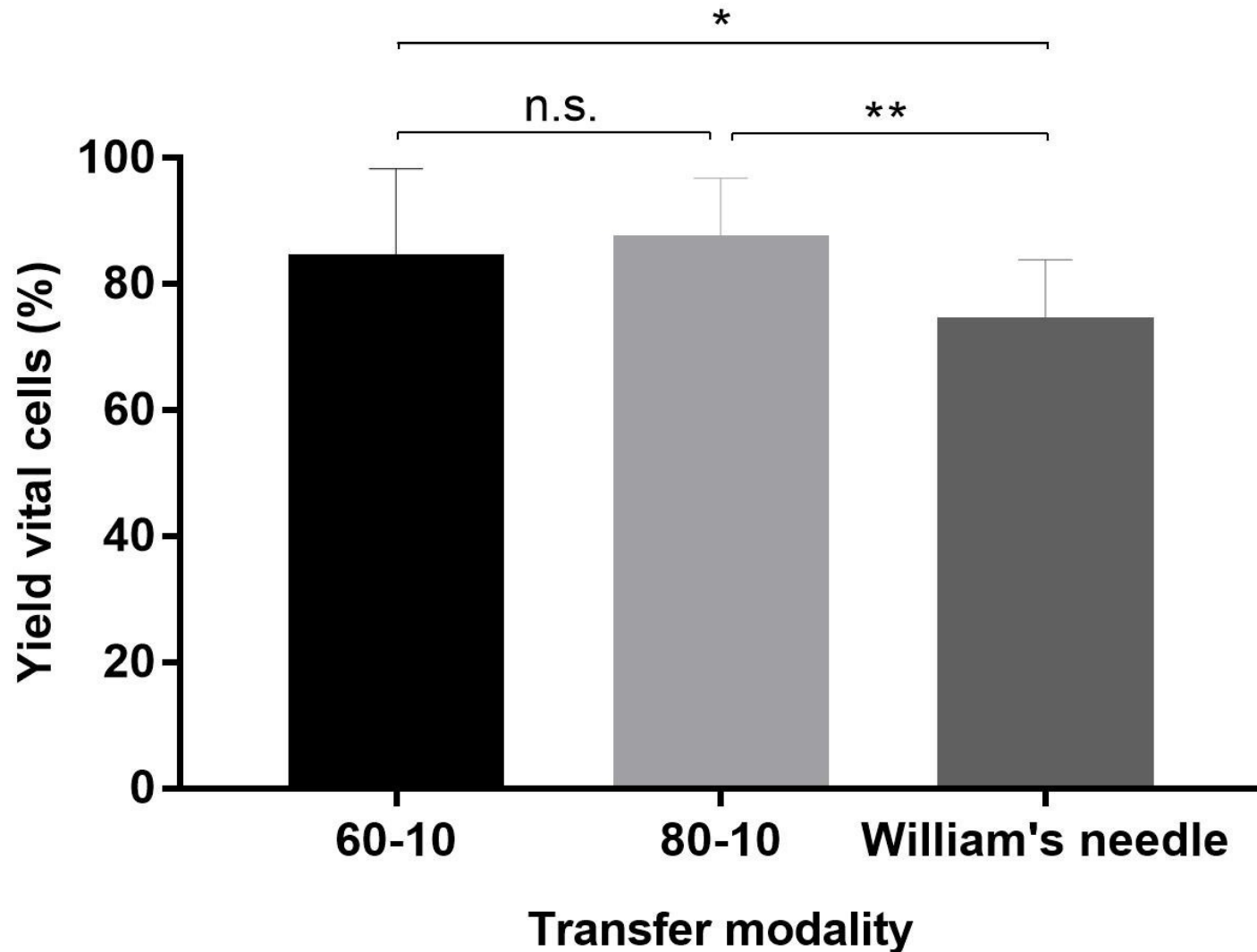
Summary

- Preselection of pressure, volume and transport medium facilitates a precise yet gentle delivery of ink, nano- and microparticles in the submucosa of the urethra
- targetting the mucosa of the sphincter requires pressure levels above E40
- targetting the musculat layer requires even higher pressure levels, approx. E60 – E80

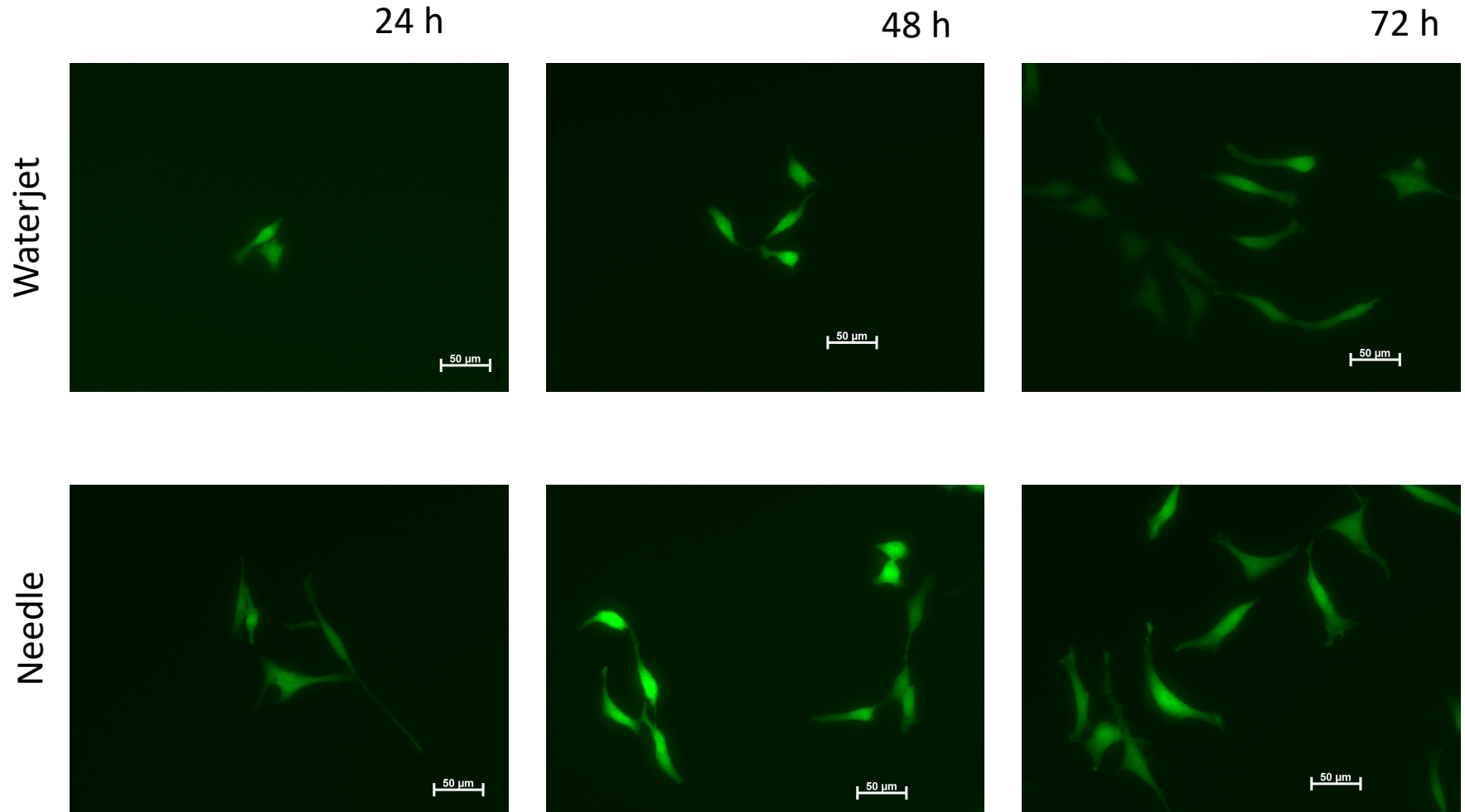
Investigating Cell Viability upon Waterjet Injection at Different Effects / Pressures



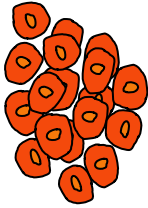
Designing a 2-level Pressure Profile for Controlled Cell Injections in Tissues



Expansion of GFP-expressing HeLa Cells after Injection in Urethral Sphincter Tissue



Going *in vivo*



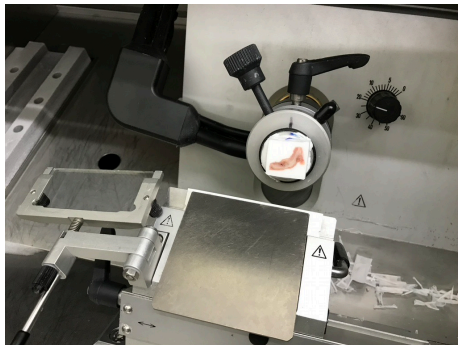
Collect homologous cells
from male littermates



Expand in vitro
Quality management



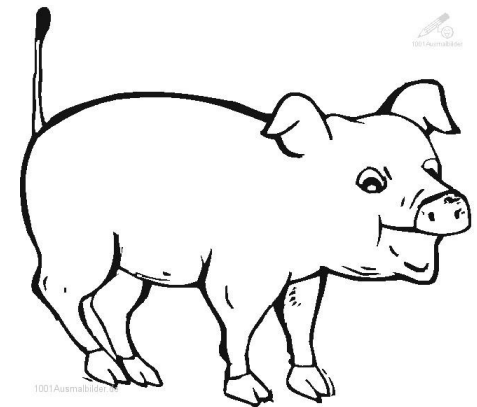
Fluorescence label
male cells



Generate cryosections
from ROI

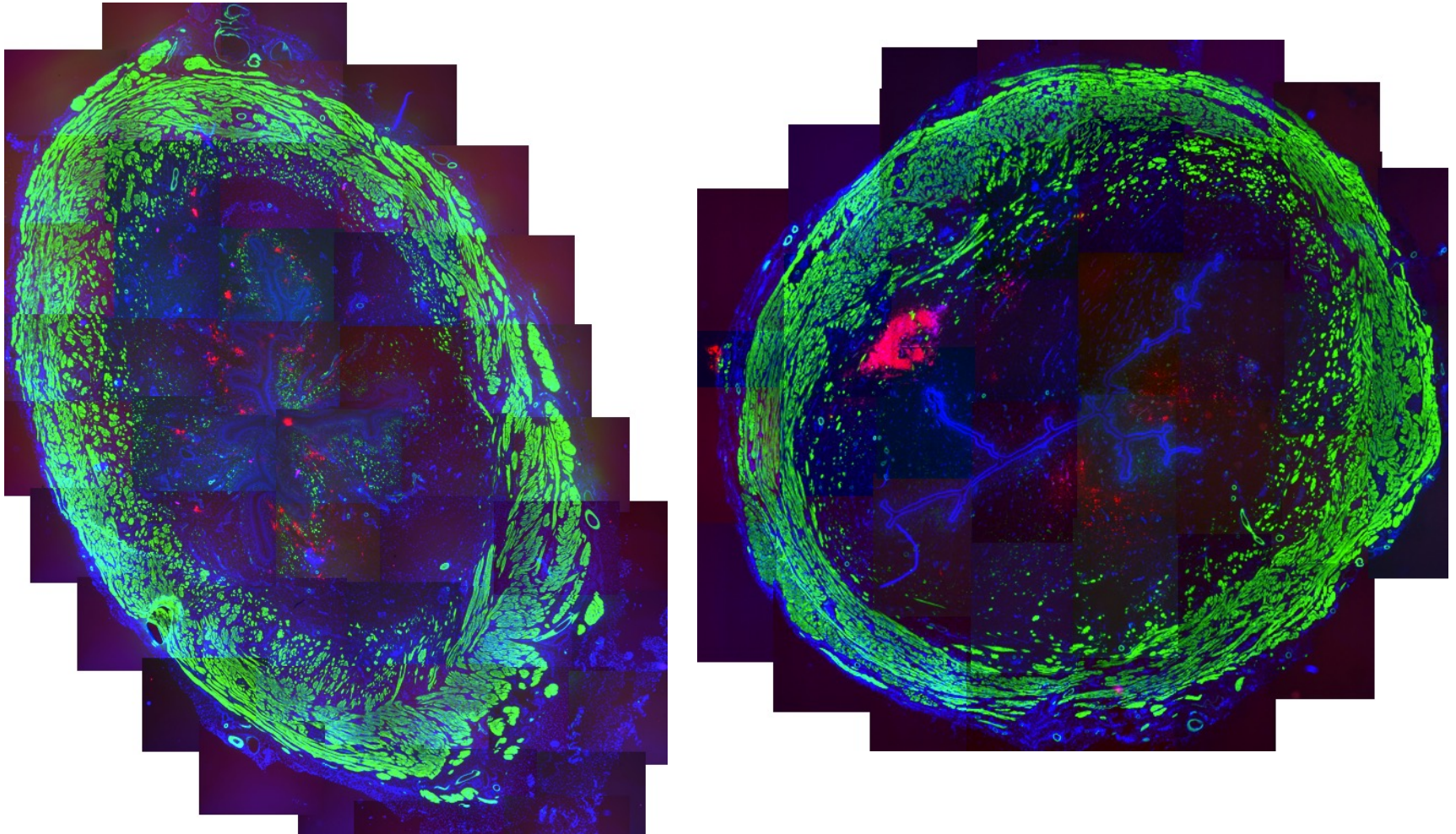


Visualize injected
cells by IVIS

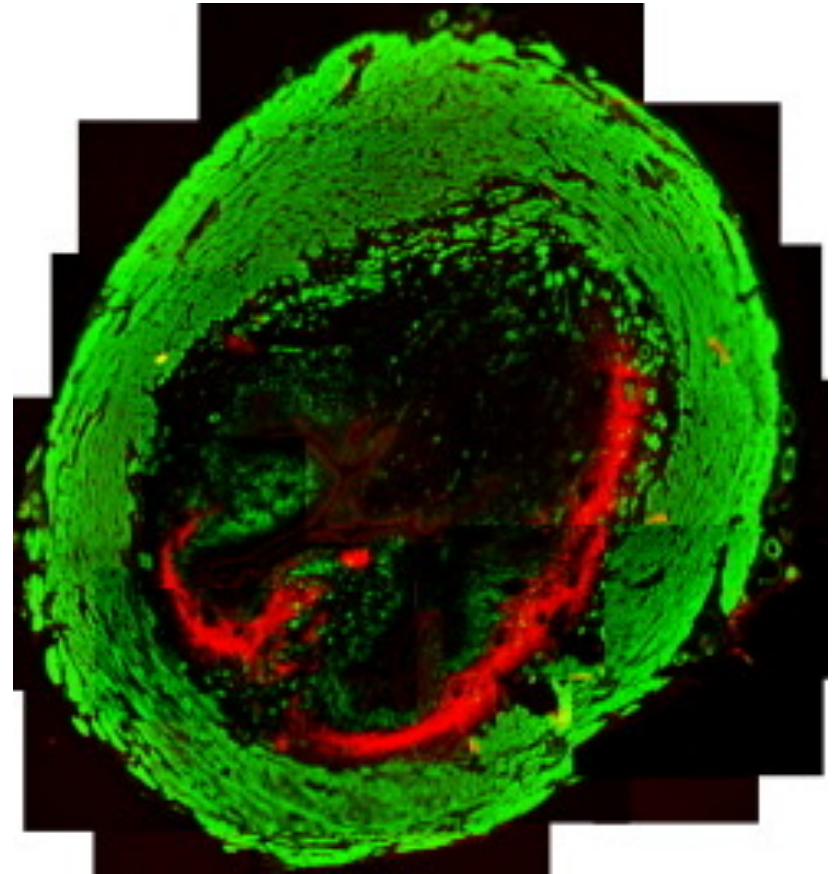
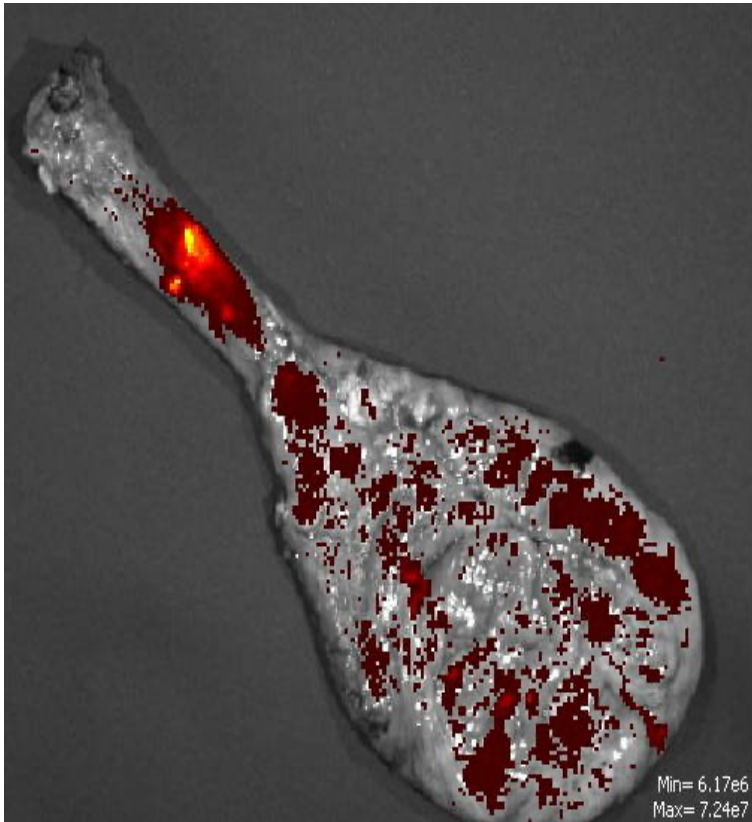


Inject in female
littermates

Detection of Needle-Injected Cells by Immunofluorescence



Detection of Waterjet-Injected Cells by IVIS and Immunofluorescence



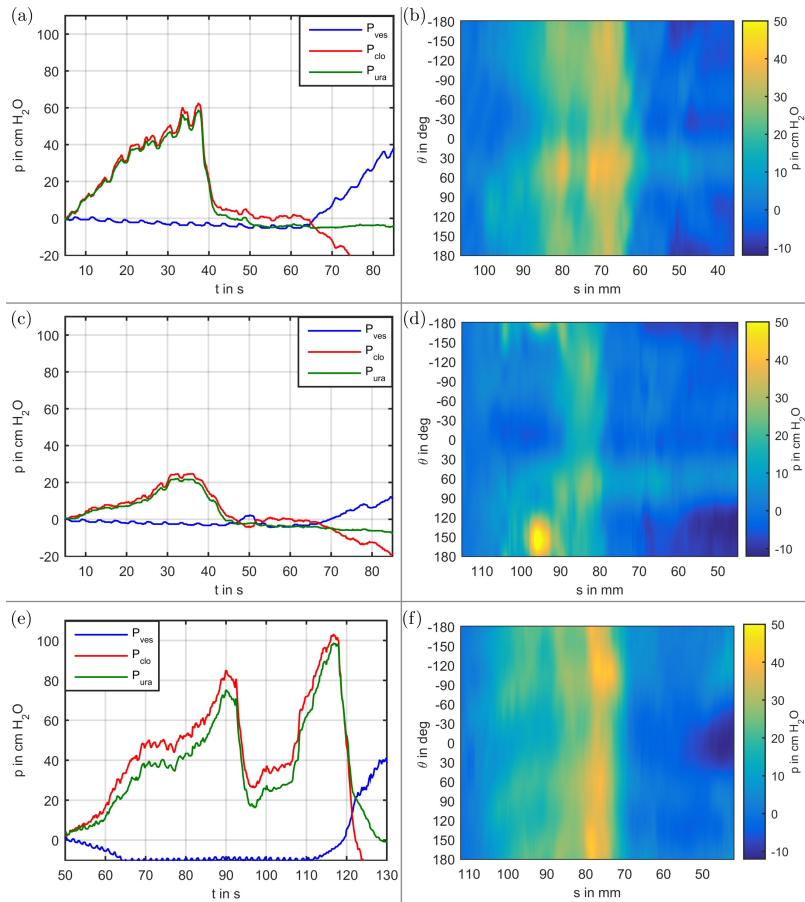
Summary:

The novel WJ technology opens possibilities for fast and precise transurethral cell injections.

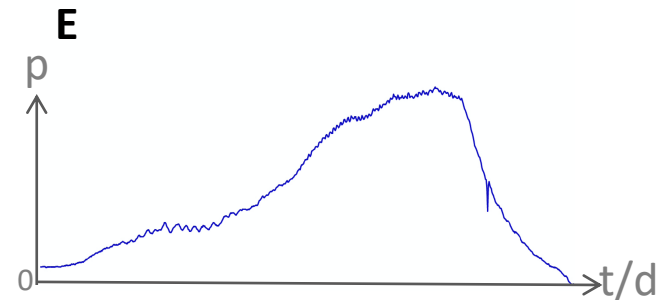
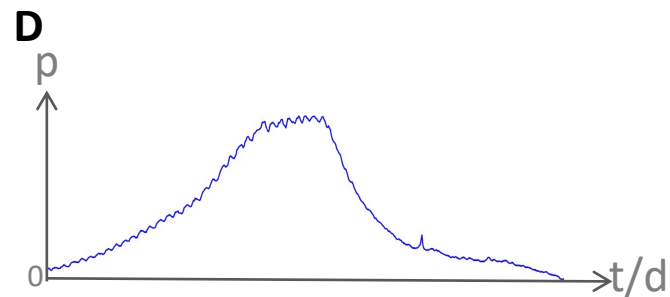
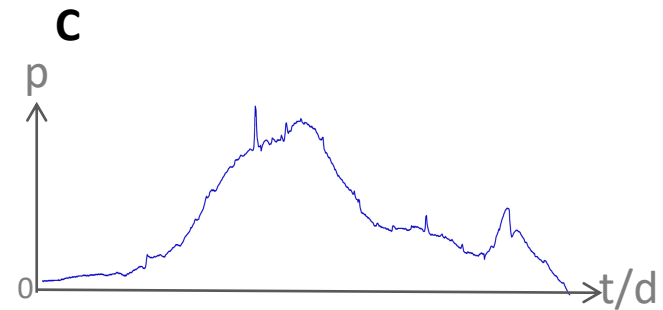
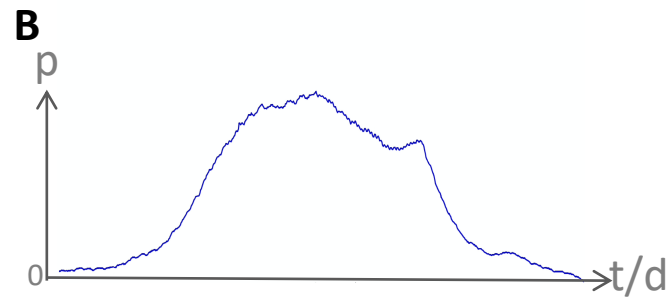
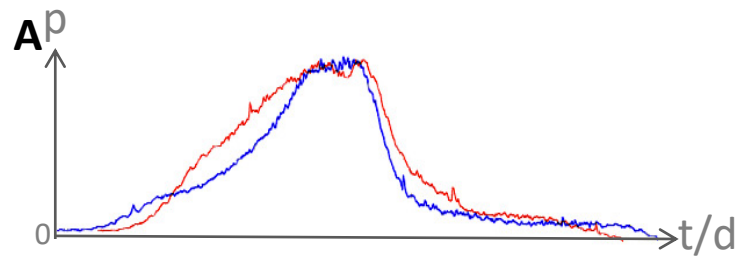
The WJ grants equal or better viability of the cells after injection and most important:

The WJ yields better distribution in the tissue targeted.

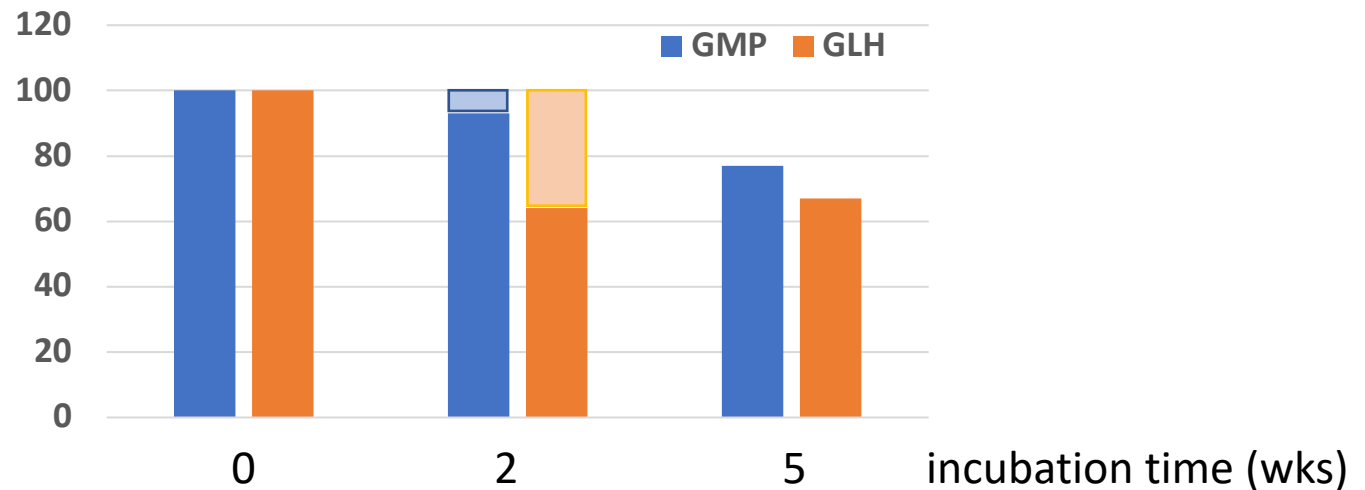
The Animal Model of Stress Urinary Incontinence



Localization of the Position of the Bladder-controlling Muscle in Pigs by Urodynamics



Developing an Incontinence Model to Investigate Functional Recovery by Injection of Cells in the Urethra in Göttingen Minipigs vs. German Landrace Hybrids



Results

Transient sphincter deficiency can be established

Efficacy of sphincter deficiency and time period available for testing cell therapy should be improved

Efficacy on incontinence induction and kinetics of recovery differ significantly between the two porcine races investigated

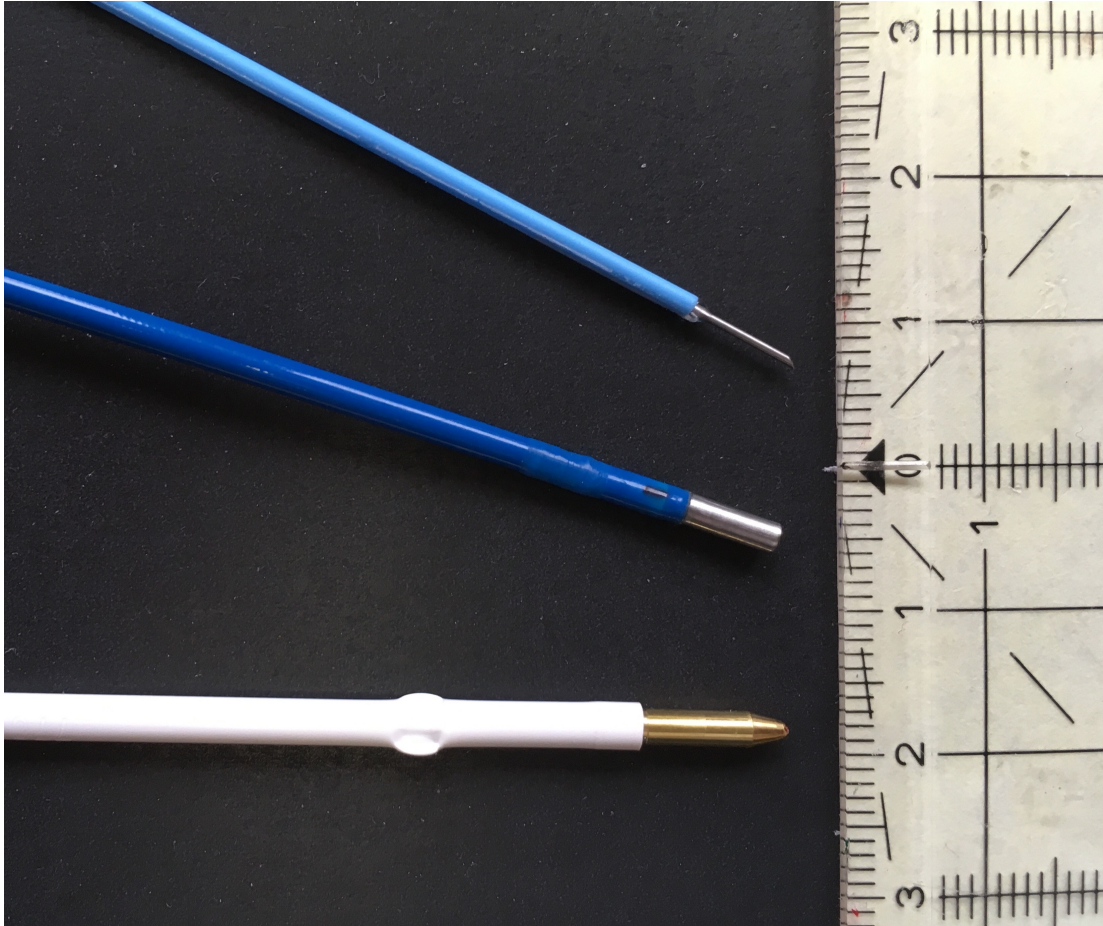
-> this variability of results may contribute to the inconsistency reported in the literature as well

Current state of the art and future perspectives:

- Preclinical Study (ADSCs / healthy pig) finished in summer 2019
- Preclinical Study (MPCs / healthy pig) closed in summer 2021
- Current DFG project:
Use an animal with sphincter deficiency and investigate if cells regenerate sphincter function after needle injection - and if so - which cells repair SUI best (ends 2022)

Apply protocols to the new waterjet injection technology
employing pigs with sphincter deficiency

Combine the SUI Large Animal Model with *two* Cell Injection Protocols

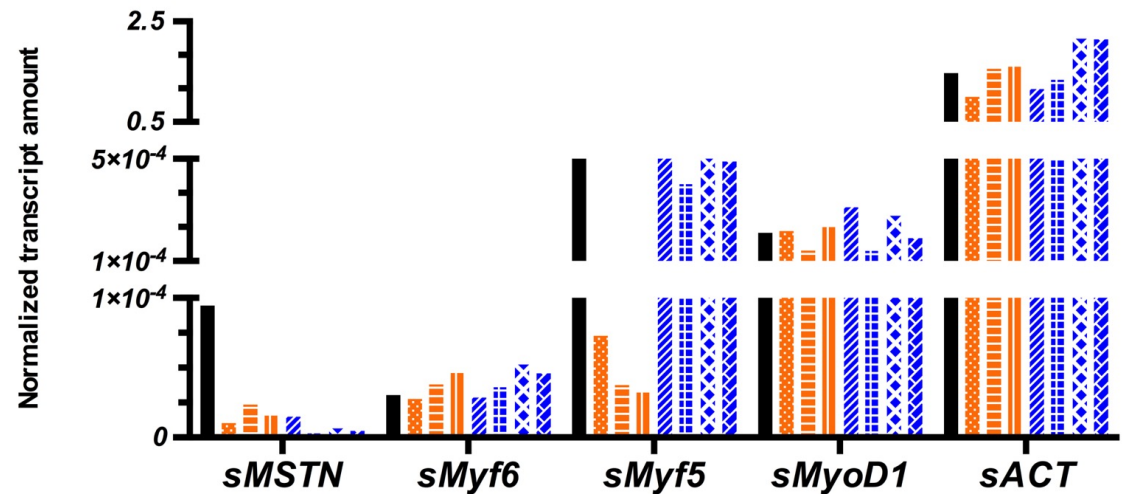
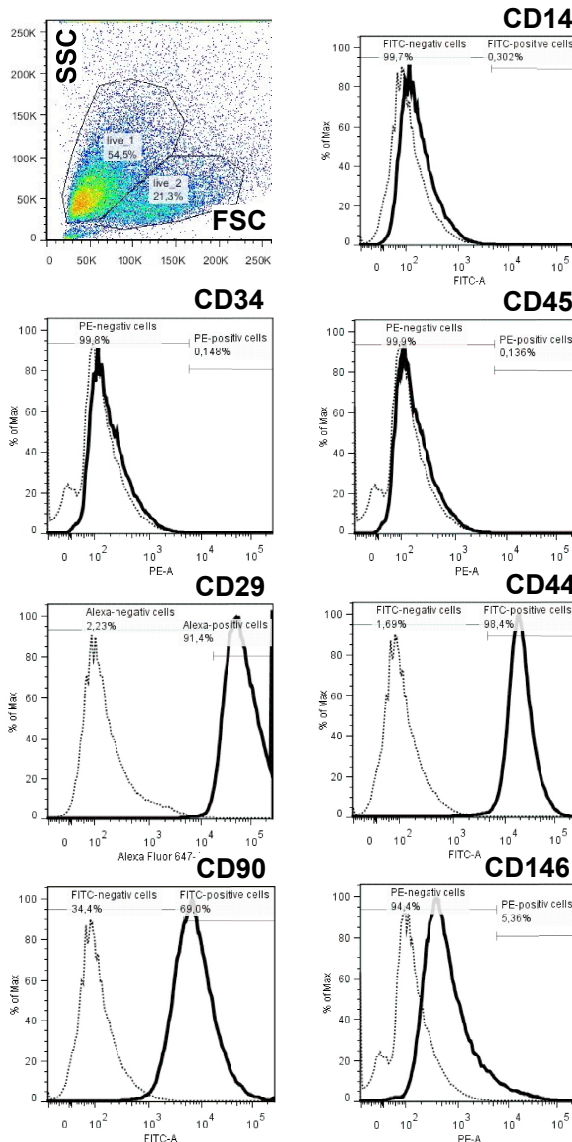


Transurethral Cell Injection by:

Williams Needle

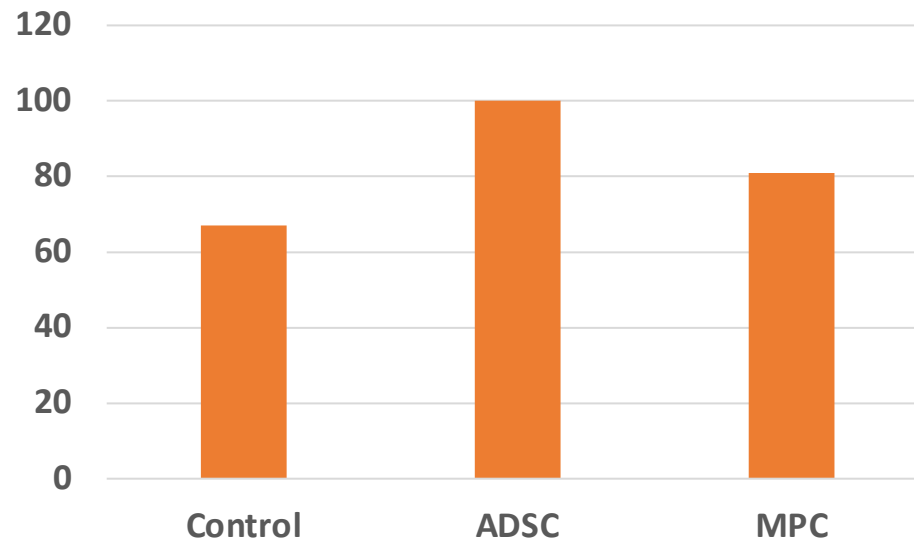
Waterjet Device

Characterise the Regenerative Cells – ADSCs vs. MPCs - Prior to Injection



- Newborn WT myoblast
- Newborn t.g. myoblast 25
- Newborn t.g. myoblast 26
- Newborn t.g. myoblast 27
- Adult t.g. myoblast 28
- Adult t.g. myoblast 29
- Adult t.g. myoblast 29R
- Adult t.g. myoblast 30

Compare Head-to-Head Sphincter Regeneration by Porcine Mesenchymal Stromal Cells vs. Myoblasts in the SUI Animal Model after Needle Injection



Compare Head-to-Head Sphincter Regeneration by Porcine Mesenchymal Stromal Cells vs. Myoblasts in the SUI Animal Model after WJ Injection

DFG grant Ai16/29-1, project starts Oct. 2022

Preliminary Results

Loss of cells in incontinent pigs upon needle injection observed at frequencies comparable to healthy animals

ADSCs seem to regenerate in the model applied better when compared to equal amounts of MPCs

Quality of MPCs must be improved to possibly facilitate the sphincter regeneration



Questions?