



Minimally Invasive Transport of Cells To Remote Sites of Patients

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Disclosure:

Data are in part the results of a collaboration with Erbe Electromedizin GmbH Tübingen. The projects have been are are supportes in part by grants from the BMBF and from the EU, and in part from the institutional budget.

Prof. Aicher has no other conflict of interests to report

Overview

Urinary incontinence. The clinical situation and challenges

Lessons from pre-clinical studies

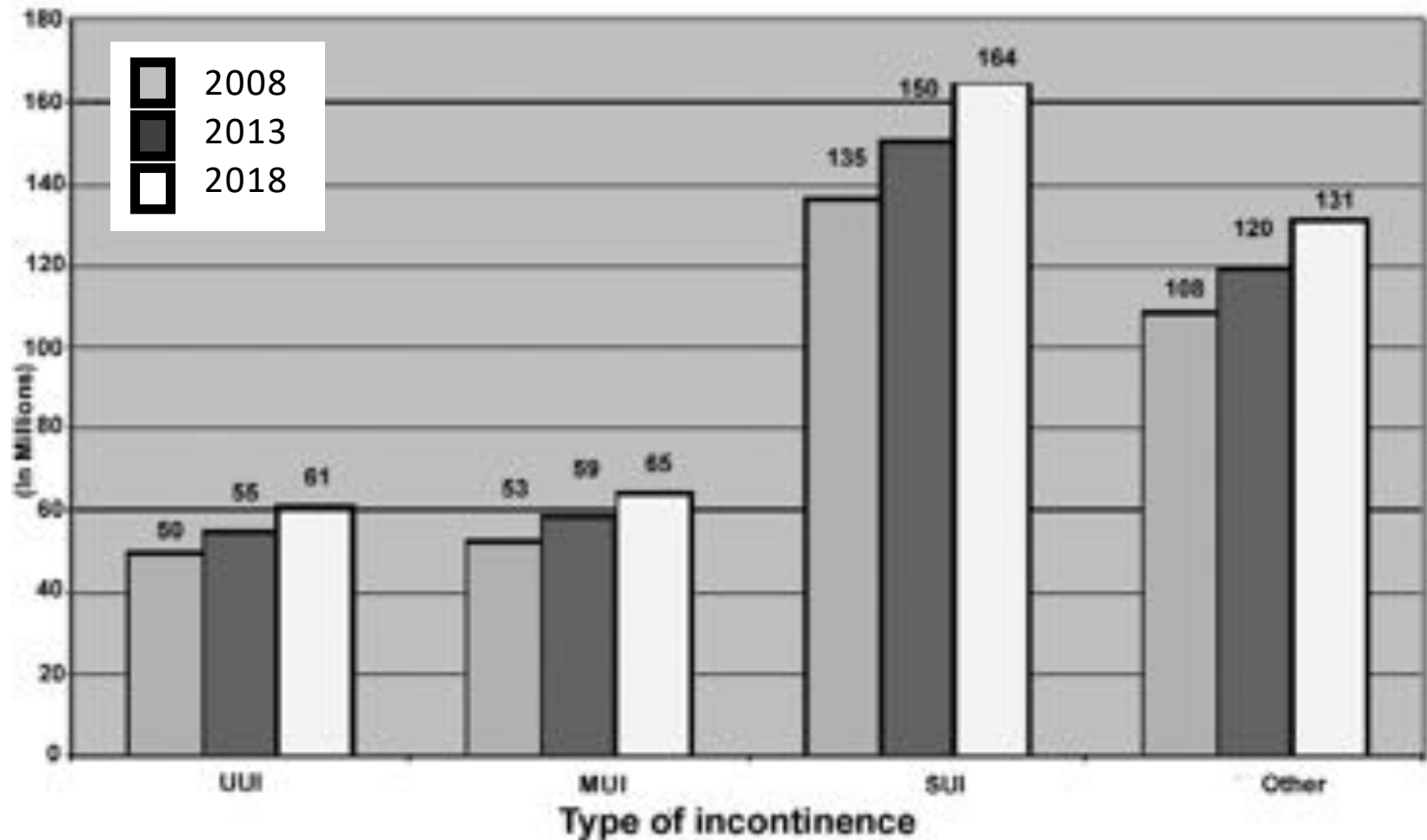
Principles of the waterjet technology

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

Future perspectives

The clinical situation: Prevalence of Urinary Incontinence in Western Countries



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

Risc Factors to Become Incontinent

Men:

Prostate surgery

Women:

Pregnancy

Childbirth

Both Gender

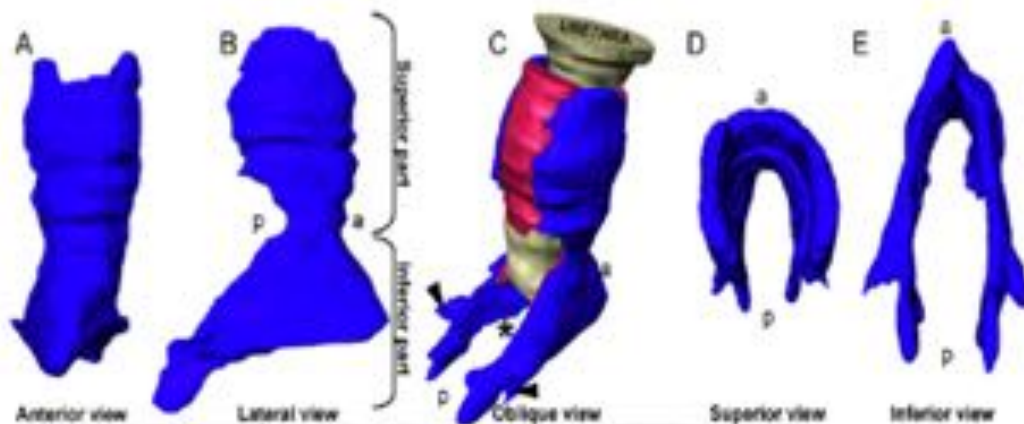
Adipositas per magna

Diabetes

Ageing

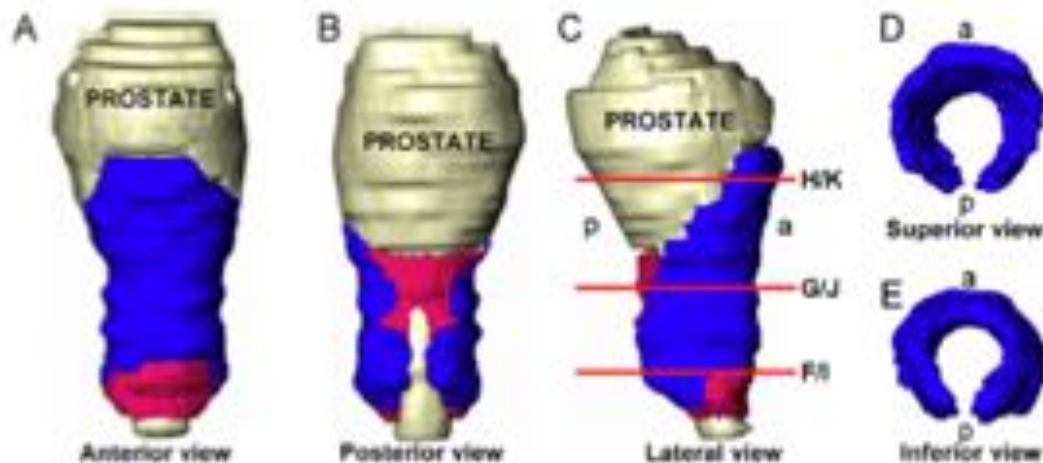
Mental Illness

The anatomical situation of the urethral sphincter complex

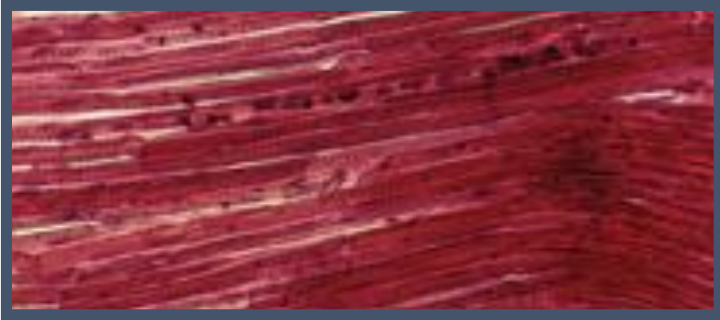


The urethral sphincter consists of:

- smooth muscle
- striated muscle

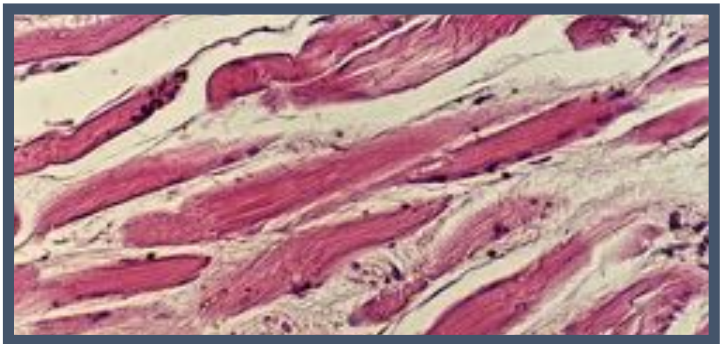


The histological situation of the sphincter tissue in time

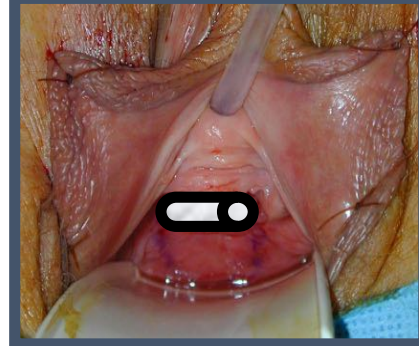
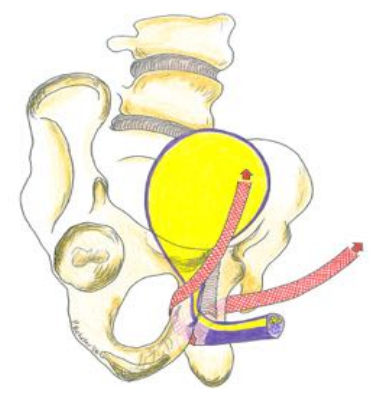
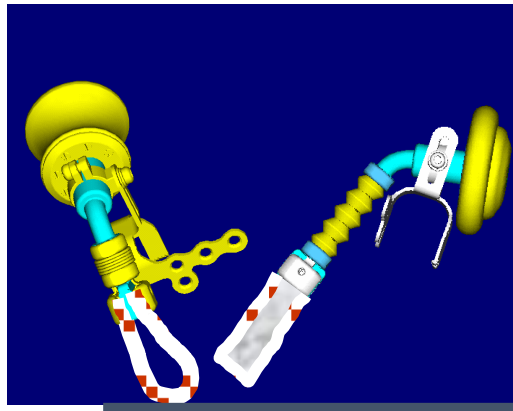
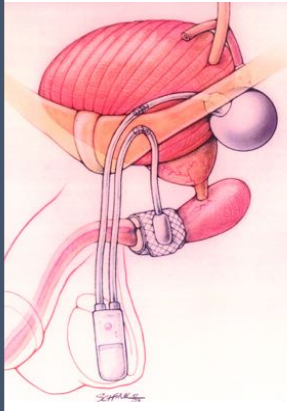
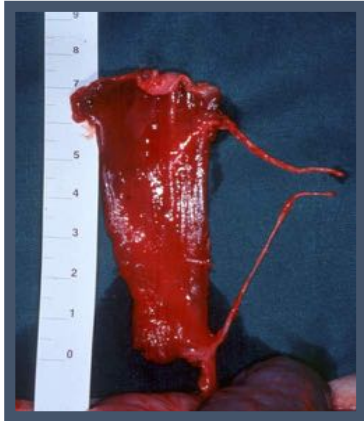


insufficient contractile function of the muscle

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



Overview on Current Treatment Options



Most current therapies applied for significant SUI
ameliorate the sequela

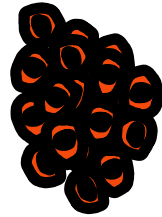
... but do not cure the conditions

Hyopthesis:

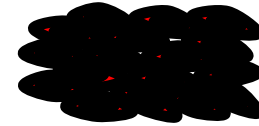
Loss of innervation and/or loss functional muscle fibres contributes to further **degeneration** of the sphincter muscle and consequently contributes to stress urinary incontinence.

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

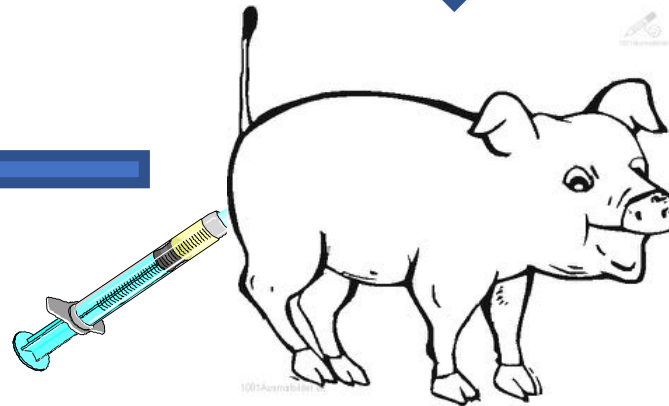
Design of the Envisioned Pre-clinical Animal Study



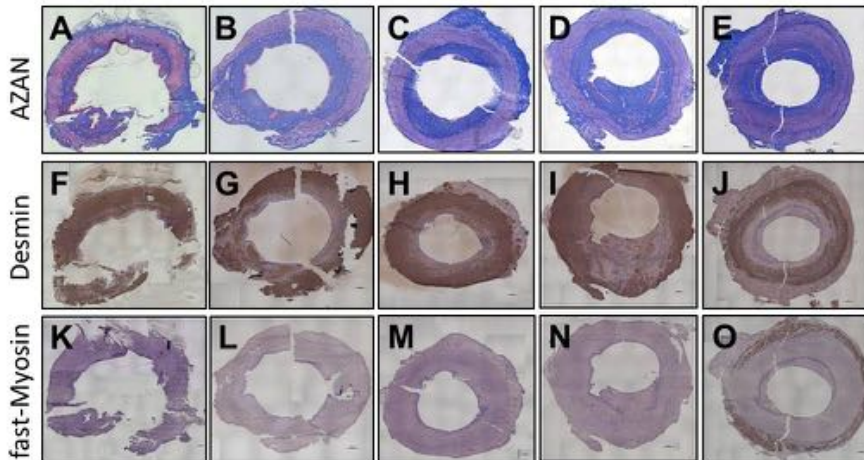
Collect Autologous
Cells



Expand in vitro
Quality Management



Inject in
Incontinent Animals



Analysis

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

significant improvement of sphincter function

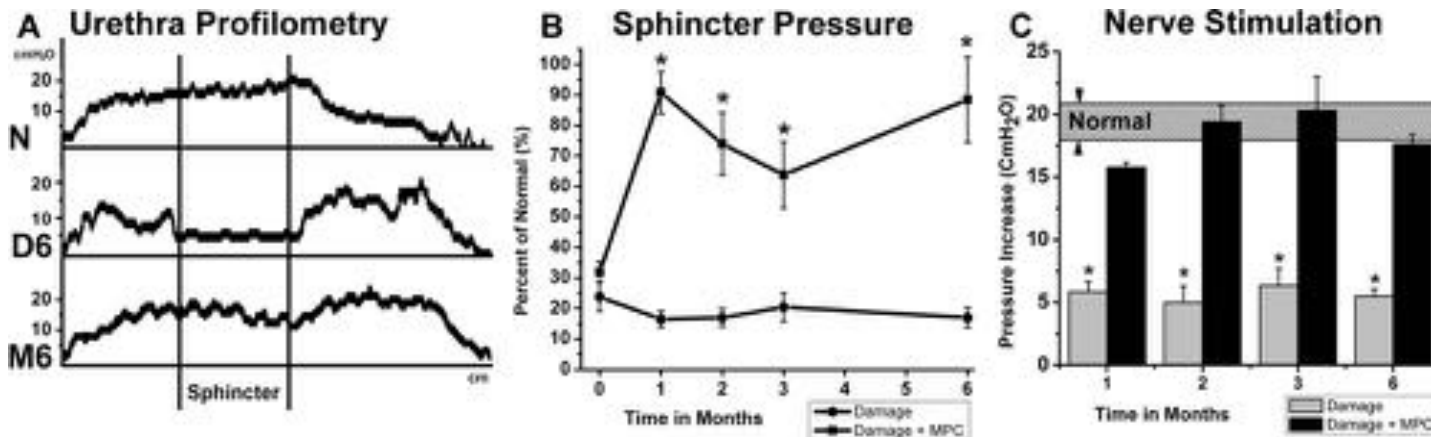


Figure 2. Functional assessment of sphincter function. (A) Representative urethra profiles showing the increase of sphincter pressure in the sphincter area after cell treatment. N, normal control; D6, damage-only control at 6 months; M6, MPC-treated animal at 6 months. (B) Graph showing sphincter pressures 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 ($p < 0.003$).

> very good recovery, promising pre-clinical data

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

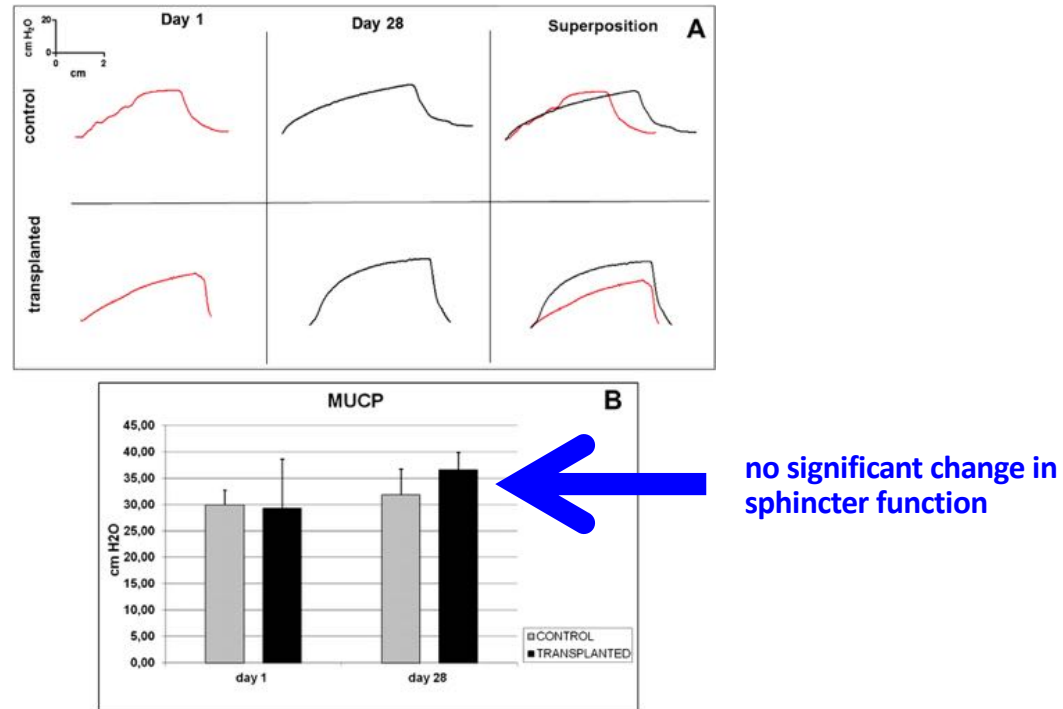
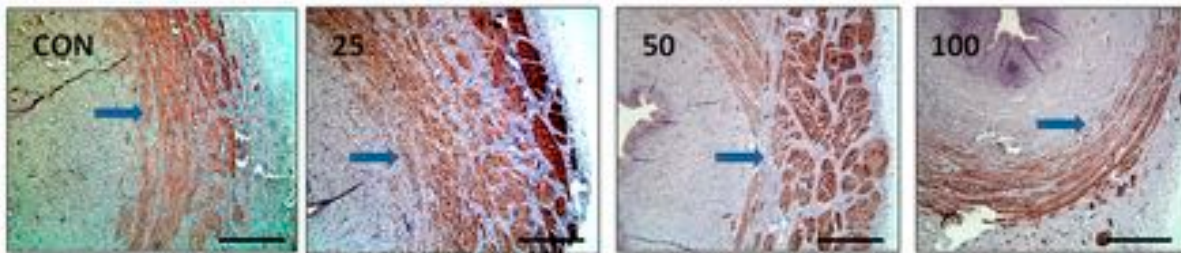


Figure 3. The functional evaluation of the porcine muscle-derived cells (pMDCs) transplantation effect. **(A)** The urethral profiles recorded during urodynamic evaluation. The profiles recorded at day 1 and day 28 of the same pig from the representative control animal and experimental animal. All profiles 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 ($P = .2$).

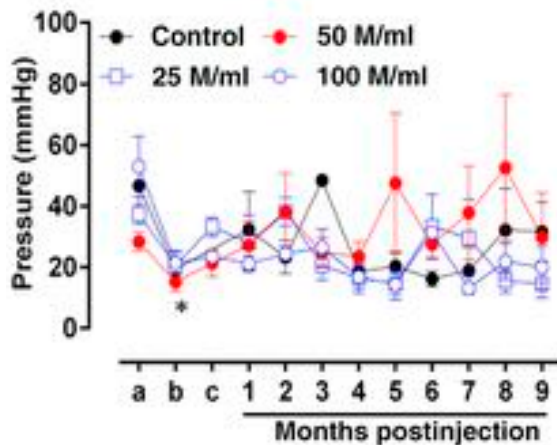
> results depend very much on the model used

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

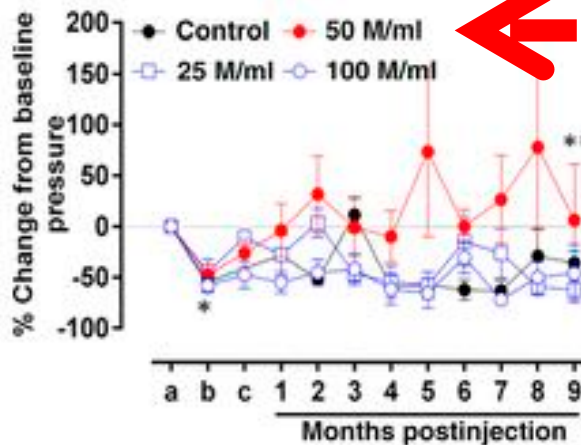
A



B Maximal Urethral Pressure (MUP)



C MUP: % Change from baseline pressure



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

> results depend very much on the cell /dosage used

Ambivalent Situation in the Current Literature on Cellular Therapies

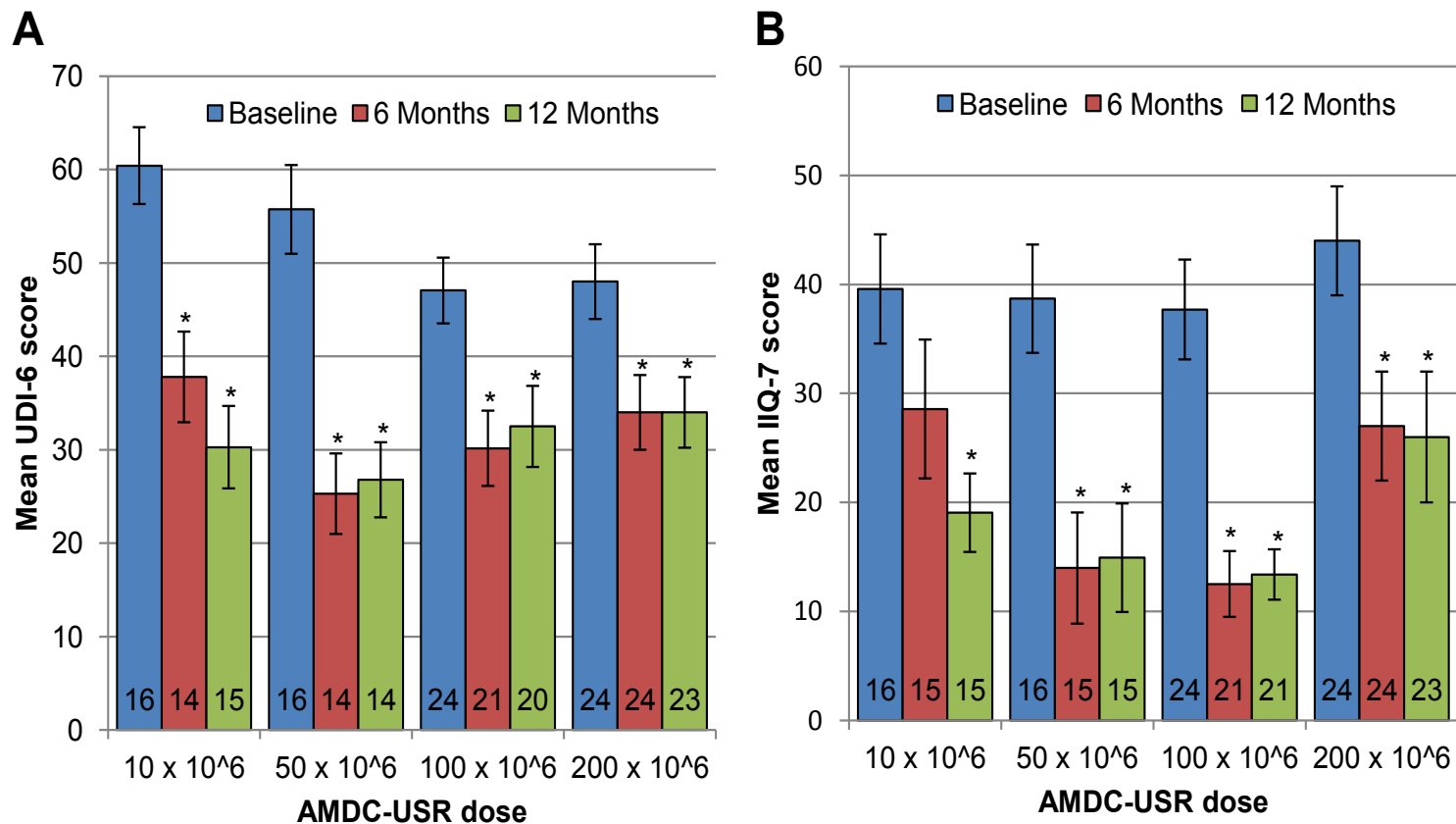
Many pre-clinical studies utilizing animal models of SUI reported on success of cell based therapies, but others reported no improvement*

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

ported on success but were retracted thereafter,



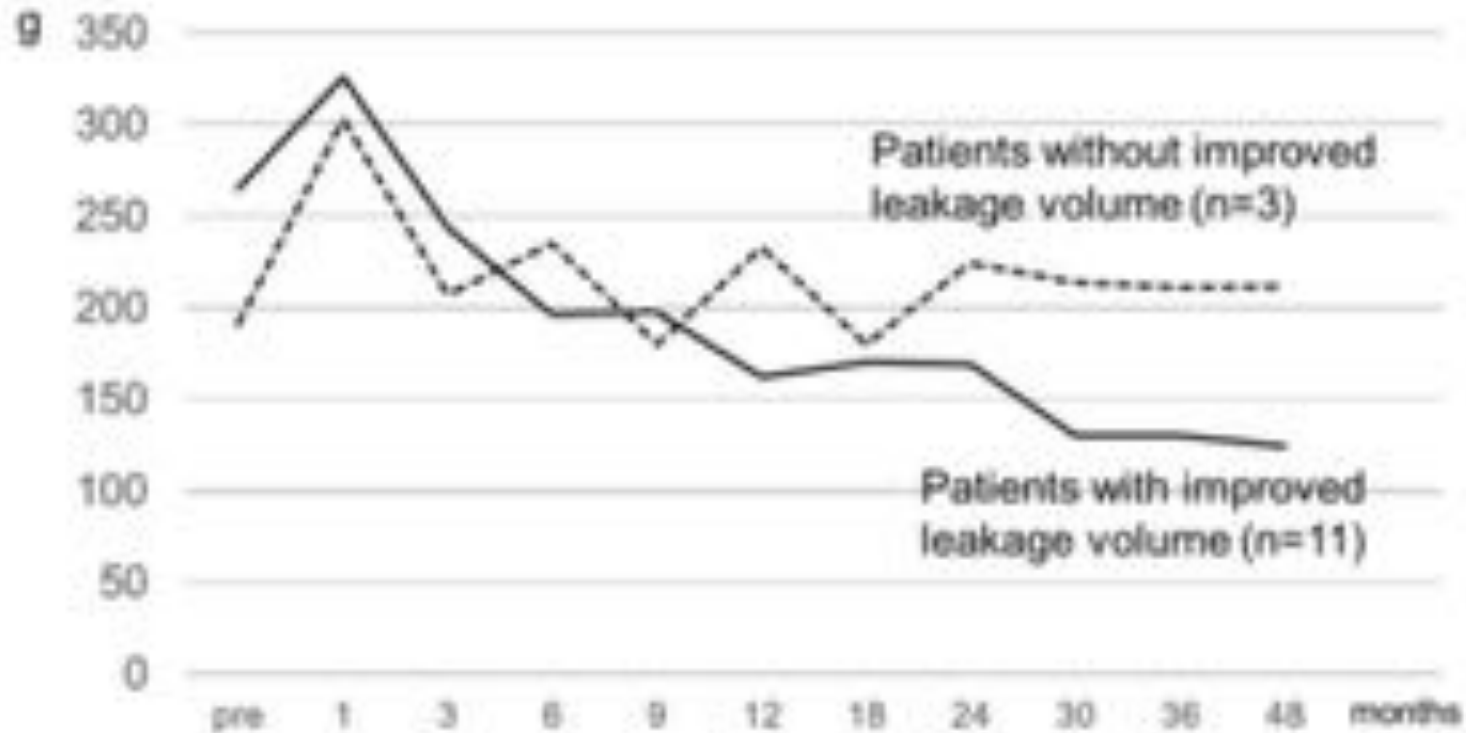
Clinical Application of Muscle-derived Cells



Result:

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

Clinical Application of Adipose Tissue-derived Cells



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

Many studies have not (yet) published their results

Some studies with larger cohorts suffered from significant drop-out

Overall cohorts investigated have been small

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

The Surgical Method

Navigation by Ultrasound

Sphincter



Bladder

Urethra



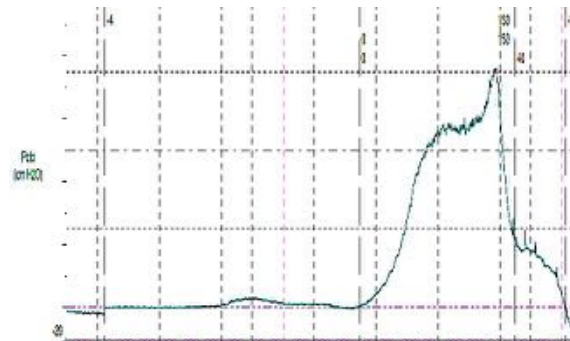
Vagina/Anal

Injection of human MSC in the urethral sphincter muscle of Göttingen minipigs

Application *in vivo*

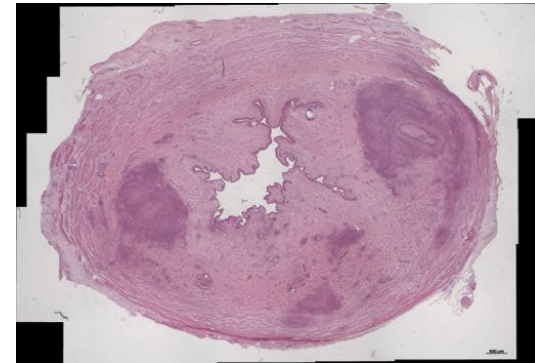


Urodynamical evaluation



Incubation 3 wks - 12 months

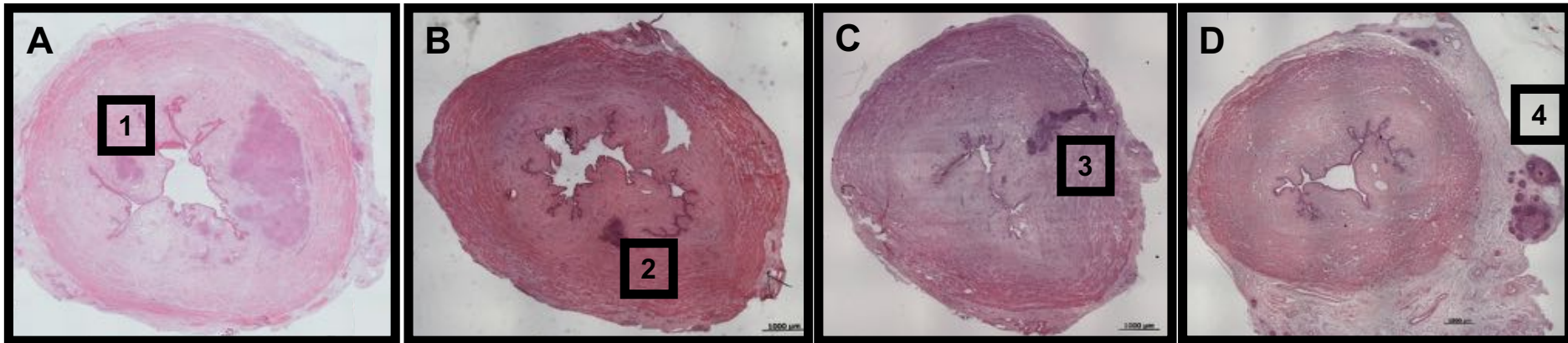
Follow-up by histology



Follow-up by MRI

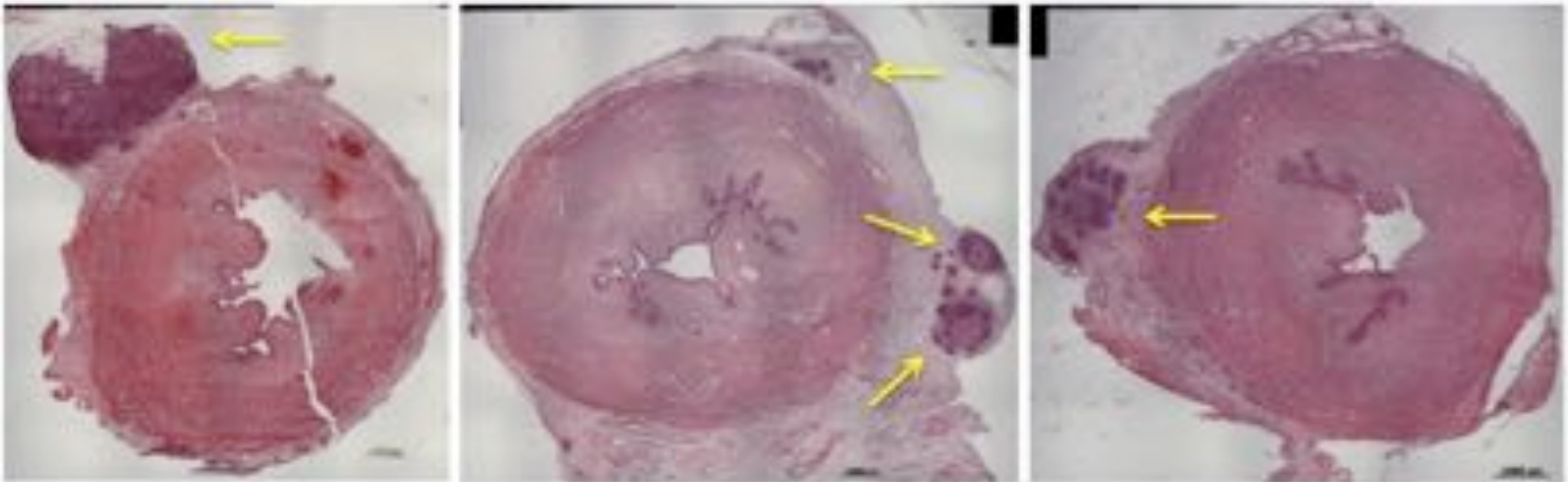


Technical challenges for transurethral injection of cells by cystoscope under visual control

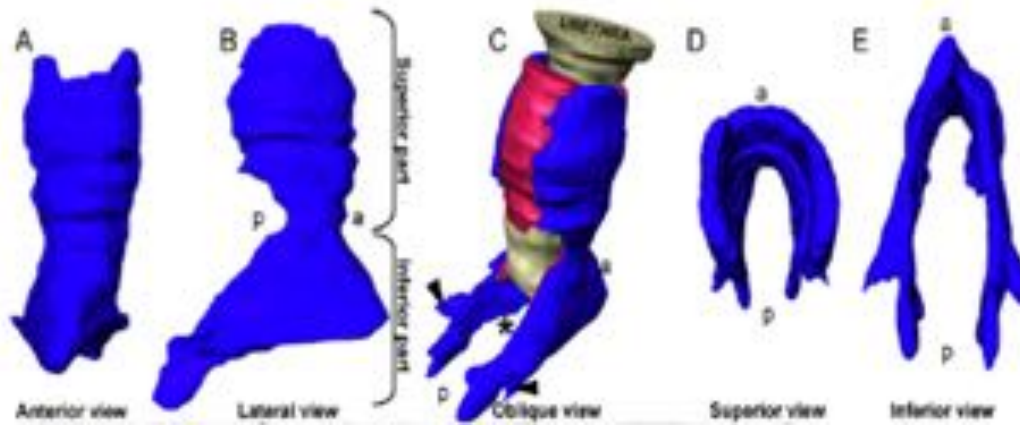


localisation	close to lumen / urothelial cells	connective tissue / intermediate	muscular layer	outside urethra
8 mm	8%	20%	13%	59%
4 mm	12%	45%	16%	27%

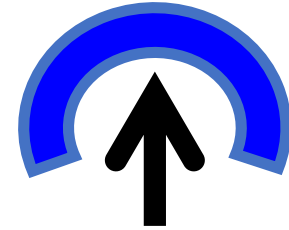
Frequent Loss of Cells Due to Improper Needle Injection



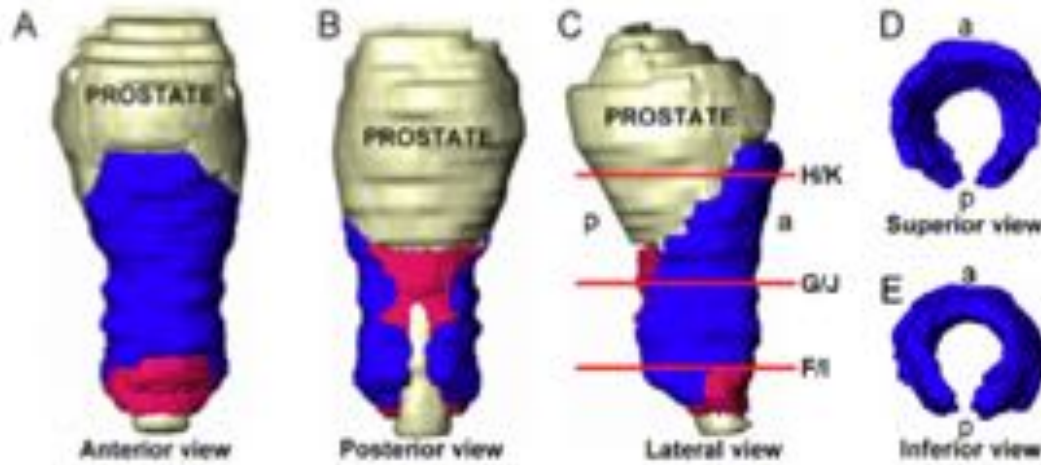
Cell Injection Strategy: Single Shot vs. Multiple Sides



female embryo, 18wks p.g.



less invasive, less distribution

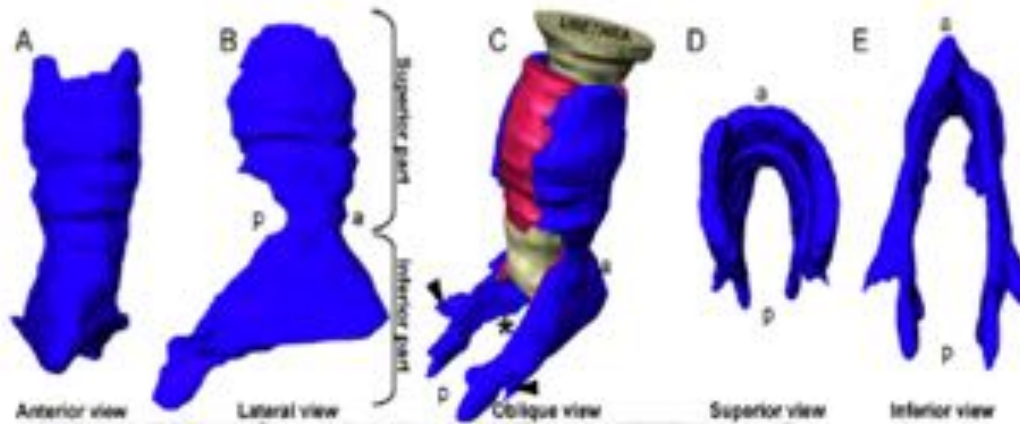


male embryo, 12wks p.g.



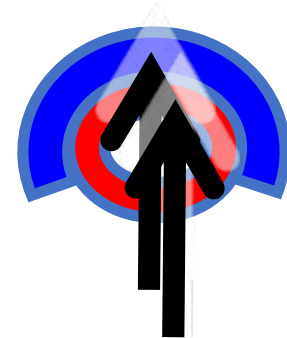
more invasive, better distribution

Cell Injection Strategy: Targeting the **Rhabdosphincter** or the **Lissosphincter**

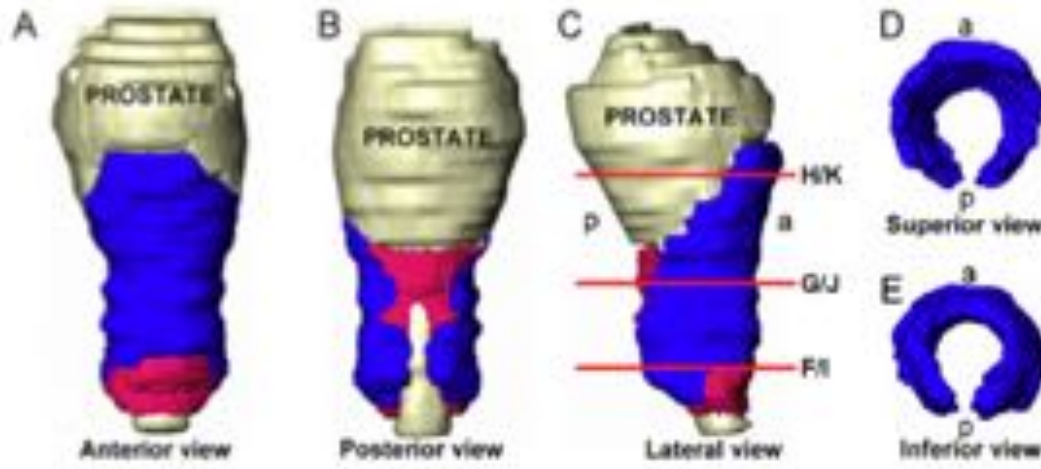


female embryo, 18wks p.g.

target the rhabdosphincter



target the lissosphincter



male embryo, 12wks p.g.

How to Resolve these Issues?

Replace the Injection Needle by a WaterJet Device

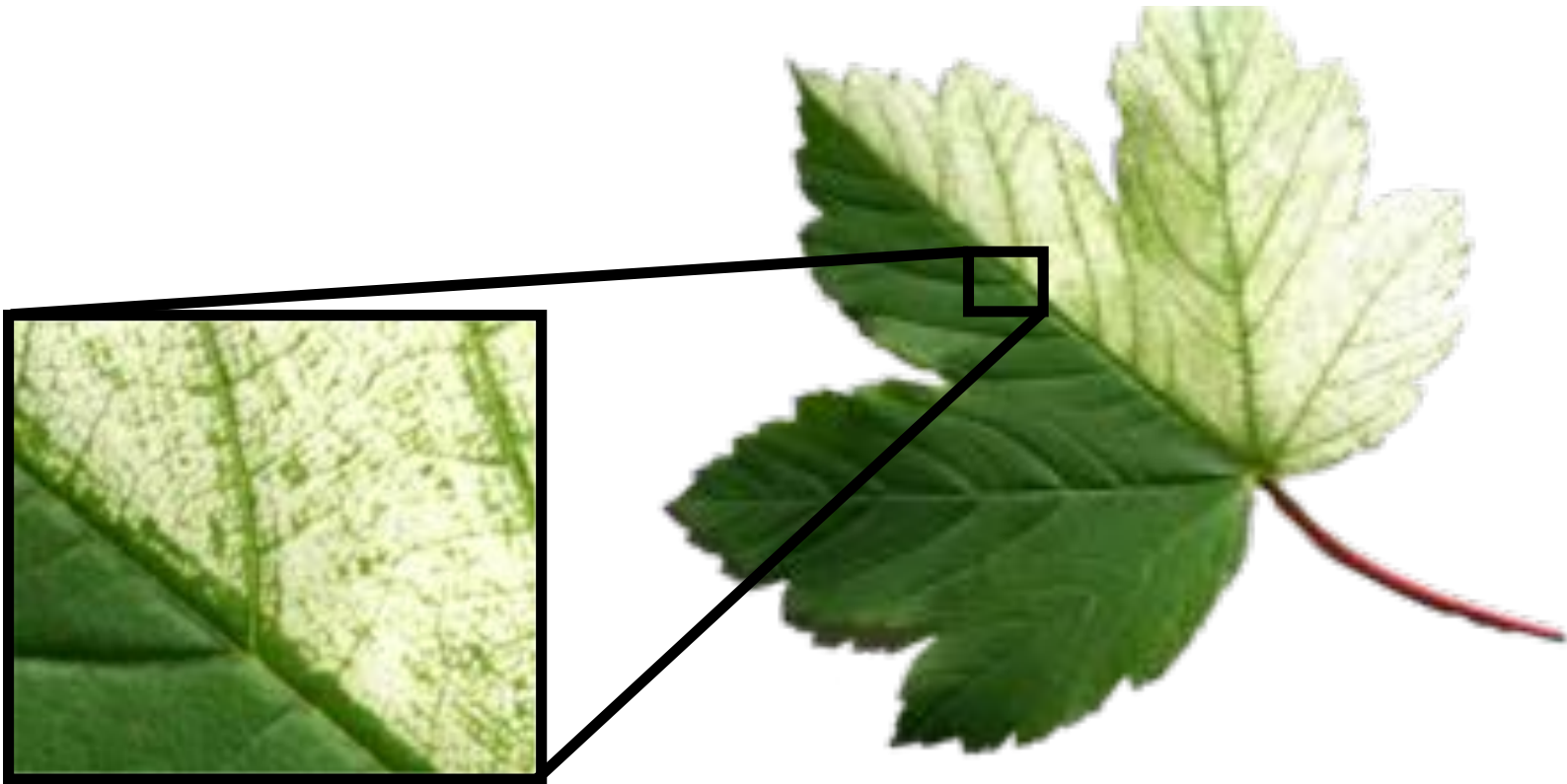
- Define the Energy of the Jet (sc. pressure)
- Define the Geometry of the Jet (sc.geometrie)
- Define Optimal Transportation Media

Pressure and Geometry of the Jet will Determine the Depth and Distribution of the Cells *in* the RoI

WaterJets in our Everyday Life

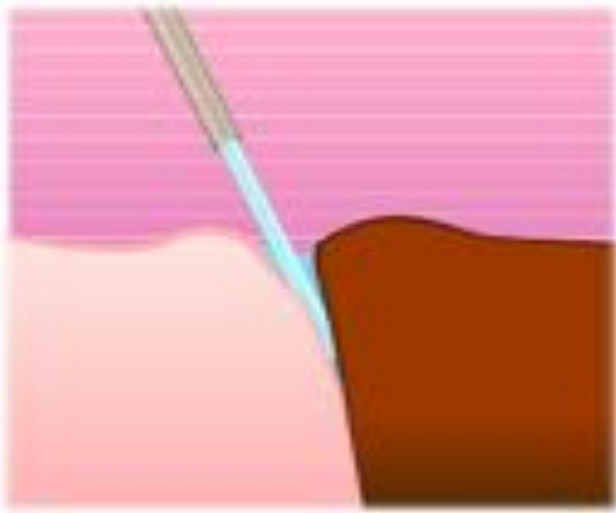


Basics of the WaterJet Technology

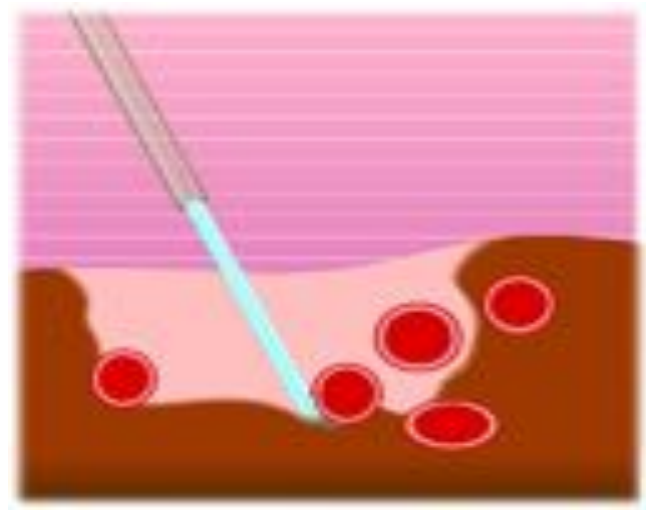


Depending on the volume, pressure and solvent one can cut, remove, or spare a given material

Basics of the WaterJet Technology in Medicine

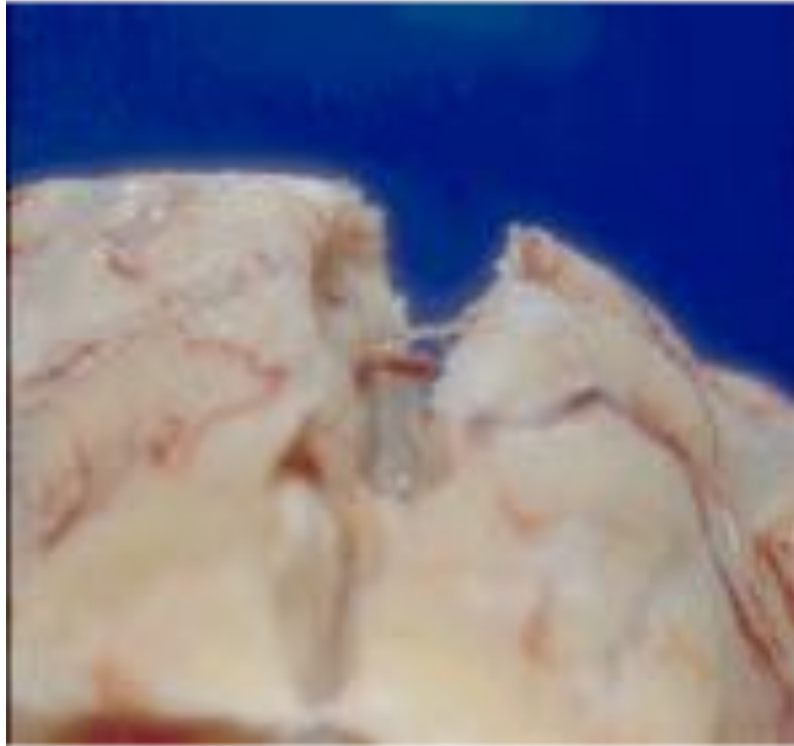


Separate layers of tissue along
density/elasticity borders



Remove soft tissue, spare
blood vessels / nerves

Experimental Cut in Rodent Brain Tissue by the WaterJet Technology

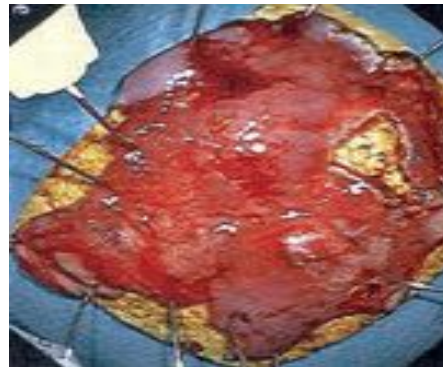


Blood vessel and nerve remain intact

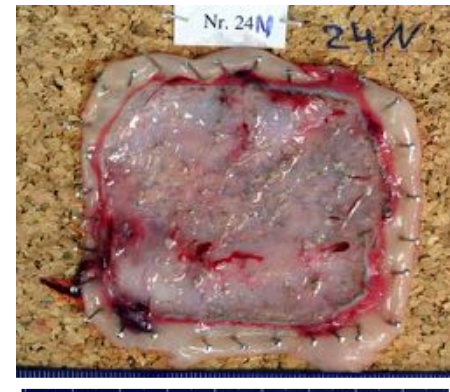
Endoscopic Removal of Inflamed Gut Tissue by WaterJet to Improve Wound Regeneration



Endoscopic view on a suspicious lesion (esophagus)



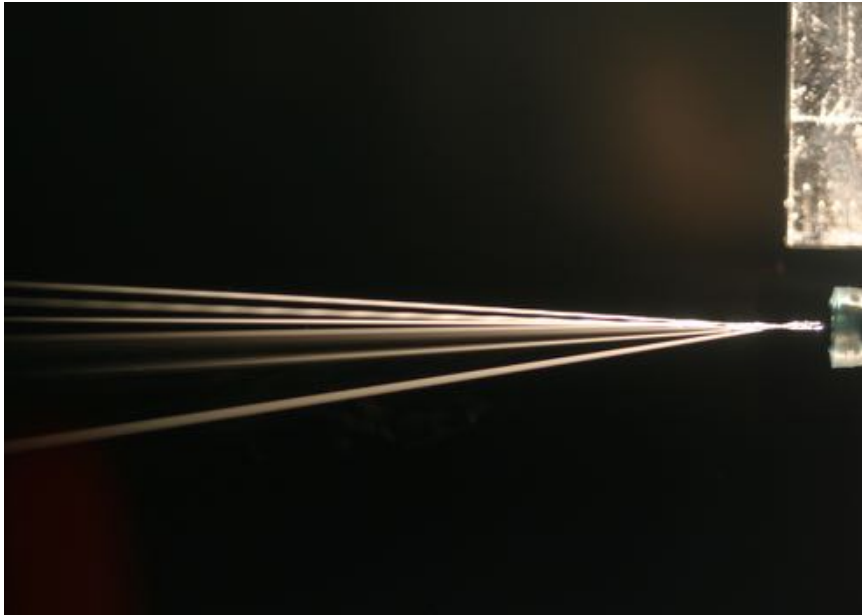
Resected lesion



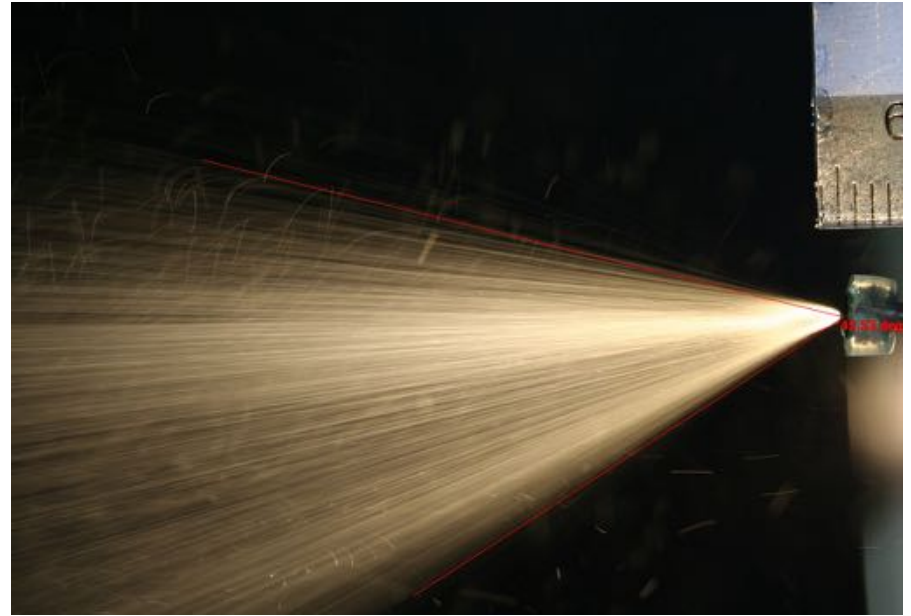
Resection bed

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

Modification of the Jet from Cutting to Application of Biomaterials

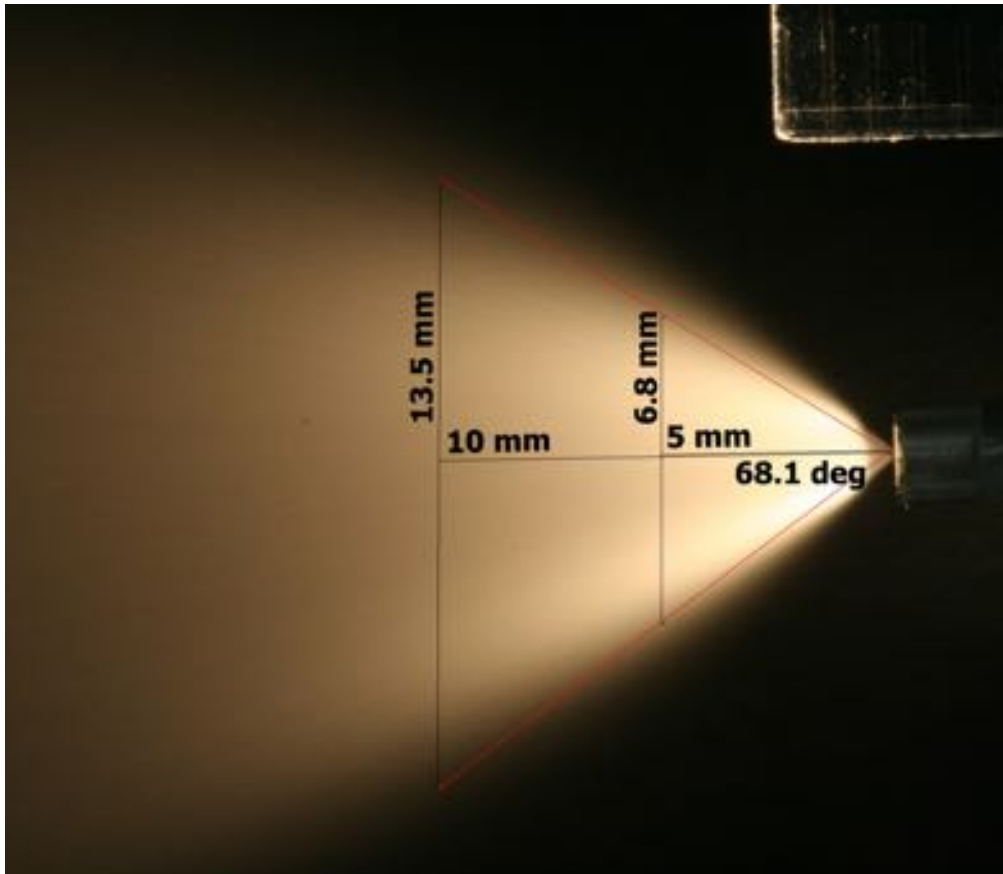


Narrow jet:
Precise cut



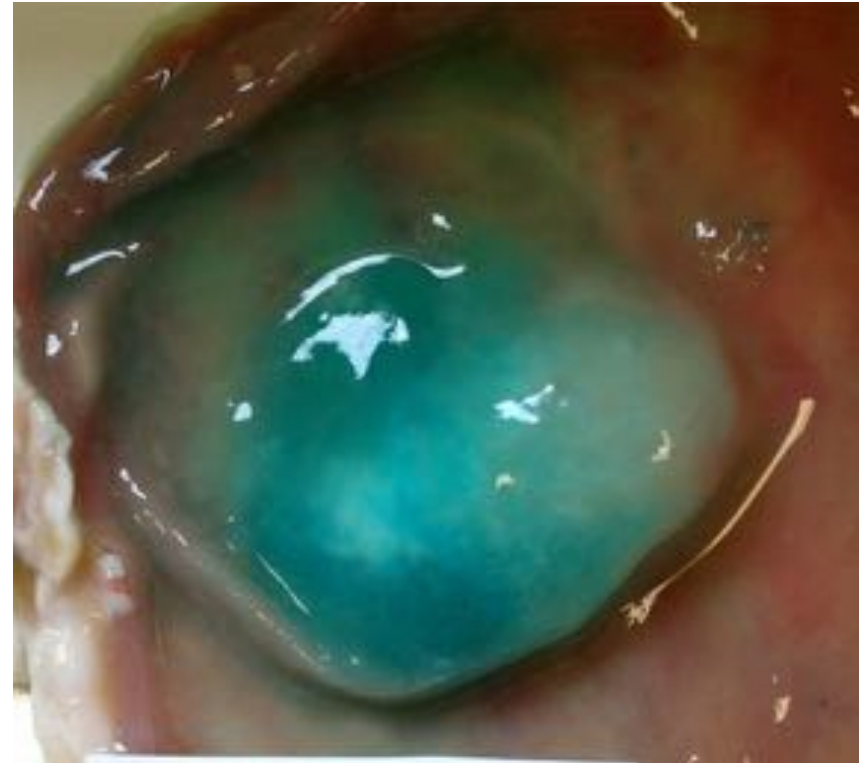
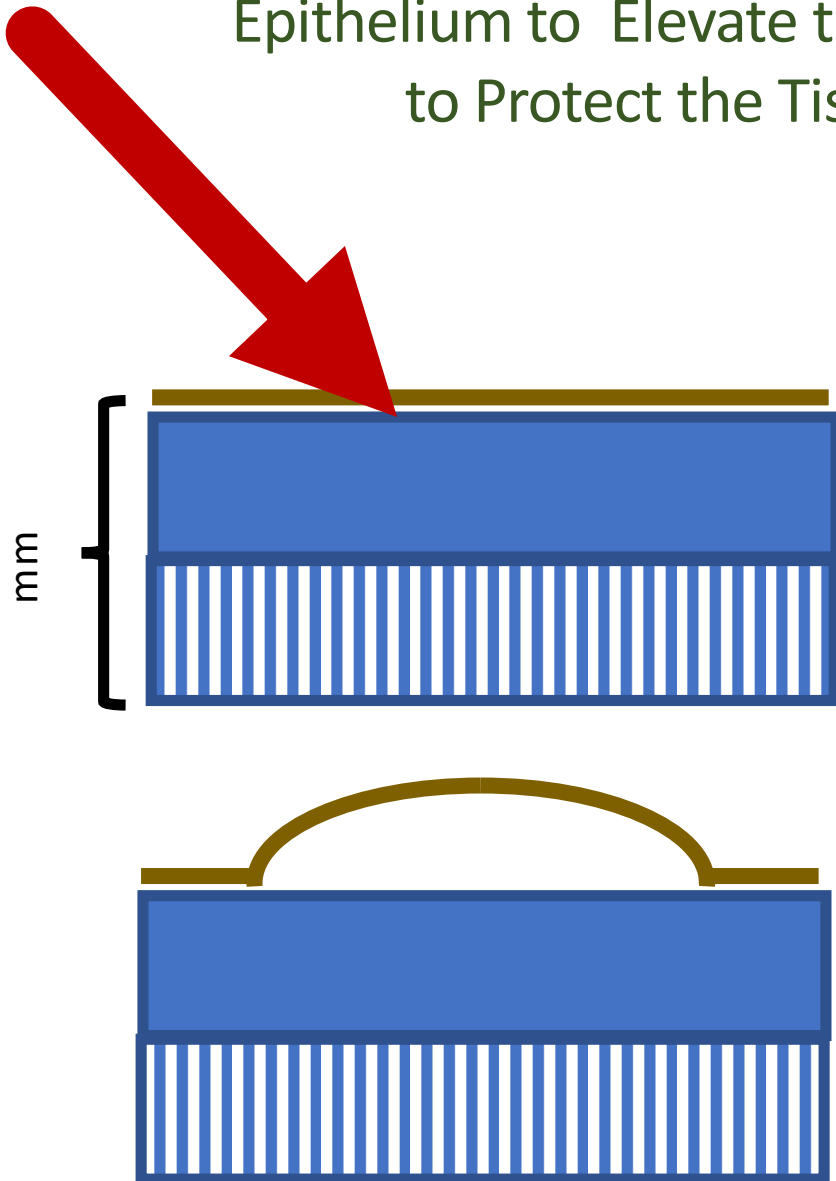
Wide Spray
Application of biomaterials

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



Modification of the Spray Fluid
Breeze by Biomaterial

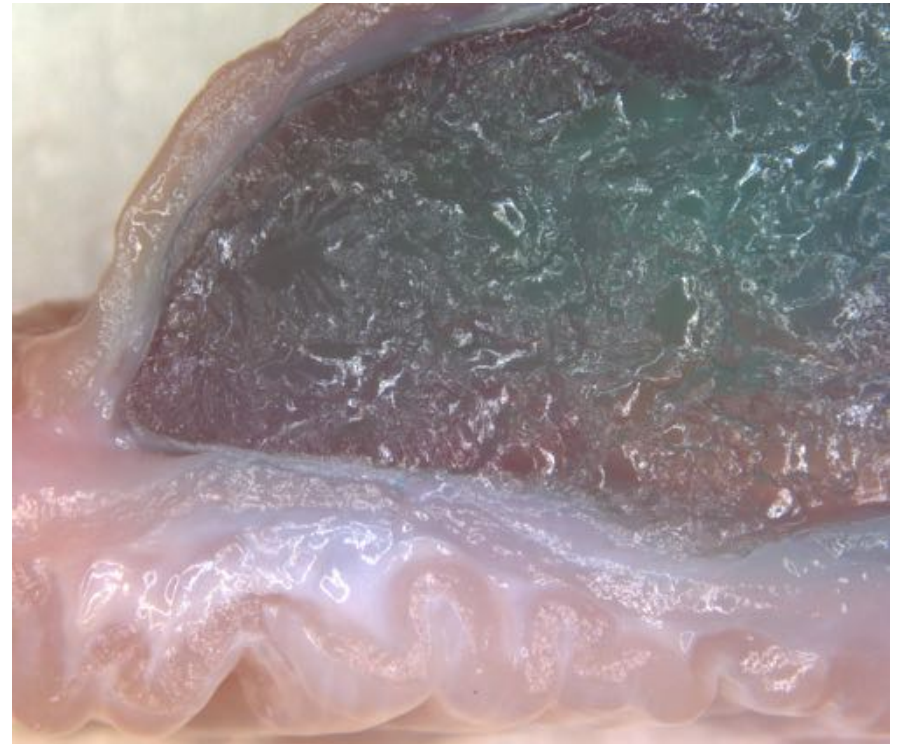
Injection of Colored Buffer in the Cadaveric GI
Epithelium to Elevate the Area of Surgery and
to Protect the Tissue Underneath



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



Top view
Injection into the
submucosa (stomach, cardia, swine)



Cross-section
Selective enrichment in the
submucosa (stomach, cardia, swine)

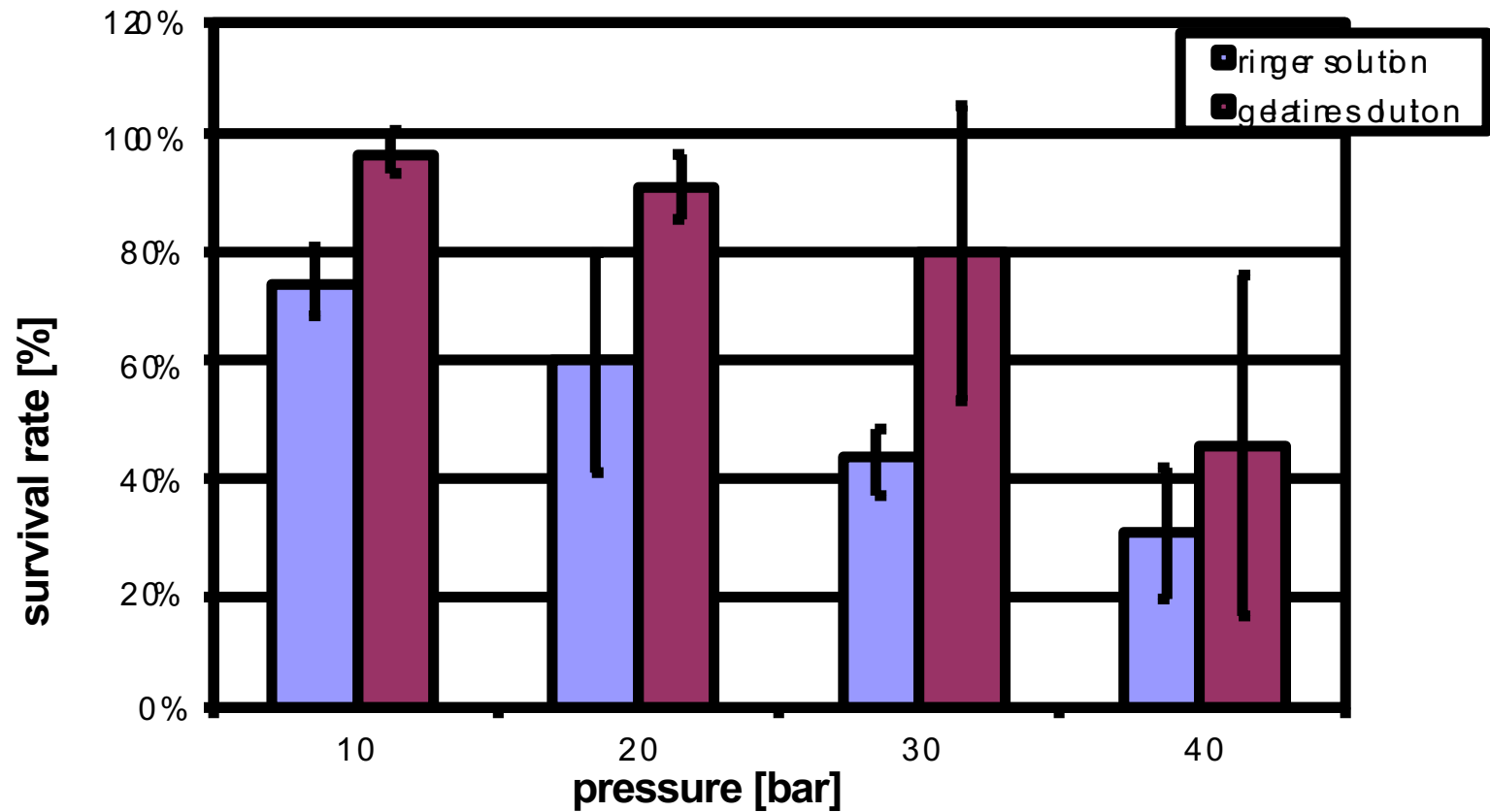
Hypotheses:

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?

Gelatine – a Clinically Approved Biomaterial

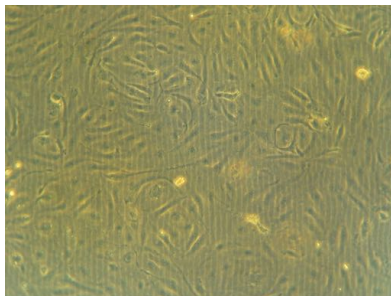
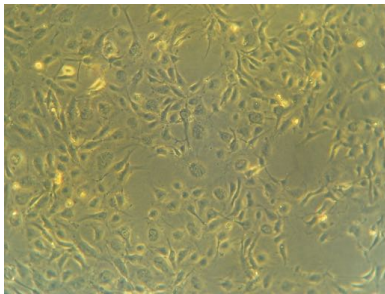
Spray of HeLa cells resuspended in gelatine with a **GI** WaterJet device



Candidate Carriers for Facilitation Cell Transport by Water Jet: Collagen > Gelatine

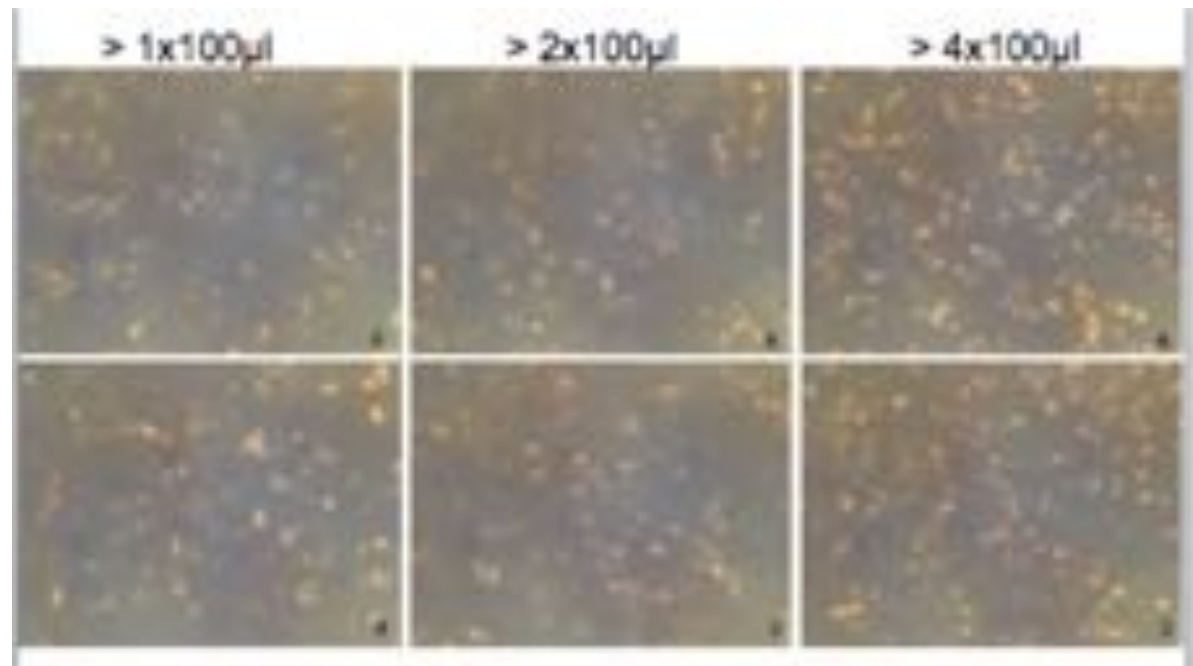
Attachment of HeLa cells on gelatine-coated slides

Control: CellBind



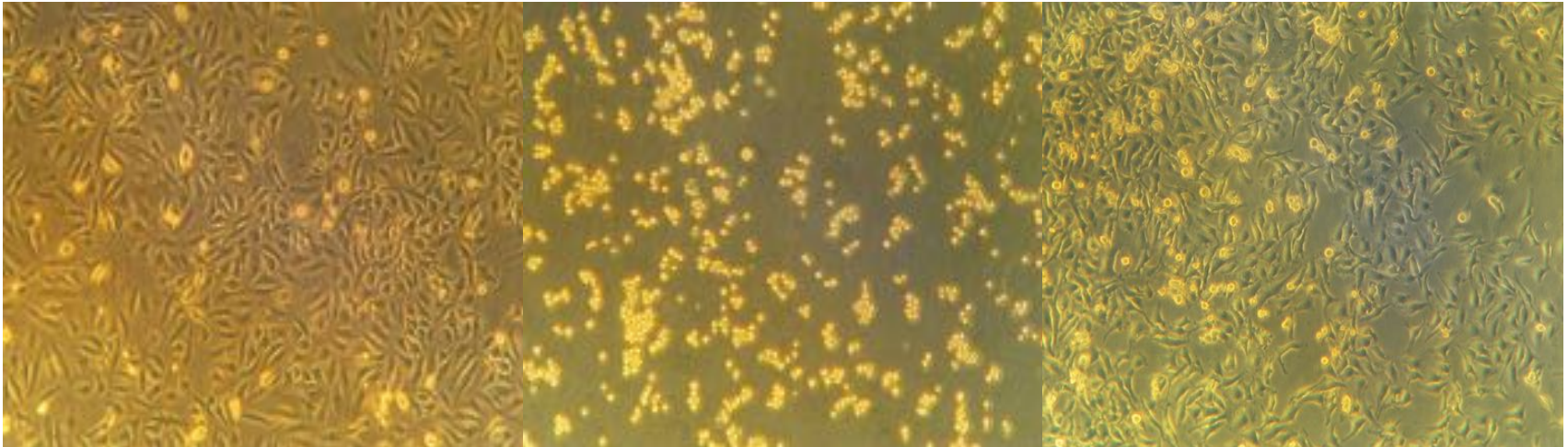
Type I collagen coat

Gelatine dilutons



Limitations of Gelatin Employed in Waterjet Applications

Seeding blends of HeLa cells resuspended in gelatine



Control (ø gelatine)

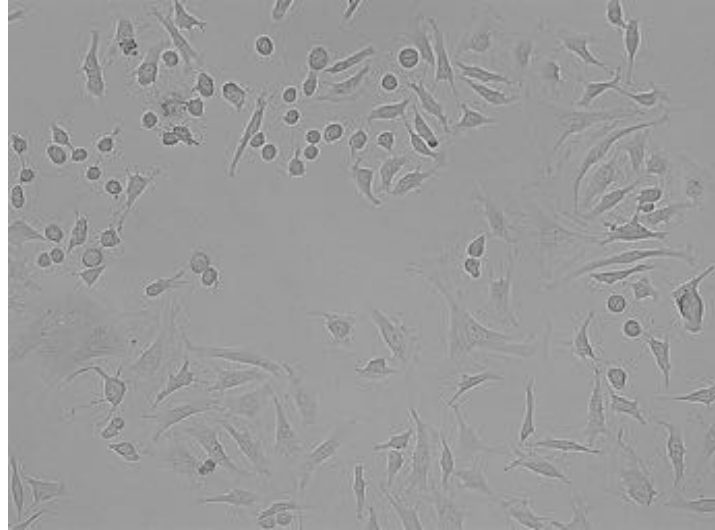
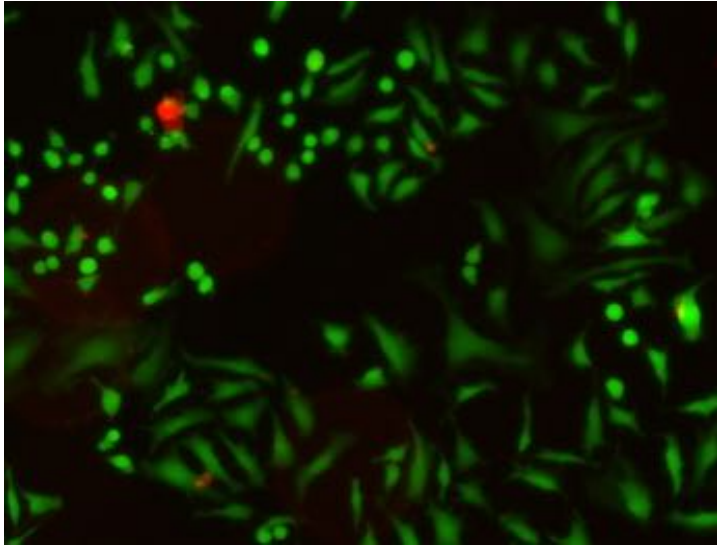
1:1 diluted gelatine

1:4 diluted gelatine

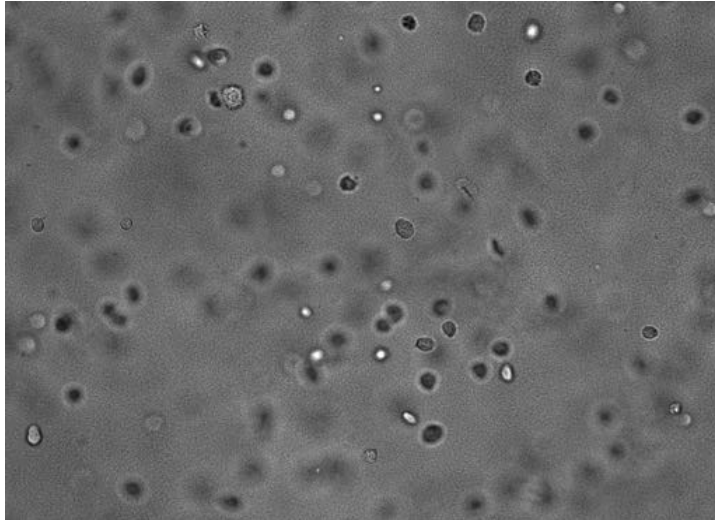
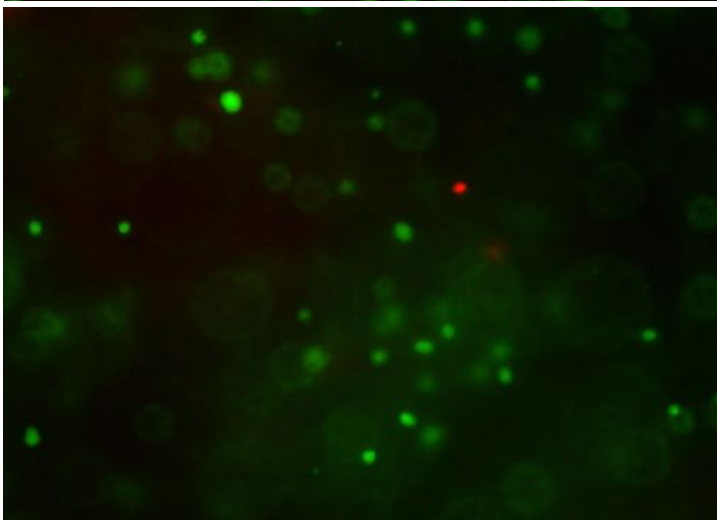
- Gelatine hardens at low temperature
 - > requires high concentrations to yield a solid matrix for cell attachment in vivo
- > High concentrations block pipe and require heated system
- High concentrations are not elastic

Investigating Fibrin Glue as Carrier for WaterJet Applications

Viability test of HeLa cells with fibrin glue

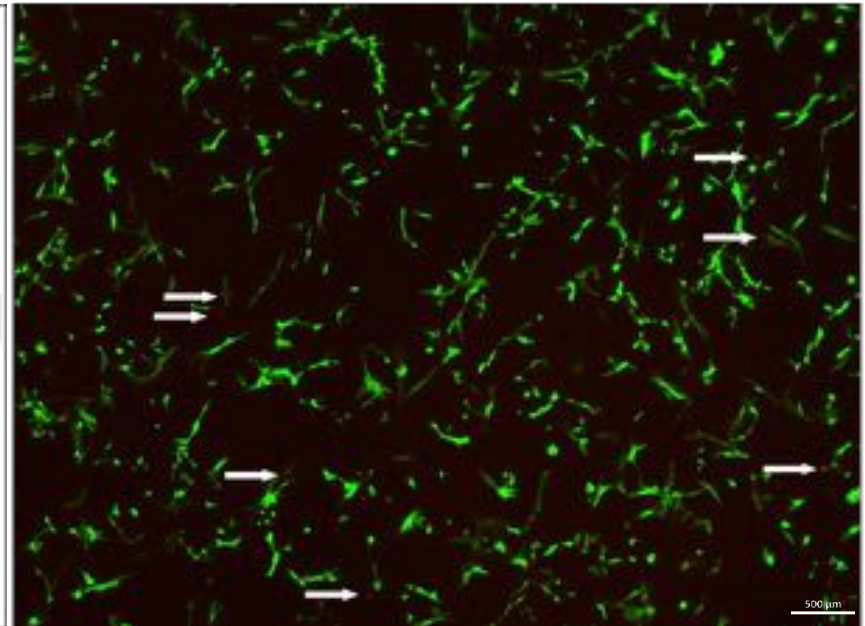
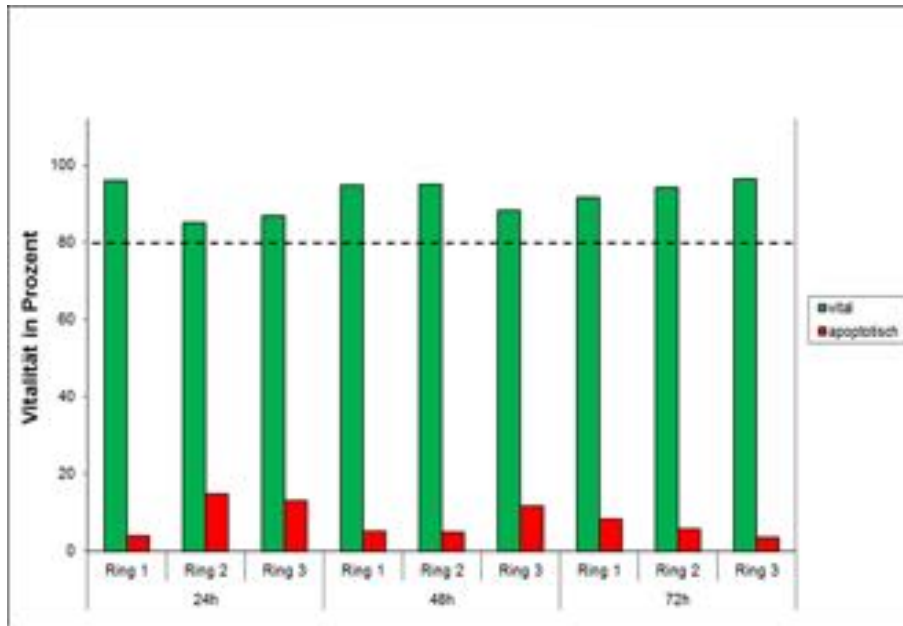


HeLa on fibrin glue



HeLa in fibrin glue

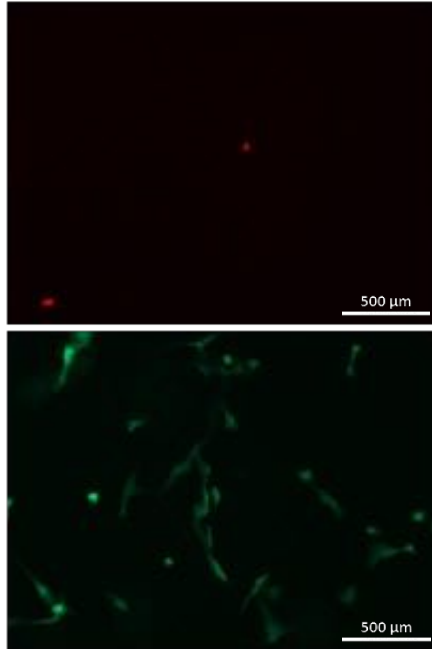
Long-term Survival and Attachment of MSCs upon Injection with Fibrin Glue



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

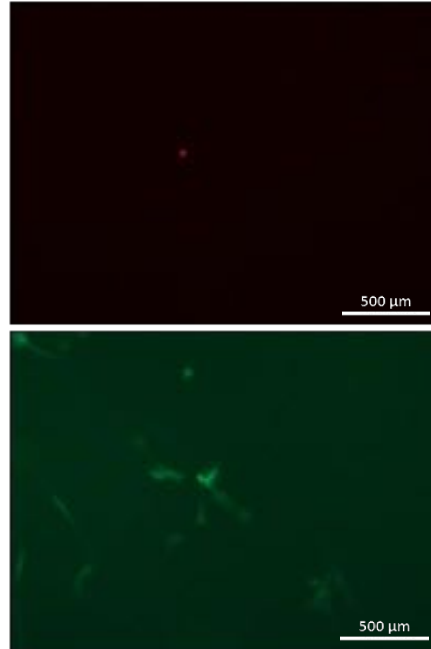
A

380 μ l (2mm)
Thrombin/Fibrin 1/20

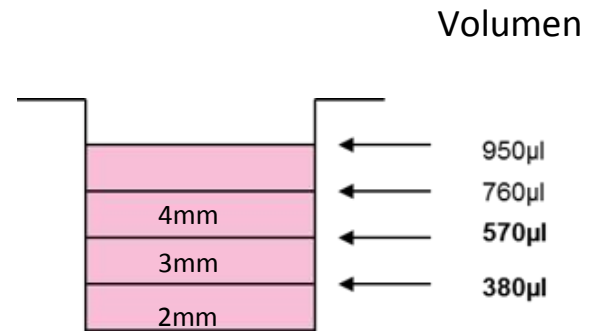


B

570 μ l (3mm)
Thrombin/Fibrin 1/20

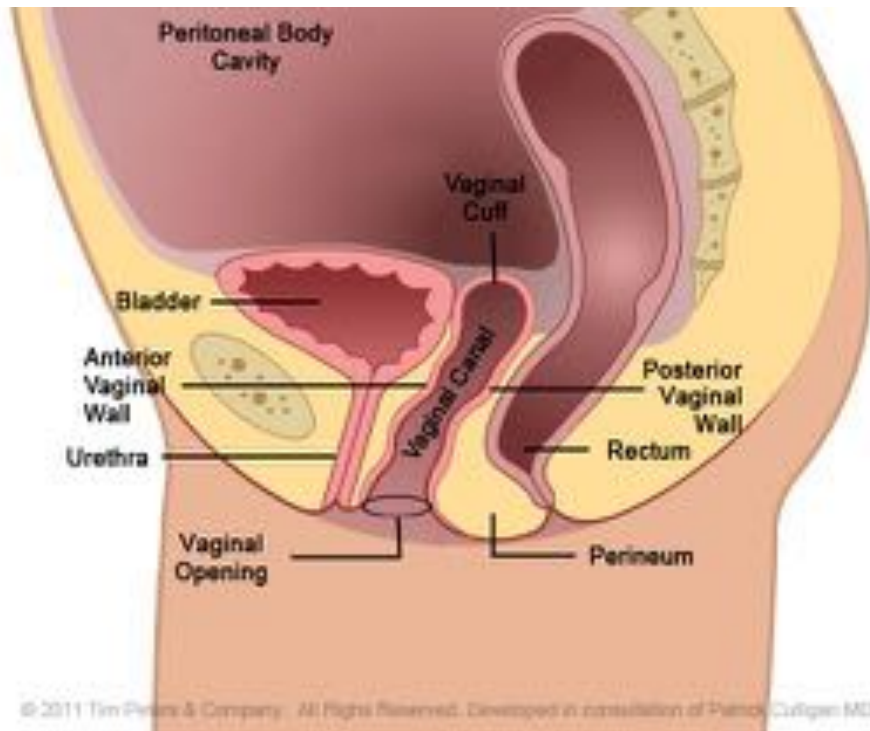


C



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

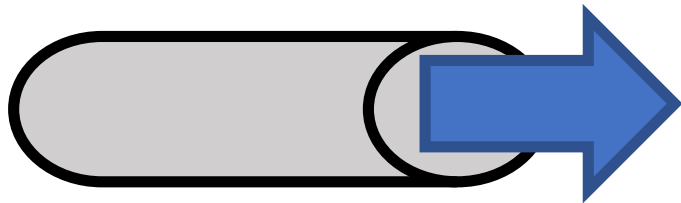
GI Application:

Wide tube

Straight Nozzle

Spray Cells **ON** Surface

-> Low Pressure



Urological Application

Narrow tube

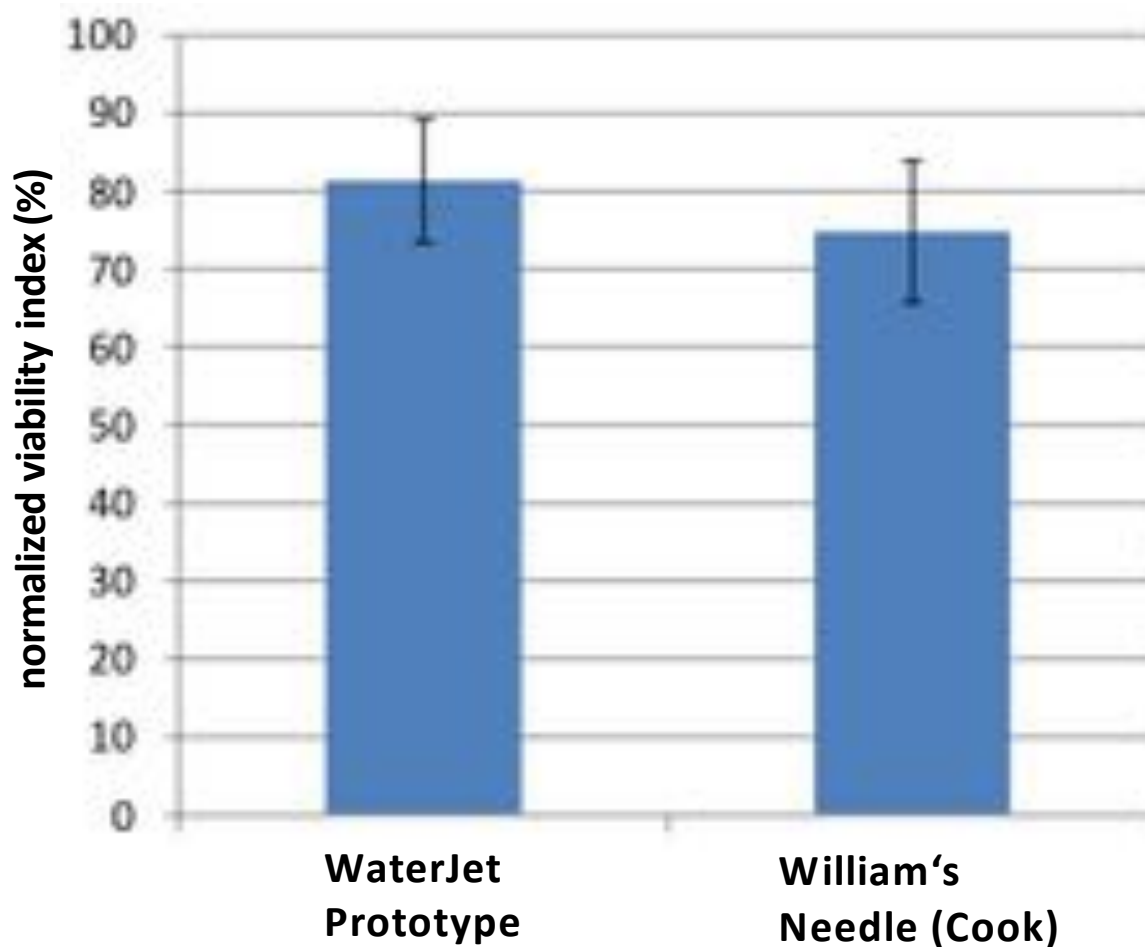
Angular Nozzle

Inject Cells **IN** Tissue

-> High Pressure

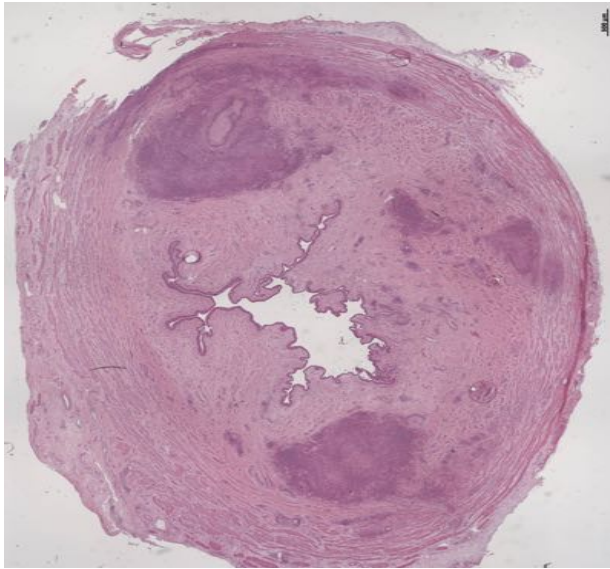


Current Injection Device Delivers MSCs with a Higher Yield of Viable Cells Compared to the Standard William's Needle

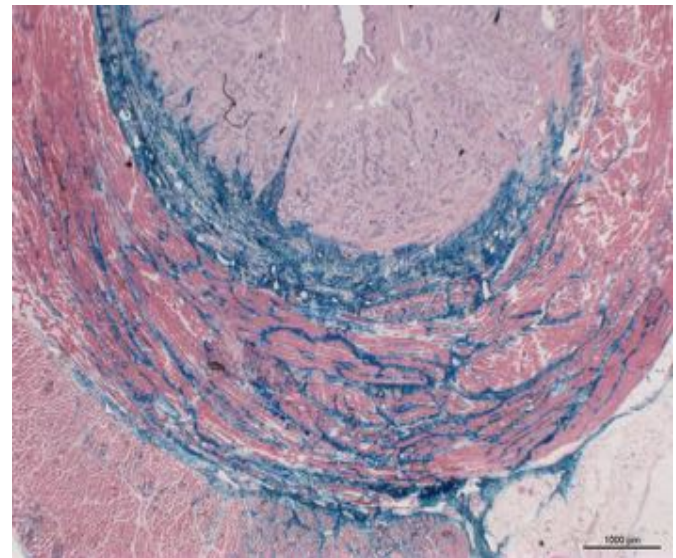


Exploring the conditions in cadaveric tissue samples

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

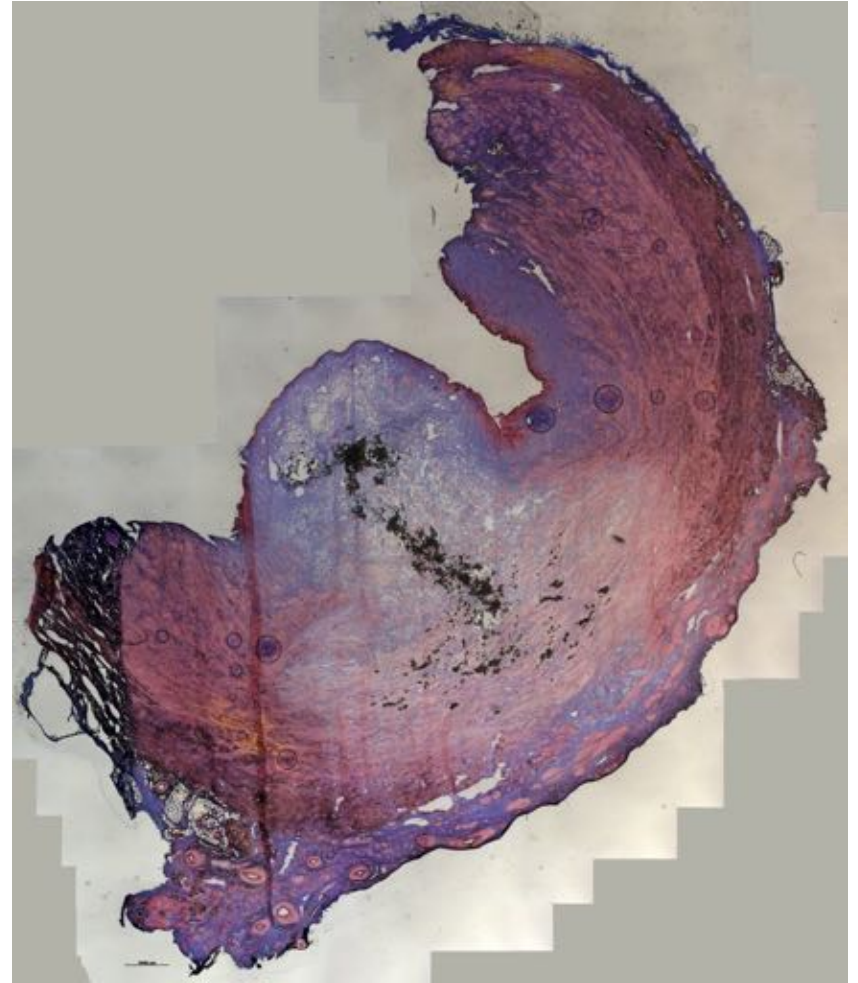
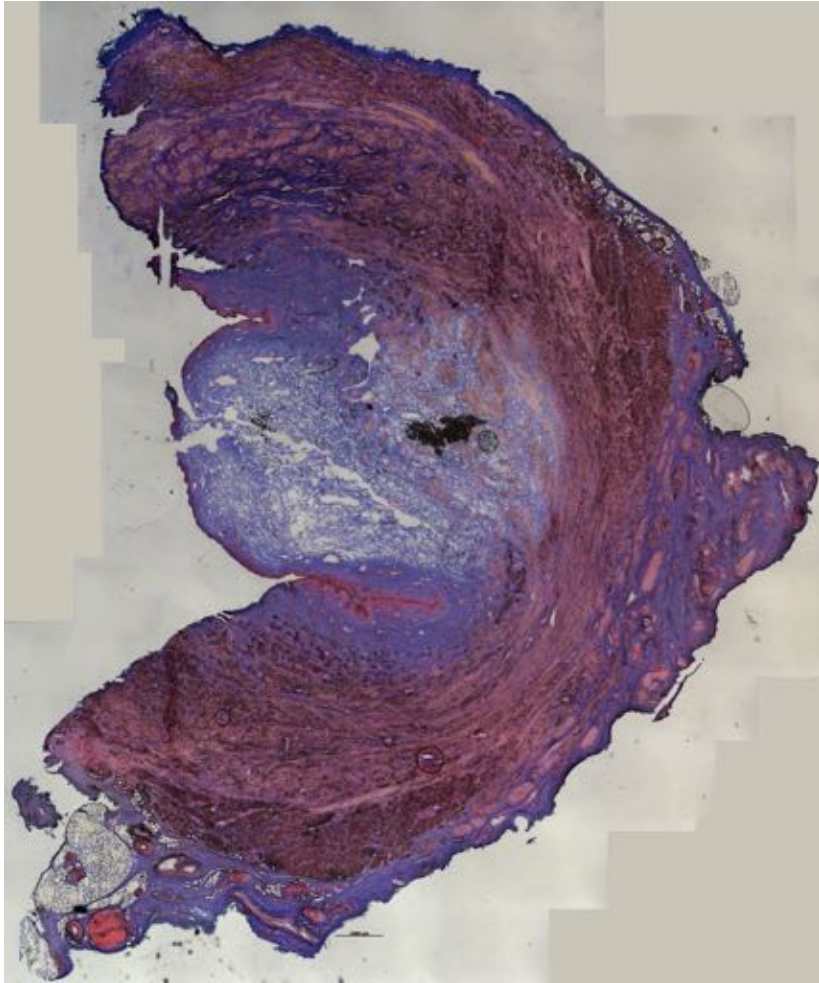


Injection of MSCs
by Needle

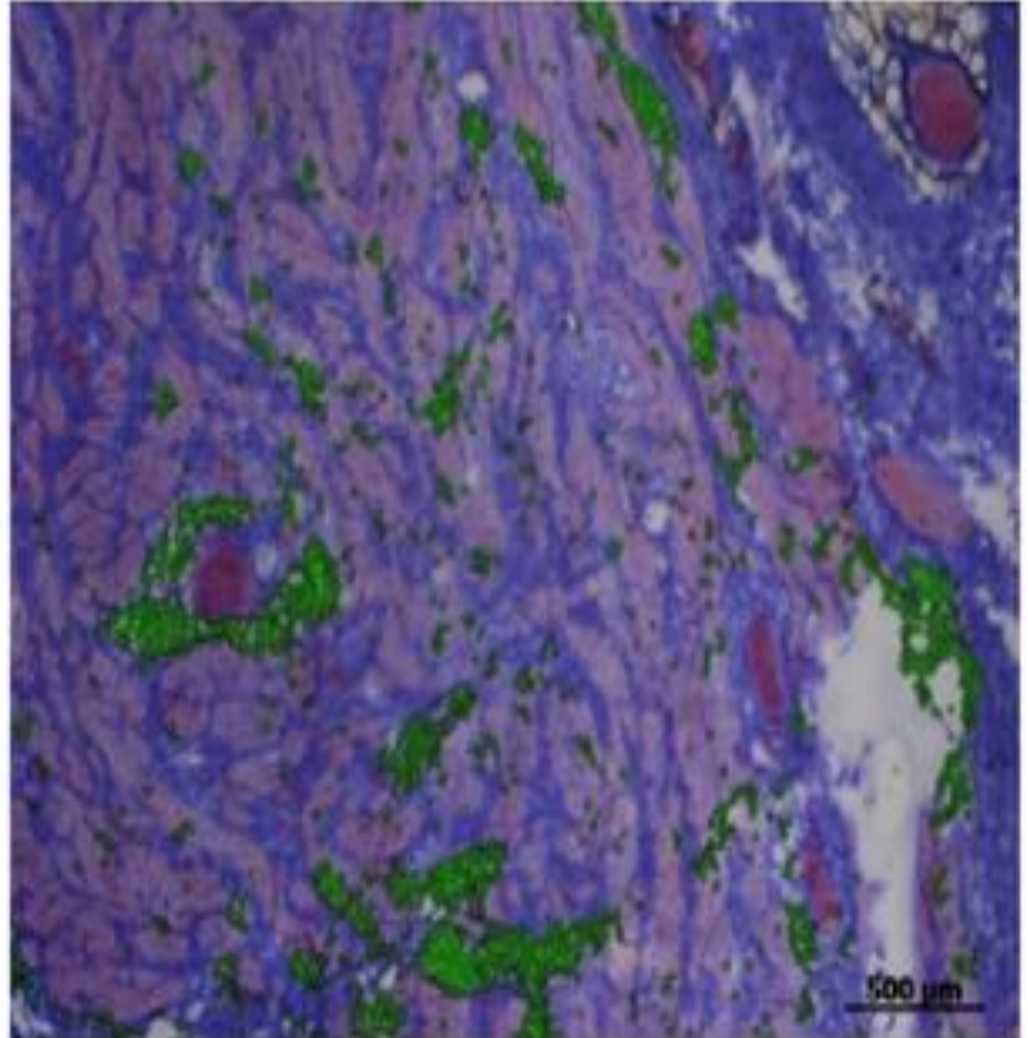
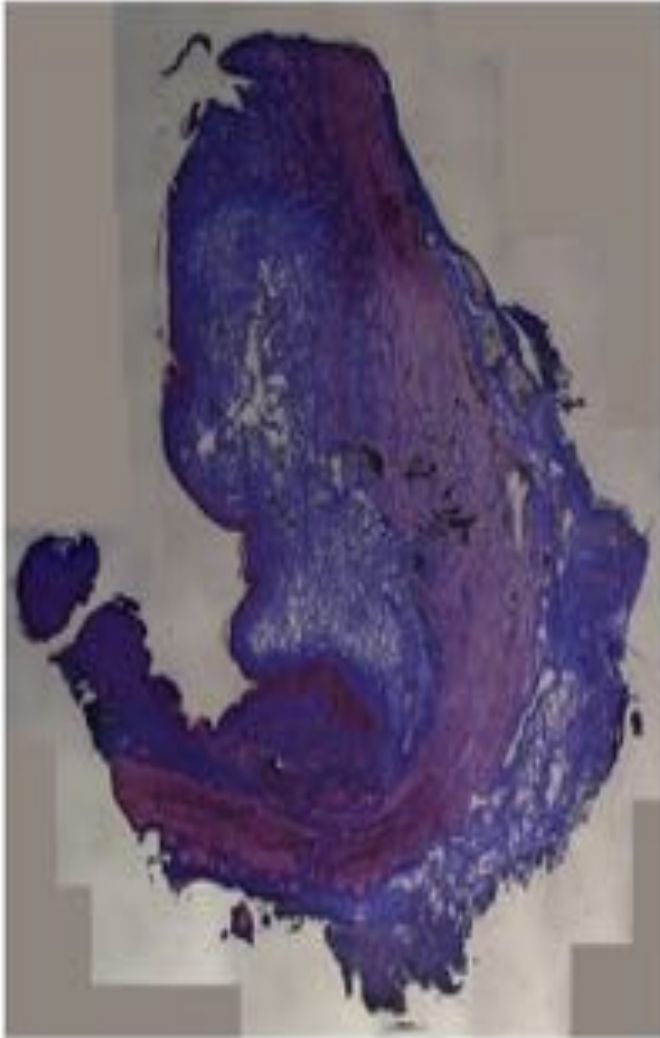


Injection of ink
by WaterJet

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



Depth of Injection Depends on the Pressure Applied During WaterJet Application

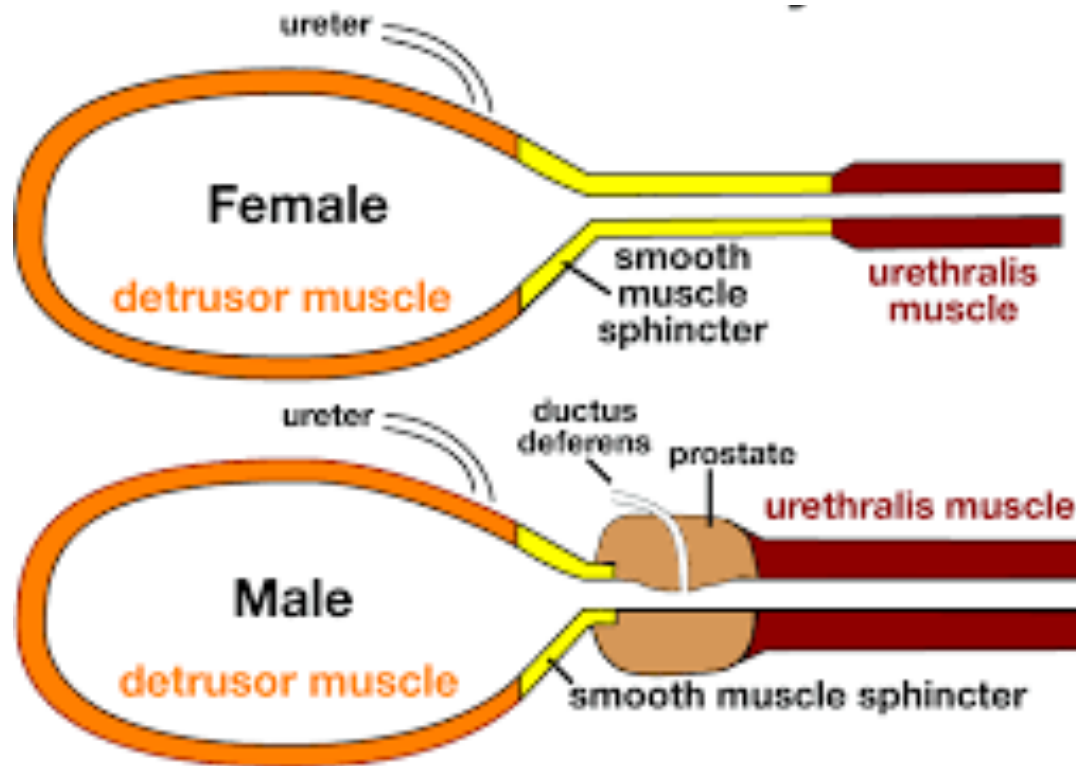


Summary

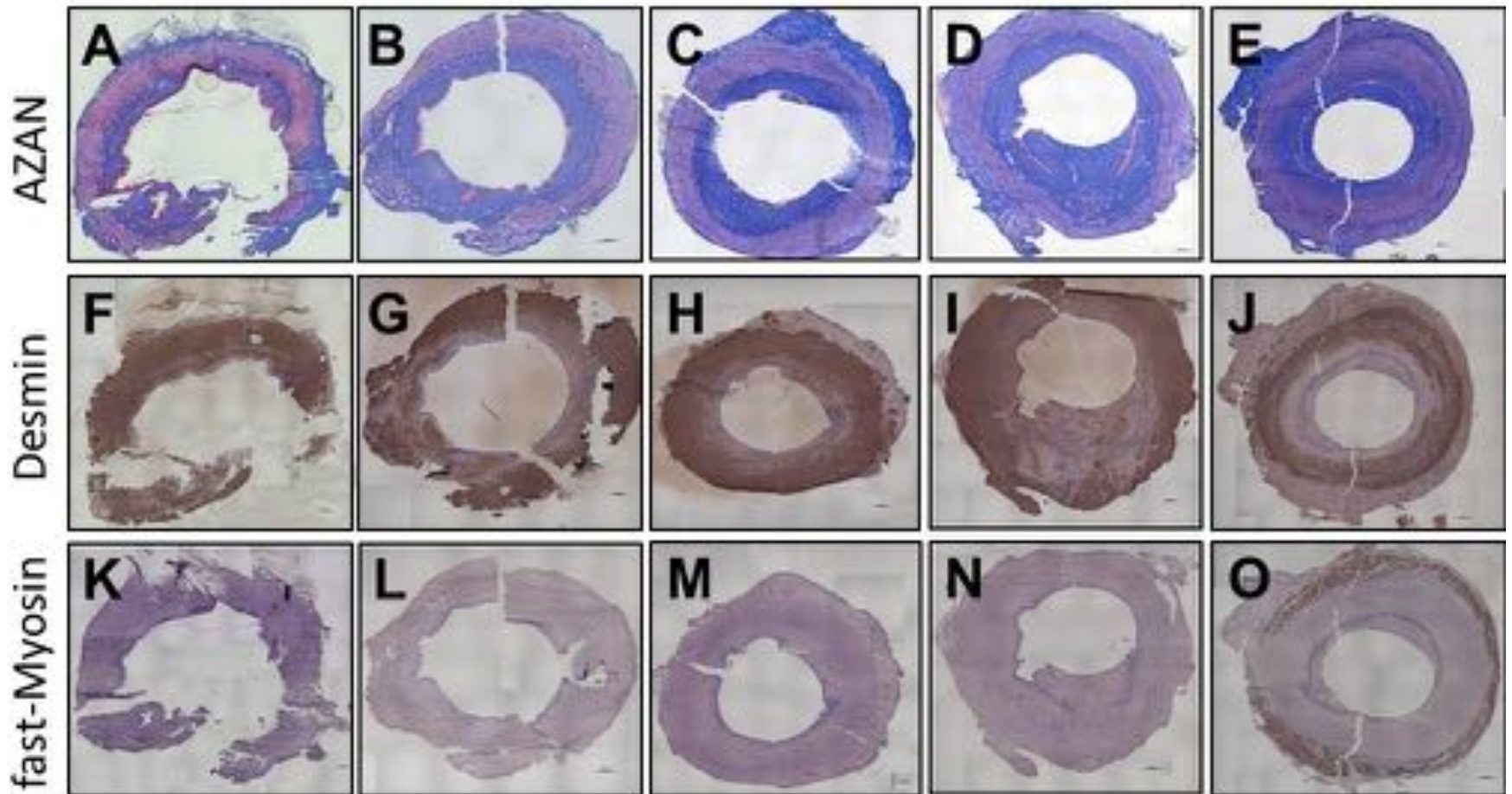
Preselection of pressure, volume and transport medium facilitates a precise yet gentle delivery of microparticles in the submucosa of the urethra

Developing a Model to Investigate the Injection of Cells in the Porcine Urethra in vivo

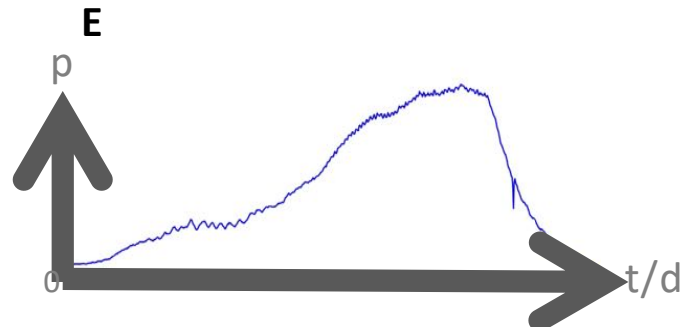
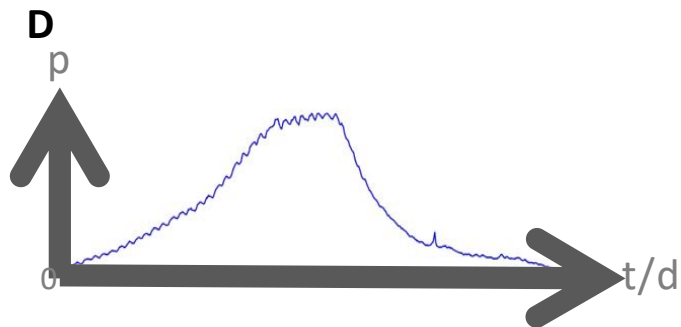
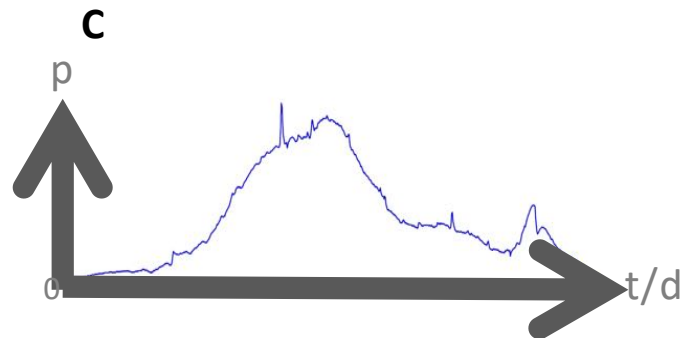
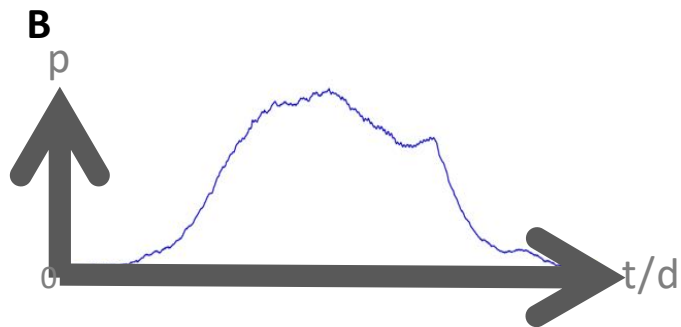
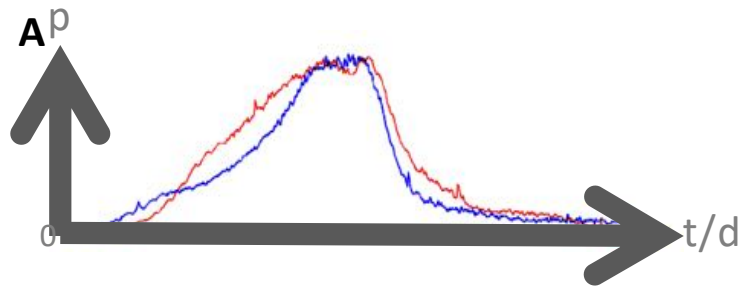
Bladder and Urethra in Quadruped Animals



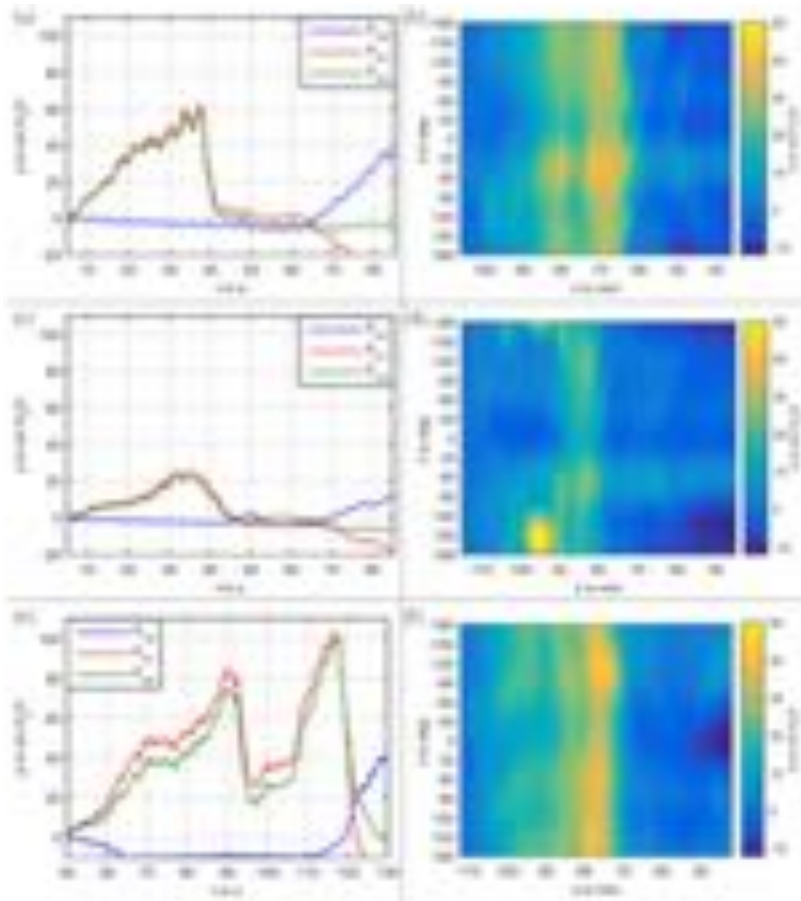
Analysis of the Urethral Sphincter Complex by Histochemistry and Immunohistochemistry



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



The Animal Model of Stress Urinary Incontinence



That is where we are right now.

Future perspectives:

Investigate injection of cells under visual control by waterjet through the urethra using an endoscope

This will take place in summer / fall 2018

Current Grant Proposal:

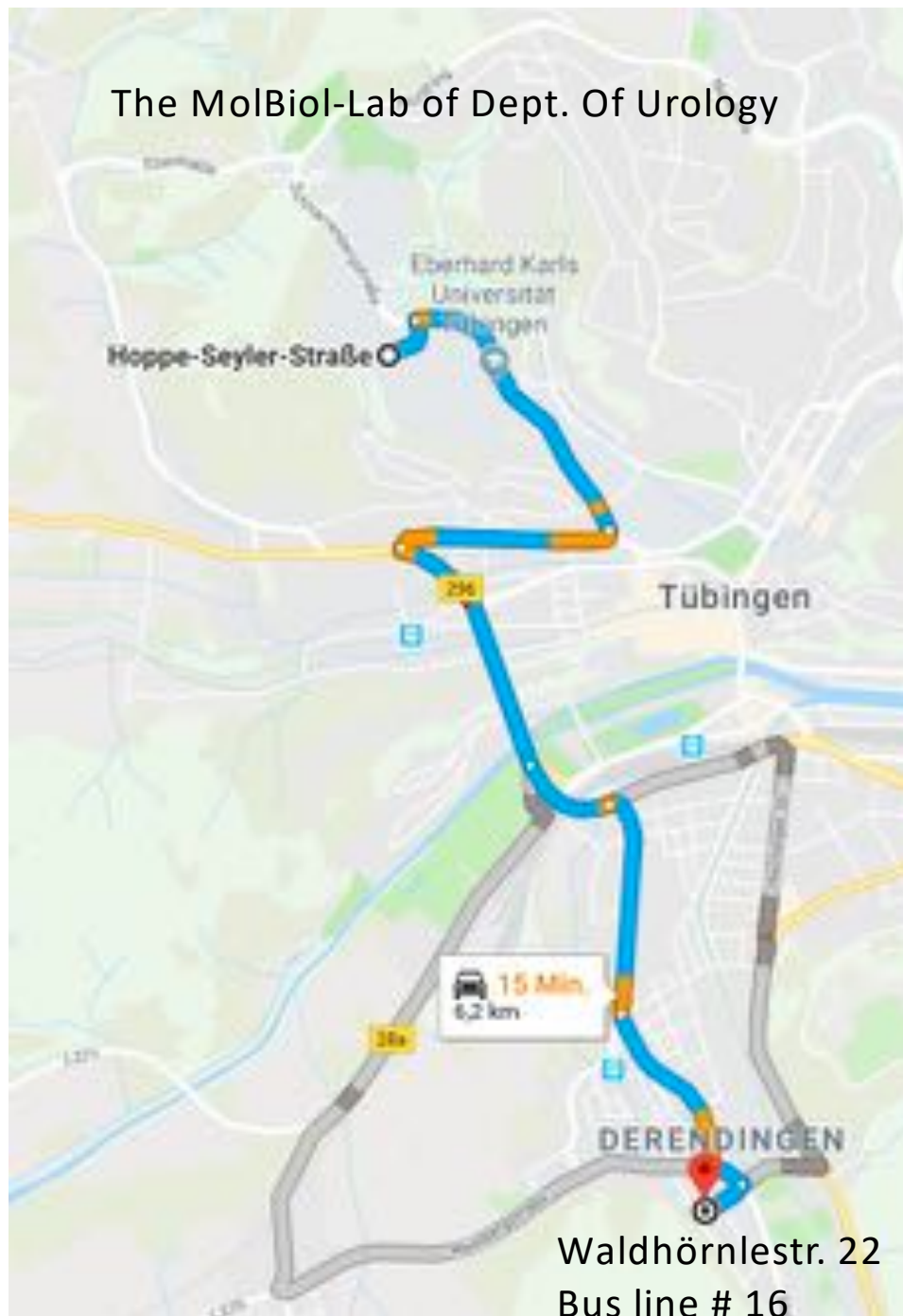
Use an animal of sphincter deficiency and investigate if cells regenerate - and if so - which cells repair SUI best

Thanks to my team for their various contributions



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The MolBiol-Lab of Dept. Of Urology



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