UROGRAFT: Implant for the urinary bladder reconstruction

There are many congenital and acquired conditions that require urinary bladder reconstruction. The most frequent cases are neurological dysfunctions, chronic inflammations, congenital and developmental defects of the urinary tract and traumas. The most frequently used material for the urinary bladder reconstruction is an intestinal wall. However, the use of this material is associated with many complications. Currently, there is no commercially available medical device or Advanced Therapy Medicinal Product (ATMP) for the reconstruction of the urinary tract which would replace autologous material (intestinal wall).

The UROGRAFT is an innovative product for human urinary bladder reconstruction. It is composed of a biocompatible, biodegradable composite scaffold inducing urinary bladder wall regeneration.

The UROGRAFT can be available in both:
· an acellular product (medical device)
· or can be combined with mesenchymal stem/stromal cells (ATMP).

The product is characterized by unique bio-configuration, favourable 3D structure, very good biocompatibility and high regenerative potential.

UNIQUE BIO-CONFIGURATION
The UROGRAFT shape, which was obtained using computer modelling based on the Finite Element Method (FEM), was designed to **minimize the area of an unfavourable environment for regeneration (scar formation in the graft centre)**, allowing for the colonization of the implant by urothelial and smooth muscle cells and revascularization. Furthermore, the shape memory of the implant allows its' insertion through a **laparoscopic or robotic trocar**. This is a clear advantage compared to the state-of-the-art surgical technique, which involves using a wall of the gastrointestinal tract, requiring multiple surgeries.

FAVOURABLE 3D STRUCTURE
The unique UROGRAFT composite **structure reduces urine permeability** and allows the growth of cells not only on the surface of the graft but also inside its three-dimensional structure (Fig.1).

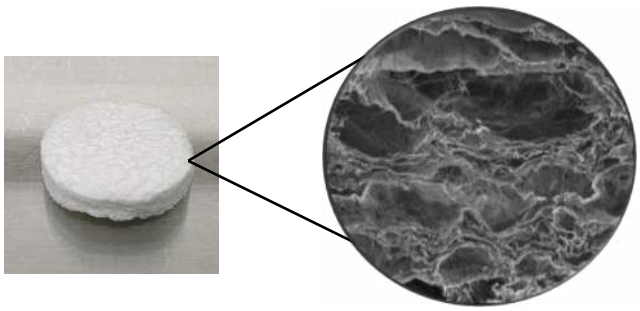


Figure 1: UROGRAFT implant structure.

It was shown that adipose-derived mesenchymal stem/stromal cells (AD-MSCs) seeded on the implant according to the invention **remain viable, attach and proliferate not only on its surface but also deep into the 3D structure of the implant.** Cell viability studies on UROGRAFT scaffolds confirmed that human AD-MSCs cultured on the UROGRAFT have a high >80% viability (Fig.2).

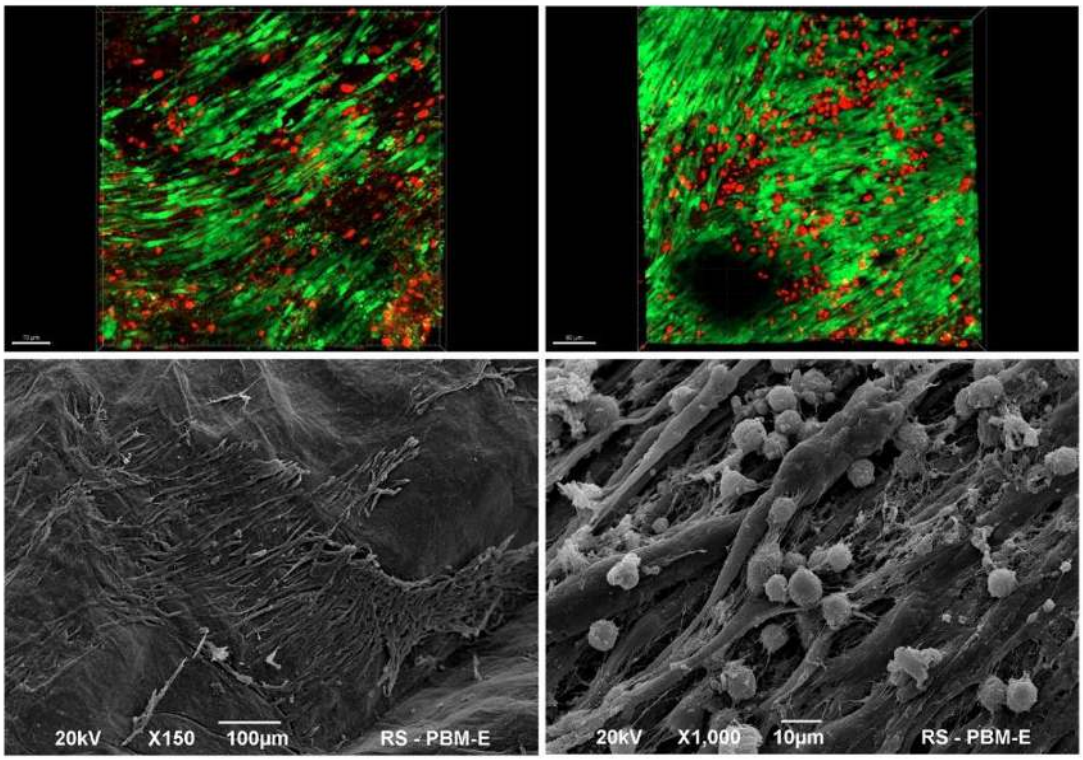
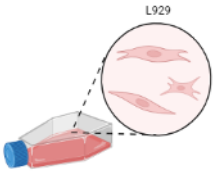


Fig. 2. Human AD-MSCs seeded on the UROGRAFT implant - live-dead staining - confocal microscope image and SEM (Scanning Electron Microscopy). The SEM results showed that the AD-MSCs cells have a correct morphology, attach to the UROGRAFT scaffold, creating a compact monolayer. The live-dead staining results showed high >80% viability of AD-MSCs on the UROGRAFT scaffold.

VERY GOOD BIOCOMPATIBILITY

The UROGRAFT was demonstrated to be **biocompatible**, *in vitro* and *in vivo;* i.e.

1. **non-cytotoxic**,
2. **non-genotoxic**,
3. **non-inducing systemic toxicity**
4. **non-inducing intradermal irritation/reactivity**.

Biocompatibility testing of UROGRAFT according to ISO 10993 biological evaluation of medical devices			
10993-5 Tests for in vitro cytotoxicity	10993-3 Tests for genotoxicity – AMES method	10993-10 Tests for irritation and skin sensitization	10993-11 Test for systemic toxicity
			
non-cytotoxic	non-genotoxic	no irritant effect	no acute systemic toxicity potential in intraperitoneal and intravenous administration

HIGH REGENERATIVE POTENTIAL

To assess the regenerative potential of the UROGRAFT preclinical study on a large animal model was performed. For this purpose, the UROGRAFT was used for urinary bladder reconstruction for 20 pigs. The observation period lasted 6 months.

Computed tomography, USG and cystoscopy reviled that the reconstructed urinary bladder wall was smooth without visible thickness, stratification and incrustations. Urodynamic analysis showed that urinary bladder function was proper, with no overactive bladder or signs of detrusor instability. The compliance of the bladder wall was also normal (Fig.3).

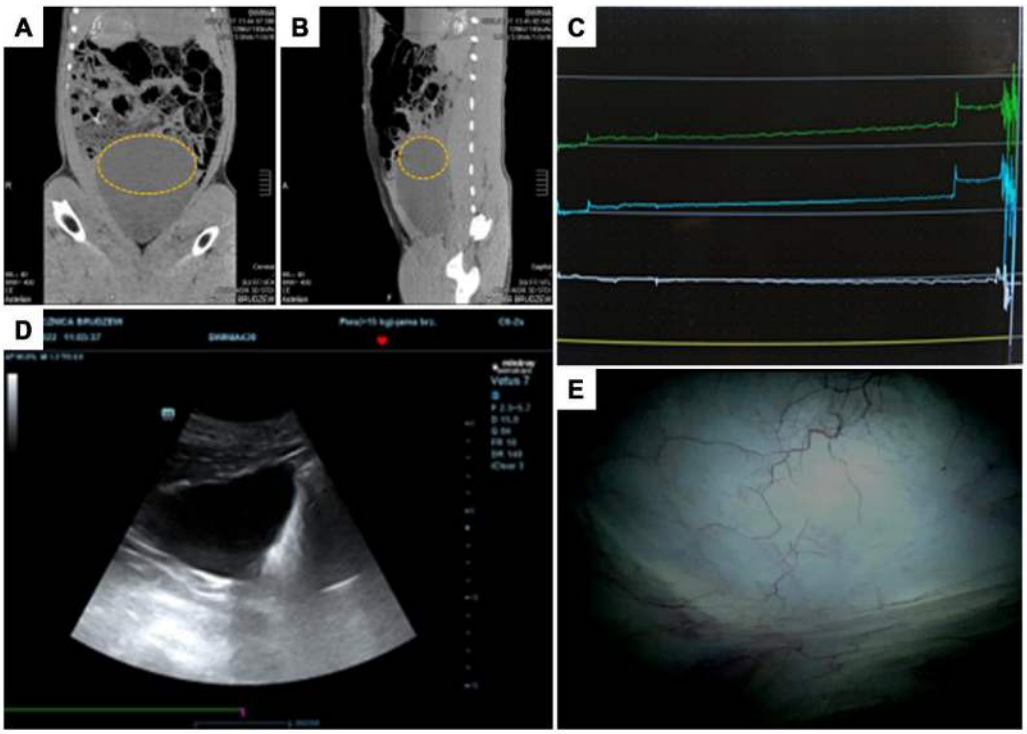


Figure 3. Imaging and functional studies in a reconstructed bladder with URGORAFT seeded with AD-MSCs (UG+AD-MSCs); AB - tomography; C- cystometry; D - USG; E - cystoscopy.

The implanted graft was very well integrated with the recipient's native tissue. Macroscopically the implant was identical to the native bladder and the border of the implant was not visible. No adhesions of the reconstructed area with the surrounding organs were observed (Fig.4).

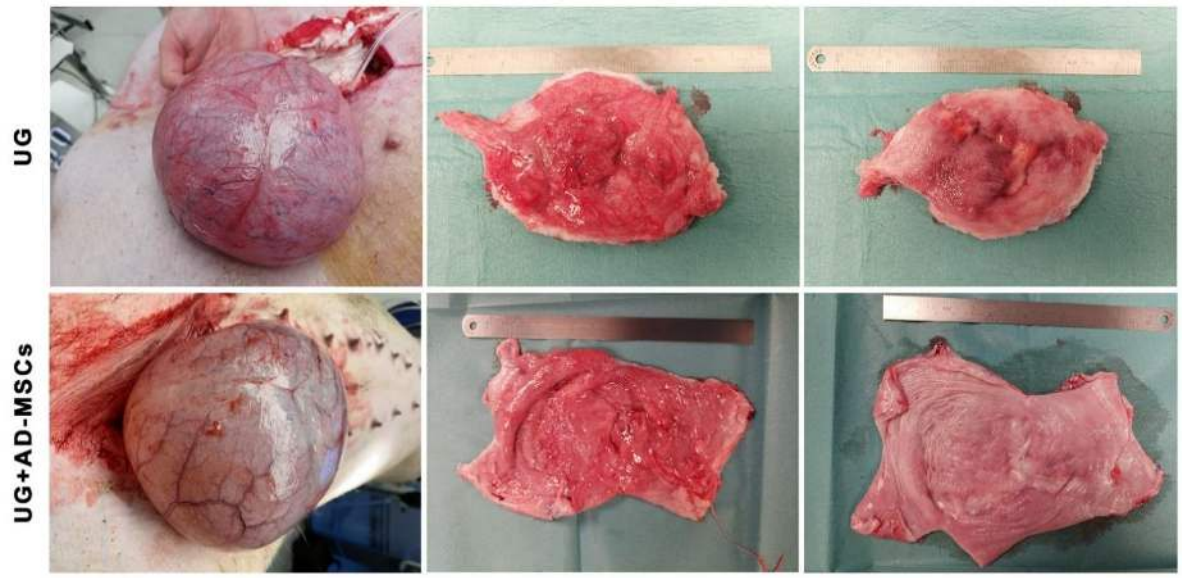


Figure 4. Porcine urinary bladders reconstructed with UROGRAFT (UG) and URGORAFT seeded with AD-MSCs (UG+AD-MSCs). Macroscopic images after 6 months follow-up. Very good integration of the UROGRAFT with the native tissue can be seen.

The regeneration process of the reconstructed bladder wall using the UROGRAFT was **more intense and was characterized by a much smaller scarring area** compared to the control group.

A well-developed mucosa was observed on the inner surface of the reconstructed area. Histological analysis using HE staining showed regeneration of the multilayered urothelium, smooth muscles and blood vessels. Trichrom-Masson staining and anti-smooth muscle immunohistochemical staining confirmed the regeneration of the muscular layer. In the central part, there were places where the regeneration process was less advanced compared to the arms and the muscle fibres were smaller and less organized (Fig.5).

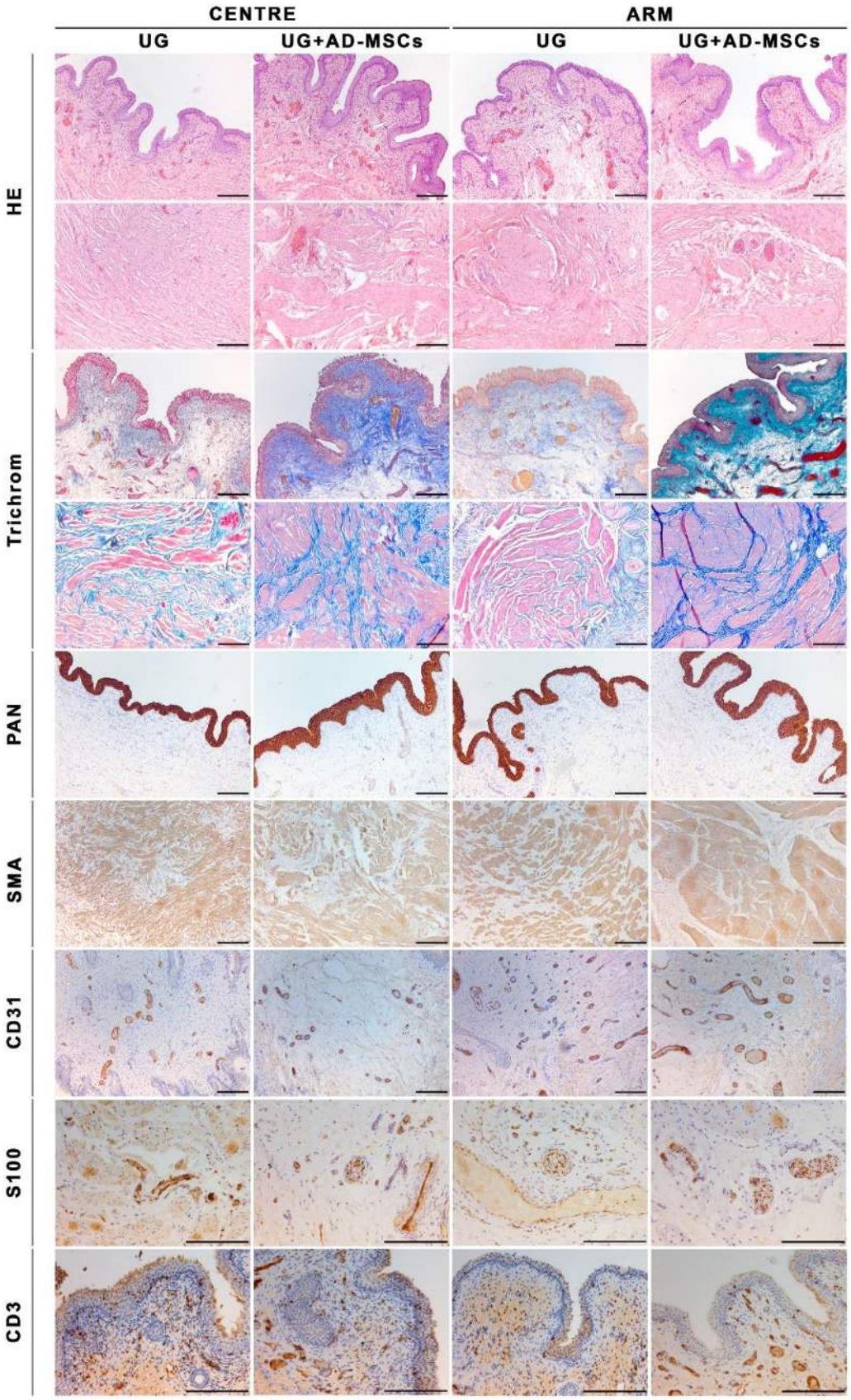
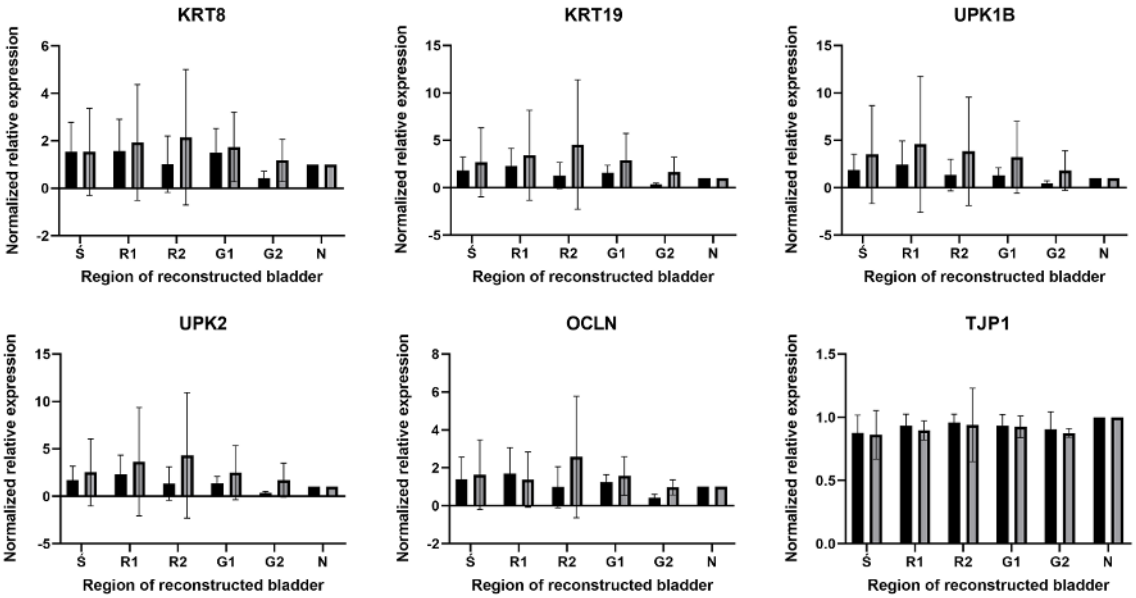


Figure 5. Histological and immunohistochemical analysis of reconstructed urinary bladder.

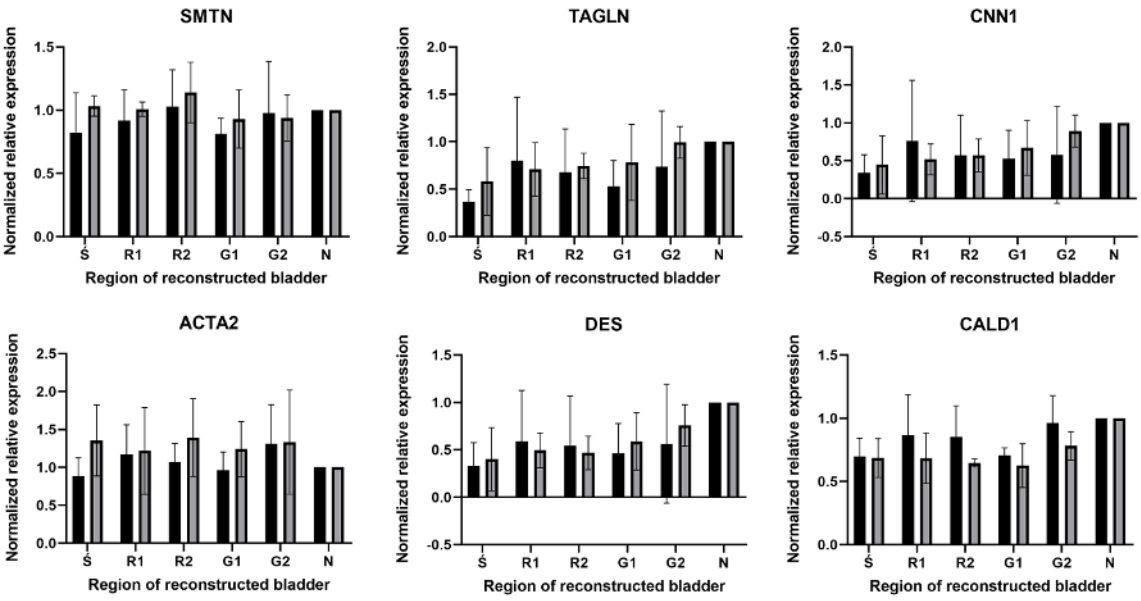
The analysis of vascularization confirmed previous observations made with the cystoscope and macroscopic analyses, in which an extensive vascular network was observed. In addition, the formation of a few nerve fibers was observed within the examined regions. Gene expression analysis was performed using real-time PCR to assess the expression of tissues-specific genes: urothelium (KRT8, KRT19, UPK1B, UPK2, OCLN, TJP1), smooth muscle (SMTN, TAGLN, CNN1, ACTA2, DES, CALD1), blood vessels (VWF, PECAM1, CDH5), nerves (UCHL1, GPM6A) and fibroblasts (VIM, FAP). These analysis showed no statistically significant differences in the analyzed fragments compared to native tissue of the bladder wall (Fig.6). The study indicated a greater regenerative capacity of the bladder wall reconstructed using the UROGRAFT implant seeded with AD-MSCs.

UROGRAFT

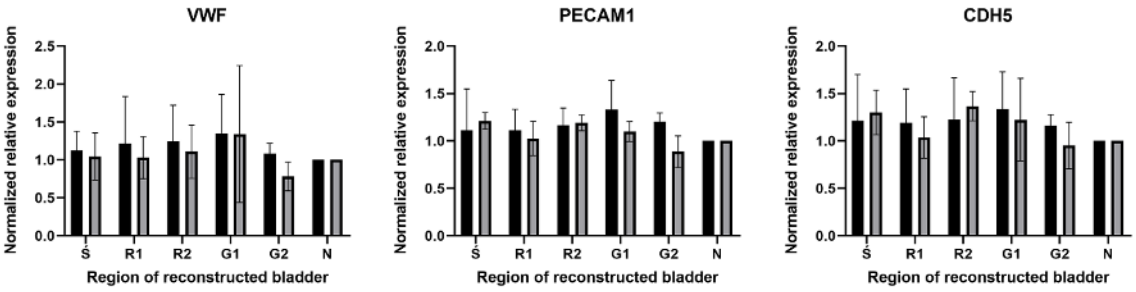
UROTHELIUM



SMOOTH MUSCLE



BLOOD VESSELS



UROGRAFT + AD-MSCs UROGRAFT

NCBR National Centre for Research and Development

LIDER

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ

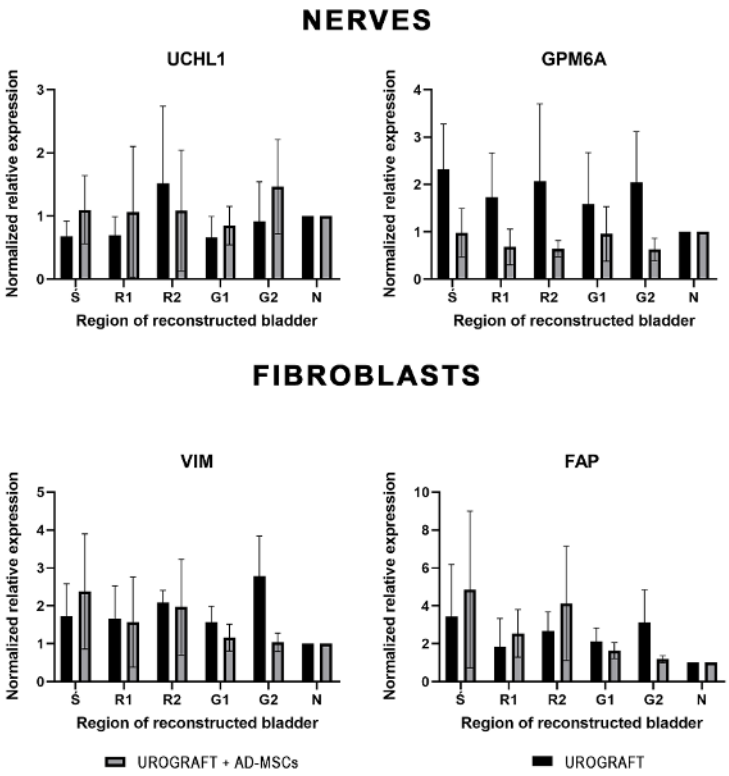


Figure 6. Gene expression analysis using real-time PCR of tissues-specific genes: urothelium (KRT8, KRT19, UPK1B, UPK2, OCLN, TJP1), smooth muscle (SMTN, TAGLN, CNN1, ACTA2, DES, CALD1), blood vessels (VWF, PECAM1, CDH5), nerves (UCHL1, GPM6A) and fibroblasts (VIM, FAP) in different regions of the reconstructed bladder with UROGRAFT and UROGRAFT + AD-MSCs.

UROGRAFT combined with mesenchymal stem/stromal cells (combined ATMP)

The UROGRAFT can be available in both: an acellular product (medical device) or can be combined with mesenchymal stem/stromal cells (combined ATMP). In order to elaborate the method of UROGRAFT seeding with human AD-MSC the process was validated in clean rooms of ATMP Manufacture according to GMP (Fig.7).

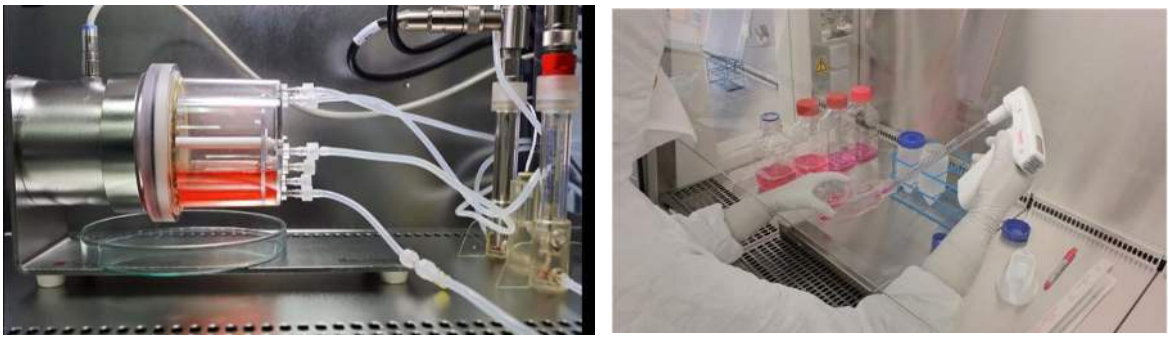


Figure 7. Preparation process of the UROGRAFT implant seeded with AD-MSCs in a clean room laboratory, ATMP Manufacture, Department of Urology and Andrology, Collegium Medicum UMK, Bydgoszcz.

The UROGRAFT is a product fully tested *in vitro* and *in vivo*, ready for use in a clinical trial.

The present work was supported by the National Center for Research and Development (NCBR) in Poland under Agreement no. LIDER/48//0195/L-9/17/NCBR/2018.

Highlight the benefits of the innovation in comparison with existing solutions

The most common indication for reconstruction of the bladder wall (cystoplasty) is the small high-pressure urinary bladder which can be results of:

- congenital and developmental defects of the urinary tract;
- chronic inflammation;
- neurogenic dysfunction;
- radiation damage;
- injuries;
- other diseases.

The most commonly used technique for bladder reconstruction utilize the autologous gastrointestinal tract tissues. However this technique carry the risk of many complications resulting from the additional surgical procedure on the intestine and the different properties of the wall of the gastrointestinal and urinary tract.

It results in:

- electrolyte and osmotic imbalance;
- neurological disorders;
- bone demineralization;
- infections;
- stone formation;
- an increased risk of carcinogenesis at the anastomotic sites of urothelium and intestinal epithelium.

At the moment, there is **no implant for the reconstruction of the urinary tract to replace the currently used autologous material** - the wall of the patient's gastrointestinal tract. Currently, various biomaterials are used in **experimental research** on the regeneration of tissues and organs using tissue engineering techniques. A special role is played by biomaterials obtained by decellularization, i.e. removal of cells from tissues or organs of animal organisms. The decellularization process consists in the complete removal of cells and their remains (nucleic acids) from tissues or entire organs using physical, chemical and biological methods. The matrix obtained in this way is made of structural proteins of the extracellular matrix, mainly collagen and elastin.

The best-known commercially available products used in clinical practice, based on cell-free matrices, are:

- **SIS (Small Intestinal Submucosa):** a cell-free scaffold made from the submucosa of the porcine small intestine wall, commercially available as Surgisis® from Cook Medical. This material is used in the treatment of hernias, perianal fistulas, dural plastic surgery, otological procedures (myringoplasty, tympanoplasty) and gastroenterological procedures (rectal prolapse surgery);
- **Decellularized bovine or equine pericardium:** commercially available under the name Veritas® or OrthAdapt® from Synovis Life Tech. This material is used in cardiac surgery in the repair of cardiovascular defects;
- **Decellularized human or porcine skin:** commercially available as AlloDerm® or Strattice™ from LifeCell Corp. as a material used as a skin substitute in the treatment of burns and breast reconstruction procedures;
- **Bladder Acellular Matrix (BAM):** commercially available as Matristem UBM. This material is intended for the treatment of difficult-to-heal wounds.
- Noticeably BAM matrices are used in experimental research for **bladder reconstruction**.

The main problems associated with the use of cell-free unmodified matrices in bladder reconstruction are:

- insufficient biocompatibility;
- urinary permeability;
- implant fibrosis.

THE URGRAFT ADVANTAGES:

Surgical procedure:

- elimination of the need of gastrointestinal tissues
- hence, elimination of the need to perform additional surgery
- shortening of the total operation time
- lack of complications associated with gastrointestinal tract surgery
- insertion of the implant through a laparoscopic or robotic trocar

Regarding the implant itself:

Main advantage: *Biological and mechanical properties similar to the native bladder wall*

In more details:

- **Shape of the implant**:
  - minimizes the area of an unfavourable environment for regeneration
  - avoid the need to create additional cavities in the bladder wall
  - accepts urine storage pressure between 20-60 ml/cm H_2O

- **Impermeability to urine**:
  - unique composite implant structure reduces urinary permeability

- **Full biocompatibility:**
  - conducted *in vitro* and *in vivo* studies have showed that the implant is biocompatible - non-cytotoxic, non-genotoxic, non-inducing systemic toxicity and non-inducing intradermal irritation/reactivity.

- **Preclinical experiments on a large animal model** (on 20 pigs) has demonstrated the efficacy of the UROGRAFT to induce urinary bladder wall regeneration.

- **"Straight off the shelf" product**: The product is available immediately and does not need to be specially made to suit a particular purpose
- **ATMP:**
  - UROGRAFT can be used in combination with mesenchymal stem/stromal cells
  - the unique composite structure of the implant allows the cells to grow not only on the surface of the implant, but also inside its three-dimensional structure

Technology Readyness Level (TRL): The UROGRAFT product is at the TRL level 6: "technology demonstrated in a relevant environment". It has been demonstrated in-vivo on 20 pigs which were operated on. However, clinical trials have not been started yet.

It should be mentioned at this point that Prof. Pokrywczyńska has the necessary infrastructure
(Cell and Tissue Bank and ATMP Manufacture) to operate the clinical trial phase on-site at Ludwik Rydygier Medical College in Bydgoszcz, University Nicolaus Copernicus.

The invention UROGRAFT has been applied for in the Polish Patent Office: patent for the invention entitled: "Wszczep do rekonstrukcji pęcherza moczowego, sposób jego otrzymywania oraz rusztowanie do wszczepu do rekonstrukcji pęcherza moczowego" application number: P.443324 [WIPO ST 10/C PL443324] (2022) and to the European Patent Office application no.: 2246008.9/EP22460080 (2022). In addition, a trade mark UROGRAFT (word mark) has been claimed trade mark no: 018816326 (2022) at the European Union Intellectual Property Office (EUIPO) and trade mark UROGRAFT (figurative mark with word elements) trade mark no: 018816337 (2022) at the European Union Intellectual Property Office (EUIPO).

Certificates, approvals, quality marks, expert opinions, recommendations, etc.:

- Certificate of sterility of UROGRAFT product seeded with hAD-MSCs cells
- Cytotoxicity test report according to ISO10993-5
- Genotoxicity study report according to ISO10993-3
- Test report for systemic toxicity in accordance with ISO 10993-11
- ISO 10993-10 Irritancy/intradermal reactivity test report
- Scientific recommendation for classification of Advanced Therapy Medicinal Products from the European Medicines Agency
- Positive expert opinion of Dr. Michal A. Skrzypczyk FEBU, President of the Reconstructive Section of the Polish Urological Association

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

23 March 2023
EMA/CAT/54798/2023
Committee for Advanced Therapies (CAT)

SCIENTIFIC RECOMMENDATION ON CLASSIFICATION OF ADVANCED THERAPY MEDICINAL PRODUCTS
Article 17 – Regulation (EC) No 1394/2007

The present scientific recommendation refers exclusively to the case as presented to the Agency without prejudice to future evaluations by the Agency.
It is stressed that the scientific recommendation on advanced therapy classification does not amount to any endorsement of the plausibility of the product, including the mode of action or therapeutic indication(s) claimed by the applicant.

1. CAT OUTCOME SUMMARY

Proposed product invented name or identifier ("the Product")	UROGRAFT	
Company developing the Product (applicant)	Nicolaus Copernicus University, Chair of Urology and Andrology, ATMP Manufactory, Bydgoszcz, Poland	
Brief description (common name or international non proprietary name where available) of Active substance(s)	Allogenic or autologous adipose-derived stromal cells	
Brief description of the finished Product	[redacted] scaffold seeded with allogenic or autologous adipose-derived stromal cells	
Proposed Indication (as proposed by the applicant)	Urinary bladder wall augmentation in patients with small capacity high pressure urinary bladder	
Advanced therapy medicinal product classification (as agreed by the CAT)	Not ATMP	
	Gene therapy medicinal product	
	Somatic cell therapy medicinal product	
	Tissue engineered product	X
	Combined ATMP	
CAT Co-ordinator	Maura O'Donovan	
ITF Co-ordinator	Nino Mihokovic	

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Opinia ekspercka

UROGRAFT - wszczep do rekonstrukcji pęcherza moczowego człowieka opracowany przez Zespół pod kierunkiem Pani Profesor Marty Pokrywczyńskiej z Katedry Urologii i Andrologii Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy, Uniwersytetu Mikołaja Kopernika w Toruniu to obiecujący wynalazek, który może znaleźć w przyszłości zastosowanie w operacjach rekonstrukcyjnych dróg moczowych i zastąpić obecnie stosowany materiał autologiczny (fragment przewodu pokarmowego). Zastosowanie wszczepu UROGRAFT podczas rekonstrukcji pęcherza moczowego ma wiele potencjalnych korzyści, np.:

- możliwość wyeliminowania etapu operacji podczas, którego izolowano fragment przewodu pokarmowego i odtwarzano jego ciągłość;
- skrócenie czasu zabiegu;
- uniknięcie powikłań wynikających z wykorzystania fragmentu przewodu pokarmowego.

Dodatkowym atutem produktu UROGRAFT jest możliwość jego zastosowania w połączeniu z mezenchymalnymi komórkami zrębowymi/macierzystymi tkanki tłuszczowej.

Na podstawie przedstawionych przez Zespół wyników przedklinicznych *in vitro* i *in vivo* mogę poświadczyć, że UROGRAFT jest bardzo atrakcyjnym produktem o dużym potencjale do zastosowania klinicznego.

Dr n. med. Michał A. Skrzypczyk, FEBU
Adiunkt Kliniki Urologii CMKP w Warszawie
Przewodniczący Sekcji Urologii Rekonstrukcyjnej
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