Bioprinting and regenerative medicine: a new approach for reconstructive urology

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Urethral stricture: A common disorder with complex consequences

Urethral dysfunction is a common condition in men, with one particular problem standing out due to its prevalence and impact on quality of life: urethral stricture. A urethral stricture is a condition in which a section of the urethra becomes narrowed due to various causes, including congenital abnormalities present at birth, injuries from bicycle accidents, sexually transmitted infections such as chlamydia, or damage resulting from medical procedures such as urinary catheterisation or cystoscopy.[1,2] Among different urinary tract disorders, urethral strictures stand out. It is estimated that this condition affects between 6 and 25 million men worldwide, making it a significant global health problem. Men suffering from urethral strictures may experience a range of symptoms, including weak urinary stream, straining to urinate, incomplete emptying of the bladder, involuntary urine leakage immediately after urination, urinary retention, as well as recurrent urinary tract infections[3].

Graphic of a stricture in the male urethra. Source: STRONG-UR

To better understand how urethral strictures affect men's daily lives, the STRONG-UR team interviewed Lucas, a 23-year-old, who was diagnosed with the condition after developing a urinary tract infection. Since urinary infections are relatively uncommon in men, his doctors performed a cystoscopy to examine his bladder. It was during this procedure that they discovered Lucas had a congenital urethral stricture. Lucas recalls being very surprised by the diagnosis, as he had never suspected anything was wrong. He shared that he had always needed to squeeze his penis to get a proper flow of urine but had assumed this was normal. After treating the infection, his clinical team recommended surgery to restore normal urinary flow. This intervention not only improved his symptoms but also reduced the risk of future infections and the loss of urinary function later in life.

[1] Cytoscopy is a procedure where a flexible tube is inserted through the urethra. A cystoscope consists of a camera and a light source that are used to observe the urethra and the bladder.

[2] Urethral strictures guidelines. European Association of Urology. https://uroweb.org/guidelines/urethral-strictures/chapter/definition-epidemiology-aetiology-and-prevention

[3] Epidemiology of urethral strictures, Amjad Alwaal, Sarah D Blaschko, Jack W McAninch, Benjamin N Breyer, National library of Medicine, 2014: https://pubmed.ncbi.nlm.nih.gov/26813256/

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Another patient, Oliver, now in his 20's, shared that the stricture developed when he was about 16 years-old, without any identifiable cause. He decided to consult a doctor due to pain during urination, blood in his urine, and a weak urinary stream. His case is not unusual. In fact, many strictures appear without a clear cause, but gradually develop, especially in young men.

To better understand the challenges of treating strictures, the STRONG-UR team spoke with two surgeons who meet stricture patients regularly. "*While good treatments exist, the success rate significantly decreases with repeated interventions, leading to patient anxiety and low self-esteem*", explains Dr. Grazvydas Tuckus, a consultant urologist at Aalborg University Hospital in Denmark. "*The first treatment would be cutting or dilating the stricture accordingly to its dimensions, [such as the intervention received by Lucas and Oliver]. In 30% of the cases, this does not work, and we see patients coming back because they are in pain or develop infections*." Dr. Marco Bandini, urologist specialised in the treatment of complex urethral strictures at the Hospital of San Raffaele in Milan, Italy, explained: "*Most of my patients had multiple unsuccessful stricture surgeries or complications from previous surgeries (removal of prostate tissue[4] or frequent catheterisation) creating strong strictures. Those patients are generally in their 60s or 70s. These situations often require urethroplasty, a procedure that reconstructs the urethra using the patient's own healthy tissue. The primary challenge is selecting the best tissue source. The most common options are tissue from inside the patient's cheek or, in some cases, the foreskin. For very long strictures (over 10 cm), both sources may be needed but can only be used once, and there is a risk of infection in these areas. Recovery is longer and painful for patients*".

Addressing the clinical challenge with 3D bioprinting

The urgent need for innovative alternatives in urethral reconstruction is clear – not only to enhance patients' quality of life but also to reduce long-term healthcare costs for society. One promising solution lies in regenerative medicine, specifically 3D bioprinting using the patient's own cells. This is the vision behind our project: the STRONG-UR team aims to revolutionise urethral repair by developing customised tissue constructs using a regenerative medicine approach. The heart of this new technology is the combined use of a 3D bioprinter and a specially developed bioink used in combination to produce customised tissues that mimic the natural structure and functions of the urethra. But what is bioink and why has it been chosen for urethra reconstruction?

[4] BPH: benign prostatic hyperplasia

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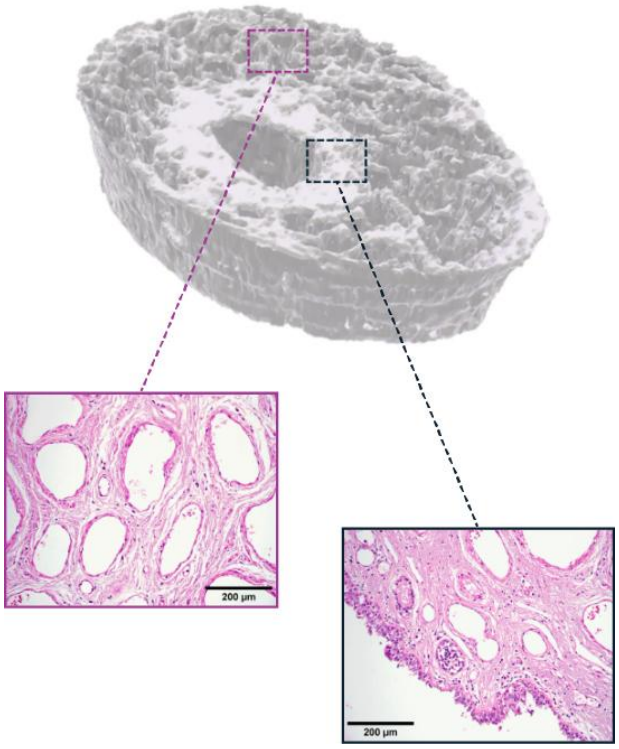
A bioink is a special mixture that contains living cells and specially formulated biocompatible hydrogels. This bioink is then printed layer by layer to form tissue structures that mimic the natural architecture and function of the urethra. It works like any other object designed with a 3D printer. Since the gels respond to specific stimuli, the microenvironment of the cells in the bioprinted tissues can be controlled more precisely by this method. This precision is crucial for replicating the complex, multilayered structure of the male urethra. A fundamental step in the development of the bioink is understanding the architecture and composition of the urethral tissue, specifically, which cells comprise the tissue and how they are organised.

Understanding the male urethra's complexity

The male urethra is far more complex than is often realised. It is not simply a tube to pass urine and semen out of the body; it is a living vascularised tube. All living tissue in the body is connected to the bloodstream, which is responsible for the delivery of nutrients and oxygen as well as for waste removal. This connection to the bloodstream is particularly crucial for the urethra, as it is surrounded by a highly vascularised spongy tissue known as the corpus spongiosum[5]. Like an "airbag", this erectile tissue surrounds and protects the urethra during urination and sexual activity. Despite its importance, this tissue has often been overlooked in traditional urethral replacement surgeries and more generally in urology research.



3D-reconstruction of the urethral surface and the spongy tissue. This 3D-model is based on microscopy images such as those shown in the right panels. The model will be used to guide the 3D bioprinting process. Source: UMC

Our team of researchers is now working to shift the paradigm towards a replacement not only of the urethra, but of the complex multilayered structure which broadly consists of:

[5] de Graaf P, Ramadan R, Linssen EC, Staller NA, Hendrickx APA, Pigot GLS, Meuleman EJH, Bouman M, Özer M, Bosch JLHR, de Kort LMO. *The multilayered structure of the human corpus spongiosum*. Histol Histopathol. 2018 Dec;33(12):1335-1345. doi: 10.14670/HH-18-022. Epub 2018 Jul 9. PMID: 29985521.

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- The first layer: a tightly packed layer of cells that prevents urine from leaking into surrounding tissues.
- The second layer: full of small blood vessels for tissue viability and healing.
- The final layer: where the tissue changes to become more sponge-like in form (the so-called corpus spongiosum).

STRONG-UR's holistic approach represents a step change from previous attempts to bioengineer urethras since the team looks at the more complete picture, taking into consideration not only the thin inner layer, but all the layers that compose the urethra.

To better support patients, the STRONG-UR team must adapt treatments to their specific and individual needs. This requires a deep understanding of three key aspects of the urethral tissue:

- How does each urethral layer work?
- What is the precise geometry of each layer?
- What are the unique structural and compositional features of each layer?

To answer these questions, our team uses advanced microscopy techniques to analyse a large bank of previously preserved urethral tissue samples.

Another important part of our research focuses on the mechanical properties of urethral tissues. During sexual activity and urination, they undergo significant degrees of elongation or compression. Understanding how the tissue responds to these forces helps us link the structure and composition to the functional properties of the urethra. This knowledge is essential for designing bioprinted constructs that not only replicate the urethra's appearance but also its mechanical resilience, ensuring long-term success and comfort for patients.

A section of the urethra and spongy tissue decellularised (all cells removed from the tissue) and stained to identify the composition of the tissue. Source: UMC

A section of the urethra and spongy tissue visualised under a microscope. Source: UMC

The STRONG-UR team observed that different urethral layers require different types of cells each with its own role, but all working together as a team. Petra de Graaf, a researcher in regenerative medicine in urology at UMC Utrecht, The Netherlands, explains that "*we focus on finding a way to precisely place the right cells at the right location, within the defined layer, and have them happy inside and effectively communicating with each other. Identifying the cells has been a long process. Developing a gel that fits them, in which they proliferate and can communicate with each other is yet another one. Our final challenge for the cells is to find a way to make sure they survive during the complex bioprinting of the multilayered structure*".

In fact, printing cells is not easy. The gel used must be expelled under high pressure to settle properly on the support platform. Because the cells are very sensitive to pressure, this creates a major challenge: to find the right printing parameters while balancing the gel's properties to minimise cell damage. The key lies in combining the right shape, the right properties, and the right cells with an optimised printing protocol. This requires involving patients, surgeons and researchers from different backgrounds, all contributing to the development of a viable alternative for urethral replacement. But you might wonder whether this is safe? This is a crucial question, so before testing on living animals and eventually humans, researchers must ensure that the bioink formulations used in bioprinting are safe and compatible with living tissue.

A bioink for medical use: searching for a safe formulation

Bioinks offer two major advantages for organ reconstruction:

- They allow the direct fabrication of living tissue constructs, using various 3D printing techniques such as extrusion or stereolithography (a method that uses a UV laser to selectively solidify layers of liquid resin) to generate customised shapes.
- They stimulate tissue healing and regeneration, unlike conventional inert materials that may trigger chronic inflammation or fibrous encapsulation - a defence response where the body forms a dense tissue layer around a foreign material.

Did you know that bioink formulations are made primarily from biological or renewable sources? For medical applications, bioinks typically contain polymers, which are long, chain-like molecules. These polymers can be tailored to have specific physical and chemical properties, making them suitable for human use. Some of these polymers are designed to absorb and retain water, forming materials known as hydrogels. Hydrogels are especially useful in regenerative medicine because their water-rich structure supports cell survival and promotes tissue formation, making them ideal for creating new tissue. To ensure biocompatibility and support cell growth, the STRONG-UR team uses gelatine as a primary component of the bioink. Gelatine is a protein derived from collagen, which is found in animal

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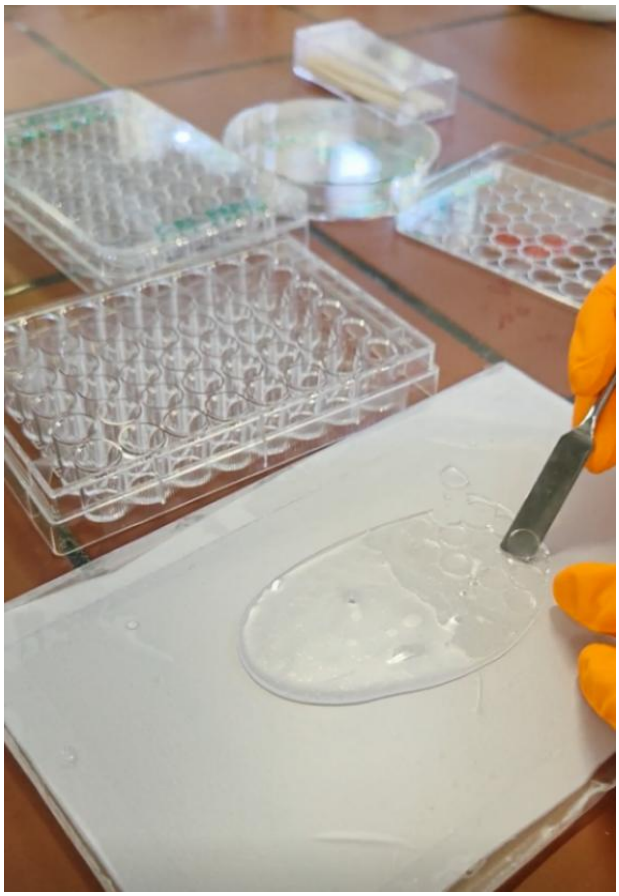
connective tissues. It is widely used in biomedical applications. Gelatine-based hydrogels show the most promise, due to their ability to mimic the natural cellular microenvironment.

Hydrogels used in bioinks can be tailored to meet the specific needs of urethral tissue, which include flexibility (i.e. swelling ratio and mechanical strength), and bio-degradation rate (i.e. how long the hydrogel remains in the body) which can be further fine-tuned for the application. This last property is particularly important since the hydrogel must support the cells and act as a scaffold for the tissue to regenerate. Once the new tissue has formed the hydrogel should gradually degrade as the new tissue replaces it, minimising risks from long-term foreign materials in the patient's body. These functionalities are crucial for providing an optimal microenvironment for cell regeneration while also allowing for successful integration into the test subject (be it animal or human). But the question now is, how will the 3D-printed hydrogel-based urethra be designed and placed in the body?



Hydrogel flexibility test. Source: 4Tissue

Surgical strategies toward clinical success

While the bioink formulation must meet the biological and mechanical needs of the application, its clinical implementation in the body needs to follow certain procedures that ensure the successful integration and acceptance of the reconstructed organ. STRONG-UR proposes two different approaches for clinical application: A single stage “fast-track” approach, and a multistage approach.

A single stage “fast track” approach

At the Catholic University of the Sacred Heart (UCSC) in Rome, Italy, pioneering successful treatments were performed that demonstrate the huge value of bioprinting in the clinical settings. The team at UCSC treated patients with intractable fistulas (i.e. abnormal openings between tubular structures), which often involve reoperations, complications and prolonged

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hospital stays, poor healing, and increased patient morbidity. Our team successfully treated a chronic *oesophago-pleural fistula*, an abnormal connection between the oesophagus and trachea, in a patient for whom conventional treatments had failed. Their method involved extracting the patient's own fat tissue and reinjecting it into the fistula, a technique known as Stromal Vascular Fraction (SVF) treatment. This treatment has also been used in patients with chronic perianal fistulas in inflammatory bowel disease (Crohn's disease). In one case, a 74-year-old patient with a recurrent rectovesical fistula (between the bladder and the rectum), received a personalised 3D-bioprinted tissue implant made from his own cells. The implant was inserted via an endoscopy into natural openings. After just fourteen days, new vascularised tissue growth was observed, demonstrating the success of the procedure.

The patient cases described earlier were performed in the context of compassionate cases, meaning that the patients only had access to an experimental treatment because they were affected by a serious or life-threatening condition lacking satisfactory alternatives. The STRONG-UR team aims to move beyond these exceptional cases to be able to offer this regenerative solution to a wide range of patients with standard authorisations. To achieve this, specific regulatory approvals are required, which demand pre-clinical evidence of treatment efficacy.

The method described above provides a less invasive and faster alternative to traditional surgery by promoting natural tissue regeneration. For this reason, our STRONG-UR team has chosen to replicate this fast-track approach to treat fistulas that may develop from urethral strictures. A urethral stricture can cause a fistula when increased pressure and tissue damage create a hole in the urethra, allowing urine to exit through unintended openings. The proposed procedure uses a handheld bioprinter (BioPen) developed by STRONG-UR partner AdBioink, which will allow injecting the bioink directly into the affected urethra, enabling precise placement and better adaptation to the irregular surface of the tissue defect.

Multistage approaches for complex strictures

For larger or longer strictures, a multistage approach, will be used. This approach uses bioprinted tissue grafts produced in the operation room with bioinks tailored to match the structural and mechanical properties of the urethral tissue. Two types of repairs are being tested: an "onlay" reconstruction, for partial urethral repair, and a tubular reconstruction, for complete urethral replacement. Both procedures use customised 3D bioprinters from STRONG-UR partner Brinter AM, adapted specifically for graft production.

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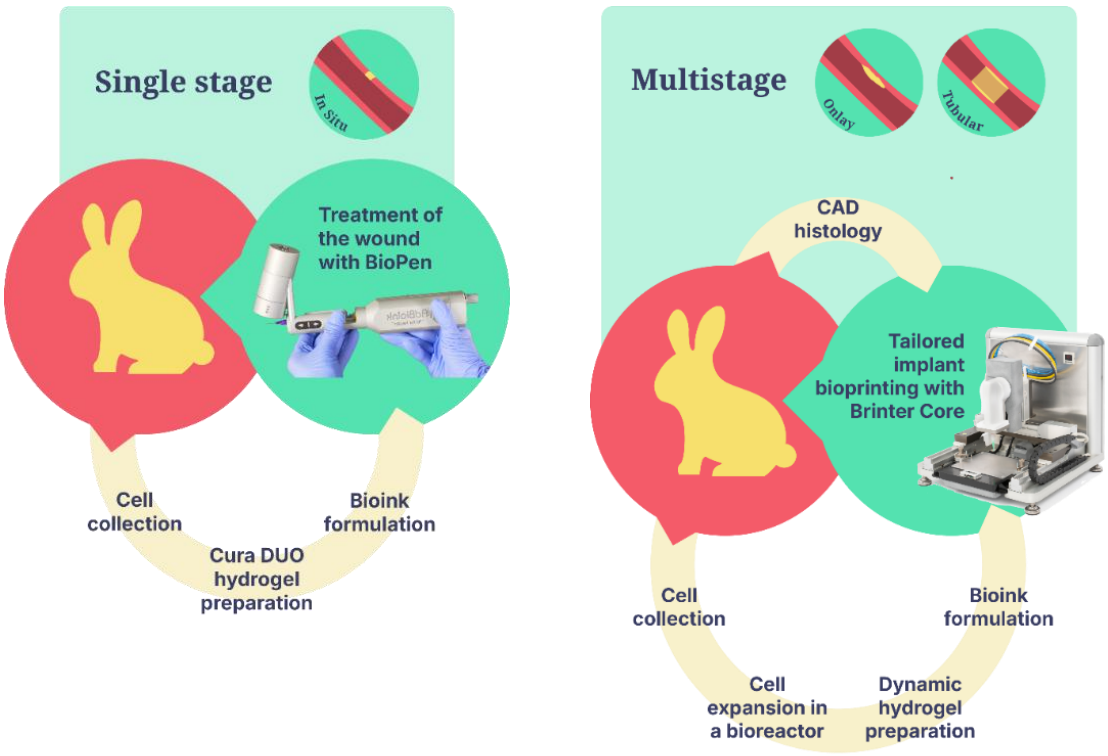
In vivo



In vivo experiments planned by STRONG-UR team to test the two approaches. Source: STRONG-UR

To test the two approaches described above, our team of researchers plan to conduct a series of animal studies using rabbits to validate the bioprinting approach and pave the way for clinical trials. Animal studies remain essential in medical research, ensuring that new innovations are both safe and effective before being tested in humans. To minimise animal suffering, our team has obtained all necessary ethical approvals and certifications and strictly follow the three “Rs" principle:

- **Replacement:** Using in *vitro* models (i.e. outside of a living organism) as an alternative to animal testing, including the use of 3D tissue engineering of the human urethra.
- **Reduction:** Applying non-invasive imaging techniques to monitor animal health over time, thereby reducing the number of animals required.
- **Refinement:** Ensuring animals are housed in authorised facilities and provided with the highest standards of care, including the use of minimally invasive techniques and ensuring humane treatment throughout the research process.

By following these steps, our team strive to balance scientific progress with the ethical treatment of animals, ensuring the highest level of care while advancing medical knowledge.

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Controlling the bioink stability

As previously explained, gelatine-based bioinks offer key advantages such as biocompatibility and biodegradability. However, gelatine temperature sensitivity can pose challenges during processing, leading to premature gelation at lower temperatures or the loss of shape fidelity at higher temperatures, which affects printing stability and accuracy. To overcome this, AdBioink has developed the BioPen, a portable, compact and ergonomic handheld bioprinter. The device includes a heat/cool function that maintains the gelatine-based bioinks at the ideal consistency during printing. It also ensures optimal temperature control preserving high cell viability during 3D bioprinting process.

Ayça Bal Öztürk, founder of AdBioink in Istanbul, Turkey, describes it: "*preliminary studies with gelatine-based hydrogels have already been conducted using this device. The result showed smooth extrusion, consistent performance, and reliable flow control while bioprinting of temperature-sensitive bioinks with stable and functional structures. The developed handheld bioprinter is an example of how STRONG-UR can make bioprinting more convenient and easier to handle across different applications and environment*".

The BioPen handheld device prototype. Source: AdBioink

By involving experts with diverse skill sets our project bridges the existing gap between biomedical researchers, clinicians and industry partners. Another important focus of the project is the development of a technology that not only meets clinical needs but also complies with regulatory requirements.

A long road to clinical use

Since STRONG-UR technology involves living cells and bioprinted tissues, the technology falls under the category of Advanced Therapy Medicinal Product (ATMPs), a regulatory category defined by the European Medicines Agency (EMA). ATMPs follow a more complex and time-intensive regulatory pathway, which has limited the number of approved treatments to date.

ATMPs often face a significant gap between basic research and clinical application, which limits the development and use of effective treatments for patients. As a result, only a few ATMPs have received market approval.

To move forward, extensive testing is required to ensure product safety and efficacy. This should at the very least include:

- In vitro chemical and physical characterisation.
- Biocompatibility testing including toxicity assessments to ensure the product does not harm living cells.
- Evaluation of local and systemic responses in one or more reliable animal models guided by ISO 10993 standards for biological evaluation.

Once robust preclinical evidence is established, clinical trials are needed. At this stage, regulatory requirements diverge significantly:

- A single, large-scale clinical trial may suffice for CE[6] approval of a medical device.
- Medicinal or combinatorial products typically require multiple trial phases, larger participant groups and longer timelines before market authorisation.

Despite extensive research on bioinks and hydrogels, only a few products have achieved market authorisation. Bridging this gap requires greater academic and industrial efforts in material characterisation, in vitro evaluation, and preclinical model development. Our STRONG-UR team is committed to scaling up production to Good Manufacturing Practises (GMP) standards and conducting proof-of-concept studies in the preclinical phase.

STRONG-UR: the road toward the next-generation of regenerative therapies

The STRONG-UR team is paving the way for a new generation of regenerative medicine therapies by developing cutting-edge bioprinting technology for the treatment for urethral strictures. The goal is to develop personalised therapies that address the limitations of traditional surgical options.

Through the development of advanced bioinks and innovative devices such as the BioPen, the team addresses the complexity of urethral tissue, including its multilayered structure. This approach – printing living cells directly into the damaged area – offers a faster, less invasive and more customisable solution tailored to each patient."

Pablo Pennisi, Associate Professor at Aalborg University and STRONG-UR coordinator explains that "*the project seeks to develop a treatment that is faster and more cost-effective. The in situ bioprinting technology, in which the bioink is printed directly onto the damaged area of the*

[6] CE marking is a certification indicating that a product meets EU safety, health, and environmental protection standards and can be marketed within the European Economic Area.

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urethra, will lead to a tissue graft that fits the injury more precisely. Since the bioink contains freshly isolated stem cells, there is no need for extensive cell expansion procedures associated with traditional cell-based regenerative therapies".

Although regulatory and clinical hurdles remain, our team is committed to bridging the gap between laboratory research and clinical application. With ongoing preclinical studies and a strong collaborative network of researchers, clinicians and industry partners, the project is moving closer to delivering safe, effective and accessible treatments that could transform the future of reconstructive urology.

Funded by the EU Horizon Europe programme, STRONG-UR began in November 2023 and is funded until October 2028. The second year promises key results including:

- Completion of studies of the structure function relationships in the male urethra.
- In vitro performance of synthesised hydrogels according to ISO standards.
- Synthesis of base components for hydrogels formulations.
- Finalisation of bioprinting protocols for reproducing tissue-specific characteristics of the urethra for in vivo reconstruction.
- Successful testing of the multilamellar bioprinting module for in vivo reconstruction.

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