REVIEW PAPER

TRAUMA AND RECONSTRUCTIVE UROLOGY

# Artificial urinary conduit construction using tissue engineering methods

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**Introduction** Incontinent urinary diversion using an ileal conduit is the most popular method used by urologists after bladder cystectomy resulting from muscle invasive bladder cancer. The use of gastrointestinal tissue is related to a series of complications with the necessity of surgical procedure extension which increases the time of surgery. Regenerative medicine together with tissue engineering techniques gives hope for artificial urinary conduit construction *de novo* without affecting the ileum.
**Material and methods** In this review we analyzed history of urinary diversion together with current attempts in urinary conduit construction using tissue engineering methods. Based on literature and our own experience we presented future perspectives related to the artificial urinary conduit construction.
**Results** A small number of papers in the field of tissue engineered urinary conduit construction indicates that this topic requires more attention. Three main factors can be distinguished to resolve this topic: proper scaffold construction along with proper regeneration of both the urothelium and smooth muscle layers.
**Conclusions** Artificial urinary conduit has a great chance to become the first commercially available product in urology constructed by regenerative medicine methods.

Key Words: urinary diversion ‹› urinary conduit ‹› tissue engineering ‹› regenerative medicine

## INTRODUCTION

Bladder cancer is the seventh most common malignancy in the world in men and the third most common in Poland. In women, this cancer occurs less frequently (17 in the world and 15 in Poland), but in contrast to men, an increasing trend of morbidity can be observed [1–5]. In Poland in 2010, 6296 incidents of bladder cancer were noticed (4919 men, 1377 women). Mortality of this cancer is high, reaching 50.2% for men and 46.5% for women [6]. Approximately 15–20% of bladder cancers infiltrate the bladder muscle layer; in such cases, the treatment of choice is radical cystectomy, which connects with the necessity of urine diversion after bladder removal [7]. In 2010, 1260 patients required urinary diversion. In Europe, each year 140 000 new cases of bladder cancer are noticed, which gives 25 000 patients for urinary diversion [8]. Urinary diversion can be divided into incontinent and continent (formed orthotopically or non–orthotopically). Incontinent ileocutaneostomy is most commonly used among surgeons and this type of urinary diversion has the greatest chances to be constructed using tissue engineering methods [9]. The use of an ileal segment, beside advantages such as maintaining ureter continuity together with urine outflow using autologous material with well developed blood supply, has number of disadvantages especially in the long–term follow–up [10, 11, 12]. This method is also associated with the necessity of additional surgical procedure conduction on the ileum. The use of tissue engineering techniques gives opportunities to construct artificial conduits *de novo* in the laboratory


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without affecting the ileum. Such an approach will reduce the time of surgery and eliminates intestinal complications [13].

## History of incontinent urinary diversion

The first attempt at incontinent urinary diversion was carried out in 1851 in London by John Simon, who conducted two ureterosigmoidal fistulas using a specially designed silver catheter. The procedure was conducted on a 13–year old boy suffering from bladder extrophy. The patient died one year after the procedure due to peritonitis [14]. In 1891, Ernst Küster performed the first time radical cystetctomy in a patient suffering from localized urinary bladder cancer. Urine was diverted by implantation of ureters into the anterior rectal wall. The patient died five days after the procedure due to an infection. Robert Coffey developed a new method of ureter implantation into the bowel wall which was used in clinical practice by Charles Mayo in 1912 (Coffey–Mayo technique). This method used a bowel submucosal tunnel which protects against urine outflow (reflux). With some modifications, this method was used till the end of World War II. In 1936, Frank Hinman and Henry M. Weyrauch analyzed 740 urinary diversion procedures performed using 60 different methods. The average perioperative mortality was only 30%. The most effective incontinent urinary diversion method with the highest survival rate in these years was cuteneous ureterostomy. The first attempt at a unilateral cuteneous ureterostomy was performed in 1889 by Jean F. A. Le Dentu. In 1892, Ludwik Rydygier performed the first bilateral cuteneous ureterostomy. In 1905, Rowsing modified Rydygier's technique by constructing a nipple after ureter exteriorization, over which a sliver urine reservoir was placed. Other incontinent urinary diversion techniques included ureter anastomosis with the urethral groove (Eduard Sonnenburg 1881) or ureter implantation into the vagina (Karl Pawlick 1888) [15]. The use of an ileal segment as a conduit for urine diversion was performed for the first time in 1911 by Zaayer on two patients [16]. The first patient died because of cancer and the second because of peritonitis. In 1950, Eugene M. Bricker presented 307 incontinent urinary diversion procedures using an ileal segment reaching 12.4% mortality; only 3.4% directly involved urinary diversion [17]. This method was considered a gold standard for 35 years and with some modification is used even now [18]. Over the past 65 years, a more effective method of incontinent urinary diversion was not developed. Tissue engineering and regenerative medicine give opportunities to change this status.

## Current attempts in artificial conduit construction

The first attempt at artificial urinary conduit construction using tissue engineering methods was performed by Drewa in 2007 [19]. In this experiment, small intestine submucosa (SIS), acellular or seeded, with 3T3 fibroblast cell line was used on rat models. The experiment was performed on 6 animals; patent conduits were observed in three rats at the end of follow–up. No differences in cell layer regeneration were observed in seeded and unseeded groups. Additionally, acellular matrices induced less severe inflammatory responses. Five years later, two other groups conducted urinary conduit construction on a porcine model. Gutjes et al. used scaffold build from collagen type I and synthetic Vypro®II mesh on 10 pigs. Animals were divided into 2 groups: acellular matrices (n = 4) and matrices seeded with urothelial cells (n = 6). Patent urostomy was obtained in 5 animals, with no differences between seeded and unseeded matrices [20]. Another group performed the experiment on 32 mini pigs divided into 4 tested groups. They used polyglicolic acid coated with poly(lactide–co–glicolide) scaffold (PGA/PLGA) unseeded or seeded with smooth muscle cells derived from different origin (bladder, adipose tissue, blood). In contrast to previously presented experiments, in this study, the bladder was removed and both ureters were transplanted to the conduit. Obtained results showed that the use of smooth muscle cells from different origins led to regeneration of a neo–organ resembling native bladder tissue composed of urothelium and smooth muscle layers. The use of acellular scaffold resulted in fibrous connective tissue development with a small number of smooth muscle cells [21]. Another study was performed on 30 rabbits using bladder acellular matrix (BAM). Acellular matrices were used in 6 animals and scaffolds seeded with urothelial cells were used in 24 rabbits. In this study, the bladder was also removed and both ureters were transplanted to the conduit. In the group where matrices were seeded, all the animals survived follow–up, conduit lumen was covered with multilayer urothelium and no severe complications were observed. In the unseeded group, 4 animals died one month before the end of follow–up and the two remaining animals had fistulas and lack of urothelium regeneration [22]. This same research group obtained similar results in a study published later. They once again used a rabbit model and BAM. Scaffold seeded with urothelial cells was protected against scar and kidney stone formation, atresia and hydronephrosis [23]. In our study, we compared two acellular matrices form different origins: autologous naturally derived acellular aortic arch and synthetic policap-

rolactone (PLCL) produced using the electrospining method. The experiment was conducted on 12 Wistar rats divided into 2 equal groups, six rats for each tested scaffold. Obtained results indicated that acellular aortic arch is an unsuitable scaffold for urinary conduit construction in a rat model. In all animals with acellular aortic arch conduit, atresion was observed. In a second group, in which PLCL scaffold was used in 3 cases, constructed conduits were patent at the end of follow–up (4 weeks), but only in one case intense urine flow, without the presence of pus in the urinary tract, was observed [24]. Autologous acellular aortic arch, despite easy accessibility and proper extracellular matrix composition, is unsuitable for urinary conduit construction because of its small diameter and too elastic structure, which leads to atresion about one week after the surgical procedure. PLCL is more rigid and its diameter can be regulated during the electrospining method, which is why better results were obtained using this scaffold. Unsatisfactory results with PLCL were probably caused by open urinary tract during follow–up, as use of urostomy bags on rat models is impossible. Such complications were not observed when a ureter segment was regenerated using this same scaffolds. Use of PLCL resulted in ureter segment reconstruction in 4 cases (n = 6), which was confirmed by urography. In that group, continuity of the ureter was preserved [24]. All currently performed attempts of experimental artificial urinary conduit construction using tissue engineering techniques are presented in Figure 1.

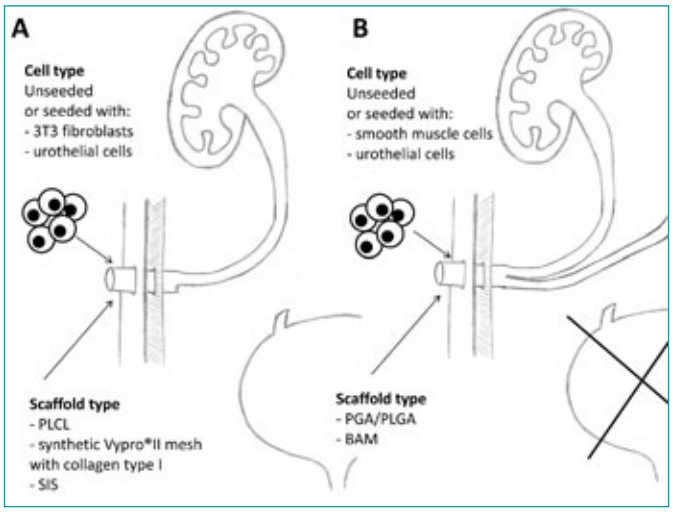


**Figure 1.** *Different approaches currently used in experimental urinary conduit construction using tissue engineering techniques. A – experiment carried out only on one ureter; bladder was preserved [19, 20, 24]. B – two ureters were anastomosed with artificial conduit; urinary bladder was removed [21, 22, 23]. PLCL – poly (L–lactide–co–caprolactone); PGA/PLGA – polyglicolic acid coated with poly (lactide–co–glicolide) scaffold; SIS – small intestine submucosa; BAM – bladder acellular matrix.*

## Future perspectives

In literature there is still a small number of works describing the use of tissue engineering for urinary conduit construction. In the paper by Sloff et al., all experiments describing the use of tissue engineering in urinary diversion construction conducted so far were evaluated [9]. They analyzed 8 works, of which 5 were related to urinary conduit and 3 to neo–bladder construction. This shows how little is known about ureter segment regeneration including artificial urinary conduit construction, which continues to be an unresolved problem. Three main factors can be distinguished to resolve this topic.

Proper scaffold is essential for urinary conduit construction. Comparison of all scaffold types were described elsewhere [13]. Synthetic polymers have promising properties because these materials can be produced *de novo* and different shapes, porosity and degradation time can be obtained. The best solution for a patient would be the use of an acellular scaffold without the necessity of cell seeding. Such an approach will eliminate invasive collection of tissue for cell isolation and long *in vitro* culture (about 2 weeks). Construction of an artificial urinary conduit without cell seeding makes it the ideal off–the–shelf product which could be purchased from the pharmacy directly before the surgical procedure [25]. Scaffold which protects seeded cells from the toxic influence of urine is an important issue. In an *in vitro* culture study, urine acted as a cytotoxic agent against urothelial cells and bone–marrow mesenchymal stem cells [26, 27]. Success in artificial urinary conduit construction will depend on appropriate scaffold production.

Urothelial cells build the inner layer of the ureter, protecting against urine components reabsorption. Urothelium can self–regenerate on a scaffold surface after transplantation by migration from surrounding tissue [28, 29, 30]. Epithelial regeneration should be potentially easier in the case of ureter segment regeneration in which urothelial cells can migrate from two edges, compared to urinary conduit. Data available in literature gives contrary results about the use of urothelial cells in tissue engineering applications. Some studies showed no differences in seeded and unseeded group [20]; on the other hand, other researches obtained positive results only when scaffold was seeded with urothelial cells [22, 23]. Dorin et al. regenerated the urethra using acellular

scaffolds of different lengths. They concluded that after 4 week follow up, the epithelial layer regenerated only on 0.5 cm scaffold, which is not a clinically important segment. On longer scaffolds, regeneration was observed only at the anastomotic edges with dense fibrosis throughout the grafts [31]. In previous experiments, only one scaffold type was analyzed. In our work, we compared two scaffold types: natural and synthetic [24]. Obtained results showed better regeneration of urothelium on synthetic biodegradable polymer compared to natural derived acellular blood vessel matrix [24]. It should be noted that material for artificial urinary conduit construction should be produced from components that will protect from fibrosis and calcification of urine substrates on the scaffold surface. Additional experiments are necessary to find scaffold with relevant properties enabling regeneration of urothelium.

The crucial point is regeneration of the smooth muscle layer and restoration of peristaltic waves on the reconstructed segment. The urinary conduit must be constructed from rigid material in order to protect from scaffold occlusion at the site of anastomosis with skin. Such properties can result in peristaltic wave arrest at the site of ureter anastomosis with scaffold, which can lead to urine reflux and development of hydronephrosis. Quick regeneration of smooth muscle layer should prevent the development of the side effects mentioned above. Despite the fact that acellular scaffold might be the best solution, some papers indicate that scaffold pre–seeded with cells (autologous form bladder biopsy or mesenchymal stem cells from different origin) showed better smooth muscle layer regeneration compared to unseeded controls [32]. The best type of cells seems to be mesenchymal stem cells from fat tissue or bone marrow and from promising sources like amniotic fluid or hair follicles [13, 33]. Use of differentiated autologous cells from bladder biopsy is limited due to the risk of cancer development in the case of bladder cancer patients, who are the main candidates for urinary conduit construction [34]. The number of cells seeded on scaffold is also very important and the conception seems to be simple: the more cells on $cm^2$ of scaffold, the better the results that can be achieved [35]. The achievement of such large cell numbers is challenging issue because the average bladder cancer patient is 65 years old and mesenchymal stem cells proliferation capacity decreases with the age of the patient and with increasing passage numbers [36, 37]. On the other hand, some authors suggested that stem cell proliferation capacity is not dependent on the donors age, which increase the chances of tissue engineering therapy use in artificial urinary conduit construction [38]. Despite that, such a large number of cells necessary for regeneration is hard to obtain and very costly because of the price of culture media containing appropriate growth factors. That is why an efficient cell culture method has to be developed to provide success of this procedure. For many years, atypical smooth muscle cells (ASMCs) localized in proximal regions of the renal pelvis were considered a peacemaker of peristaltic waves responsible for urine passage into the bladder. Recent studies indicated that interstitial cells of Cajal like cells (ICC–LC) expressing the c–kit gene, which are sparsely distributed within the lamina propria and muscle layer of upper urinary tract, play an important role in promoting pyeloureteric peristalsis [39]. ICC–LC are electrically active and responsible for conduction of slow–wave potentials for peristaltic movements [40]. Contraction waves generated in the renal pelvis are probably propagated, coordinated and modulated in the upper urinary tract by ICC–LC [41, 42]. ICCs, thanks to their automatism, are able to replace discontinued atypical SMCs impulses and maintain peristaltis of lower ureter parts [40, 43]. *In vitro* isolation and culture of ICC–LC from the urinary tract has not yet been established, but taking into consideration previous works and our experience on urinary conduit construction, addition of these cells or coculture of them with smooth muscle cells can potentially accelerate the restoration of peristaltic waves on the reconstructed segment, which could prevent development of hydronephrosis.

## CONCLUSIONS

In conclusion, success in ureter conduit construction using tissue engineering techniques depends on finding the proper scaffold. We believe that implementation of this procedure is possible and urinary conduit should be the first commercially available product in clinical practice constructed using regenerative medicine. Confirmation of this opinion is registered in a ClinicalTrial.gov study about incontinent urinary conduit construction using PGA/PLGA scaffold seeded with autologous smooth muscle cells derived from adipose tissue biopsy.

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