Results of Use of Tissue-Engineered Autologous Oral Mucosa Graft for Urethral Reconstruction: A Multicenter, Prospective, Observational Trial

Gouya Ram-Liebig a,⁎, Guido Barbagli b, Axel Heidenreich c, Dirk Fahlenkamp d, Giuseppe Romano e, Udo Rebmann f, Diana Standhaft f, Hermann van Ahlen g, Samer Schakaki g, Ulf Balsmeyer d, Maria Spiegeler h, Helmut Kissel b

a UroTiss Europe GmbH, Otto-Hahn-Str.15, 44227 Dortmund, Germany
b Centro Chirurgico Toscana, Via dei Lecci, 22, 52100 Arezzo, Italy
c University Clinic and Polyclinic for Urology, Kerpener Str. 62, 59037 Cologne, Germany
d Zeisigwald Clinics Bethanien, Department of Urology, Zeisigwaldstrasse 101, 09130 Chemnitz, Germany
e Urology Unit, Ospedale del Valdarno, Santa Maria alla Gruccia, Piazza del Volontariato, 1, 52025 Montecatini-Arezzo, Italy
f Diakonissen Clinics Dessau, Department of Urology, Gropiusallee 3, 06846 Dessau-Roßlau, Germany
g Osnabrueck Clinic, Department of Urology, Am Finkenhügel 1, 49076 Osnabrück, Germany
h St. Hedwig Hospital, Department of Urology, Große Hamburger Strasse 5-11, 10115 Berlin, Germany

Article history:
Received 30 June 2017
Received in revised form 30 July 2017
Accepted 15 August 2017
Available online 16 August 2017

Abstract

Background: Harvest of oral mucosa for urethroplasty due to urethral stricture is associated with donor-site-morbidity. We assessed functionality and safety of an authorized tissue-engineered oral mucosa graft (TEOMG) under routine practice in stricture recurrences of any etiology, location, length and severity (real-world data).

Methods: 99 patients from eight centers with heterogeneous urethropast experience levels were included in this prospective, non-interventional observational study. Primary and secondary outcomes were success rate (SR) and safety at 12 and 24 months.

Findings: All but one patient had ≥77.1% (64 of 83) ≥2 and 31.3% (26 of 83) ≥4 previous surgical treatments. Pre- and postoperative mean ± SD peak flow rate (Qmax) were 8.3 ± 4.7 mL/s (n = 57) and 25.4 ± 14.7 mL/s (n = 51). SR was 67.3% (95% CI 57.6–77.0) at 12 and 58.2% (95% CI 47.7–68.7) at 24 months (conservative Kaplan Meier assessment). SR ranged between 85.7% and 0% in case of high and low surgical experience. Simple proportions of 12-month and 24-month SR for evaluable patients in all centers were 70.8% (46 of 65) and 76.9% (30 of 39). Except for one patient, no oral adverse event was reported.

Interpretations: TEOMG is safe and efficient in urethropasty.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Urethral stricture affects up to 0.6% of the male population with significant disease burden (Alwaal et al., 2014; Liu et al., 2016; Wessells et al., 2017; Latini et al., 2014). Except for the guidelines of the American Urological Association, no therapeutic recommendations exist (Wessells et al., 2017; Latini et al., 2014). These guidelines are mainly based on expert opinions and publications of lower evidence strength grades due to the lack of data obtained from prospective multicenter trials under good clinical practice (GCP) standard (Latini et al., 2014; Mundy, 2006; Tritschler et al., 2013). Consequently, different surgical techniques are applied according to the surgeon’s preference and previous experience. Over the last two decades, buccal mucosa became the tissue of choice for urethral reconstruction (Wessells et al., 2017; Ram-Liebig et al., 2015; Markiewicz et al., 2007). However, oral mucosa harvest may lead to donor-site morbidity (Ram-Liebig et al., 2015; Jang et al., 2005; Fasolis et al., 2014; Markiewicz et al., 2008).

Tissue-engineered oral mucosa graft (TEOMG) represents an alternative material for urethropasty. It helps to avoid morbidities associated with graft harvesting at the oral site and provides substitution tissue for urethral reconstruction in any size required (Ram-Liebig et al., 2015). We conducted an observational study with a TEOMG, with market authorization in Germany (MukoCell®), to expand the knowledge about feasibility, safety, and efficacy when used under routine real-world conditions in non-preselected adult male patients with surgically unsuccessful pretreated urethral stricture. The current data from our observational trial are reported to the Paul-Ehrlich-Institut, the regulatory body in Germany, responsible for marketing authorization of advanced therapy medicinal products (ATMP) - among others - and approval of clinical trials, as well as to the European Medicine Agency (the European Union agency for the evaluation of medicinal products).
2. Materials and Methods

2.1. Study Design and Patients

The study is a prospective, observational survey conducted at eight German urologic centers, with <10 to >80 urethroplasties/year. This study is registered in Germany at the Paul-Ehrlich-Institut observational trial registry, NIS number 110.

Enrolled were adult male patients with recurrent urethral stricture. Decision for treatment of an individual patient with the autologous TEOMG was met solely by the treating surgeon.

All data captured during the observation were obtained from routine clinical care assessments which were done by the investigators according to their local medical practice (Real-world data), without additional, study-mandated examinations or clinic visits. The study was monitored by an independent licensed German Contract Research Organization.

The trial was designed in accordance with the Declaration of Helsinki with all its amendments. The study was approved by the local ethics committees and the competent national supervisory authority (Paul-Ehrlich-Institut, Langen, Germany). The trial followed GCP guidelines, European guidelines on ATMPs, and German Transplant Act. The patients signed informed consents for biopsy and blood taking, as well as for urethroplasty with TEOMG. The TEOMG implanted in the context of this study (MukoCell®) was provided by UroTiss GmbH, Germany.

2.2. Coordination and Schedules

For the manufacture of MukoCell®, a tiny oral biopsy is required. For being authorized to take biopsies, the urologist needs an approval according to German Drug Law from the authority, who granted the Good Manufacturing Practice (GMP) license for the TEOMG. For this, the clinic has first to provide documents to show that it has an appropriate facility. A hygiene plan, complying with the medical standards and suitable for carrying out biopsy procedures and blood collection is also required. The urologist who will have the primary responsibility for biopsy taking and blood collection, as well as the medical staff, who will be involved in the procedures must be trained for biopsy taking and blood collection and their storage as well as documentation of the procedure according to standard operating procedures, in compliance with good professional practice. Once the tissue collection authorization is available, and the patient agrees for the urethroplasty with MukoCell®, the urologist contacts the company by phone or email and informs it about the date, planned for biopsy taking and urethroplasty surgery. Within a few days, or if necessary within hours, he gets a biopsy kit from the Good Manufacturing Practice (GMP) laboratory. The biopsy kits are stable for 6 months. Together with the biopsy kit, the patient gets a unique identification code. This code is the first step of patient recruitment into the study. For tissue collection, a donor record, containing documentation of donor suitability and a patient consent form, should be completed. Once the biopsy is taken, it is put into the specific package, which is picked up at the same day. On the day after, upon arrival in the GMP laboratory, the manufacture begins. For safety reasons, the serologic examination must be negative for specific infectious agents (Human Immunodeficiency Virus, Hepatitis B and C, Treponema Pallidum), to allow release of the tissue for manufacture. Once manufacture begins, the date for urethroplasty is already fixed. According to this date, MukoCell® is placed in a sterile double package, and sent within a qualified transport container to the hospital. It must be used within 48 h.

TEOMG is an industrial product. The manufacturing processes therefore cannot be disclosed in all details. All procedures (identification code, biopsy taking, manufacturing, shipment) are standardized, validated and certified, respectively.

3. Procedures

For manufacture of TEOMG, a tiny oral mucosa biopsy of 0.5 cm² (Fig. 1A) was taken from patient’s buccal mucosa and sent to the GMP laboratory for aseptic manufacturing of the graft, which has been described elsewhere (Ram-Liebig et al., 2015). In the manufacture site, all culture flasks, materials and documents were identified for each patient with the unique identification code, which was the same, as on the biopsy kit. All manufacture steps took place in an isolator and the culture steps in an incubator (37 °C, 5% CO₂) in the GMP laboratory. After separation from submucosa, the mucosa tissue was used for setting the cell cultures in flasks and their incubation. The expansion of cells took about two weeks. Once the epithelial cells were confluent, primary cultures were detached form the flasks and the non-splitted cells of passage 1 were seeded on a biodegradable membrane. Subsequently, the final TEOMG, consisting of oral epithelial cells from first passage cultured on biodegradable protein containing scaffold, was placed in a sterile container, packaged and pharmaceutically released for therapeutic use, after a final check of properness of quality control results and completeness of documentation. The manufacture of each batch was documented in an according protocol. All remaining materials and wastes were disposed according to specific Standard Operating Procedures. Timing of the whole procedure (3 weeks) was highly reliable, allowing to settle the surgery date as soon as the biopsy is taken from the patient. After release, the TEOMG was sent to the hospital for implantation into the patient’s diseased urethra (Ram-Liebig et al., 2015). The shipment of TEOMG is a validated process, ensuring stability and viability of the tissue for 48 h.

Before urethroplasty, information on demographic and medical history was gathered. Pre- and post-operatively, results from physical examination, vital signs measurements, electrocardiogram, serological examinations, concomitant medication, and conventional urological examinations (e.g. urethroscopy, urethrogram Fig. 1E, or uroflowmetry) were collected.

The TEOMG was implanted in accordance with the substitution urethroplasty technique routinely applied by the surgeon (Fig. 1B–D) when native buccal mucosa was used. After the operation, an 18 to 20 Ch. Foley silicon catheter was left in the urethra. Suprapubic catheter was placed in the urinary bladder in some cases about 3–6 weeks later, the catheters were removed and the patient underwent voiding urography (Fig. 1F).

Routine urological examinations such as uroflowmetry, urethroscopy and/or urethrogram were usually repeated every 3 months during the first year and every 6 months during the second year unless there were symptoms of urethral re-stricture (e.g. decreased urinary flow).

4. Outcomes

The primary outcome was the success rate (SR), defined as the absence of stricture recurrence, at 12 months after TEOMG implantation. The prospectively selected definition of stricture recurrence was: evidence of a postoperative peak flow rate (Qmax) < 15 mL/s on uroflowmetry plus the urethra is not passable with a catheter (di-ameter = 16–18 Ch) or during standard urethroscopy. However, these diagnostic criteria did not correspond to the actual routine diagnostic practice at the participating sites, precluding the use of this definition for stricture recurrence. Therefore, a consolidated assessment of stricture recurrence was made post hoc, based on investigator rating, patient-reported spontaneous micturition after urethroplasty, and uroflow rate following urethroplasty (i.e. Qmax < 15 mL/s). The physician’s assessment “treatment successful = yes” was used to exclude stricture recurrence, except in cases where patients reported difficulty of spontaneous micturition, where later re-stricture was detected, where there was need for further instrumental intervention, or where the physician’s statement
was missing in the case report form. In the latter situation, any evidence of re-stricturing detected during monitoring was used to assess treatment failure, unless re-stricturing was clearly outside the operated urethral area (i.e. heterotopic recurrence). In the absence of such evidence in these patients, the outcome was considered successful (i.e. no stricture recurrence). As a sort of sensitivity analysis, available flow rates separately based on uncensored data were analyzed, thereby using the objective outcome component of the primary endpoint (i.e. Qmax < 10 mL/s) as measure for stricture recurrence (Mundy, 2006). For this paper, uncensored data analysis was also assessed utilizing age-related Qmax (Abrams et al., 1987) as evidence of stricture recurrence.

Secondary outcomes were SR at 3, 6, 18, and 24 months after TEOMG implantation, proportion of patients with spontaneous urination at 24 h after removal of the intraoperatively inserted catheter, and Qmax at catheter removal and 3, 6, 12, 18, and 24 months after urethroplasty. Safety endpoints included adverse events, frequency of perioperative complications (at oral and urethral sites), vital signs and electrocardiogram data. In addition, oral pain was recorded within 10 days postoperatively, and at 3, 6, 12, 18, and 24 months as assessed by the patients on a 4-point Likert scale (1 = none, 2 = mild, 3 = moderate, 4 = severe pain). All adverse events, either local or systemic, were handled according to GCP guidelines of the International Conference on Harmonization (ICH-GCP guideline E6 [R1]).

4.1. Statistical Analysis

We calculated Kaplan-Meier estimates of stricture-free survival with corresponding 95% confidence intervals (CIs) for median event times. In the Kaplan-Meier analysis, no evidence of treatment failure at the time of last observation was classified as censored observation. In case of doubt, treatment failure was assumed. The proportion of patients (crude rate) with treatment success was also assessed, accompanied by the 95% CI, for all time points evaluated. For this uncensored analysis, missing data were imputed by using the last observation carried forward method. For patients with no evidence of treatment failure at a time of last observation occurring before the analysis time point, absence of stricture recurrence during the observation gap was confirmed by retrospectively screening hospital records, and assessed a recurrence in unclear cases. Otherwise, all data analyses were done using summary descriptive statistics. Data from all sites were pooled and analyzed based on the full analysis set (FAS; all enrolled patients who received the TEOMG [MukoCell®] and had at least one assessment post-surgery) and safety population. The FAS set was identical to the safety analysis set. A Cox

Fig. 1. Urethroplasty with the autologous tissue-engineered oral mucosa graft MukoCell®. A small oral mucosa biopsy is taken from the cheek of the patient (A) which is used for the manufacture of the graft. The latter is cut into the desirable size (B), transferred to the opened urethra (C) and sutured as a ventral onlay graft (D). Pre- (E) and postoperative (F) voiding urethrography, before and 3 weeks after the implantation of autologous tissue-engineered oral mucosa graft. The strictured (S) and grafted (G) area are indicated in (E) and (F).
were excluded from the study. Positive serology for screened infectious agents such as patient’s decision changing, non-conveniently treated at the study centers, or being already treated with TEOMG. On average, 5% of evaluable patients had at least one, and approximately 77% of evaluable patients had at least two previous surgical treatments (urethroplasty and/or urethrotomy) for their urethral stricture.

The overall SR at 12 months (primary outcome) varied between centers with the tendency that sites recruiting fewer patients had a higher proportion of stricture recurrence cases (Table 2). Three surgeons operated in the centers 1, 3, and 4 (2 surgeons/center). In the centers 2, 5, 6, and 7 always one surgeon performed all operations.

In the majority of patients, we observed no stricture recurrence during the observation period with SR of 67.3% (95% CI 57.6–77.0) at 12 months, and 58.2% (95% CI 47.7–68.7) at 24 months (Kaplan-Meier analysis (Fig. 2A)). The success rate ranged between 85.7% in the case of high and 0% in the case of low experience in the surgical method.

The uncensored analysis provided similar SR with 69.4% (68 of 96 patients, 95% CI 59.3–78.3) at 12 months and 62.2% (61 of 98, 95% CI 51.9–71.8) at 24 months. In the uncensored sensitivity analysis, utilizing a Qmax of <10 mL/s as evidence of stricture recurrence, 12-month and 24-month SR were 72.8% (67 of 92, 95% CI 62.6–81.6) and 67.4% (62 of 92, 95% CI 56.8–76.8), respectively, indicating that results from the primary analysis are conservative in nature. In this paper, due to the high average patient age, we also assessed uncensored data analysis utilizing age-related Qmax (Abrams et al., 1987) as evidence of stricture recurrence as shown in Fig. 2B. Simple proportions of 12-month and 24-month SR for evaluable patients were 70.8% (46 of 65) and 76.9% (30 of 39), respectively.

The majority of stricture recurrences (70.3%) developed within 8 months of substitution urethrolasty, and diminished gradually thereafter (Fig. 2). In the case of recurrence, it was not stated, if the new stricture was in the graft itself, or only at the proximal or distal anastomotic end.

After catheter removal, 92.6% (75 of 81 evaluable patients) were able to spontaneously micturate compared with 70.8% (52 of 72) at baseline. Preoperatively, mean ± SD Qmax was 8.3 ± 4.7 mL/s (n = 57) increasing to 25.4 ± 14.7 mL/s (n = 51) following catheter removal.

Presence of comitant diseases (cardiovascular, pulmonary, oncological, inflammatory, orthopedic, diabetes mellitus), and smoking habits (never, stopped, active) did not significantly affect outcome as confirmed by Kaplan-Meier analyses (data not shown).

By Kaplan-Meier analysis, the comparison of RFS at 12 months in strictures with localization in the bulbar versus penile stricture was in the graft itself, or only at the proximal or distal anastomotic end.

Results are available for 99 patients. In total, 65 patients (65.7%) and 39 patients (39.4%) reached 12 and 24 months of follow-up, respectively. Patients’ baseline characteristics are shown in Table 1.

The etiology of strictures was most frequently iatrogenic but was unknown in approximately one-third of study participants. In most patients, the stricture was located in the bulbar urethra (bulbar: 82.0%; penile: 18.0%); stricture length ranged between 5 and 130 mm with an overall mean of 38.0 mm. All but one evaluable patient had at least one, and approximately 77% of evaluable patients had at least two previous surgical treatments (urethroplasty and/or urethrotomy) for their urethral stricture.

5. Results

Table 1

| Site of stricture | Total population (n = 99) |
|------------------|--------------------------|
| Site of stricture | Age (years) | Body-mass index (kg/m²) |
|------------------|-------------|-------------------------|
| Bulbar* | 36 (42.4%) | 27.9 (4.2) |
| Penile* | 5 (5.9%) | 14 |
| Missing data | 10 |

Table 2

| Previous surgical intervention (urethroplasty and/or urethroplasty) | Total population (n = 99) |
|---------------|--------------------------|
| None | Length of stricture (mm) |
| 1 | 21 (21.7%) |
| 2–3 | 38 (45.8%) |
| ≥41 | 26 (31.3%) |

Data are mean (SD) or number (%); missing data are excluded from all percentage calculations.

| Previous surgical intervention (urethroplasty and/or urethroplasty) | Total population (n = 99) |
|---------------|--------------------------|
| None | Missing data |
| 1 | 1 (1.2%) |
| 2 | 38 (45.8%) |
| ≥4 | 26 (31.3%) |

Table 2

| Center | N | Success | 95% CI |
|--------|---|---------|-------|
| Overall | 98 | 67.3% | 57.6–77.0 |
| 1 | 8 | 85.7% | 59.7–100 |
| 2 | 10 | 80.0% | 55.2–100 |
| 3 | 27 | 72.3% | 54.7–89.9 |
| 4 | 23 | 69.3% | 50.3–88.3 |
| 5 | 6 | 66.7% | 28.9–100 |
| 6 | 13 | 56.4% | 27.2–85.6 |
| 7 | 6 | 50.0% | 10.0–90.0 |
| 8 | 5 | 0% | – |

Cl = confidence interval.

a Including 3 patients with involvement of the membranous urethra.

b Including patients with bulbo-penile strictures (n = 6) and patients with multiple strictures including a penile one (n = 6).

c Including patients with involvement of the membranous urethra.

Proportion of patients in full analysis set without stricture recurrence.

One patient with first assessment after 12 months was excluded.

3 of 5 patients failed, 2 were censored.
with stricture recurrence at 12 months and the number of prior surgeries as risk factor for re-stricture at 24 months, showing a hazard ratio (HR) (95% CI) of 2.23 (1.20–4.10; p = 0.010) and 1.74 (1.02–2.93; p = 0.039), respectively (Table 3). On multivariate analysis, timing of catheter removal and number of prior stricture surgeries remained statistically significantly associated with treatment failure, indicating that both are independent risk factors for the development of stricture recurrence.

Fig. 3 shows the Kaplan–Meier plot of re-stricture-free survival by number of prior surgeries. Long-term SR were best in patients with 1 previous surgical treatment and worst in patients with a history of ≥4 surgical treatments (urethrotomy or urethroplasty) (p = 0.019 for 1 vs. ≥4 prior treatments, log-rank test). Only one patient had no prior treatment rendering it impossible to do comparisons against patients without surgical pre-treatments. Kaplan–Meier analysis of re-stricture-free survival by duration of catheterization showed that patients with catheter removal ≥28 days post-surgery exhibited the highest risk of treatment failure (p = 0.018 for ≤20 vs. ≥28 days, log-rank test).

Stricture length, age, and body mass index were not statistically associated with stricture recurrence.

No adverse event occurred at the oral mucosa harvesting site. Upon inquiry, three patients reported transient mild pain at the donor site. One patient was reported to have an unspecified complaint.

Treatment-related adverse events and serious adverse events other than stricture recurrence, which were reported by the investigators, are presented in Table 4.

Ninety patients were assessed for urethral pain within 10 days of urethroplasty surgery. Upon inquiry, 57 (63.3%) reported no pain, 33 (36.7%) had mild pain, and 4 (4.4%) experienced moderate pain. Immediately after catheter removal, 10% of patients reported mild urethral pain which subsided in most patients by month 3. At and beyond 3 months after urethroplasty, transient mild urethral pain was reported.

---

**Table 3**

Univariate analysis of risk factors for stricture recurrence (based on uncensored data).

| Factor                  | N  | 12 months HR (95% CI) | p value | 24 months HR (95% CI) | p value |
|-------------------------|----|-----------------------|---------|-----------------------|---------|
| Age                     | 96 | 0.85 (0.52–1.36)      | 0.492   | 0.98 (0.63–1.51)      | 0.918   |
| Number of prior surgeries* | 82 | 1.42 (0.81–2.48)      | 0.211   | 1.74 (1.02–2.93)      | 0.039   |
| BMI                     | 94 | 1.39 (0.81–2.40)      | 0.231   | 1.31 (0.80–2.14)      | 0.282   |
| Stricture length        | 90 | 1.02 (0.63–1.64)      | 0.942   | 1.19 (0.76–1.85)      | 0.438   |
| Duration of catheterisation | 93 | 2.23 (1.20–4.10)      | 0.010   | 1.47 (0.88–2.50)      | 0.135   |

BMI = body mass index; CI = confidence interval; HR = hazard ratio.

* Urethrotomy and/or urethroplasty.
sporadically only (1–3 patients). Vital signs measurements and electrocardiogram assessments did not reveal clinically relevant findings. Physical examination findings were consistent with the underlying disease and the applied surgical procedure, or were considered unrelated to TEOMG implantation.

6. Discussion

We have shown that use of a TEOMG for reconstruction of the bulb and penile urethra is feasible, safe, and efficacious in a heavily pre-treated population. In our multicenter, prospective, observational study in a non-preselected cohort, we observed a satisfactory clinical outcome after 12 and 24 months in the majority of patients.

Prospective multicenter studies of urethroplasty are rare (Mundy, 2006; Tritschler et al., 2013). A recent meta-analysis of 10 cohort studies of buccal mucosa graft urethroplasty and end-to-end anastomosis in short segment bulbular urethral stricture reported a recurrence rate of 30% (Yuri et al., 2016). The current survey, evaluated by an independent CRO, included complex penile and longer strictures (Liu et al., 2016; Ortega & Pena, 2009; Bello, 2016; Breyer et al., 2010; Yalcinkaya et al., 2015), and excluded end-to-end anastomosis (known to have a very high SR) (Ortega & Pena, 2009; Ivaz et al., 2017). One can therefore consider the results of our study in general accordance with buccal mucosa urethroplasty (Yuri et al., 2016; Chauhan et al., 2016).

Previous studies in urethroplasty usually were retrospective, conducted at specialized single institutions, and operations were done by well-experienced surgeons (Liu et al., 2016; Chauhan et al., 2016; Barbagli et al., 2005; Kulkarni et al., 2012; Andrich & Mundy, 2008). Consequently, the reported SR was expectedly high. In our study, clinics with varying levels of experience in urethral reconstruction surgery were included, displaying the heterogenic national urethral stricture population and routine practice patterns. Therefore, our data may describe more closely the use of urethroplasty and its effectiveness under real world conditions. Data were collected prospectively, by using a standardized case report form, and, if necessary, followed-up by phone. They were monitored and evaluated by an independent clinical research organization and therefore represent a higher level of evidence (Latini et al., 2014; Bellomo & Bagshaw, 2006) (level 2, as adapted by the International Consultation on Urological Disease from the Oxford Centre for Evidence-Based Medicine) (Latini et al., 2014), compared to preceding studies not exceeding level 3 (Wessells et al., 2017; Latini et al., 2014; Mundy, 2006; Tritschler et al., 2013), or recommendations mostly based on expert opinion (evidence level 4) (Latini et al., 2014). From these, a MukoCell® drug registry was established, comprising real-world urethroplasty results.

The patient population in our study mostly had undergone multiple previous urethral surgeries (urethrotomy and/or urethroplasty) which failed. A high number of dilatations were also reported (127 preoperative dilatations in 10 patients, multiple dilatations in 8, no dilatations in 6 and unknown in the remaining patients) but not assessed in our statistical evaluations. Due to the high number of prior failed treatments in the study cohort and the known correlation with stricture recurrences (Liu et al., 2016; Bello, 2016; Breyer et al., 2010; Kulkarni et al., 2012), the presented overall success rate is, as can be expected for urethroplasty with native oral mucosa. The subgroup analysis regarding number of prior surgeries (Fig. 3) confirms this, displaying a much higher success rate of 83.3% in the patients with one prior surgery.

There were notable differences in SR between centers with the general tendency that sites recruiting more patients had a higher SR. This may be attributable to the learning curve in performing TEOMG implantation as reported for other urological procedures (Abboudi et al., 2014). Heterogenous levels of urethroplasty experience (SR ranging between 0% to 85.7%) as well as varying post-surgical management could also have an influence. Additionally, some of the investigators have included patients with less complex urethral stricture diseases (Alwaal et al., 2016) while other surgeons used TEOMG implantation as the very last therapeutic option, which was associated with an increased risk of stricture recurrence. Unclear filling of some report forms by urologists have resulted in the statistical rating of some successful outcomes as recurrences. Heterotopic strictures, postoperative urinary flows of 16 ml/s and 27 ml/s, and missing case report forms, in a total of six patients, have been assessed as stricture recurrences in the statistical analysis, due to the conservative GCP approach. These exemplarily stand for the difficulties and limitations of applying GCP principles to an observational trial, with its intrinsic grades of freedom. Finally, during the post-operative follow-up time, 1 urinary tract infection (Navai et al., 2008) with febrile temperature, may have caused a recurrence.

Among urologists, some controversy exists about the time for catheter removal following urethroplasty (Al-Qudah et al., 2005). Based on the presented results, we suggest that after TEOMG implantation, the indwelling catheter should not be left in place for longer than 3–4 weeks.

We could not identify stricture length as independent risk factor for treatment failure, which may indicate that a TEOMG implant tailored according to individual needs favors successful outcome largely independent from stricture length.

We did not detect unexpected adverse events related to TEOMG implantation, and particularly, the reported serious adverse events were evidently unrelated to the TEOMG itself, but rather to the implantation surgery in general. Only one adverse event (mild, transient pain) occurred at the donor site of oral mucosa, indicating the virtual absence of donor-site morbidity.

The excision of large segments of native oral mucosa results in long-term (>12 months) side-effects in about 20% of the cases, e.g. scars and oral contractures (Jang et al., 2005; Fasolis et al., 2014; Markiewicz et al., 2008). Chronic mechanical irritations from ill-fitting dentures and dental rubbings (Perry et al., 2015) as well as parafunctional bitings of oral mucosa (Piemonte et al., 2010) bulges have a high association in the development of oral cancer. The use of TEOMG would provide the

| Table 4 |
|----------|
| Adverse event | n | Time point |
| Local dermal infection | 2 | Postoperatively (Liu et al., 2016) |
| Serious adverse event | 0 | Time point |
| Urinary tract infection | 2 | 6 weeks and 16 months |
| Ureter stone | 1 | 2 months |
| Crohn’s disease | 1 | 2 months |
| Pulmonary embolism | 1 | Postoperatively |
| Epileptic seizure | 1 | Postoperatively |
| Death | 1 | 34 months |
For ATMPs, as for all therapeutic measures, the benefit-cost relation must be positive and must be assessed for each product individually. In the case of the TEOMG the benefit consists in the avoidance of a second operation, namely the excision and potentially damage of healthy oral mucosa. Today, tissue engineering technology helps to avoid the additional oral intervention. Nevertheless, ATMPs cannot be delivered for free. In fact, unlike for native oral mucosa, strict rules for quality and safety standards must be kept for ATMPs like TEOMG. Moreover, to gain market access, a high level of clinical evidence is required, and therefore cost-intensive and time-consuming clinical studies must be carried out. We are now faced with the decision between a graft, which indeed is cost-free but requires the excision and more or less the damage of healthy pieces of the body, partly with severe complications, and a product, which is not cost-free, but helps to avoid the additional intervention. From an ethical point of view, physical integrity is a precious good and has a high value. To our opinion we should try, not to sacrifice the integrity of the body just because of cost saving. The best solution in the case of TEOMG would therefore be the short-term reimbursement of this product by health insurances.

In conclusion, we have shown that TEOMG represents a safe and efficient alternative to native oral mucosa as a graft for surgical substitution of narrowed urethra, which may spare the patients risk and discomfort at the intra-oral donor site. Furthermore, our results suggest that surgical substitution should be performed early in disease before interventions repeatedly have failed, and that the surgeon's experience and appropriate post-surgical management (e.g. early catheter removal) are key for a favorable outcome.

Funding Source

The study was sponsored by UroTiss Europe GmbH, Dort mund, Germany. The funder is the owner of the rights of the study results. It had no role in data collection, data analysis, or data interpretation. The study was monitored and analyzed by an independent German Clinical Research Organization. All authors had access to all data in the study and held final responsibility for the decision to submit for publication. The source data can be provided upon request.

Conflict of Interests

GRL is an employee of UroTiss Europe GmbH. Guido Barbagli is advisor for UroTiss Europe. The other authors declare no competing interests.

Registration

Paul-Ehrlich-Institut observational trial registry, NIS number 110.

Author Contributions

GRL did the literature search and wrote the report. The remaining authors performed surgical operations and were involved in patient recruitment, follow-up, data collection and patient care. They also contributed to the design of the study and assisted with data interpretation. GB and GR operated at German centers. All authors revised the report and approved the final version.

References

Abboudi, H., Khan, M.S., Guru, K.A., et al., 2014. Learning curves for urological procedures: a systematic review. BJU Int. 114 (4), 617–629.
Abrams, P., Feneley, R., Torrens, M., 1987. Urodynamik für Klinik und Praxis. Springer, Berlin–Heidelberg, Germany, p. 39.
Al-Qudaih, H.S., Cavalcanti, A.G., Santucci, R.A., 2005. Early catheter removal after anterior anastomotic (3 days) and ventral buccal mucosal onlay (7 days) urethroplasty. Int. Braz. J. Urol. 31 (5), 459–463.
Alwaal, A., Blaschko, S.D., McNinch, J.W., Breyer, B.N., 2014. Epidemiology of urethral strictures. Transl. Androl. Urol. 3 (2), 209–211.
Alwaal, A., Sanford, T.H., Harris, C.R., Osterberg, E.C., McNinch, J.W., Breyer, B.N., 2016. Urethral stricture score is associated with anterior urethroplasty complexity and outcome. J. Urol. 195 (6), 1817–1821.

Andrich, D.E., Mundy, A.R., 2008. What is the best technique for urethroplasty? Eur. Urol. 54 (5), 1031–1041.

Barbagli, G., Palminteri, E., Guazzoni, G., Montorsi, F., Turini, D., Lazzeri, M., 2005. Bulbar urethroplasty using buccal mucosa grafts placed on the ventral, dorsal or lateral surface of the urethra: are results affected by the surgical technique? J. Urol. 174 (3), 955–957.

Bello, J.O., 2016. Impact of preoperative patient characteristics on post-urethroplasty recurrence: the significance of length and prior treatments. Niger. J. Surg. 22 (2), 86–89.

Bellomo, R., Bagshaw, S.M., 2006. Evidence-based medicine: classifying the evidence from clinical trials—the need to consider other dimensions. Crit. Care 10 (5), 232.

Breyer, B.N., McNinch, J.W., Whitson, J.M., et al., 2010. Multivariate analysis of risk factors for long-term urethroplasty outcome. J. Urol. 183 (2), 613–617.

Butler, C.E., Ong, D.P., 2005. Simultaneous in vivo regeneration of neodermis, epidermis, and basement membrane. Adv. Biochem. Eng. Biotechnol. 94, 23–41.

Chauhan, S., Yadav, S.S., Tomar, V., 2016. Outcome of buccal mucosa and lingual mucosa graft urethroplasty in the management of urethral strictures: a comparative study. Urol. Ann. 8 (1), 36–41.

Fasolis, M., Zavattiero, E., Sedigh, O., et al., 2014. Oral mucosa harvest for urologic reconstruction: role of maxillofacial surgeon and donor-site morbidity evaluation. J. Craniofac. Surg. 25 (2), 604–606.

Ivaz, S., Bugeja, S., Frost, A., Andrich, D., Mundy, A.R., 2017. The nontransecting approach to bulbar urethroplasty. Urol. Dent. Clin. N. Am. 44 (1), 57–66.

Jang, T.L., Erickson, B., Medendorp, A., Gonzalez, C.M., 2005. Comparison of donor site intraoral morbidity after mucosal graft harvesting for urethral reconstruction. Urology 66 (4), 716–720.

Kulkarni, S.B., Joshi, P.M., Venkatesan, K., 2012. Management of panurethral stricture disease in India. J. Urol. 188 (3), 824–830.

Latini, J.M., McNinch, J.W., BRANDES, S.B., Chung, J.Y., 2005. SIU/ICUD consultation on urethral strictures: epidemiology, etiology, anatomy, and nomenclature of urethral stenoses, strictures, and pelvic fracture urethral disruption injuries. Urology 83 (3 Suppl.), 1–7.

Liu, J.S., Dong, C., Gonzalez, C.M., 2016. Risk factors and timing of early recurrence after urethroplasty. Urology 95, 202–207.

Markiewicz, M.R., Lukose, M.A., Margarone, J.E., Barbagli, G., Miller, K.S., Chuang, S.K., 2007. The oral mucosa graft: a systematic review. J. Urol. 178 (2), 387–394.

Markiewicz, M.R., DeSantis, J.L., Margarone, J.E., Pogrel, M.A., Chuang, S.K., 2008. Morbidity associated with oral mucosa harvest for urological reconstruction: an overview. J. Oral Maxillofac. Surg. 66 (4), 739–744.

Meneghini, A., Caccia, A., Cavallari, L., Abatangelo, G., Ferrarrese, P., Tasca, A., 2001. Bulbar urethral stricture repair with buccal mucosa graft urethroplasty. Eur. Urol. 39, 264–267.

Mundy, A.R., 2006. Management of urethral strictures. Postgrad. Med. J. 82, 489–493.

Navai, N., Erickson, B.A., Zhao, L.C., Okotie, O.T., Gonzalez, C.M., 2008. Complications following urethrotomy: a six year experience. Int. Braz. J. Urol. 34 (5), 594–600.

Ortega, J.L., Pena, C.P., 2009. Surgical treatment of urethral stenosis. Results of 100 urethroplasties. Arch. Esp. Urol. 62 (2), 109–114.

Perry, B.J., Zinnit, A.P., Lewandowski, A.W., Bashford, J.J., Dragovic, A.S., Perry, E.J., Hayathabkhi, R., Perry, C.F., 2015. Sites of origin of oral cavity cancer in nonsmokers vs smokers: possible evidence of dental trauma carcinogenesis and its importance compared with human papillomavirus. JAMA Otolaryngol. Head Neck Surg. 141 (1), 5–11.

Piemonte, E.D., Lazos, J.P., Brunotto, M., 2010. Relationship between chronic trauma of the oral mucosa, oral potentially malignant disorders and oral cancer. J. Oral Pathol. Med. 39 (7), 513–517.

Ram-Liebig, G., Bednarz, J., Stuerzebecher, B., et al., 2015. Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe. Adv. Drug Deliv. Rev. 82–83, 181–191.

Tritschler, S., Rosen, A., Pfullhase, C., Stief, C.G., Röbben, H., 2013. Urethral stricture: etiology, investigation and treatments. Dtsch Arztebl Int 110 (3), 220–226.

Wessells, H., Angermeyer, K.W., Elliott, S., et al., 2017. Male urethral stricture: American urological association guideline. J. Urol. 197 (1), 182–190.

Yalcinakaya, F., Zengin, K., Sertel, N., Yigitbas, O., Bokurt, H., Sarikaya, T., Karabacak, R., 2015. Dorsal onlay buccal mucosal graft urethroplasty in the treatment of urethral strictures - does the stricture length affect success? Adv. Clin. Exp. Med. 24 (2), 297–300.

Yuri, P., Wahyudi, I., Rodjani, A., 2016. Comparison between end-to-end anastomosis and buccal mucosa graft in short segment bulbar urethral stricture: a meta-analysis study. Acta Med. Indones 48 (1), 17–27.