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Safety and mid-term surgical results of anterior urethroplasty with the tissue-engineered oral mucosa graft MukoCell®: A single-center experience

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Abbreviations & Acronyms
AE = adverse event
AUA-SS = American Urological Association Symptom Score
BMI = body mass index
CI = confidence interval
FU = follow-up
IQR = interquartile range
ND = non-definable
NOMG = native oral mucosa graft
OR = odds ratio
PVR = post-void residual
Q\textsubscript{\text{max}} = maximum urinary flow rate
RUG = retrograde urethrography
SD = standard deviation
SR = success rate
TEOMG = tissue-engineered oral mucosa graft
UTI = urinary tract infection
VCUG = voiding cystourethrography

Objective: To assess the mid-term efficacy and safety of anterior urethroplasty using an autologous tissue-engineered oral mucosa graft (MukoCell®).

Methods: The data of 77 patients with anterior urethral strictures undergoing treatment with MukoCell® at a tertiary center from June 2016 to May 2019 were analyzed. Patients' characteristics, pre- and postoperative diagnostics, perioperative complications, and follow-up data were obtained. The overall recurrence-free survival, outcomes of the different surgical techniques, stricture localizations, stricture length, early complications of the procedure and risk factors of recurrence were assessed.

Results: The median follow-up period was 38 months (interquartile range 31–46). The overall recurrence-free rate of anterior urethroplasty using MukoCell® was 68.8%, 24 patients (31.2%) developed a recurrence of the stricture. The stricture recurrences were observed at a median of 7 months (interquartile range 3–13) only in patients with at least one previous surgery or repeated dilatations in their medical history. No oral-urethral adverse events related to the use of MukoCell® were observed, except for a urethrocystaneous fistula (1.3%) requiring reoperation.

Conclusions: Anterior one-stage urethroplasty using MukoCell® showed in our hands a mid-term success rate of up to 68.8% without significant adverse events after a median follow-up period of 38 months. This procedure might be an alternative option for long-segment urethral reconstruction.

Key words: anterior urethral stricture, autologous transplantation, male urological surgery, oral mucosa, tissue engineering.

Introduction

The use of autologous NOMG in urethral reconstruction was first reported in 1993,1 and has been established as the gold standard of anterior medium/long-range urethroplasty for adults over the last two decades.1 This surgical technique relies on augmentation of the narrow urethral lumen through a free mucosal graft in “onlay,” “inlay” or a “combined ventral-dorsal” technique2 depending on the location and length of the stricture, the quality of the pararectal tissue and the extent of the accompanying spongiosis. Long-term surgical outcomes of 80–95% have been reported.3 However, intraoral morbidity due to harvesting of buccal or lingual mucosa is widely reported.4–6 Against this background, an alternative to the excision of large segments of native oral mucosa was required.

In 1990, Romagnoli et al. published the first application of autologous urethral tissue-engineered cultured cells in two children with penoscrotal hypospadias.7 In 1993, the application of a polytetrafluoroethylene scaffold for cultured cells was used in eight children with hypospadias.8 In 2008, Bhargava et al. introduced an autologous TEOMG for urethroplasty in five adults with lichen sclerosus, using sterilized de-epidermized dermis as a scaffold.9 In 2015, Ram-Liebig et al. published the application of a new TEOMG with market
authorization in Germany (MukoCell®) in 21 adult patients with anterior strictures. In 2017, a multicenter, prospective, monitored observational study with 99 adult patients having anterior urethral strictures of any etiology, location and length was carried out by the same group of authors. Finally, in 2018, Barbagli et al. published a retrospective multicenter report on the long-term surgical results (median 55 months) with MukoCell® in 38 patients.

Within the scope of the present retrospective single-arm and single-center observational study, we re-evaluated the feasibility and safety of the one-stage anterior augmentation urethroplasty using this new TEOMG (MukoCell®) in the context of the previously reported literature.

**Methods**

**Patient population**

After approval by the local ethics committee (No. 20-1424), a retrospective analysis of men undergoing urethroplasty with MukoCell® at our tertiary institution between June 2016 and May 2019 was carried out. Inclusion criteria were anterior urethral strictures ≥2 cm independent of etiology and previous surgical procedures. The exclusion criteria included short anterior strictures of <2 cm length, as resection with primary anastomosis up to this length was preferred, as well as posterior strictures after pelvic fracture. The eligible patients were informed about the procedure and provided signed detailed written consent form with guarantees of confidentiality.

**Preoperative evaluation**

The clinical history, including the AUA-SS Questionnaire, was assessed, and a urine test, urine culture, ultrasonographic PVR urine measurement, uroflowmetry and RUG occasionally combined with VCUG were obtained preoperatively. In equivocal cases, a rigid urethroscopy or urethral sonography was additionally carried out for stricture visualization. In the case of confirmed symptomatic UTIs or positive urine cultures, tailored antibiotic treatments were started preoperatively.

**Manufacturing the TEOMG**

A requirement for the manufacture of MukoCell® was an oral biopsy (approximately 0.5 cm³) from the recipient patient. Biopsy was obtained under local anesthesia on an outpatient basis by a trained urologist of our department following the required specific approval by the German Drug Law from the German national authority (Fig. 1a,b). The tissue was sent to a certified laboratory for cell isolation and cultivation. The entire standardized manufacturing procedure following the Good Manufacturing Practice principles lasted 3 weeks and has already been published elsewhere. After 2 weeks of incubation, the epithelial cell cultures were seeded on a protein containing biodegradable scaffold of animal origin of 2.8 × 3.8 cm (Fig. 1c). Subsequently, the final TEOMG, containing at least 1 million autologous living epithelial cells, packaged in a sterile container was sent back to our hospital for its therapeutic use. The pharmacology, pharmacokinetics, toxicology and tumorigenicity of MukoCell® have already been also fully analyzed and reported.

**Surgical technique**

The surgeries were carried out by two senior surgeons with extensive experience in anterior urethroplasty. Four established surgical techniques were carried out according to the surgeon’s preference and stricture characteristics. The ventral or dorsal onlay grafting technique was carried out as described by Morey and McAninch and Barbagli et al., respectively. The dorsal inlay technique was carried out as described by Asopa et al., and the combined ventral onlay and dorsal inlay technique was carried out as described by Palminteri et al., for bulbary and by Hudak et al. for pendulous strictures. All techniques were used as described in the literature, with the only difference being the MukoCell®-Graft instead of a NOMG (Fig. 2). In long strictures, the graft was divided in the middle and sutured by continuity or multiple grafts were used. Intraoperatively, bringing a suction device in contact with the TEOMG was avoided to prevent damaging or removing the cell architecture off the scaffold.
Postoperative evaluation

All patients received single-shot antibiotics perioperatively and were maintained on low-dose oral antibiotics until the catheter was removed. At 3 weeks, a VCUG was obtained to exclude extravasation implicating delayed healing, and the catheter was removed. In the case of fistula, we prolonged the catheterization for 1 week and repeated the VCUG. All patients’ charts were reviewed for emergency clinic visits, and reported UTIs, oral and surgical site complications (based on the Clavien–Dindo classification) within 90 days of surgery to determine the safety of the procedure.

Follow up

The patients underwent FU examinations using the AUA-SS Questionnaire, uroflowmetry and PVR measurement at 3, 6, 12, 18 and 24 months postoperatively, and annually thereafter. If obstructive symptoms or recurrent UTIs were reported, or uroflowmetry showed a $Q_{\text{max}} < 15$ mL/s, additional imaging (RUG or VCU) was initiated.

Study design

The primary outcome was the overall recurrence-free surgical result. Success was defined as normal voiding without the need for any further instrumentation. Failure was defined as the need for any further treatment for recurrent stricture or a $Q_{\text{max}} < 15$ mL/s. Secondary end-points were outcomes of the different surgical techniques, stricture localizations and length, as well as early complications, as a surrogate of the procedure’s safety.

Statistical analysis

Demographic, perioperative and FU data of the cohort were analyzed using SPSS version 25 (IBM, Armonk, NY, USA). For each variable, normal distribution was tested with the Kolmogorov–Smirnov test. Normally distributed continuous variables are reported as mean ± SD, otherwise as the median with IQR. Categorical variables were calculated as absolute and relative frequencies.

In addition, univariate and multivariate regression analyses were carried out to determine risk factors. Age at the time of surgery, stricture length, stricture site, surgical technique, previous interventions, diabetes (yes/no), smoking (yes/no) and fistula complications (yes/no) were included in the multivariate model, as they are established risk factors for recurrence after NOMG use. All tests were based on a significance level of $\alpha = 0.05$.

Results

A total of 77 male patients were enrolled in the present study. The median age was 60 years (IQR 44–72 years), and the mean BMI was 27.9 kg/m² (SD 4.2 kg/m²). The median stricture length was 4 cm (IQR 4–6 cm), with nine patients having long strictures (8–16 cm). The median preoperative $Q_{\text{max}}$ was 5 mL/s (IQR 3.2–8.2 mL/s), median PVR 85 mL (IQR 50–120 mL) and median AUA-SS 23 points (IQR 17–23 points). Successful harvesting of oral mucosa and tissue culture for the TEOMG graft was 100%. No patient reported donor-site pain, numbness, oral tightness or salivary changes at the time of urethroplasty.

The median operative time was 104 min (IQR 85–131). No intraoperative complications were recorded. The median FU was 38 months (IQR 31–46). We reported an overall SR of 68.8% ($n = 53$); 24 patients (31.2%) developed a recurrence (Fig. 3). Table 1 summarizes the SR according to patient and stricture characteristics.

We noted a 100% SR in virgin cases with no previous intervention, 85.7% by one previous urethrotomy or urethralplasty and 73.8% by two or more previous surgeries. In patients with a history of repetitive (>10) dilatations, our SR was surprisingly low at 31.3%. The stricture recurred at a median time of 7 months (IQR 3–13). The recurrence occurred in 11 patients (45.8%) in the first 6 months, in seven (29.2%) after 6–12 months, in four (16.7%) during the...
second year and in two (8.3%) during the third year after urethroplasty (Fig. 4).

The postoperative median $Q_{\text{max}}$ was elevated to 18.9 mL/s (IQR 15.2–25.4), PVR was reduced to median 0 mL (IQR 0–20 mL) and AUA-SS to median 5 points (IQR 2–8 points).

VCUG 3 weeks postoperatively showed incomplete patency of anastomosis in seven patients (9.1%), which required a prolonged catheterization of 2–4 further weeks. Three of these patients (42.9%) developed a recurrence. AEs included five cases of perineal wound hematoma or dehiscence (6.5%), which healed in secondary intention, five febrile UTIs (6.5%) treated with antibiotics and one urethral fistula (1.3%) requiring surgical revision (Clavien–Dindo 3b).

No oral complications at the biopsy site were reported.

The treatment of recurrences was individual, regarding patients’ wishes and stricture characteristics. A successful single urethrotomy was carried out in three patients with short recurrent strictures located at the proximal or distal anastomosis. Two patients received a second MukoCell® urethroplasty, as they developed strictures along the whole transplant length, without further recurrence. A total of 13 patients were treated with urethroplasty using NOMG, as they could not afford a second TEOMG manufacture, only one of them developed a second recurrence. Three patients declined further surgery and repeated dilatations. Finally, three patients were lost to FU. In total, 18 of 24 patients with recurrent strictures could be successfully salvaged.

The univariate as well as the multivariate logistic analysis detected stricture length $\geq 6$ cm ($P = 0.048$), as well as repetitive dilatations ($P < 0.001$), as statistically significant risk factors for stricture recurrence (Table 2).

**Discussion**

We presented the largest single-center clinical experience with TEOMG urethroplasty using the majority of established surgical techniques, re-evaluating the feasibility and safety of MukoCell®, with slightly lower, but comparable, SR to the classic augmentation urethroplasty with NOMG after a median FU of 38 months. This approach appears to be versatile and straightforward, as it avoids the harvesting of oral mucosa, resulting in reduced oral complication rates, reduced operating time and preserving sufficient extent of ipsilateral

![Fig. 3 Kaplan–Meier plot of overall SR (re-stricture-free survival). Time calculated from the date of urethroplasty surgery. Urethral strictures of any etiology, location, length and severity are included.](image-url)
cheek mucosa for future graft harvesting in case of salvage urethroplasty.

Ram-Liebig et al. first published preliminary clinical data of MukoCell® urethroplasty with 80.9% SR at a median of 18 months FU \((n = 21)\).\(^{10}\) Another multicenter, prospective, monitored observational trial on TEOMG urethroplasty with MukoCell® reported 70.8% SR by 65 patients in 12 months’ FU, and 76.9% by 39 patients in 24 months’ FU.\(^{11}\) The retrospective multicentric series of Barbagli et al. \((n = 38)\) showed an overall SR of 84.2% at a median 55 months’ FU.\(^{12}\) Comparing their SR distribution among different urethral segments with the present study showed better SR for bulbar strictures (93.1% vs 75.6%), but similar SR for penile (66.7% vs 63.6%) and penobulbar strictures (50% vs 53.3%).

The present subgroup analysis showed excellent SR (100%) in primary surgical reconstructions, such as in cases with one previous intervention (85.7%), with decreasing SR for cases after two to five endoscopic/open surgeries (73.8%) becoming surprisingly low after repeated dilatations (31.3%). Only repetitive dilatations could be identified as a statistically significant risk factor for stricture recurrence, the regression analysis was underpowered to prove an association of recurrence with multiple previous surgeries \((P = 0.258)\). Such an association, however, has been proven for NOMG urethroplasty, taking into account that every manipulation provokes a denser, more extensive spongiosis, and alters the vascular supply of urethral and periurethral tissue, providing less healthy tissue to use for graft take.\(^{19,21}\) Additionally, a previous study could identify the number of previous interventions as an independent risk factor for recurrence after TEOMG urethroplasty in a multivariable analysis.\(^{11}\)

The present cohort included patients who are often excluded from group analysis of urethral stricture disease, such as those with lichen sclerosus, pelvic radiation therapy and prior hypospadias repairs. However, because the sample size was minimal \((n = 4)\), no conclusions about the SR of TEOMG use for such indications could be withdrawn. A lower SR can yet be expected, extrapolating the increased likelihood of urethroplasty failure and the reconstructive challenge presented by men with such medical history using buccal grafts, which is well recognized in the literature.\(^{22-24}\)

A distinct decrease at SR was noticed with increasing stricture length, with strictures ≥6 cm achieving statistical significance for recurrence \((P = 0.048)\). In the study of Breyer et al., the association between increasing stricture length (>4 cm) and failure of NOMG trended toward statistical significance. Furthermore, Chapman et al. reported a significantly high risk of bulbar stricture recurrence with increased length.\(^{19,25}\) Finally, although smoking and diabetes have been proven well-established risk factors for stricture recurrence through their contribution to urethral microvascular damage, surprisingly, they did not achieve statistical significance in the present study \((P = 0.112\) and 0.254, respectively).

Recurrences occurred mainly in the first 6 months after surgery (45.8%), followed by decreasing rates over time. Early recurrence corresponds with the previous findings of Ram-Liebig et al., reporting 70.3% of stricture recurrences within 8 months and diminishing gradually thereafter,\(^{11}\) and Barbagli et al. recording the time of recurrence within 3 months in 33.3%, 6 months in 16.7%, 10 months in 16.7% and 12 months in 33.3% of patients.\(^{12}\) However, we noted two recurrences 27 and 29 months after surgery, proving the necessity of long-term FU equivalently to findings of studies with NOMG urethroplasties.\(^{19,24}\)

Comparing our SR distribution among different urethral segments with the series of Barbagli et al. showed worse SR for bulbar strictures (75.6% vs 93.1%), but similar SR for penile (63.6% vs 66.7%) and penobulbar strictures (53.3% vs 50%).\(^{12}\) Excluding the recurrences of bulbar strictures with a history of multiple dilatations (5/11), though, we noticed an acceptable SR (86.7%) approximating previous literature reports.

Regarding TEOMG safety, we noticed only a few minor AEs and one single major AE of urethral fistula requiring surgery. The first clinical safety data on this TEOMG urethroplasty have been reported in the form of a moderated poster at the AUA Conference 2014, noticing no peri- or postoperative AE related to MukoCell®.\(^{26}\) Rahm-Liebig et al. reported two local dermal infections and two UTIs.\(^{11}\) Barbagli et al. observed no local (oral-urethral) or general AE related to TEOMG.\(^{12}\) Concluding the use of TEOMG did not increase postoperative complications or patient emergency clinic visits 1 month after surgery in our cohort, a fact that strengthens the previous statements and verifies urethroplasty with MukoCell® as a safe surgical procedure.

The most important factor for establishment of the current TEOMG urethroplasty is the optimal patient selection, which remains unanswered in the literature. Although stricture length ≥6 cm was associated with recurrence, we suggest TEOMG use for long strictures, which would otherwise require large segments of oral mucosa being harvested. Especially in redo-urethroplasty patients, when excessive oral mucosa has already been used and patients refuse to have mucosa removed again, TEOMG could prevent further buccal scarring and related complications, as well as the use of a skin graft or flap, with inferior aesthetic and functional results.
A current drawback of the described procedure is that the costs of TEOMG manufacture are usually not covered by public health insurance, resulting in patients having to finance these costs themselves. An authorization of MukoCell® by the European Medical Agency and reimbursement by the public care system are anticipated to spread its use among other countries apart from Germany and facilitate its clinical assessment.

The current study was a retrospective evaluation of patients undergoing a urethroplasty with MukoCell® in our...
department during the past 5 years. Its most significant limitation was its retrospective character and the absence of a control group. In this direction, we initiated a multicentric pivotal prospectively randomized clinical phase III study to confirm the efficacy of MukoCell® in direct comparison with NOMG. identify further risk factors for treatment failure and determine the appropriate indications for this procedure. Furthermore, our population cannot be considered homogeneous regarding the stricture etiology and characteristics, as well as patient characteristics. Inevitably, a heterogeneity also exists in patients who were initially treated elsewhere, regarding the technique and expertise of that procedure, which is reflected in the intraoperative quality of urethral and periurethral tissue. These parameters could bias the statistical analysis presenting inferior SRs.

Additionally, due to the small sample size, our comparative statistical analysis to identify possible risk factors for recurrence disease was underpowered, noticing, however, a significant negative influence of repetitive dilatations and stricture length ≥6 cm. Thus, further studies are required to answer this important clinical question. Furthermore, the FU time of some patients was short, limiting our ability to capture all late recurrences. Hence, the current results should be treated as preliminary and will be re-evaluated over time.

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Conflict of interest

None declared.

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