available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Review – Reconstructive Urology

Editorial by Karl-Dietrich Sievert on pp. 607–609 of this issue

Tissue Engineering of the Urethra: A Systematic Review and Meta-analysis of Preclinical and Clinical Studies

Luuk R.M. Versteegden [a,†], Paul K.J.D. de Jonge [b,†], Joanna IntHout [c], Toin H. van Kuppevelt [a], Egbert Oosterwijk [b], Wout F.J. Feitz [b,d], Rob B.M. de Vries [e], Willeke F. Daamen [a,]*

[a] *Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands;* [b] *Department of Urology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands;* [c] *Department for Health Evidence, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands;* [d] *Radboudumc Amalia Children's Hospital, Radboud university medical center, Nijmegen, The Netherlands;* [e] *SYRCLE (SYstematic Review Centre for Laboratory Animal Experimentation), Department for Health Evidence (section HTA), Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands*

Article info

Article history:
Accepted March 17, 2017

Associate Editor:
Christian Gratzke

Keywords:
Urethra
Reconstructive surgery
Tissue engineering
Systematic review
Meta-analysis
Clinical studies
Preclinical studies

Abstract

Context: Urethra repair by tissue engineering has been extensively studied in laboratory animals and patients, but is not routinely used in clinical practice.
Objective: To systematically investigate preclinical and clinical evidence of the efficacy of tissue engineering for urethra repair in order to stimulate translation of preclinical studies to the clinic.
Evidence acquisition: A systematic search strategy was applied in PubMed and EMBASE. Studies were independently screened for relevance by two reviewers, resulting in 80 preclinical and 23 clinical studies of which 63 and 13 were selected for meta-analysis to assess side effects, functionality, and study completion. Analyses for preclinical and clinical studies were performed separately. Full circumferential and inlay procedures were assessed independently. Evaluated parameters included seeding of cells and type of biomaterial.
Evidence synthesis: Meta-analysis revealed that cell seeding significantly reduced the probability of encountering side effects in preclinical studies. Remarkably though, cells were only sparsely used in the clinic (4/23 studies) and showed no significant reduction of side effects. In 21 out of 23 clinical studies, decellularized templates were used, while in preclinical studies other biomaterials showed promising outcomes as well. No direct comparison to current clinical practice could be made due to the limited number of randomized controlled studies.
Conclusions: Due to a lack of controlled (pre)clinical studies, the efficacy of tissue engineering for urethra repair could not be determined. Meta-analysis outcome measures were similar to current treatment options described in literature. Surprisingly, it appeared that favorable preclinical results, that is inclusion of cells, were not translated to the clinic. Improved (pre)clinical study designs may enhance clinical translation.
Patient summary: We reviewed all available literature on urethral tissue engineering to assess the efficacy in preclinical and clinical studies. We show that improvements to (pre)clinical study design is required to improve clinical translation of tissue engineering technologies.

† These authors contributed equally to the study results.
* Corresponding author. Post Office Box 9101, 6500 HB Nijmegen, The Netherlands.
Tel. +31 24 36 10557; Fax: +31 24 35 40339.
E-mail address: Willeke.Daamen@radboudumc.nl (W.F. Daamen).

http://dx.doi.org/10.1016/j.eururo.2017.03.026
0302-2838/

1. Introduction

Congenital birth defects of the urethra, such as hypospadias (1 in every 300 births) [1,2], and acquired urethral abnormalities, such as urethral strictures (1 in every 1000 men > 65 yr of age [3]), represent major clinical entities. Treatment usually involves a surgical procedure with risk of (recurrence of) strictures or fistula requiring additional care or reintervention. Whenever possible, local tissue flaps or stricture resection in combination with end-to-end anastomosis are used for urethra reconstruction [4,5]. Generally, two surgical approaches exist for urethral reconstruction: partial replacements using onlay or inlay techniques or the full circumferential procedure, which is used in rare cases with significant urethral scarring or lichen sclerosis. Depending on patient and local factors, procedures can be performed as one-stage procedure or as planned multistage procedure [3].

Autologous tissue transplantation such as buccal mucosa or free skin grafts are the standard treatments [6–9]. However, due to the limited quantity of available donor tissue, accompanying donor site morbidity (16–32% for buccal mucosa grafts) and complications (eg, recurrences or infections), alternative treatment options are needed to improve long-term outcomes [10]. Tissue engineering may overcome some of the aforementioned disadvantages by providing a temporary template to guide tissue regeneration [11]. In general, tissue engineered templates include decellularized tissue or de-novo prepared materials from natural or synthetic origin [12–14]. Templates can be seeded with (stem) cells from the patient prior to implantation. These cells may stimulate tissue remodeling by excreting cytokines and growth factors and contributing to cellular population of the template [15,16].

Despite the potential of tissue engineering shown in in vitro research and preclinical studies, clinical translation is limited. To improve translation, an evidence-based approach, such as systematic reviews, can be applied when designing new tissue engineering strategies. This will avoid unnecessary replication of studies and will help to select the most optimal experimental design and model. We are the first to perform a comprehensive systematic review of evidence for the efficacy of urethral tissue engineering in preclinical and clinical studies. A meta-analysis was used to compare different experimental designs based on clinically relevant outcomes. This systematic review aims to improve the translation of urethral tissue engineering from bench to bedside.

2. Evidence acquisition

2.1. Literature search

To identify all available studies on urethral tissue engineering published and indexed up until June 1, 2016, a systematic search strategy was applied in PubMed (Supplementary data 1) and Embase (via OvidSP; Supplementary data 2). This strategy combined a tissue engineering search component containing synonyms for tissue engineering related terms [17] with a customized search component for urethra or urethra-related diseases. Medical Subject Headings terms and EMTREE terms were used in PubMed and Embase, respectively, together with separate words or word combinations in title or abstract. Next, either an animal filter designed by Hooijmans et al (PubMed) [18] or de Vries et al (Embase) [19] was applied (Supplementary data 1 and 2, search component 3A) or a custom filter for clinical studies (Supplementary data 1 and 2, search component 3B). In addition, retrieved reviews were screened for primary studies not found using the search strategy. Clinical studies found during animal search strategy were marked and screened for relevance and vice versa.

2.2. Study selection

Duplicates in retrieved articles were removed in EndNote (Version X7.2, Thomson Reuters, PA, USA). Studies were assessed independently by LV and PdJ. First, clearly irrelevant studies were excluded based on title. Next, titles and abstracts of the remaining articles were screened for relevance in Early Review Organizing Software (Buenos Aires, Argentina, www.eros-systematic-review.org) using the following exclusion criteria: (1) no urethra, (2) no tissue engineering, (3) no animals or patient, (4) no primary study. A study was considered to be about tissue engineering when a processed template was used. Studies on tissue transplants or reconstructive surgery without the use of a template or without a urethra defect were excluded. Of the remaining studies, full texts were screened using the same exclusion criteria. Articles not available as full text were excluded at this stage. No language restrictions were applied in the screening phase. If necessary, Google translate was used. Retrieved studies from search updates were directly screened in Endnote according to the same principles. In all stages of the selection process, discrepancies between reviewers were discussed until consensus was reached.

2.3. Study characteristics

From all included studies, general information (author, year) and study characteristics (age range of patients, animal species, sex, surgical procedure, type of biomaterial, type of cells) were extracted and listed in Table 1 for preclinical studies and Table 2 for clinical studies. For languages other than English, German, and French, Google Translate was used to retrieve study characteristics.

2.4. Extraction outcome data

Three outcome measures were used to evaluate study outcome: (1) incidence of side effects, for example, strictures, stenosis, fistulae, and infections, (2) functionality, defined as the ability to void with continence, and (3) study completion, for animals defined as survival until predetermined endpoint and for clinical studies as available for follow-up or no additional urethroplasty required. Only English, German, and French studies were

Table 1 – Study characteristics of all 80 preclinical studies sorted on inclusion in meta-analysis, surgical procedure, and biomaterial

	Author	Yr	Animal model	No. of animals	Sex	Surgical procedure (defect length in mm)	Biomaterial (category)	Type of added cells	Quality assessment + meta-analysis
1	Feng C	2011	Rabbit	28	M	Inlay (15)	Acellular corpus spongiosum, porcine (D)	Autologous corporal SMCs and lingual keratinocytes	Yes
2	Ayyildiz A	2006	Rabbit	10	M	Inlay (5)	Alloderm + acellular pericardium, bovine (D)	–	Yes
3	Chen F	1999	Rabbit	10	M	Inlay (10)	BAM, porcine (D)	–	Yes
4	Chun SY	2015	Rabbit	10	M	Inlay (20)	BAM, porcine (D)	Autologous minced urethral muscle and urothelial tissue	Yes
5	Sayeg K	2013	Rabbit	18	M	Inlay (35)	BAM, porcine (D)	Autologous bladder SMCs	Yes
6	Huang JW	2014	Rabbit	30	M	Inlay (15)	BAM, rabbit (D)	–	Yes
7	Li C	2008	Rabbit	24	M	Inlay (20)	BAM, rabbit (D)	Autologous oral keratinocytes	Yes
8	Li C	2013	Rabbit	27	M	Inlay (20)	BAM, rabbit (D)	Autologous oral keratinocytes and TGF-β siRNA transfected fibroblasts	Yes
9	Li H	2014	Rabbit	36	M	Inlay (20)	BAM, rabbit (D)	Epithelial-differentiated rabbit adipose-derived stem cells	Yes
10	Wang F	2014	Rabbit	12	M	Inlay (10)	Denuded amnion, human (D)	Rabbit urothelial cells	Yes
11	Kajbafzadeh AM	2014	Rabbit	12	M	Inlay (5)	Preputial acellular matrix, human (D)	–	Yes
12	Kawano PR	2012	Rabbit	24	M	Inlay (10)	SIS, 1- and 4 –layer, porcine (D)	–	Yes
13	Guo H	2015	Rabbit	24	M	Inlay (20)	SIS, porcine (D)	Autologous keratinocytes and TIMP siRNA transfected fibroblasts	Yes
14	Kropp BP	1998	Rabbit	8	M	Inlay (10)	SIS, porcine (D)	–	Yes
15	Rotariu P	2002	Rabbit	7	M	Inlay (25)	SIS, porcine (D)	–	Yes
16	Villoldo GM	2013	Rabbit	15	M	Inlay (10)	SIS, porcine (D)	–	Yes
17	Shokeir A	2003	Dog	21	M	Inlay (30)	UAM, dog (D)	–	Yes
18	Huang JW	2015	Rabbit	30	M	Inlay (20)	Cellulose (N)	Rabbit lingual keratinocytes	Yes
19	Xie M	2013	Dog	10	F	Inlay (50)	Silk fibroin (N)	Autologous oral keratinocytes and fibroblasts	Yes
20	Xie M	2013	Dog	9	F	Inlay (30)	Silk fibroin (N)	Dog urothelial cells	Yes
21	Sun D	2014	Rabbit	21	M	Inlay (5)	Subcutaneous implanted autologous minced muscle (N)	Human umbilical cord MSCs	Yes
22	Xu Y	2014	Rabbit	21	M + F	Inlay (5)	Subcutaneous implanted autologous muscle microsomes (N)	Human umbilical cord MSCs	Yes
23	Zhang K	2015	Rabbit	12	M	Inlay (20)	P(LA/CL) + type I collagen in combination with ICG-001 (Wnt-pathway inhibitor) (S)	Rabbit bladder urothelial cells	Yes
24	Wang DJ	2015	Rabbit	24	M	Inlay (5)	Polylactid acid (S)	Rabbit AdSCs	Yes
25	Kelami A	1971	Dog	10	M	Inlay (30)	PTFE (S) + lyophilized dura, human (D)	–	Yes
26	Chung YG	2014	Rabbit	8	M	Inlay (20)	Silk fibroin (N) + SIS (D)	–	Yes
27	Lv X	2016	Rabbit	18	M	Inlay (15)	Silk-Keratin-Gelatin-Calcium peroxide (N) + SIS, porcine (D)	–	Yes
28	Nuininga JE	2003	Rabbit	18	M	Inlay (10)	SIS, 1 - and 4 - layer, porcine (D) + Type I collagen (N)	–	Yes
29	Zhang Q	2008	Rabbit	12	M	Full (10)	Acellular amnion, human (D)	Homologous endothelial progenitor cells	Yes
30	Parnigotto PP	2000	Rabbit	12	M	Full (10)	Acellular aorta, rabbit (D)	–	Yes
31	DeFilippo RE	2002	Rabbit	24	M	Full (10)	BAM (D)	Autologous bladder SMCs and urothelial cells	Yes
32	El-Tabey N	2012	Dog	14	F	Full (30)	BAM (D)	Autologous bladder SMCs and urothelial cells	Yes
33	Wang JH	2013	Rabbit	18	M	Full (30)	BAM with polylactid acid –glycolic acid with VEGF (D)	–	Yes
34	DeFilippo RE	2015	Rabbit	15	M	Full (30)	BAM, porcine (D)	Autologous bladder SMCs and urothelial cells	Yes
35	Dorin RP	2008	Rabbit	12	M	Full (5–30)	BAM, porcine (D)	–	Yes
36	Orabi H	2012	Dog	21	M	Full (60)	BAM, porcine (D)	Autologous bladder SMSs and urothelial cells	Yes

Table 1 (*Continued*)

	Author	Yr	Animal model	No. of animals	Sex	Surgical procedure (defect length in mm)	Biomaterial (category)	Type of added cells	Quality assessment + meta-analysis
37	Fu Q	2007	Rabbit	18	M	Full (15)	BAM, rabbit (D)	Autologous foreskin epidermal cells	Yes
38	Fu Q	2008	Rabbit	18	M	Full (15)	BAM, rabbit (D)	Autologous foreskin epidermal cells	Yes
39	Gu GI	2012	Rabbit	18	M	Full (15)	BAM, rabbit (D)	Autologous mesothelial cells	Yes
40	Li CL	2013	Rabbit	30	M	Full (30)	BAM, rabbit (D)	Autologous bone-marrow derived MSCs and SMCs	Yes
41	Li B	2013	Rabbit	12	M	Full (15)	Frozen-thawed bladder mucosa, dog (D)	—	Yes
42	Kjaer TB	1976	Dog	9	M	Full (30)	Lyophilized vein, dog (D)	—	Yes
43	Shokeir A	2004	Dog	14	M + F	Full (30)	UAM, dog (D)	—	Yes
44	Sievert KD	2001	Rabbit	14	M	Full (8–11)	UAM, dog and rabbit (D)	—	Yes
45	Sievert KD	2000	Rabbit	30	M	Full (8–11)	UAM, rabbit (D)	—	Yes
46	Lv X	2016	Dog	18	F	Full (20)	Bacterial cellulose + potato starch (N)	Dog lingual muscle cells	Yes
47	Gu GI	2010	Rabbit	9	M	Full (15)	De-novo created tissue in peritoneal cavity (N)	—	Yes
48	Jia W	2015	Dog	10	M	Full (50)	Type I collagen scaffold + /-3VEGF (N)	—	Yes
49	A Da Silva LF	2014	Rabbit	16	M	Full (10)	Type I collagen, bovine (N)	Autologous bladder SMCs	Yes
50	Nuininga JE	2010	Rabbit	32	M	Full (10)	Type I collagen, bovine (N)	—	Yes
51	Kanatani I	2007	Rabbit	28	M	Full (15)	Type I collagen, porcine + P(LA/CL) (N)	—	Yes
52	Micol LA	2012	Rabbit	16	M	Full (10)	Type I collagen, rat tail (N)	Autologous bladder SMCs	Yes
53	Mikami H	2012	Dog	10	M	Full (20)	Type I collagen, rat tail (N)	Autologous oral epithelial and muscle cells	Yes
54	Italiano G	1997	Rabbit	14	M	Full (15)	Hyaluronan benzyl ester (S)	—	Yes
55	Italiano G	1998	Rabbit	4	M	Full (15)	Hyaluronan benzyl ester (S)	—	Yes
56	Fu Q	2014	Dog	18	M	Full (15)	PGA (S)	Oral mucosal epithelial cells and AdSCs	Yes
57	Hakky SI	1977	Dog	15	M	Full (50)	Polyethylene terephthalate (S)	—	Yes
58	Hakky SI	1977	Dog	9	M	Full (50)	Polyethylene terephthalate (S)	—	Yes
59	Olsen L	1992	Dog	6	M	Full (30–40)	Polyglactin fiber coated with polyhydroxybutyric acid (S)	—	Yes
60	Anwar H	1984	Dog	10	?	Full (25)	PTFE (S)	—	Yes
61	Dreikorn K	1979	Dog	12	M	Full (30–80)	PTFE (S)	—	Yes
62	Xie H	2007	Rabbit	34	M	Inlay + full (15)	Elastin and collagen, porcine (N)	—	Yes
63	El-Assmy A	2004	Rabbit	18	M	Inlay + full (15)	SIS (D)	—	Yes
64	Wang YQ	2005	Rabbit	14	M	Inlay (10)	BAM, human (D)	—	No (CN)[a]
65	Beintker M	2007	Rat	20	M	Inlay (?)	SIS (D)	—	No[b]
66	Glybochko PV	2014	Rabbit	Unknown	M	Full (?)	Acellular artery, human (D)	—	No (RU)a
67	Peng WB	2013	Rabbit	Unknown	M	Full (25)	BAM (D)	Rabbit hair follicle stem cells	No (CN)a
68	Hu YF	2008	Rabbit	30	M	Full (10–15)	UAM, rabbit (D)	—	No[c]
69	Hu YF	2009	Rabbit	20	M	Full (10–15)	UAM, rabbit (D)	—	No (CN)[a]
70	Yang SX	2004	Rabbit	30	M	Full (10–15)	UAM, rabbit (D)	—	No[c]
71	Lebret T	1994	Rat	7	F	Full (?)	Type IV collagen, human (N)	—	No[b]
72	Fu WJ	2009	Rabbit	32	M	Full (10–15)	PLLA (S)	Autologous urothelial cells	No[c]
73	Verit A	2003	Dog	2	M	Full (10)	PTFE (S)	—	No (TR)[a]
74	Huang X	2006	Rabbit	12	M	Inlay + full (?)	SIS, porcine (D)	—	No (CN)[a]
75	Fu Q	2006	Rabbit	12	M	Unclear (10–30)	BAM, rabbit (D)	—	No (CN)[a]
76	Xu LS	2007	Rabbit	48	M	Unclear (?)	UAM, porcine (D)	—	No[c]
77	Han P	2009	Rabbit	24	M	Unclear (20)	UAM, rabbit (D)	Rabbit bladder SMCs	No (CN)[a]
78	Huang HJ	2007	Rabbit	48	M	Unclear (?)	UAM, rabbit (D)	Rabbit bone marrow derived MSCs	No (CN)[a]
79	Zhang Y	2011	Rabbit	Unknown	M	Unclear (?)	Silk fibroin (N)	Rabbit AdSCs	No (CN)[a]
80	Liu C	2008	Dog	12	M	Unclear (15–30)	Silk fibroin (N)	—	No (CN)[a]

? = unclear; AdSC = adipose-derived stem cells; BAM = bladder acellular matrix; CN = Chinese; D = decellularized; F = female; M = male; MSC = mesenchymal stem cells; N = natural; P(LA/CL) = copoly(L-lactide/ε-caprolactone); PTFE = polytetrafluoroethylene; RU = Russian; S = synthetic; SIS = small intestinal submucosa; siRNA = small interfering RNA; SMC = smooth muscle cell; TGF-β = transforming growth factor-β; TR = Turkish; UAM = urethral acellular matrix; VEGF = vascular endothelial growth factor.

[a] Excluded from meta-analysis due to language restrictions defined in Section 2.5.

[b] Excluded from meta-analysis because only two studies used rats (insufficient for statistical analysis).

[c] Excluded from meta-analysis due to unclear experimental setup.

Table 2 – Study characteristics of all 23 clinical studies sorted on inclusion in meta-analysis, surgical procedure, and biomaterial

	Author	Yr	No. of patients	Sex	Age range	No. of patients with prior surgery	Surgical procedure (defect length in mm)	Biomaterial (category)	Availability biomaterial	Type of added cells	Quality Assessment + meta-analysis
1	Atala A	1999	4	M	4–20 yr	4	Inlay (50–150)	BAM, human (D)	Exp	–	Yes
2	El-Kassaby AW	2003	28	M	2–61 yr	Unknown	Inlay (15–160)	BAM, human (D)	Exp	–	Yes
3	El-Kassaby AW	2008	15	M	21–59 yr	9	Inlay (20–180)	BAM, human (D)	Exp	–	Yes
4	Le Roux PJ	2005	9	M	15–56 yr	5	Inlay (10–50)	SIS, porcine (D)	Com	–	Yes
5	Palminteri E	2006	20	M	20–74 yr	16	Inlay (20–80)	SIS, porcine (D)	Com	–	Yes
6	Donkov II	2006	9	M	26–45 yr	5	Inlay (40–60)	SIS, 4-layer, porcine (D)	Com	–	Yes
7	Fiala R	2007	50	M	45–73 yr	Unknown	Inlay (40–140)	SIS, 4-layer, porcine (D)	Com	–	Yes
8	Orabi H	2013	12	M	1.5–15 yr	3	Inlay (15–35)	SIS, 4-layer, porcine (D)	Com	–	Yes
9	Xu YM	2013	28	M	2–69 yr	28	Inlay (35–70)	SIS, 4-layer, porcine (D)	Com	–	Yes
10	Hauser S	2006	5	M	61–80 yr	5	Inlay (35–100)	SIS, 1- and 4 –layer, porcine (D)	Com	–	Yes
11	Osman NI	2014	5	M	36–66 yr	4	Inlay (?)	De-epidermised dermis, human (D)	Exp	Autologous buccal mucosa-derived keratinocytes and fibroblasts	Yes
12	Fossum M	2012	6	M	14–44 mo	Unknown	Inlay (?)	Acellular skin, human (D)	Exp	Autologous urothelial cells	Yes
13	Raya-Rivera A	2013	5	M	10–14 yr	2	Full (40–60)	Polyglycolic acid and poly-(lactide-co-glycolide acid) (S)	Exp	Autologous bladder SMCs and urothelial cells	Yes
14	Bhargava S	2008	5	?	Unknown	5	Inlay (?)	De-epidermised dermis, human (D)	Exp	Autologous buccal mucosa-derived keratinocytes and fibroblasts	No[a]
15	Carpenter CP	2012	1	M	68 yr	1	Inlay (25)	Alloderm (D)	Com	–	No[b]
16	Kim JY	2005	1	M	48 yr	0	Inlay (40)	Alloderm (D)	Com	–	No[b]
17	Mantovani F	2003	1	M	72 yr	1	Inlay (?)	SIS, porcine (D)	Com	–	No[b]
18	Lin J	2005	16	M	18–46 yr	Unknown	Full (?)	Acellular skin, human (D)	Exp	–	No (CN)[c]
19	Villavicencio H	1989	22	?	28–80 yr	Unknown	Inlay + full (?)	Lyophilized dura, human (D)	Exp	–	No (ES)[c]
20	Glybochko P	2015	1	M	64 yr	Unknown	Unclear (?)	Acellular artery, human (D)	Exp	Autologous buccal mucosa-derived keratinocytes	No (RU)[c]
21	Yang WZ	2011	8	M	4–23 yr	0	Unclear (?)	Acellular skin human (D)	Exp	–	No[d]
22	Mantovani F	2002	5	M + F	70–79 yr	Unknown	Unclear (?)	SIS, porcine (D)	Com	–	No (IT)[c]
23	Li P	2009	8	M	8–36 mo	Unknown	Unclear (25–45)	Gelating sponge (N)	Exp	–	No (CN)[c]

? = unclear; BAM = bladder acellular matrix; CN = Chinese; Com = commercial availability; D = decellularized; ES = Spanish; Exp = experimental availability; IT = Italian; MSC = mesenchymal stem cells; N = natural; RU = Russian; S = synthetic; SIS = small intestinal submucosa; SMC = smooth muscle cell.

[a] Excluded from meta-analysis because same patients were included in long-term follow-up study by Osman et al., 2014 (#11).

[b] Excluded from meta-analysis due to case study.

[c] Excluded from meta-analysis due to language restrictions defined in Section 2.5.

[d] Excluded from meta-analysis due to unclear surgical procedure.

considered for quality assessment and meta-analysis. When critical information needed (eg, surgical procedure or number of animals/patients) was incomplete, studies were excluded. As only two studies used rats these were also excluded at this stage.

2.5. Quality assessment

Due to the nonrandomized, noncontrolled nature of most preclinical and clinical studies, no standard risk of bias analysis could be performed as validated tools are unavailable for these types of studies. Instead, overall quality was independently scored by PdJ and LV based on the reporting of specific key information (Fig. 2). Discrepancies were discussed until agreement was reached.

2.6. Meta-analysis

The following main research question was considered: "What is the evidence for the efficacy of urethral tissue engineering in preclinical and clinical studies?" Subquestions included the effects of the addition of (stem) cells to the template, the type of biomaterial, as well as potential differences between animal species on the separate outcome measures. Analyses for preclinical and clinical studies were conducted separately, as were full circumferential and inlay procedures. Statistical analyses were performed with SAS/STAT software version 9.2 for Windows, copyright 2002–2008 by SAS Institute Inc., Cary, NC, USA.

2.6.1. Preclinical studies

The following preclinical data were extracted for all available time points per study: the total number of animals as well as the number of animals without side effects, with functionality, and alive at the study endpoint. Time points were categorized in three periods: 0–4 wk, 5–11 wk, and 12 wk or longer.

Per study, the probability of response (eg, having no side effects) with a corresponding 95% exact (Clopper-Pearson) confidence interval (CI) was estimated per outcome. An additive random-effects logistic meta-regression model was fitted by means of a generalized linear mixed model approach. The number of responding animals out of the total was used as outcome parameter. In addition, the following independent parameters were used: treatment (combining the addition of cells and the type of biomaterial) and animal species. Random effects for study and for treatment grouped by study, were added. The Akaike Information Criterion [20] showed that models based on combined study data were preferable to models based on the period data (period as factor), therefore all time points per study were combined. When possible, the maximum likelihood approach with adaptive quadrature was used as estimation method. If this did not converge, the maximum likelihood with the Laplace approximation was applied. The resulting estimated odds were backtransformed into percentages and corresponding 95% CIs. In addition, the marginal effects of the treatments were estimated by combining the estimated percentages for rabbits and dogs, including 95% logit-based CIs, as described by Zou [21]. All p values were based on these CIs.

2.6.2. Clinical studies

For the analyses of the clinical outcomes, the following data per study were extracted: total number of patients, and numbers of patients without side effects, with functionality, and completing the study. No separate time points were analyzed in the human studies. For each study, the probability of response with corresponding 95% exact CIs was estimated per outcome. Due to limited study diversity, meta-regression models similar to preclinical studies were only fit for inlay repair and biomaterial type decellularized. A compound symmetry random effect was added for the addition of cells, grouped by study. Estimated odds from meta-regression were backtransformed into probabilities and corresponding 95% CIs.

3. Evidence synthesis

3.1. Literature search and screening

Fig. 1A and B show the results of the literature search and screening of collected studies. After the search, 1524 unique preclinical and 5361 unique clinical studies were identified. During title and abstract screening of these studies, 1349 and 5282 were excluded, respectively. After full text screening, 80 preclinical studies and 23 clinical studies were included in the study characteristics table (see Section 3.2). Only 63 preclinical and 13 clinical studies were eligible for the quality assessment (Section 3.3) and meta-analysis (Section 3.4).

3.2. Study characteristics

3.2.1. Preclinical studies

Preclinical study characteristics are summarized in Table 1 (see Supplementary data 3 for references of listed studies). Only three animal species, rabbits (59/80), dogs (19/80), and rats (2/80) were used, which were predominantly males (72/80). Full circumferential repair was investigated in 41 studies, inlay repair in 30 studies, both methods in three, while the procedure was unclear in the remaining studies (6/80). In dogs, primarily full defect repairs were performed (14 full vs 4 inlay), while in rabbits both inlay (25) and full repairs (26) were employed.

Due to the wide variety of materials used, they were categorized into three categories: decellularized templates (46/80), de novo prepared templates from natural materials (18/80), and de novo prepared templates from synthetic materials (12/80). Four (4/80) studies used multiple material types in different groups and these were assessed separately in the meta-analysis. Synthetic materials were almost exclusively used for full repair (10 full vs 3 inlay). Cells were incorporated into templates in 34 studies, of which bladder smooth muscle cells and urothelial cells were mostly used (13/34), followed by keratinocytes and fibroblasts from oral tissue (6/34) or a combination thereof (2/34), foreskin epidermal cells (2/34) and omental

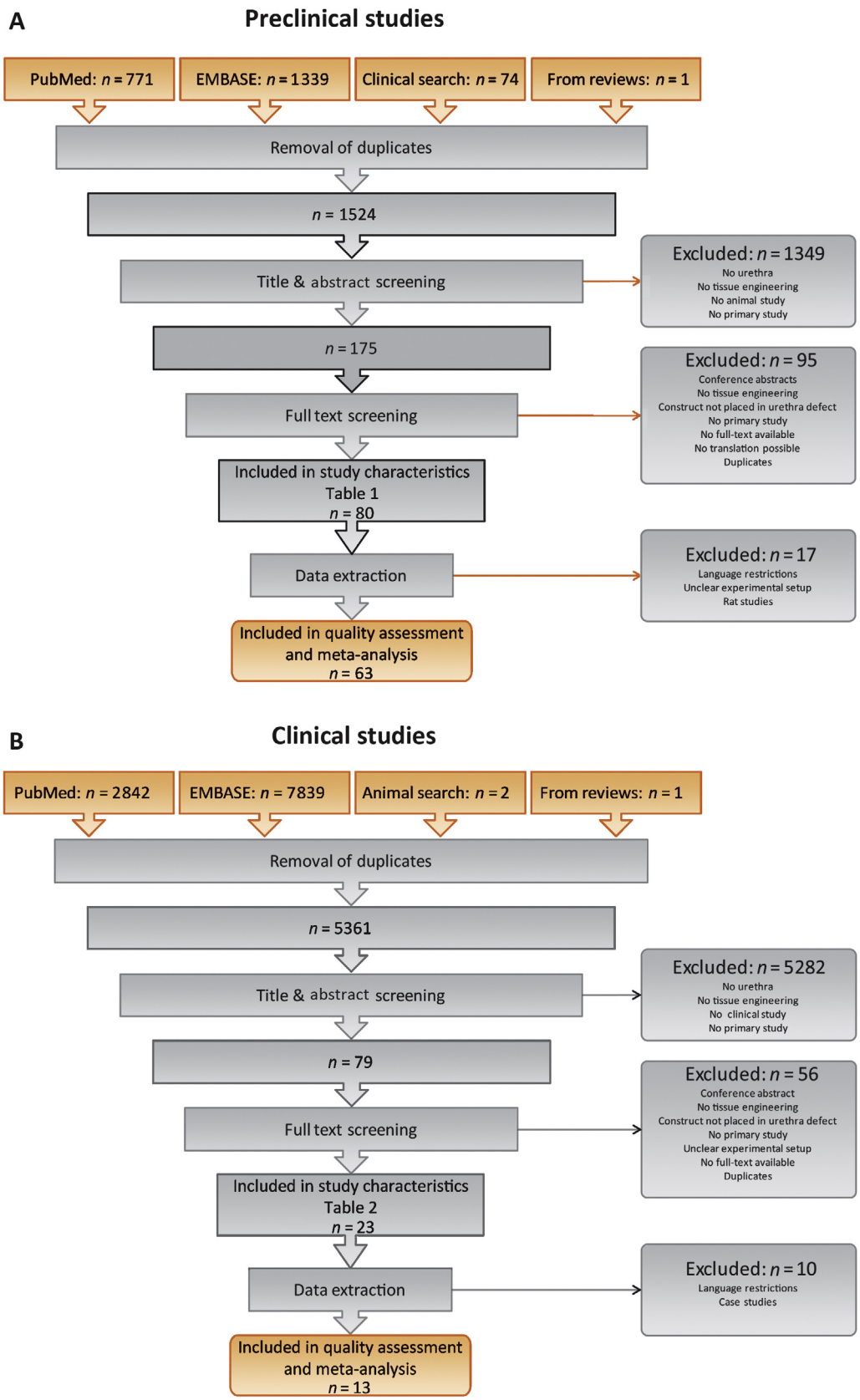

Fig. 1 – Flowchart of search and screening process of (A) preclinical studies and (B) clinical studies. The search was updated until June 1, 2016.

mesothelial cells (1/34). Stem cells, mostly derived from adipose tissue, bone marrow or human umbilical cord, were used in 10 studies.

3.2.2. Clinical studies

Study characteristics of clinical studies are listed in Table 2 (see Supplementary data 4 for references of listed studies). Clinical studies were performed with males, except for one study (Table 2). From 23 studies, 16 used an inlay approach, two a full circumferential procedure, one used both approaches, while in four studies the procedure was unclear. The majority of studies (21/23) used decellularized templates, while natural and synthetic templates were both used once. Four studies used cell-seeded templates; 2/23 buccal mucosa keratinocytes and/or fibroblasts and 2/23 bladder smooth muscle cells and/or urothelial cells.

3.3. Quality assessment

The quality of reporting was assessed for 63 preclinical and 13 clinical studies from which outcome data could sufficiently be extracted for inclusion in the meta-analysis (Fig. 2). Results per study are listed in Supplementary data 5. Reporting of information regarding included animals/patients, such as species and strain, sex, number of animals/patients, age/weight, and patient inclusion criteria, were generally well described.

Overall quality of the experimental setup was poor. Although the different experimental groups were well described, hardly any control groups were present, and randomization and blinding were seldom mentioned in both preclinical and clinical studies. Also, clinical study protocols were not published. However, surgical procedure, composition, size, and preparation of the implants were clearly described in most studies. Reporting of outcome measures was good for both preclinical and clinical studies with respect to the description of outcome measures, follow-up time, and side effects. The number of drop-outs was clearly mentioned in clinical studies, but only in half of the preclinical studies. For preclinical studies, histological sampling location and representativeness of the results were poorly described.

3.4. Meta-analysis

3.4.1. Preclinical studies

For full circumferential repair (Fig. 3A), the addition of cells significantly reduced the probability of side effects, independent of the type of biomaterial used ($p = 0.001$). Exact point estimates including CI are given in Supplementary data 6. Regarding the type of biomaterial, when no cells were used, estimates show that synthetic materials had a higher probability for having no side effects compared to decellularized and natural materials. With cells seeded, estimated probabilities were similar for all materials. For functionality and study completion, estimated probabilities were similar for all study conditions.

For inlay repair (Fig. 3B), the addition of cells significantly reduced the probability of side effects ($p = 0.003$), albeit less than for full repair. Estimated probabilities were similar for all types of biomaterial regardless of the addition of cells. For functionality and study completion, estimated probabilities were similar for all study conditions. It was impossible to estimate study completion probability per biomaterial as almost all animals survived inlay repair (statistical model did not converge).

Although estimated probabilities for dogs and rabbits were slightly different, differences were not statistically significant. Consequently, the animal species had only marginal influence on outcome (data not shown).

3.4.2. Clinical studies

For clinical studies, a similar meta-analysis was performed (Fig. 3C). Only inlay repair using decellularized materials with or without cells could be analyzed due to the limited number of other combinations. No statistically significant differences were found for the inclusion of cells for any of the outcome measures ($p = 0.5$ for side effects, $p = 0.7$ for functionality, and $p = 0.08$ for study completion).

When comparing preclinical and clinical estimated probabilities, point estimates for absence of side effects after inlay repair seem to be higher in clinical studies for both acellular and cellular templates. For functionality, the point estimates were similar. The estimated probability for study completion was much lower in clinical studies compared to preclinical studies regardless of the addition of cells, but these cannot be directly compared due to distinctive definitions for study completion and differences in disease status.

4. Discussion

Reconstructive surgery using biomaterials has been studied as an alternative approach for urethral repair since the early seventies and efforts along these lines expanded rapidly in the nineties when the term "Tissue Engineering" was introduced (Fig. 4) [11]. Nowadays, preclinical studies have been readily performed, but clinical studies have not followed this trend. Although many (pre)clinical studies have been performed, tissue engineering is not used as an alternative treatment in routine clinical practice, except for a select patient group with a history of failed repairs [22–24]. In this systematic review, all (pre)clinical publications on urethra tissue engineering until June 2016 were analyzed to assess the evidence for the efficacy. For clinical studies, the term "effectiveness" may be more suitable, as most studies included in this review showed a heterogeneous patient population [25]. However, we used the term "efficacy" for preclinical and clinical studies throughout this systematic review. For both preclinical and clinical studies, tissue engineering had a high probability for functionality, defined as voiding with continence. Study completion was high in preclinical studies, but not in clinical studies. This may be related to the difference in our definition of study completion and in study design. In preclinical studies, animals generally only need to survive for several months to study the tissue regeneration process, compared with patients that need to show a good long term outcome

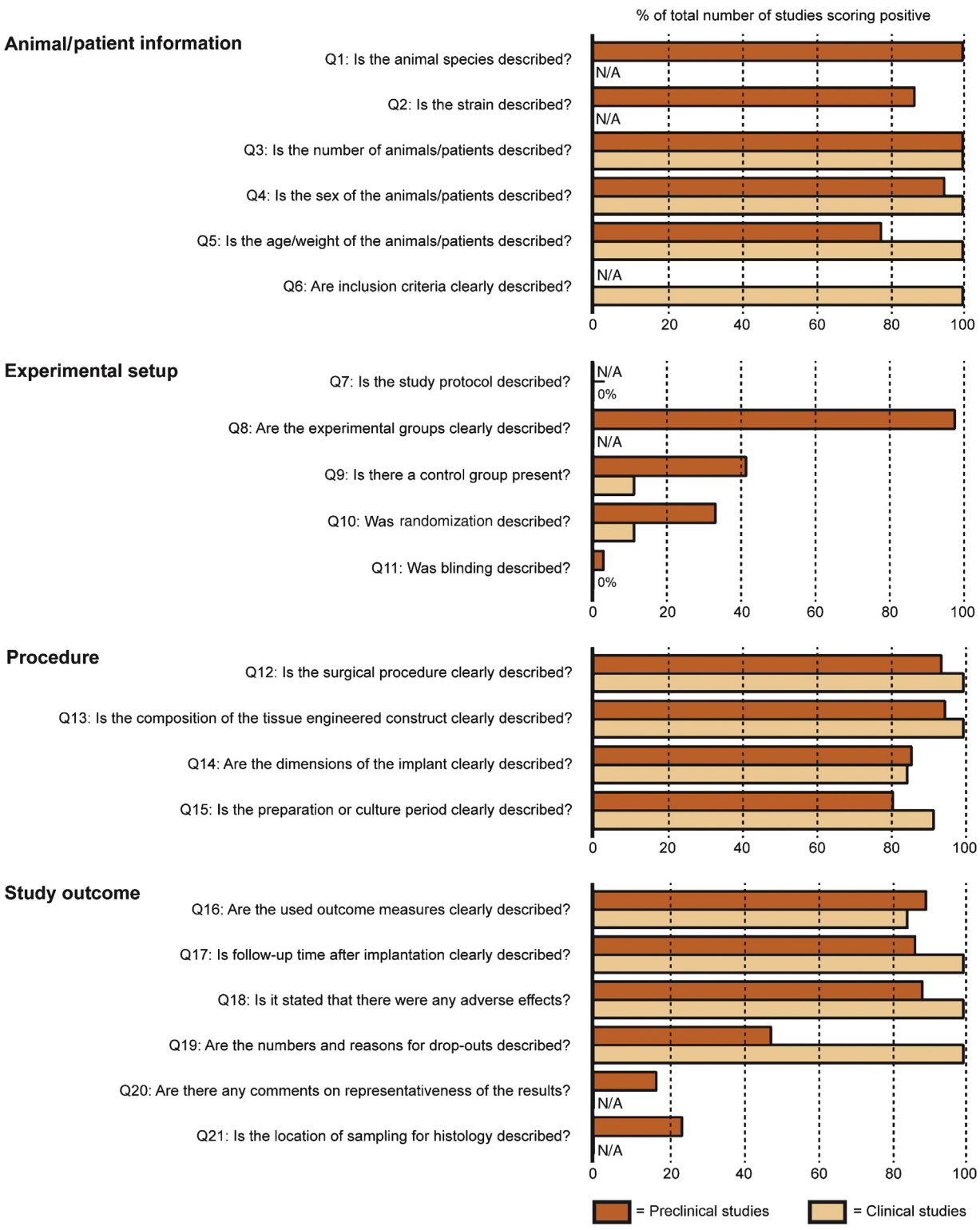

Fig. 2 – Quality assessment of preclinical and clinical studies. All studies included for meta-analysis were scored on clear reporting of several key parameters (Q1–Q21) showing that study design such as inclusion of proper control groups, associated randomization and blinding, reporting of key parameters such as representativeness of shown results, and drop-outs needs to be improved in preclinical studies.
N/A = not applicable; Q = question.

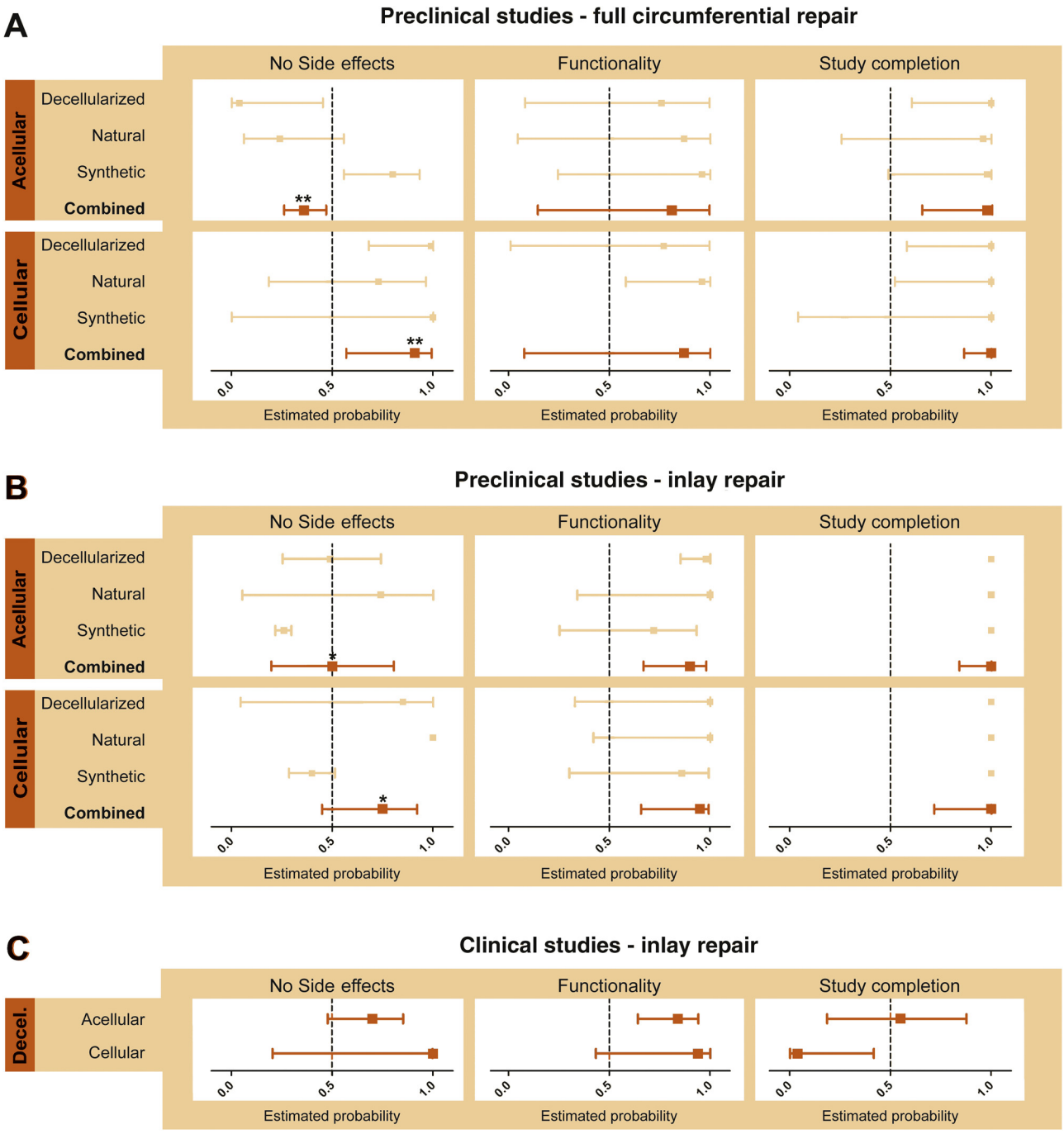

Fig. 3 – Estimated probability including 95% confidence intervals for the absence of side effects, functionality and study completion for (A) full circumferential repair and (B) inlay repair in preclinical studies, both categorized for the use of cells and the type of biomaterial. (C) For the clinical studies, only decellularized material with or without cells could be analyzed. The effect of cells on the three outcome measures was calculated in estimated probabilities. Overall differences for cellular versus acellular templates were determined for each outcome measure for both full and inlay repair. Specific point estimates and confidence interval are given in Supplementary data 6.
Decel. = decellularized.
* ***p*** **= 0.003.**
** ***p*** **= 0.001.**

without reintervention and without being lost in follow-up. Most patients had a history of failed repairs using conventional techniques, while healthy animals were used. As randomized clinical studies were lacking, for example, comparison with standard treatments (free skin graft or buccal mucosa urethroplasty) [3], no direct comparisons with current clinical practice could be made. Available literature about complex two-stage urethroplasty shows complication-free rates, functionality, and study completion of approximately 62%, 67%, and 36% [26], similar to the outcome of tissue engineered urethras (based on point estimates). This suggests that tissue engineered urethras

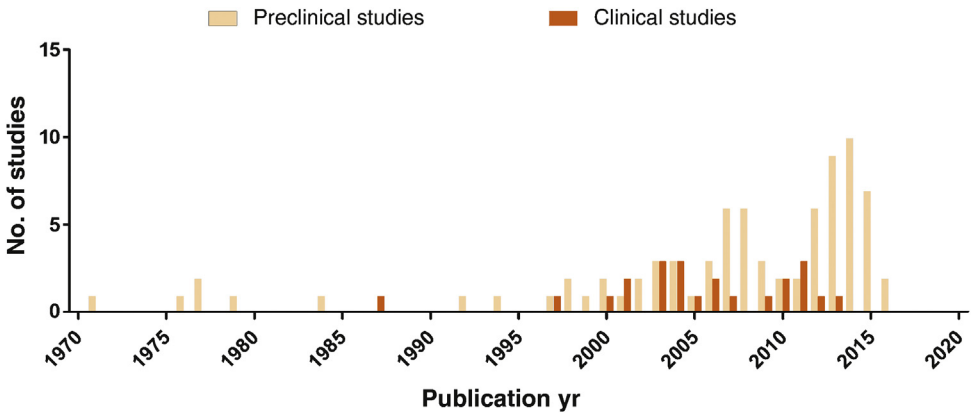

Fig. 4 – Number of publications per year for preclinical and clinical studies included in this systematic review. After several single studies between 1971 and 1994, the number of publications increased. Peaks in both clinical and preclinical studies were seen around 2005–2008 and again between 2012–2015.

may perform adequately and may be a valid alternative. Clearly, randomized controlled clinical trials are needed to clarify this issue.

4.1. *Application of cells*

There is no consensus on the potential beneficial effects of cell seeding of tissue engineered constructs for the urogenital system. For tissue engineering of the bladder, the addition of cells did not give an overall beneficial effect on tissue regeneration [27], while others claim that cells are required for urethra repair of constructs >0.5 cm [28]. For urethra tissue engineering, the inclusion of cells significantly reduced side effects in preclinical studies for both full ($p = 0.001$) and inlay ($p = 0.003$) defects. In other, less comprehensive systematic reviews, a similar outcome regarding the effectiveness of the addition of cells was shown [29,30]. For full defects, cell addition has more added value, which may be explained by the fact that cells can only infiltrate from the two urethra edges, while in inlay repair cell ingrowth can also occur from the sides, boosting cell coverage.

The effects of cell addition on functionality and study completion were not significant, regardless of surgical procedure. This may be caused by the short follow-up period underestimating long-term complications, such as complete strictures. Meta-analysis of clinical studies showed no significant effect of cells for any of the outcome measures. Consequently, the use of cells for the repair of urethra in the clinic remains debatable.

4.2. *Type of biomaterial*

Meta-analysis showed no differences in estimated probabilities for the different materials in most of the conditions, with the exception of synthetic materials showing better estimated probabilities than natural materials in full circumferential repair without cells regarding side effects. For inlay repair in preclinical studies, synthetic materials did not perform as well as in full repair, but only a limited number of studies was reported.

Decellularized materials were used in the vast majority of clinical studies. This may be related to the experience with decellularized materials in other fields of tissue engineering, such as skin tissue engineering [31]. Which type of biomaterial is superior to the current state of the art remains to be established.

4.3. *Selection of animal species*

The choice of animal species is often based on financial issues, experience of the researchers, ethical arguments and practical restrictions [32–34]. An evidence-based approach can aid in selection of the most appropriate model. In this review, differences between treatment were not notably influenced by the choice for rabbit or dog; however, a higher statistical power would strengthen this claim.

4.4. *Clinical relevance and limitations of preclinical and clinical studies*

Quality of the experimental designs and reporting of preclinical studies was generally low. Proper control groups, such as sham operation groups and standard treatment groups, were often lacking. Instead, the experimental material without cells was generally considered the control. In addition, outcome measures and drop-outs were not specifically reported for each animal, complicating data interpretation. Also, representativeness of presented data was often not mentioned. This may have hampered clinical translation of these preclinical findings. To improve this, all design parameters and outcomes should be specifically documented for individual animals similar to patients in clinical studies. The "Gold standard publication checklist to improve the quality of animal studies" by Hooijmans et al [35] would be helpful for the design and reporting of preclinical studies.

Another limitation for the level of evidence provided by the preclinical studies is the use of healthy animals, in which a created defect is immediately closed, compared to patients with a history of stricture, lichen sclerosis or hypospadias. From the patients in clinical studies 75% had

one or more previous treatments, for example, dilation, urethrotomy, or urethroplasty, before attempting the tissue engineered constructs. The requirement of animal models with injury or disease has been shown in other fields [36] and should also be considered in tissue engineering, in this particular situation by inducing strictures.

Clinical studies provided a low level of evidence due to their setup, making the true effect of tissue engineering as surrogate for the current standard treatment unclear. Only El-Kassaby et al (Table 2) performed a small randomized controlled study. To improve the level of evidence, more randomized controlled studies are needed, preferably with larger numbers of patients and longer follow-up. Compared with the preclinical studies, reporting of important parameters was much better, notably regarding drop-outs and adverse events. Nevertheless, to further improve the quality of the clinical studies, the study protocol should be published with the manuscript and a detailed description of patient inclusion criteria (eg, sex, age, and medical history) should be provided.

The level of evidence is further limited by original research's susceptibility to publication bias [37], which may lead to overestimation of the treatment effect in preclinical studies. Recognition of this bias may partly explain the poor translation of tissue engineering techniques to the clinic.

Furthermore, preclinical studies should better support the clinical need: the majority of preclinical studies involves full circumferential repair, where clinicians mainly perform inlay repair [3]. This may be explained by preclinical researchers attempting to prove the effectiveness of the experimental treatment for the most problematic (circumferential) procedures, assuming that it will also be effective in less complicated (inlay) approaches.

Finally, inclusion of cells remains challenging in a clinical setting as no beneficial effect was seen (in 11 patients), even though this significantly improved preclinical outcome. It is possible that inclusion of cells was perceived as too problematic, despite better results in a preclinical setting and that in the final assessment the choice was driven by parameters other than preclinical outcome. To consider cells for clinical applications, its efficacy has to be proven as the use of cells involves extensive regulatory requirements which may hamper clinical application [38–40]. In addition, the costs of cellular implants will be higher compared with off-the-shelf acellular implants, since two procedures are needed (cell harvesting in urine or biopsy, and urethroplasty) and in vitro cell expansion may be needed [41,42].

5. Conclusions

The efficacy of tissue engineering for urethra repair could not be determined due to a lack of controlled (pre)clinical studies. However, meta-analysis outcomes (side effects, functionality, and study completion) were comparable to current treatment options described in literature, indicating the potential of tissue engineering for urethra repair. The findings of this systematic review may result in improved study design which may aid the translation of tissue engineered urethras to the clinic as an alternative for autografts.

Author contributions: Willeke F. Daamen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Versteegden, de Jonge, van Kuppevelt, Oosterwijk, Feitz, de Vries, Daamen.
Acquisition of data: Versteegden, de Jonge.
Analysis and interpretation of data: Versteegden, de Jonge, IntHout, de Vries, Daamen.
Drafting of the manuscript: Versteegden, de Jonge.
Critical revision of the manuscript for important intellectual content: IntHout, van Kuppevelt, Oosterwijk, Feitz, de Vries, Daamen.
Statistical analysis: IntHout.
Obtaining funding: van Kuppevelt, Oosterwijk, Feitz, Daamen.
Administrative, technical, or material support: None.
Supervision: van Kuppevelt, Oosterwijk, Feitz, de Vries, Daamen.
Other: None.

Financial disclosures: Willeke F. Daamen certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: PIDON (NovioTissue Project), a combined subsidiary program of the Dutch Ministry of Economic Affairs, and the states of Gelderland and Overijssel (PID101020).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2017.03.026.

References

[1] Gallentine ML, Morey AF, Thompson Jr IM. Hypospadias: a contemporary epidemiologic assessment. Urology 2001;57:788–90.
[2] Pierik FH, Burdorf A, Nijman JM, et al. A high hypospadias rate in The Netherlands. Hum Reprod (Oxford England) 2002;17:1112–5.
[3] Mundy AR. Management of urethral strictures. Postgrad Med J 2006;82:489–93.
[4] Brouwers MM, Feitz WF, Roelofs LA, et al. Risk factors for hypospadias. Eur J Pediatr 2007;166:671–8.
[5] Santucci RA, Joyce GF, Wise M. Male urethral stricture disease. J Urol 2007;177:1667–74.
[6] Orabi H, Bouhout S, Morissette A, et al. Tissue engineering of urinary bladder and urethra: advances from bench to patients. TSWJ 2013;2013:154564.
[7] Lumen N, Oosterlinck W, Hoebeke P. Urethral reconstruction using buccal mucosa or penile skin grafts: systematic review and meta-analysis. Urol Int 2012;89:387–94.
[8] Burger RA, Muller SC, el-Damanhoury H, et al. The buccal mucosal graft for urethral reconstruction: a preliminary report. J Urol 1992;147:662–4.
[9] Barbagli G, Selli C, di Cello V, Mottola A. A one-stage dorsal free-graft urethroplasty for bulbar urethral strictures. Br J Urol 1996;78:929–32.
[10] Dublin N, Stewart LH. Oral complications after buccal mucosal graft harvest for urethroplasty. BJU Int 2004;94:867–9.

[11] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
[12] Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. Nat Mater 2009;8:457–70.
[13] Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008;60:184–98.
[14] Freed LE, Vunjak-Novakovic G, Biron RJ, et al. Biodegradable polymer scaffolds for tissue engineering. Biotechnology NY 1994;12:689–93.
[15] Guo S, Dipietro LA. Factors affecting wound healing. J Dental Res 2010;89:219–29.
[16] Bianco P, Robey PG. Stem cells in tissue engineering. Nature 2001;414:118–21.
[17] Sloff M, de Vries R, Geutjes P, et al. Tissue engineering in animal models for urinary diversion: a systematic review. PLoS One 2014;9:e98734. http://dx.doi.org/10.1371/journal.pone.0105484.
[18] Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. Lab Anim 2010;44:170–5.
[19] de Vries RB, Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. A search filter for increasing the retrieval of animal studies in Embase. Lab Anim 2011;45:268–70.
[20] Akaike H. Akaike's Information Criterion. In: Lovric M, editor. International Encyclopedia of Statistical Science. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 25.
[21] Zou GY. Assessment of risks by predicting counterfactuals. Stat Med 2009;28:3761–81.
[22] Barbagli G, Sansalone S, Djinovic R, Romano G, Lazzeri M. Current controversies in reconstructive surgery of the anterior urethra: a clinical overview. Int Braz J Urol 2012;38:307–16, discussion 16.
[23] Mundy AR, Andrich DE. Urethral strictures. BJU Int 2011;107:6–26.
[24] Subramaniam R, Spinoit AF, Hoebeke P. Hypospadias repair: an overview of the actual techniques. Semin Plast Surg 2011;25:206–12.
[25] Singal AG, Higgins PD, Waljee AK. A primer on effectiveness and efficacy trials. Clin Transl Gastroenterol 2014;5:e45. http://dx.doi.org/10.1038/ctg.2013.13.
[26] Faure A, Bouty A, Nyo YL, O'Brien M, Heloury Y. Two-stage graft urethroplasty for proximal and complicated hypospadias in children: a retrospective study. J Pediatr Urol 2016;12:286.
[27] Sloff M, Simaioforidis V, de Vries R, Oosterwijk E, Feitz W. Tissue engineering of the bladder–reality or myth?. A systematic review. J Urol 2014;192:1035–42.
[28] Dorin RP, Pohl HG, De Filippo RE, Yoo JJ, Atala A. Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration? World J Urol 2008;26:323–6.
[29] Qi N, Li WJ, Tian H. A systematic review of animal and clinical studies on the use of scaffolds for urethral repair. J Huazhong Univ Sci Technol Med Sci 2016;36:111–7.
[30] Xue JD, Gao J, Fu Q, Feng C, Xie H. Seeding cell approach for tissue-engineered urethral reconstruction in animal study: a systematic review and meta-analysis. Exp Biol Med 2016;241:1416–28.
[31] Debels H, Hamdi M, Abberton K, Morrison W. Dermal matrices and bioengineered skin substitutes: a critical review of current options. Plast Reconstr Surg Glob Open 2015;3:e284. http://dx.doi.org/10.1097/GOX.0000000000000219.
[32] de Vries RB, Buma P, Leenaars M, Ritskes-Hoitinga M, Gordijn B. Reducing the number of laboratory animals used in tissue engineering research by restricting the variety of animal models. Articular cartilage tissue engineering as a case study. Tissue Eng Part B Rev 2012;18:427–35.
[33] de Vries RB, Wever KE, Avey MT, et al. The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. ILAR J 2014;55:427–37.
[34] Oerlemans AJ, Feitz WF, van Leeuwen E, Dekkers WJ. Regenerative urology clinical trials: an ethical assessment of road blocks and solutions. Tissue Engin Part B Rev 2013;19:41–7.
[35] Hooijmans CR, Leenaars M, Ritskes-Hoitinga M. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. Altern Lab Anim 2010;38:167–82.
[36] Dixit R, Boelsterli UA. Healthy animals and animal models of human disease(s) in safety assessment of human pharmaceuticals, including therapeutic antibodies. Drug Discov Today 2007;12:336–42.
[37] Korevaar DA, Hooft L, ter Riet G. Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments. Lab Anim 2011;45:225–30.
[38] Schneider CK, Salmikangas P, Jilma B, et al. Challenges with advanced therapy medicinal products and how to meet them. Nat Rev Drug Discov 2010;9:195–201.
[39] EMA. Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. 2007.
[40] Brevignon-Dodin L, Singh P. ATMP in practice: towards a new industry landscape in tissue engineering. J Commerc Biotechnol 2009;15:59–65.
[41] Culme-Seymour EJ, Mason K, Vallejo-Torres L, et al. Cost of stem cell-based tissue-engineered airway transplants in the United Kingdom: case series. Tissue Eng Part A 2016;22:208–13.
[42] Zhang Y, McNeill E, Tian H, et al. Urine derived cells are a potential source for urological tissue reconstruction. J Urol 2008;180:2226–33.