A Systematic Review of Animal and Clinical Studies on the Use of Scaffolds for Urethral Repair

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Summary: Replacing urethral tissue with functional scaffolds has been one of the challenging problems in the field of urethra reconstruction or repair over the last several decades. Various scaffold materials have been used in animal studies, but clinical studies on use of scaffolds for urethral repair are scarce. The aim of this study was to review recent animal and clinical studies on the use of different scaffolds for urethral repair, and to evaluate these scaffolds based on the evidence from these studies. PubMed and OVID databases were searched to identify relevant studies, in conjunction with further manual search. Studies that met the inclusion criteria were systematically evaluated. Of 555 identified studies, 38 were included for analysis. It was found that in both animal and clinical studies, scaffolds seeded with cells were used for repair of large segmental defects of the urethra, such as in tubular urethroplasty. When the defect area was small, cell-free scaffolds were more likely to be applied. A lot of pre-clinical and limited clinical evidence showed that natural or artificial materials could be used as scaffolds for urethral repair. Urinary tissue engineering is still in the immature stage, and the safety, efficacy, cost-effectiveness of the scaffolds are needed for further study.
Key words: material/scaffold; urethral repair; tissue engineering/regenerative medicine; animal models; clinical studies

Recently, patients suffering from urethral stricture and hypospadias are treated with transplanted tissue or cells. A variety of transplanted tissues have been used for urethral repair, including foreskin, buccal mucosa, tunica vaginalis, bladder mucosa, and peritoneum, etc[1]. However, serious complications often occur along with the use of the graft tissue, such as inflammatory stenosis and urethral fistula. Promising materials have always been desired for urethral reconstruction. Tissue engineering, one of the major components of regenerative medicine, consists of cell and scaffold transplantation, towards the development of biological substitutes that can restore and maintain normal function of tissues[2]. This strategy avoids graft rejection and long-term use of medications usually needed after allogeneic transplantation[3].

Current scaffold researches have focused on the use of natural and artificial biodegradable materials with minimal immunogenicity and toxicity, including extracellular tissue matrix as well as synthetic polymers. Biomaterials are decellularized tissue, which can provide the perfect scaffold environment, and retain the major structural and tensile properties of the native tissue[4]. At present, material scientists have the ability to develop biocompatible scaffolds with various physical parameters, combining high porosity with mechanical integrity to promote cell infiltration and angiogenesis[5]. Synthetic materials are designed to provide a three-dimensional structure with appropriate mechanical strength that can mimic the native extracellular matrix, promoting cellular attachment, proliferation, and migration[6, 7]. These scaffolds degrade slowly *in vivo* and are gradually replaced by the extracellular matrix proteins that are secreted by the ingrown cells[8].

Most materials have been used for urethral repair in animal studies. There are two different approaches of application of scaffolds *in vivo*: use of scaffolds *per se*, and cell-seeded scaffolds. Scaffolds used for urethral repair are expected to have excellent biocompatibility, absorbability and biodegradability. The autologous cells and all kinds of stem cells seeded on scaffolds offer options for reconstructive surgery. Search for ideal urethral substitutes, however, has still been a great challenge in the field of urethral repair.

Evidence from animal studies can provide clues for further applications in clinical trials. It is necessary to conduct a systematic review to compare the results from animal studies with those of the clinical trials[9, 10]. In this study, a systematic review including animal and clinical studies was performed to evaluate scaffolds reported in the currently available studies that are appropriate for urethral repair.

1 MATERIALS AND METHODS

1.1 Search

All articles published up to June 20, 2015 were searched in PubMed and OVID databases, with the following medical subject headings (MESH) used: "tissue engineering", "regenerative medicine", "urethra", and "scaffold". Manual search was further conducted for relevant articles.

1.2 Selection Criteria

Studies were identified by two authors, independently, and inconsistency on the inclusion of studies was solved under discussion. The studies, meeting the fol-

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lowing criteria, were included: (1) studies assessing the effect of the scaffold on animals or patients; (2) animals randomly assigned in the studies; (3) *in vivo* studies of scaffolds performed in animals; (4) the original studies (reviews and duplicate publications excluded) with language limited to English and Chinese.

1.3 Data Extraction

From the included studies, the following information was extracted: (1) Animal models: first author; year of publication; animals for modeling; cell types; biomaterial/artificial tissue; surgical procedures; duration of observation; repaired length; negative/positive rate. (2) Clinical studies: first author; year of publication; number of patients; cell types; biomaterial/artificial tissue; urethral pathology; follow-up; repaired length; negative/positive rate.

1.4 Outcome Variables and Analysis

In the current review, studies in which no urethral stricture or fistula occurred were considered to have positive results and vice versa. In clinical studies, urethra with no need for further invasive or surgical interventions was regarded as success. Because of the obvious methodological and clinical heterogeneity, meta-analysis was considered inappropriate for this system review.

2 RESULTS

2.1 Description of Included Studies

A total of 555 studied were identified. On the basis of predefined criteria, 517 studies were excluded, with various reasons for exclusion listed in fig. 1. Thirty-eight studies were eventually included for the systematic review. Of the 38 studies, 27 were about the scaffolds used in animal models (no control groups in four studies) and 11 about the scaffolds used for patients (one case-control study only). A summary of the 38 included studies is given in tables 1 and 2.

The 38 studies investigated the efficacy of transplantation of material/scaffold to animals or patients for urethral repair. Different types of scaffolds were used in these studies, including small intestine submucosa (SIS), bladder acellular matrix (BAM), urethral acellular matrix (UAM), acellular aortic matrix, silk fibroin matrix (SFM) and synthetic polymer. Studies fell into two categories, namely biomaterials and artificial materials.

2.2 Animal Models

2.2.1 Biomaterials SIS was used in five studies. Nuininga[11] reported that one-layer SIS was more suitable for urethral repair than four-layer SIS. The regeneration time of urothelium in the one-layer SIS group was less than in the four-layer SIS group. But Kawano[12] observed that four-layer SIS was more advantageous than one-layer SIS and buccal mucosa for onlay urethral repair. Despite the almost same incidence of stenosis in each group, four-layer SIS group showed higher levels of collagen Ⅲ/Ⅰ, which could lead to less fibrosis. Kropp[13] showed that SIS onlay grafts promoted the regeneration of the normal epithelium. However, according to Chung's study[14], SIS was found to cause chronic inflammatory reactions in comparison to other substitutes (such as silk fibroin matrix) within three months. El-Assmy[15] found cell-free SIS used in tubular urethroplasty tended to develop urethral fistulae or strictures. Bundles of smooth muscle were replaced by abundant collagen connective tissue in the nonseeded tubularized SIS in his study.

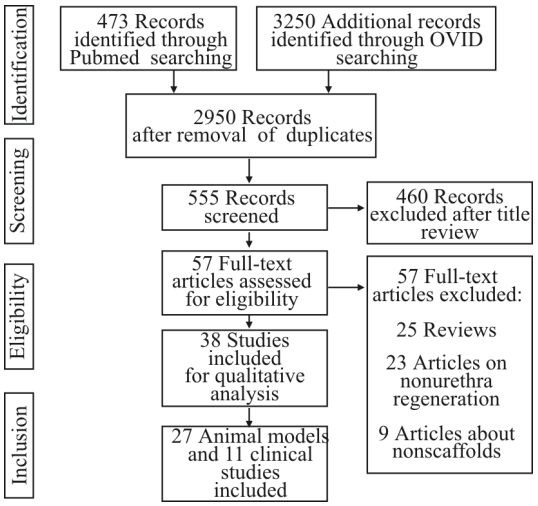

Fig. 1 Flow chart of article selection in terms of the PRISMA Statement

BAM was used in 11 studies, and different kinds of cells were seeded on the scaffolds for urethra reconstruction. Cell-seeded BAM was reported to perform better in animal studies[16–21]. Chen *et al*[22] discovered that cell-free BAM in onlay urethroplasty could maintain a wide urethral caliber without any signs of strictures. However, other authors[23, 24] found that cell-free BAM could develop strictures in onlay urethral repair. According to Huang's study[25], peracetic acid (PAA)-treated nonseeded BAM promoted urinary tract cells regeneration and neovascularization compared with the unoptimized BAM. Chun[26] reported satisfactory results by combining cell-free BAM with autologous urethral tissue. The cells from autologous urethral tissue promoted the regeneration of urothelium and smooth muscle cells. Uroepithelial cells, smooth muscle cells, mesothelial cells, bone marrow mesenchymal stem cells, epithelial-differentiated rabbit adipose-derived stem cells, oral keratinocytes and fibroblasts were seeded on BAM in these studies[16–21]. The tubularized BAM seeded with autologous cells formed new tissue, which was histologically similar to native urethra[16–21]. Nevertheless, all animals treated with cell-free BAM by tubular urethroplasty developed stricture.

Sievert *et al* reported no significant difference in urethral function between heterologous and homologous UAM for urethral repair. Urethroplasties were undertaken with tubularized UAM without cells, with no fistula developed[27, 28]. However, Shokeir[29] revealed different outcomes. Their results showed that all animals treated with tubularized UAM developed urethral fistula or stricture, which worsened, even leading to urine retention. Han[30] reported UAM enhanced with smooth muscle cells promoted the regeneration of cells for onlay urethral repair.

Parnigotto[31] took advantage of tubularized cell-seeded acellular aortic matrix to repair urethra. Three of 14 animals developed fistula or died in their study. Wang[32] found denuded human amniotic scaffold could be used as a substitute in urethral reconstruction. Cell-seeded denuded human amniotic scaffold could

minimize potential rejection and maximize the biocompatibility of amniotic membrane, suggesting that denuded human amniotic scaffold can be used as a potential ideal substitute.

2.2.2 Artificial Materials Xie[33, 34] used dogs as animal models and found that cell-seeded SFM used as a urethral substitute didn't give rise to strictures, ulceration and fistula, but cell-free SFM could cause urethra strictures to varying degrees. However, Liu[35] and Chung[14] reported SFM without seeded cells did not cause urethra strictures in rabbits for urethral repair. According to their studies, the implanted SFM for defect repair was degraded completely at last and was replaced by smooth muscle cells and urothelial cells.

Collagen-Sponge Tubes reinforced with Copoly (L-Lactide/e-Caprolactone) Fabric (PLA/PLC-I) was reported to be an urethral substitute by Kanatani[36]. PLA/PLC-I waved in a vascular stent style could cause slightly fibrosic but completely epithelialized and regenerated smooth muscle layer. High-density collagen gel tubes (hdCGT) was attempted to be a scaffold by Micol[37]. The tissues were histologically similar to normal urethra 3 months after cell-seeded hdCGT implantation. This surgical procedure might be useful as an effective treatment of congenital and acquired urethral pathologies.

Table 1 Biomaterials and artificial materials applied in animal models

First author	Year of publication	Animals for modeling	Cell types	Biomaterial/ artificial tissue	Surgical procedures	Duration of observation	Repaired length	Negative/positive rate
Kropp[13]	1998	Rabbit	NA	Porcine SIS	OU	2–3 months	4 cm	0/8
Nuininga[11]	2003	Rabbit	NA	SIS	OU	1–9 months	0.5–1.0 cm	0/6 (1-layer SIS) 1/5 (4-layer SIS)
El-Assmy[15]	2004	Rabbit	NA	SIS	OU and TU	3–12 weeks	1.5 cm	3/6 (OU) 9/0 (TU)
Kawano[12]	2012	Rabbit	NA	Porcine SIS	OU	12 weeks	1.0 cm	1/11 (1-layer SIS) 1/11(4-layer SIS)
Chung[14]	2014	Rabbit	NA	SFM Rabbit SIS	OU	3 months	2.0 cm	0/4 (SFM) 0/4 (rabbit SIS)
Chen[22]	1999	Rabbit	NA	Porcine BAM	OU	0.5–6 months	1.0 cm	0/10
Fu[16]	2007	Rabbit	UC	Rabbit BAM	TU	1–6 months	1.5 cm	0/9 (cell-seeded) 9/0 (no cell-seeded)
Fu[17]	2008	Rabbit	UC	Rabbit BAM	TU	12 months	0.8 cm	0/3
De Filippo[18]	2012	Rabbit	UC and SMC	Porcine BAM	TU	1–6 months	3.0 cm	0/9 (cell-seeded) 6/0 (no cell-seeded)
Gu[19]	2012	Rabbit	MC	Rabbit BAM	TU	1–6 months	1.5 cm	0/9 (cell-seeded) 9/0 (no cell-seeded)
Orabi[20]	2013	Dog	UC and SMC	BAM	TU	1–12 months	6.0 cm	0/15 (cell-seeded) 6/0 (no cell-seeded)
Li[21]	2013	Rabbit	BMSC and SMC	Rabbit BAM	TU	2 weeks–4 months	3.0 cm	0/24 (cell-seeded) 6/0 (no cell-seeded)
Li[24]	2013	Rabbit	OKC and TGF-β1 siRNA transfected fibroblasts	Rabbit BAM	OU	1–6 months	2.0 cm	0/18 (cell-seeded) 9/0 (no cell-seeded)
Li[23]	2014	Rabbit	Epith-rASCs	Rabbit BAM	OU	2 weeks–6 months	2.0 cm	0/12 (Epith-rASCs seeded) 12/0 (Und-rASCs seeded) 12/0 (no cell-seeded)
Huang[25]	2014	Rabbit	NA	Porcine BAM	OU	1–3 months	1.5 cm	2/13 (PAA-treated) 5/10 (none PAA-treated)
Chun[26]	2015	Rabbit	NA	BAM and autologous urethral tissue	OU	4, 8 and 12 weeks	2.0 cm	0/10

To be continued

Sievert[27]	2000	Rabbit	NA	Rabbit UAM	TU	10 days–12 months	1.0–1.5 cm	4/26
Sievert[28]	2001	Rabbit	NA	Rabbit UAM Canine UAM	TU	6–8 months	1.0–1.5 cm	0/14
Shokeir[29]	2004	Dog	NA	UAM	TU	1 week– 3 months	3.0 cm	14/0
Han[30]	2009	Rabbit	SMC	UAM	OU	2–12 weeks	2.0 cm	0/12
Parnigotto[31]	2000	Rabbit	UC	Rabbit acellular aortic matrix	TU	10 days–12 months	1.0 cm	0/14
Wang[32]	2014	Rabbit	UC	dHAS AM	OU	2 weeks–3 months	1.0 cm	2/4 (AM) 0/6 (dHAS)
Liu[35]	2007	Rabbit	NA	SFM	OU	2–16 weeks	1.5 cm	0/12
Xie[33]	2013	Dog	UC	SFM	OU	1–6 months	3.0 cm	0/9
Xie[34]	2014	Dog	OKC and fibroblasts	SFM	OU	6 months	5.0 cm	0/5 (cell-seeded) 5/0 (no cell-seeded)
Kanatani[36]	2007	Rabbit	NA	PLA/PLC- I	TU	1–6 months	1.5 cm	0/14 (vascular stent) 14/0 (urethral tube)
Micol[37]	2012	Rabbit	SMC	hdCGT	TU	1–3 months	2.0 cm	4/4 (cell-seeded) 6/2 (no cell-seeded)

SIS: small intestine submucosa; BAM: bladder acellular matrix; UAM: urethral acellular matrix; SFM: silk fibroin matrix; PLA/PLC-I: collagen-sponge tubes reinforced with copoly (L-lactide/e-caprolactone) fabric; hdCGTs: high-density collagen gel tubes; dHAS: denuded human amniotic scaffold; AM: amniotic membrane; UCs: uroepithelial cells; SMCs: smooth muscle cells; MCs: mesothelial cells; BMSCs: bone marrow mesenchymal stem cells; Epith-rASCs: epithelial-differentiated rabbit adipose-derived stem cells; OKCs: oral keratinocytes; OU: onlay urethroplasty; TU: tubular urethroplasty; NA: not available

2.3 Clinical Studies

2.3.1 Biomaterials Porcine SIS was used in five studies[38–42]. Mantovani[38] reported one patient who underwent 12 transurethral resections (TUR) for recurrence of superficial bladder tumor. Follow-up for 16 months revealed satisfactory urodynamic and subjective outcomes in the patient after treatment with SIS. The SIS graft promoted the generation of surrounding tissue in the repaired region. Nine patients with bulbar urethral strictures were treated by endoscopic urethroplasty reported by le Roux[39]. After urethroplasty, two patients maintained urethral patency without any intervention in two years; six patients developed strictures within 3 months and one patient was lost of follow-up. Hauser[40] reported that five patients underwent four-layer SIS grafting, and among them, four patients had a recurrent stricture and one patient developed bladder stones. Fiala[41] *et al* treated 50 patients with implantable SIS and the outcomes of 40 patients were satisfactory. Bulbar strictures (1/10), bulbopenile strictures (5/31) and penile strictures (4/9) occurred within 6 months post surgery. Palminteri[42] *et al* treated 20 patients with implantable SIS and the outcomes of 17 patients were satisfactory. Re-stricture developed in penile urethra (1/1) and penile-bulbar urethra (2/3). When the first try with SIS graft was successful in clinical trails, some surgeons began using SIS as a substitute for urethral repair. The success rates were different among these studies[38–42].

BAM was used in three studies[43–45]. Atala[43] treated four patients with hypospadias who needed repeated repairs with human BAM in 1999. Repaired length ranged from 5 to 15 cm. After repairing, four patients had normal function without any evidence of narrowing. el-Kassaby[44] reported 28 patients who received human BAM graft and 24 patients had successful outcomes. The rest four patients had a slight caliber decrease at the anastomotic site after urethrography and one of them developed subcoronal fistula. The four patients had to receive second operation. In el-Kassaby's study[45], 15 patients were subjected to BAM grafting. Eight out of nine patients had a healthy urethral bed (88.9%), and two out of six patients had an unhealthy urethral bed (33.3%). These studies suggested that in the absence of sufficient genital skin or mucosa, use of BAM is a feasible method for urethral repair, and it is more suitable for patients with healthy urethral bed.

Bhargava[46] implanted oral keratinocytes and fibroblasts-seeded deepidermized dermis (DED) to five patients. Two of them developed fibrosis and contraction after surgery. After intervention, four patients achieved satisfying outcomes. Fossum[47] used human urothelium (UC)-seeded acellular dermis as the transplant to repair urethra. Five of six patients succeeded at first attempt and an additional procedure (urethrotomy) was needed for the remaining patient. The use of the cell-seeded dermis provided new option for urethral repair, but more evidence is needed for its widespread application.

2.3.2 Artificial Materials Polyglycolic acid/poly (lactide-co-glycolide acid) (PGA/PLGA) meshes as a kind of synthetic material, gradually draw attentions of research scholars. Raya-Rivera[48] treated 5 patients with cell-seeded PGA/PLGA in their study for urethral repair. In this study, one patient needed a second operation, and the rest 4 patients had a wide urethral caliber without diverticula at 12th month after surgery. PGA/PLGA could be a potential scaffold used for urethral repair in the future.

Table 2 Biomaterials and artificial materials applied in clinical studies

First author	Year of publication	Patients (*n*)	Cell types	Biomaterial/ artificial tissue	Urethral pathology	Follow-up (months）	Repaired length	Negative/ positive rate
Atala[43]	1999	4	NA	Human BAM	Repeat hypospadias repair	22	5–15 cm	1/3
El-Kassaby[44]	2003	28	NA	Human BAM	Anterior strictures	36–48	1.5–16 cm	4/24
el-Kassaby[45]	2008	15	NA	Human BAM	Bulbar, pendulous and combined bulbopendulous strictures	18–36	2–18 cm	5/10
Mantovani[38]	2003	1	NA	Porcine SIS	Urethral stricture	16	Not reported	0/1
le Roux[39]	2005	9	NA	Porcine SIS	Bulbar strictures	12–24	1–4 cm	6/2 (one patient lost during follow-up)
Hauser[40]	2006	5	NA	Porcine SIS	Bulbar strictures and combined penile-bulbar strictures	3.7–12.7	3.5–10 cm	5/0
Fiala[41]	2007	50	NA	Porcine SIS	Bulbar, bulbopenile and distal penile strictures	24–36	4–14 cm	10/40
Palminteri[42]	2007	20	NA	Porcine SIS	Penile, bulbar and penile-bulbar strictures	13–15	4–10 cm	3/17
Bhargava[46]	2008	5	OKC and FC	DED	Urethral stricture secondary to lichen sclerosus (LS)	32–37	4.5 cm–entire uretha	2/3
Fossum[47]	2012	6	Human UC	Acellular dermis	Scrotal and perineal hypospadia	72–103	Not reported	1/5
Raya-Rivera[48]	2011	5	Human UC and SMC	PGA/PLCA	Complete posterior urethral disruption (3/5), failed previous posterior urethral repair (2/5)	36–76	4–6 cm	1/4

NA: not available; BAM: bladder acellular matrix; SIS: small intestinal submucosa; PGA/PLCA: polyglycolic acid/poly (lactide-co-glycolide acid) meshes; DED: deepidermized dermis; OKCs: oral keratinocytes; FCs: fibroblasts; UCs: uroepithelial cells; SMCs: smooth muscle cells

3 DISCUSSION

SIS onlay grafts used in animal studies were demonstrated to promote the regeneration of normal epithelia and smooth muscle cells[11–15]. But SIS tended to elicit chronic inflammatory reactions in comparison to other substitutes (such as SFM)[14]. Tubular urethroplasty with SIS in animal models were reported to be undesirable. Some clinical studies[38, 41, 42] showed that SIS grafts could be used as substitutes for urethral repair. However, other studies[39, 40] did not support the application of SIS grafts for urethral repair. The discrepancy may result from the different surgical approaches used and different urethral pathology between animals and humans. More evidence needs to be found before SIS is widely used.

BAM appears to be an excellent biomaterial for urethral repair, since it can be safely obtained and processed easily. No matter what kinds of cells were applied to the scaffolds, cell-seeded BAM performed better than cell-free BAM in most animal models. Cell-free BAM in some studies also generated good results. The possible reasons for the difference may result from the fact that long segmental patch was required in some studies, which limited the growth of cells around the anastomotic sites. Clinical studies[43–45] showed that use of BAM was a feasible method for urethral repair, and it was more suitable for patients with healthy urethral bed when compared with those with unhealthy urethral bed. The first attempt by using other tubular biomaterials achieved perfect results in the animal urethral repair[46–48]. Cell-seeded scaffolds are a better selection than scaffolds only.

In this review, we observed that, in both animal and clinical studies, scaffolds seeded with cells were more suitable for treatment of large segmental urethral defects, such as in tubular urethroplasty. When the repaired area was small, cell-free scaffold could also be applied. As synthetic scaffolds with optimum surface porosity and chemistry could promote cellular attachment, proliferation and differentiation, they are the potential substitute in the future[49]. Although these studies[32–37, 46–48] showed encouraging results, further improvements are still needed for finding out ideal scaffolds.

There were several limitations in this review. First, a publication bias may exist because only published literatures were selected. Second, there was a language bias in this study as languages were limited to English and Chinese only in this review.

Currently, surgery is still the treatment of choice for urethral injury caused by stricture, infection or hypospadias. Although an end-to-end anastomosis resection of the diseased tissue is feasible for short strictures, longer segmental defects necessitate use of additional tissue for repair. Autologous tissues from genitals, skin

flaps or buccal mucosa usually give rise to complications. Nondegradable grafts have often been associated with erosion, fistula, stenosis and so on[45]. Tissue engineering substitutes hold promises to resolve the difficulties and offer new options for reconstructive surgery. Biomaterials and artificial materials are good alternatives when classical urethroplasty approaches are proved fail in long and complex strictures. Biological and synthetic materials have been successfully applied in animal and clinical studies, but more basic researches are still needed before the widespread application in clinical practice. The use of these scaffolds to optimize graft material makes it possible to combine the most refined surgical techniques with the best graft material, to achieve even more reliable results[50]. Urinary tissue engineering is still in the immature stage, and the safety, efficacy and cost-effectiveness of the materials also need further study.

Conflict of Interest Statement

The authors declared no potential conflicts of interest.

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