Towards clinical application of tissue engineering for erectile penile regeneration

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Competing interests

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Key Points

- Reconstruction of the erectile tissue has a variety of indications, including trauma, surgical amputation, gender affirmation, and Peyronie's disease.
- Current surgical treatments for penile reconstruction and erectile dysfunction are associated with high complication rates, emphasizing the need for a tissue engineered approach
- The goals of penile tissue engineering are to cosmetically correct volume deficits, to accomplish urinary voiding, and to achieve erectile function.
- To date most studies investigating tissue engineering for erectile penile regeneration are preclinical trials in animal models.
- Contemporary tissue engineering strategies enable the seeding of a biomaterial scaffold and implantation to construct a neocorpus; the use of induced-pluripotent stem cells and 3D-bioprinted scaffolds can provide a patient-specific approach to penile tissue engineering.
- The use of gene therapy in erectile tissues has only been successfully performed in one human clinical trial; however, it has the potential to enhance neurogenic and vasculogenic regeneration, increasing blood flow, and increasing growth factor delivery to the corpus cavernosum.

Abstract | Penile wounds after traumatic and surgical amputation require reconstruction in the form of

autologous tissue transfers. However, currently used techniques are associated with high infection rates, implant erosion and donor site morbidity. The use of tissue engineered neocorpora provides an alternative treatment option. Contemporary tissue engineering strategies enable the seeding of a biomaterial scaffold and subsequent implantation to construct a neocorpus. Tissue engineering of penile tissue should focus on two main strategies: first, correcting the volume deficit for structural integrity in order to enable urinary voiding in the standing position and second, achieving erectile function for sexual activity. The functional outcomes of the neocorpus can be addressed by optimizing the use of stem cells and scaffolds, or alternatively, the use of gene therapy. Current research in penile tissue engineering is largely restricted to rodent and rabbit models, but the use of larger animal models should be considered as a better representation of the anatomical and physiological function in humans. The development of a cell-seeded scaffold to achieve and maintain erection continues to be a considerable challenge in humans. However, advances in penile tissue engineering show great promise and, in combination with gene therapy and surgical techniques, have the potential to substantially improve patient outcomes.

[H1]Introduction

Penile reconstruction can be required for several clinical indications, including congenital anomalies, malignancy, gender affirmation and trauma. Penile tissue defects can have a profound impact on urinary and sexual function [1]. Penile defects can have a considerable consequence on psychosexual health as the phallus is an symbol of masculinity, both anatomically and culturally[2]. Several surgical techniques have been described for phallic reconstruction, but outcomes are limited by the lack of a suitable substitute for the erectile tissue within the corpus cavernosum[3–6]. Moreover, the tissue structure and function of the corpora and the overlying tunica albuginea, make reconstruction of physiological erectile tissue challenging[7–9].

The human penis is a complex structure. A fascial layer, the tunica albuginea surrounds three cylinders of erectile tissue: two corpora cavernosa and one corpus spongiosum[10]. Despite the fact that many studies investigating penile regeneration have been performed in rodents, the murine penis differs from the human penis considerably (**Figure 1**).

The erectile process is mediated by increased arterial blood flow and reduced venous outflow[11] — relaxation of the cavernous smooth musculature results in arteriolar dilation, which increases blood flow. The tunica albuginea compresses the subtunical venular plexus reducing venous outflow and, therefore, increasing intracorporal pressure and further expansion of the tunica occludes the emissary veins between the inner circular and outer longitudinal layers further decreasing venous outflow[11]. Thus, sequestration of blood within the penile corpora results in penile erection. The complex structure of the human penis makes any form of reconstruction challenging and is the primary reason why the outcomes of current procedures are limited and why alternative approaches have been delayed.

Smooth muscle cells and endothelial cells are the primary cells of the corpus cavernosum and have an important role in maintaining and regulating penile function[12]. The cavernous spaces are irregular, tortuous and lined by endothelial cells resting on a basal lamina[13]. The predominant tissues between the cavernous vascular spaces are connective tissue and bundles of smooth muscle cells[14], composed of 40.7% collagen, 40.4% smooth muscle and 12.8% elastic fibres[15]. The erectile tissues of the human penis are surrounded by the collagenous tunica albuginea. The cellularity of the tunica albuginea is much lower than that of the corpora[16], which is an important consideration for penile reconstruction, particularly with regards to the choice of scaffold materials and cellular seeding.

Current penile reconstruction strategies are multistage procedures involving tissue transfer with or without the use of an inflatable penile prosthesis[17]. Penile reconstruction can provide functional, aesthetic and psychological benefits to patients. At present, reporting of outcomes and complications is not standardised in the literature, making clinical decisions based on evidence difficult[17]. However, the radial forearm free flap technique is the current gold standard for phalloplasty, despite considerable risks associated with this surgery, particularly associated with the use of a penile prosthesis[17]. The risks associated with penile prosthesis include infection (1.7–15.0% of patients)[18,19], erosion (1.0–8.1% of patients)[20,21], mechanical dysfunction (1.4–13.7% of patients)[18,19] and revision surgery (41.0% of patients)[20]. These complications are further increased in patients undergoing phalloplasty using free or pedicle flaps owing to the absence of the tunica albuginea, which protects from trauma and prevents erosion of the prosthesis [22]. Ultimately, none of these reconstructive procedures are able to adequately restore de novo erectile tissue function[7].

Human penile transplantation is an emerging technique to treat penile disfigurement[23–25]. In 2017, van der Merwe and colleagues described the 24-month follow-up outcomes of a successful penile transplantation, requiring lifelong immunosuppression[23]. The procedure resulted in a viable graft, spontaneous voiding and natural erections; however, the patient also developed a urinary fistula and experienced episodes of graft rejection[26]. Also in 2017, Cetrulo and colleagues reported the first US penis transplantation and described similar complications; the patient ultimately required two re-operations for a haematoma evacuation and skin debridement, but after 7 months reported recovered partial sensation of the penile shaft, spontaneous penile tumescence, and dramatic improvement of self-image[25] . Although penile transplantation seems to be a successful procedure[27], the patients will require lifelong immunosuppression to treat a non-life-threatening disease, which results in an increased susceptibility to opportunistic infections and malignancy [28]. In addition penile transplantation has also been associated with poor outcomes requiring surgical removal owing to psychological concerns due to the cosmetic appearance of transplanted penis secondary to segmental epidermal necrosis [24] . These data emphasize the need for a tissue-engineered alterative to transplantation, which is not associated with the potential effects of high-dose immunosuppression.

In the past, penile reconstruction has generally been considered a surgical endeavour — phalloplasty using local or distant tissues are used to construct a composite phallus[9]. Local flaps are most often harvested from the suprapubic or groin region or from anterolateral thigh pedicled flaps. Free flaps often arise from latissimus dorsi, osteocutaneous fibula, or radial forearm free flap. Phalloplasty is associated with urethral stricture and fistula, as well as the need for prosthesis to obtain an erection[9,29]. Tissue engineering to re-establish the anatomy and function of the corpora cavernosa and tunica albuginea is key to addressing these challenges. The process of tissue engineering neocorpora involves cellular harvesting, cellular expansion and scaffold seeding, and implantation. Aternatively a cell-free construct can be used. Unseeded scaffolds release growth factors to recruit endogenous stem cells and alter the phenotype through chemotaxic signals to differentiate into fibroblasts, smooth muscle cells and endothelial cells (Fig. 2). Tissue engineering of the corpora cavernosa should focus on three main goals: first, correcting volume deficit to ensure penile length to enable urinary voiding in the standing position; second, achieving erectile function for sexual activity; and third, cosmesis.

This Review summarizes the evolution of penile tissue engineering with a focus on the corpus cavernosum and tunica albuginea, highlighting potential developments and persistent limitations for regenerating penile tissue.

[H1] Animal models for penile regeneration

Several mammalian models are used to study tissue engineering in corpus cavernosum and tunica albuginea regeneration, most frequently rabbits, rats and mice[30–39]. Animal models are useful for penile regeneration studies; however, ethical concerns highlight that animal suffering should be replaced with in-vitro analysis and computational modelling when possible[40] .

Small mammals such as rodents are frequently used in penile tissue-engineering studies owing to their low cost and ease of handling. However, small animal models have several limitations. First, the size differences between humans (Fig. 1a) and rodents (Fig. 1b) limit the usefulness of cellular density and ICP outcomes when translating to a human study. Secondly, aesthetic outcomes of penile regeneration in rodents have not been reported in the current literature, which is a major limitation of the current literature. Finally, the composition of rodent corpus cavernosa is different from humans, as rodent corpora contain less SMC tissue and more collagen than human corpus cavernosa, which means that rodent erectile function is less reliant on dilatation of arterioles and arteries increasing blood flow[41] .

A large animal model, such as monkey or dog, which have phallic anatomy that more closely aligns with that of a human[42] should be studied to improve clinical translation; with emphasis on aesthetic outcomes and maintaining intracorporal pressure in a volume similar to that of humans[43]. Extensive research into the mechanical properties and stability of a biomaterial should be completed before scaffolds are tested in animal models, and bioreactors should be used to simulate the mechanical processes of the biological system before implantation[44]. These optimisation steps would enable reduction and refinement of animal testing by improving experimental techniques and enabling advances in penile tissue engineering.

[H1]Tissue engineering of the corpus cavernosa

In vivo studies investigating the use of tissue-engineered corpus cavernosum have been underway for the past two decades (**Table 1**). Initial studies began in 1999 by Atala's group, who seeded human corporal endothelial cells (EC) and smooth muscle cells (SMC) on biodegradable polyglycolic acid (PGA) scaffolds[30,45], which were chosen for their high tensile strength, high melting point and degradation properties. The seeded PGA scaffold was implanted into 20 athymic mice resulting in well vascularised SMC and EC growth on day 14 with polymer degradation after day 28[30]. However, polymer scaffolds are unable to mimic the unique three-dimensional penile structures, leading to the use of acellular matrix (ACM), which is similar in architecture to native tissues such as in urethroplasty[46]**.** The extracellular matrix structure is isolated to form an acellular matrix via a process of decellularisation, which removes the cellular component from tissues. The use of ACM caused increased organisation of smooth muscle and endothelial cells as they are similar in architecture to native corpora, resulting in the formation of vascularized corpus cavernosum[45]. Atala et al.[45] developed corporal tissue consisting of human cavernosal smooth muscle and endothelial cells seeded onto a acellular collagen matrix derived from donor rabbit corpora and implanted subcutaneously into athymic mice. However, as this type of engineered corpus cavernosum was formed ex situ prior to implantation, the pattern of vascularization was different from native human tissue, which is especially important as human cavernosal smooth muscle and endothelial cells were used in this study, which might be detrimental to the eventual cellular density and resulting erectile function[45]. Despite this limitation, Kwon *et al.*[47] demonstrated that segmental replacement of corporal body defects with acellular corporal collagen matrices in a rabbit model resulted in intact structural integrity, erection, and successful ejaculation**.** This study revealed that engineering of corporal tissue was possible; however, cavernosometry showed that the maximal intracavernosal pressure (ICP) of the engineered grafts reached only <50% of the ICP of normal controls, which is not sufficient to maintain an erection for successful penetration. The low corporal cellularity observed in earlier studies[45] was overcome by using dynamic cell seeding, which uses hydrodynamic forces to enhance the kinetic cell attachment without compromising the uniformity of cell distribution[48]. Dynamic seeding, in combination with the use of a bioreactor, enabled the cellular content to reach 71% of the normal corporal tissue compared with just 50% of the cellular content reached with static seeding[48]. In a subsequent study, Chen *et al.*[49] used dynamic cell seeding to engineer the entire pendular structure of penile corporal bodies in a rabbit model. The resulting bioengineered corpora demonstrated innervation of the corporal tissue without nerve grafts or tissue-engineered nervous tissue, as the neurovascular bundle was preserved in dissection[49]. After electrical field

stimulation, the male rabbits with bilateral cavernosal implants were able to achieve ICPs of 244 ± 56 mmHg, compared with 257 ± 56 mmHg in the normal control group, which enabled successful erection, copulation, and impregnation of female rabbits. Despite being nearly a decade old, these findings are currently the most advanced within the field of corporal tissue engineering.

[H1]Tissue engineering of tunica albuginea

The tunica albuginea is a fascial tissue that encases the three cavernous bodies and is essential for penile erection (**Fig. 1a**). The importance of the tunica in erection is highlighted by the prevalence of ED in men with Peyronie's disease, 25–50% of whom have ED caused by fibrosis of the tunica albuginea resulting in vascular compromise or veno-occlusive dysfunction[50]. Although various graft types, including fibroblast sheets and human tunica albuginea acellular matrix have been investigated for tissue repair to replace defective or missing tunica tissue, no ideal substitute has been identified, owing to issues with donor site morbidity and limited effectiveness, increasing the need for an alternative[51–56].

Tissue engineering of the tunica albuginea is less well explored than that of the corpus cavernosum (**Table 2**). Reports of tissue engineered tunica albuginea began with Schultheiss *et al.*[57] in 2004, who demonstrated improvements in cell proliferation and ECM synthesis of porcine fibroblasts when seeded onto collagen matrices and cultivated in a bioreactor under multiaxial stress compared with static cultures. Unfortunately this study's outcomes were presented solely as histological images without data quantification. As is the case for corporal tissue engineering, the cell type used is crucial to the outcomes of the graft.

[H1]Cell Sources for penile regeneration

Creation of a functional neocorpora requires two cell types: SMCs and ECs[58]. These cells are principally sourced from autologous, allogeneic or xenogeneic origins[12,59]. Neither allogeneic or xenogeneic cells cause donor site morbidity, which results in damage to the donor tissues during harvesting of the cell source. By contrast, autologous cells do cause donor site morbidity, but this disadvantage is outweighed by their benefits in immune compatibility[59]. Stem cells are undifferentiated and the hallmark attributes of true stem cells includes

multipotentiality and self-renewal capacity. Pluripotent stem cells are able to give rise to all cell types in the body; by contrast, multipotent stem cells are able to form more than one cell type, but are more limited than pluripotent cells as they can only form certain cells based on their lineage, for example the skeletal stem cell[60]. Totipotent, like pluripotent, stem cells are able to give rise to all cell types in the body as well as placenta and embryo cells. Unipotent cells are further limited and are able to differentiate along only one lineage. Mature cells are differentiated, functionally reliable and express the desired phenotype before transplantation, but are often unable to adapt to the *in vivo* environment or proliferate in the same way that stem cells can, which can limit their use in tissue engineering[61]. Several cell sources are used in penile tissue engineering, including mesenchymal, embryonic and adipose-derived stem cells.

[H2] Cell types

SMCs are the active unit of the corpus cavernosum as they are responsible for vessel wall tone, which controls the increased blood follow and decreased venous outflow necessary to achieve and maintain erection. SMCas are, therefore, the most vital component of a successful tissue-engineered penis[11]. However, SMCs have some peculiarities, in particular their varying embryological origins, such as the neural crest, serosal mesothelium and somites, and their ability to undergo phenotypic modulation in cell culture, affecting the activities of growth factors and cytokines, such as TGF-B, TNF-a and PDGF[62].

[H3] Mesenchymal stem cells

MSCs are one of the main sources of autologous stem cells used in neocorporal tissue engineering, as they are relatively easy to isolate from various tissues, such as fat, and expand under standard culture conditions[33,63]. MSCs have been used to successfully produce both SMC and EC[63]. However, their role in penile regeneration is not limited to differentiation into these cell types. MSCs have also been shown to improve cavernous nerve restoration when selected on the basis of p75 nerve growth factor expression and injection into the corpora, rescuing erectile function in a rat model of bilateral cavernous nerve crush injury[64]. Although the mechanism underlying $CD133^+$ MSC differentiation into neural structures remains unclear, seeding alginate scaffolds with $CD133^+$ cells in a rat corpora cavernosa defect model improved mean ICP via upregulation of endogenous nerve growth factor and vascular endothelial growth factor (VEGF) expression[34]. MSCs are abundant and

relatively easy to isolate, and their ability to affect nerve growth makes them a particularly interesting option for penile regeneration.

[H3] MDSCs

Together with MSCs, MDSCs are the most commonly used stem cell types in penile tissue engineering, owing to their prolonged proliferation[65], low immunogenicity[66], low carcinogenic risk[66], and ability to differentiate into several lineages[65]. MDSCs can differentiate into SMCs[67,68], demonstrated by their differentiation into α-smooth muscle actin-positive cells, and endothelial NOS-positive cells when seeded onto acellular corporal collagen matrices implanted within the tunica albuginea of rabbits[36]. VEGF-expressing MDSCs isolated from rat hindlimbs have better growth and attachment on acellular matrices leading to greater improvement in contractile response and also more effective repair and healing of cavernosal tissue compared to a rat model of type 2 diabetes and obesity without MSDCs administration [35,38].

[H3]ADSCs

The use of adipose-derived stem cells (ADSCs) has been well documented in soft-tissue reconstruction, owing to their relative safety and ease in harvesting, and have also been used in corporal regeneration[69]. ADSC proliferation, endothelial marker expression, and endocytosis are greatly diminished when grown in the absence of fibroblast growth factor 2 (FGF2), suggesting that FGF2 signalling mediates ADSC endothelial differentiation which is particularly important for vascularisation of the regenerated corpus[70]. When used in combination with low-energy shockwave therapy, the paracrine effects of human-ADSC have been shown to significantly improve ICP compared with groups without ADSC injections in a postprostatectomy rat model ($p<0.05$)[71].

[H3] Endothelial cells and endothelial progenitor cells

Endothelial progenitor cells (EPCs) are capable of differentiating into mature endothelial cells[72]. They are thought to have physiological roles in angiogenesis and vasculogenesis[73], and have been used in many aspects of tissue engineering, including vascular grafts[74,75], bone regeneration[76], and neovascularisation of ischaemic

limbs[77]. Thus, EPCs could be a valuable cellular strategy for corporal regeneration. To date, no studies have investigated their use in penile tissue engineering; however, mature endothelial cells have been successfully used[30,45,47,49,78]. Following further optimisation of isolation, recruitment and transplantation, EPCs have the potential to be a useful cell source for penile tissue engineering[76].

[H3] CNS-derived stem cells

Central nervous system derived stem cells, known for their plasticity and ability to differentiate into SMCs, have also been used in penile tissue engineering[79,80]. Yong *et al.*[81] demonstrated that rat foetal brain stem cells differentiate into penile SMCs when exposed to media that was conditioned in penile SMC culture; however, this study did not identify the specific factors necessary for transdifferentiation. Although this study confirms the potential of using brain-derived stem cells for penile regeneration, the difficult harvesting techniques of these cells from foetal brain tissue mean that their use is currently limited.

[H3]Other cell sources

Outside the currently small field of corporal regeneration, several other potential cell sources should be considered for their potential application in the penis. Two cell types in particular have shown potential for tissue engineering of corpus cavernosa: urine-derived stem cells (USCs), and ESCs. USCs are easily obtained from excreted urine, so harvesting USCs is less invasive and less costly than harvesting other cell types[82]. USCs have been shown to differentiate into SMCs and produce neuronal protein markers, such as S100, NF200 and GFAP, in vitro [83,84]. USCs express MSC markers [82]; however MSCs usually stop proliferating before 10 passages, whereas a single USC can proliferate for 20 passages and reach a population doubling rate of 60-70[84], which is potentially beneficial for cell expansion within the tissue construct[84].

Alternatively, ESCs could be useful; however, their use is ethically controversial as these cells can only be obtained following the termination of human embryos[85]. Neural ESCs have been investigated as an option for erectile regeneration in a rat model of bilateral cavernous nerve crush injury, whereby neural ESCs injected into the major pelvic ganglion resulted in significantly higher ICP (55.1cmH20 vs 30.5cmH2O) than the control group, with crush injury but without ESCs injections ($p<0.05$)[86]. However, the use of ESCs is hindered by a poor

understanding of the mechanisms of human ESC growth and differences in cellular mechanisms between human and rodent ESCs.

[H3] Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are mature cells taken from a patient that are reprogrammed into an immature, embryonic state, induced by Oct3/4, Sox2, c-Myc and Klf4 under embryonic stem cell culture conditions[87]. These iPSCs can then differentiated into any cell type, such as retinal pigment epithelial cells[88], oligodendrocytes[89], and hematopoietic stem cells[90]. This technique has not yet been applied to corporal or tunica albuginea regeneration; however, patient-derived iPSCs can give rise to innumerable differentiated cell types including those necessary for penile tissue engineering, providing an attractive option for patient-specific treatments for penile regeneration[91]. Although no clinical trials are currently investigating IPSCs in penile regeneration, a Japanese study in the field of cardiology has demonstrated the role of myocardial cell sheets from IPSCs for treating severe heart failure[92].

[H2]Cell sources for tunical engineering

Documented cell types used in tunica albuginea tissue engineering are limited. In the initial 2004 study, porcine fibroblast-seeded onto decellularised collagen matrices were shown to form a homogeneous multilayer with matrix infiltration and regular cell alignment after 7 days, as well as de novo production of extracellular matrix proteins, such as collagen for regeneration of the tunica[57]. A 2012 study by Ferretti *et al.*[93] treated 5mm x 2mm sections of tunica albuginea with autologous fibroblast-seeded PGA scaffolds or PGA alone in a rat model of Peyronie's disease. At 4 months, recurrence of Peyronie's disease in the cell-seeded group was lower than in the unseeded scaffolds, and cavernous nerve stimulation was improved. Human-derived stem cells have also been used in a rat model of tunica albuginea pathology[86]. ADSCs were injected into the tunica of a rat model, resulting in a significant improvement in ICP ($p=0.03$) . ADSCs are easily obtained and expanded, and are capable of forming a multitude of tissues[94]. Intratunical injections of human ADSCs in men during the active phase of Peyronie's disease prevents the formation of fibrosis and elastosis in the tunica albuginea and corpus cavernosum[95], targeting the pathogenesis of, Peyronie's, which is caused by fibrosis of the tunica affecting the flow of blood into the penis resulting in ED[96]. ADSCs have also shown promise for use in tunica albuginea reconstruction when seeded on biological scaffolds. ADSCs seeded onto porcine small

intestinal submucosa (SIS) and grafted onto tunica albuginea defects in rats were shown to increase transforming growth factor B1 and FGF2 protein expression levels maintaining erectile[97] . Despite some promising observations, the use of ADSCs in penile regeneration still faces various limitations, such as low cell survival and engraftment when differentiated into cardiomyocytes and vasculature cells in a heart disease model in vitro [98]. No data are currently available regarding survival of ADSCs in the tunica.

Even with substantial advances in cellular sourcing for tunica albuginea reconstruction, the optimal cell type remains elusive. The regenerated tunica albuginea must act as a tough fibrous layer of connective tissue that surrounds the corpora cavernosa of the penis preventing venous drainage of the penis and sustaining the erect state[21]. To date, fibroblasts and human ADSCs seem to provide the most promising cell sources owing to their ability to form native extracellular collagen and result in sustained penile erection[93–95].

[H2]Donor suitability

The clinical setting is important when considering the suitability of the cell source. Cells used for neocorporal engineering should not be harvested from the autologous corpus of patients with neoplastic disease owing to the risk of cancer cell propagation into newly formed tissue[99]. Furthermore, cell-based therapies involving totipotent human embryonic stem cells (ESCs) can themselves form teratoma formation when injected into severe combined immunodeficient- beige mice[100], raising clinical concerns[101,102]. Ultimately no cases of tumour formation from multipotent stem cell-seeded scaffolds have been reported following transplantation for penile regeneration, but further investigation of their malignant potential is required in biological models[103]. Neoplasia is not the only factor that needs consideration when determining the clinical appropriateness of the cell source. Kovanecz *et al.*[31] have demonstrated that corporal regeneration using muscle-derived stem cells (MDSCs) taken from a long-term uncontrolled diabetic rat donor resulted in incomplete corporal tissue repair capacity when implanted into rat corpus cavernosa, owing to imprinted effects from hyperglycaemia and the dyslipidaemic environment. As a result some human donors might not suitable to provide autologous cell source owing to their environmental effects on the harvested cells.

The corpus cavernosum is a highly perfused structure, which means that injected stem cells can often be flushed away shortly after injection, failing to provide the desired local role in penile regeneration[58]. Stem cells have been shown to have paracrine effects [104]. This property was found to be particularly beneficial in cavernous-nerve-injury-induced erectile dysfunction (ED) due to chemokine–chemokine receptor interaction[105] . Fandel *et al.*[105] established the paracrine effects of stem cells by demonstrating that adipose-derived stem cells (ADSCs) initiate neural regeneration and recruitment towards the major pelvic ganglion after cavernous injury. Similarly, Cengiz et al.[32] have demonstrated the paracrine effects of stem cells by showing that the administration of human umbilical cord blood can enhance the recovery of erectile function and promote hypoxia-inducible factor-1α (HIF1α) and von Willebrand pathways when injected into the corpora of diabetic rats with ED [32,105].

[H1]Scaffolds for penile regeneration

In addition to the choice of optimal cell type, progress in engineering the ideal corporal tissue also relies on optimal scaffold selection. Designing scaffolds specifically for use in corporal tissue is a challenge, owing to the structural difference between the flaccid and erect penis. Biomaterials for penile scaffolds must, therefore, reproduce the mechanical properties of native penile tissues, such as elasticity, hardness, and lack of fatigue that are essential for facilitating sexual function while also providing a site-specific cell-delivery system.

[H2]Scaffolds for the corpora

[H3]Acellular scaffolds

The challenges of introducing stem-cell therapy into clinical practice — such as malignancy, immune response and ethical concerns — have shifted the focus towards using a cell-free tissue engineered scaffold for tissue reconstruction[106]. A non-seeded scaffold provides a surface that should mimic extracellular matrix (ECM), regulating cell function of migrating cells *in vivo*[106]. Multiple studies have investigated the effectiveness of ACMs, including human acellular dermis and porcine bladder ACM[39,45,47,48], which mimic natural ECM as a structural template to support cell adhesion[107], migration, proliferation and differentiation without cell

seeding in penile tissue engineering[30,39,45,47–49]. However, growth factors and signalling molecules, such as FGF and VEGF are required to maintain cell differentiation, and these studies did not add these complementary signalling components. Thus, the outcomes of these studies of non-seeded scaffolds were poor[108,109]. Although some of these factors, such as VEGF and platelet-derived growth factor exist in the ACM[110], fibronectin was required to enhance the regenerative effects of growth factors in vivo in a diabetic mouse model of chronic wounds and a rat model of bone defect[109,110].

Polyglycolic acid (PGA), a synthetic polymer with high tensile strength, high melting point and degradable properties, was the first scaffold type used in penile tissue engineering[30]. Synthetic biomaterials are economically viable to produce, nonimmunogenic, and can be tailored specifically to meet functional needs by altering the polymer[111]. However, they are associated with a risk of a foreign body reaction and cell damage can occur during polymer resorption, as degradation products can activate macrophages, reactive oxygen intermediates, and degradative enzymes at the cell membrane and biomaterial interface[112–114]. Consequently, allogenic, autogenic or xenogeneic ACM have been adopted[36,38,47,49]. ACMs have cellular antigens removed for immunological tolerability, but extracellular components, such as collagen are preserved. ACMs are the principal scaffolds used in tissue engineering of corpora cavernosa and have demonstrated success in corporal regeneration in rodent and rabbit models owing to the preservation of naturally occurring growth factors — such as FGF and VEGF— which further encourage cell survival, attachment and tissue generation[45,47,49].

However, ACMs are not without their own challenges. Reproducibility is a problem owing to a shortage of donor options[115]. Controlling the biological properties is another challenge of ACM as removing cellular components can alter the ECM. Manufacturing costs associated with producing ACMs are high as they are acquired from living tissue and the decellularization process is expensive [68]. Despite these limitations, acellular scaffolds are currently the best option available to meet the unique demands of engineering the erectile tissue of the penis[58].

[H2]Current status of corporal scaffolds

Thus far, corpus cavernosum tissue-engineering strategies have used scaffolds both with and without cell seeding to facilitate the incorporation of the graft by native tissue (Table 1). At present, studies using cell-

seeded acellular corpus cavernosal scaffolds have demonstrated the best results regarding ICP in mouse and rabbit models[45,47,49]. However, advances in bioprinted scaffolds and polymer hybrids have considerable potential for future clinical application[116].

[H2]Scaffolds for the tunica

Acellular matrix from porcine bladder tissue has been used for tissue engineering of the tunica albuginea, but preclinical results have been mixed and not encouraging[57,117–119] (Table 2). Human tunica albuginea ACM isolated from patients undergoing male-to-female gender affirmation surgery has been used as a potential to bridge a corpora cavernosa tunica albuginea defect with current studies limited to the in vitro stage[120]. Dermal grafts have been created using human fibroblasts isolated from a skin biopsy and cultured *in vitro* to form fibroblast sheets to cover corporeal defects — Chun et al.[117] compared dermal graft harvested from the lower abdomen and cadaveric pericardium in 27 males, and reported comparable patient satisfaction and functional outcomes in the two groups. Human fibroblast sheets have also been seeded with human umbilical vein endothelial cells and placed in a bioreactor to form endothelialized tunica albuginea[121]. The tissue-engineered endothelialised tunica albuginea graft was shown to be structurally similar to normal tunica, in terms of collagen 1 staining and presented superior burst pressure 584mmHg compared to 719mmHG (n=3)[121]. Sheets were seeded with human umbilical vein endothelial cells and placed in a bioreactor to form endothelialized tunica albuginea.

Overall, the optimal scaffold for tunica albuginea tissue engineering remains unknown. Natural biomaterials such as acellular porcine small intestinal submucosa have been shown to provide viable 3D architecture and supply instructive cues such as upregulating iNOS to direct cell phenotype for tunical regeneration and restoration of erectile function[97] . Synthetic polymers could potentially be further engineered to release growth signalling molecules for enhanced tunical regeneration. The role of synthetic polymers in tunica albuginea regeneration needs to be explored.

[H2]3D-bioprinted scaffolds

3D bioprinting technology involves the printing of ECM together with the cell-containing scaffold[122]. Bioprinting can produce biocompatible materials and distribute cells within the scaffold for regenerative medicine[123]. The discovery of iPSCs enables the creation of patient-specific stem cell lines from mature cells obtained from venepuncture or skin biopsy, which can then be bioprinted[124]. Microextrusion bioprinting, inkjet bioprinting, and laser-assisted bioprinting techniques have been used to produce various tissues necessary for corpus cavernosa and tunica albuginea regeneration[116], including cardiovascular[125], musculoskeletal[126] and neural tissue[127]. However, 3D bioprinting has not been applied in penile tissue engineering to date because it can be slow and expensive as layer-by-layer assembly with bio-glue is required and suitable bioinks with good biocompatibility and appropriate mechanical strength are limited[128].

[H1]Gene therapy for penile erection

Gene therapy is the replacement or upregulation of genes intended to ameliorate a defect or terminate a tissue insult[129]. Clinical trials of gene therapy have shown efficient gene editing[130] and successful correction of inherited mutations[131], engagement of stem cells to regenerate tissues[132], and cancer treatment[133]. Gene therapy has been investigated in animals models as a means to restore normal function to the penis, but to date only one trial on gene therapy for ED in humans has been published[134]. In this study, Melman et al.[134] administered a single-dose of DNA plasmid carrying human cDNA encoding *hSlo* to the corpus cavernosum of 11 men. Unfortunately efficacy conclusions cannot be drawn from this phase I trial it did not include a control group; however, improves in the International Index of Erectile Function were reported by patients. Gene therapy has been suggested as a potential for the treatment of ED, especially those unresponsive to first-line therapy with phosphodiesterase type 5 inhibitors[134,135].

Different viral vectors, such as adenovirus, herpes simplex virus have been used as vehicles to deliver genes to penile tissue for the treatment of ED owing to their efficiency in delivering their genetic information to the target cell and because they are readily produced. Viral vectors are associated with immunogenicity, which can potentially be avoided by using synthetic delivery vectors; lipid-based and polymer-based vectors have been used to deliver genes to the penis and other tissues[136]. Nonviral vectors are less efficient at delivering their genetic material to the target cell, and production is limited by sufficient quantities of DNA for transduction[137].

Gene therapy for ED uses three primary therapeutic gene transfer approaches: targeting the nitric oxide (NO) system, targeting potassium channels, or targeting growth factors .Targeting the NO system, such as NOS synthase, affects cavernous smooth muscle relaxation resulting in penile erection[138]. Similarly, genes related to potassium channels, such as *pcDNA/hSlo* functionally enhance smooth muscle relaxation, promoting erection[139] . Growth factors such as brain derived neurotrophic factor (BDNF) and VEGF have been used in neurogenic ED to enhance nerve regeneration[138]. Adenoviral vector-mediated *BDNF* transfer into the corpora of a cavernous-nerve-injured rat model improved ICP using cavernous nerve stimulation and increased nNOS staining in pelvic ganglion neurons[140]. Adenovirus-mediated gene transfer of *VEGF* coadministered with angiopoietin-1 to diabetic rats with ED increases eNOS phosphorylation and cGMP expression in cavernous tissues, resulting in complete restoration of erectile function[141].

The use of these different vectors to target different factors can be applied to corpus cavernosal regeneration, through enhancing neurogenic and vasculogenic regeneration, increasing blood flow, stabilising membrane potential and increasing growth factor delivery.

[H1]Translational potential of animal studies

As yet, no human studies have been performed that inserted tissue-engineered matrices into the corpus cavernosum or tunica albuginea. However, one study reported use of a synthetic poly lactide-co-glycolide biodegradable scaffold coated in autologous fibroblasts, which was successfully inserted underneath the deep fascia to in humans to improve penile girth (measured by distance and patient satisfaction analogue scale)[142]. In ths study, mean erect penile girth increased from 10.26cm preoperatively to 13.18 cm postoperatively ($p<0.001$).. A similarly successful penile enhancement procedure used Regen Biotech scaffold seeded with autologous scrotal dartos cell, in which the construct was implanted superficial to the deep fascia[143].

In studies of corporal reconstruction, stem cells have been harvested from humans and have been successfully implanted into rabbits and mice, forming erectile tissues[30,34,45,144]. In 2009, human SMCs and ECs were implanted into rabbits by Chen *et al*. resulting in regeneration of the entire corpus cavernosum and enabling erection, copulation and impregnation [49]. Decellularised human corpus cavernosum was used as a tissue-

engineered scaffold in a rat model, but the translational value of this study is limited as the scaffold was implanted into a rat omentum and ICP was not measured[39].

Although advances in regeneration of the corpus cavernosum have enabled impregnation in in vivo animal models, as yet, no human studies have been performed using tissue-engineered corpus cavernosum or tunica albuginea. The use of large animal models with comparable size and intracavernosal pressure to humans will help to support the translation of small animal studies of corpus cavernosum and tunica albuginea tissue engineering into clinical use. Furthermore, developments in gene therapy and the use of iPSC might also help to overcome difficulties in clinical translation, as both techniques have been studied using human-derived cells and can be used to enhance cellular proliferation and differentiation in the damaged corpora and tunica. The increased use of autologous cells will also help to optimise regenerative outcomes.

[H1]Conclusions

Currently, penile reconstruction techniques include use of a variety of free or pedicle flaps with or without an inflatable penile prosthesis, or penile transplant to enable sexual intercourse . The high complication rates, including infection, erosion and revision surgery demand an alternative option. Contemporary tissue engineering strategies enable the seeding of a biomaterial scaffold and implantation of this scaffold to construct a neocorpus. However, testing in large animal models, which have a penis size and ICP range similar to that of a human penis, are required to confirm its applicability in clinical practice. Preclinical research using iPSCs in penile reconstruction should also be expanded as this cell type enables patient-specific treatment. Furthermore, 3D bioprinting of scaffolds should be further developed, as it has the capability to produce precise cell-laden structures. 3D bioprinting could be developed for use in combination with advances in gene therapy . Thus far, the *in vitro* and *in vivo* findings support a promising role of tissue engineering in human penile reconstruction, and improved human translation is awaited with great interest.

Author contributions

T.A, M.K., L.M. and D.M.K. researched data for the article. All authors made substantial contributions to discussions of content and writing the manuscript. T.A, M.K., A.M. D.M.K. and A.A. reviewed and edited the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

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Publisher's note

Toc blurb

Despite improved surgical techniques and the development of transplants, therapeutic options for men who have penile disorders or who have sustained trauma to the penis are limited and erectile function can rarely be rescued. In this Review, the authors discuss regenerative approaches to tissue engineering the tunica and corpora to improve outcomes in these patients.

Terms
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Figure 1|Anatomy of penis in humans and animal models. a| In human males, the tunica albuginea surrounds the three cylinders of erectile tissue: two corpora cavernosa and one corpus spongiosum. Relaxation of the cavernous smooth musculature and increased arterial blood flow to the corpus cavernosum initiate penile erection. The tunica albuginea is essential in maintaining erection by compressing the subtunical venular plexus, reducing venous outflow. b| The murine penis has several differences from the human penis[145]. First, the murine penis has a cartilaginous MUMP and ossified bone, the os penis. Second, the murine penis has several corpora cavernosa, including the MUMP corpus and urethral corpus. Third, in the flaccid state the murine penis is positioned internally beneath the external prepuce.

Figure 2|The process of tissue engineering neocorpora. 1. Cellular harvesting- Cells are isolated and cultured from autologous, allogeneic or xenogeneic tissue sources. Cells are expanded *in vitro* and are used in their harvested form as differentiated downstream progenitors or as reprogrammed IPSCs. 2. Scaffold seeding- Cells are seeded onto the scaffold. The scaffold should regulate cellular adhesion, proliferation and differentiation.

3. Implantation of seeded construct- The construct is surgically implanted into the recipient site. 4. Implantation of unseeded construct- Alternatively, scaffolds can be implanted without cellular seeding. Unseeded scaffolds release growth factors which recruit SMCs and ECs for penile regeneration.

Table 1| *in vivo* studies of corpus cavernosum tissue engineering

Year	Model	Scaffold	Seeded?	Cell type seeded	Cell type formed in vivo	Reference
1999	Mouse	PGA	Yes	SMC/EC	SMC/EC	[30]
2002	Rabbit	AM	Yes	SMC/EC	SMC/EC	[47]
2003	Mouse	AM	Yes	SMC/EC	SMC/EC	[45]
2007	Rat	-	No	MSC	SMC/EC	[63]
2008	Mouse	AM	Yes	EC	SMC/EC	[48]
2008	Rat	-	No	NCSC	SMC	[144]
2008	Rat	-	No	MDSC	SMC	[146]
2010	Rabbit	AM	Yes	SMC/EC	SMC/EC	[49]
2011	Rabbit	AM	Yes	MDSC	SMC/EC	[36]
2012	Rabbit	AM	Yes	MDSC	SMC	[105]
2013	Rabbit	AM	Yes	MDSC	SMC	[38]
2016	Rat	-	No	MDSC	SMC	[31]
2016	Rat	-	No	ADSC	SMC	[71]
2017	Rat	-	No	HUBC	SMC	[32]
2017	Rat	-	No	BMC	SMC/EC	[33]
2017	Rat	Alginate	Yes	BMC	SMC/EC	[34]
2017	Rat	AM	Yes	EC	SMC/EC	[39]

AM, Acellular Matrix; BMC, Bone Marrow Cells; CC, Corpus Cavernosal Tissue; EC, Endothelial Cells; FBSC, Foetal Brain Stem Cells; HUBC, Human Umbilical Cord Blood Mononuclear Cells; MDSC, Muscle-Derived Stem Cells; NCSC, Neural Crest Stem Cell; PGA, Polyglycolic Acid; SMC, Smooth Muscle Cells

Table 2 / *In vivo* studies of tunica albuginea tissue engineering applications

Year	Model	Scaffold	Seeded?	Cell type seeded	Cell type formed in vivo	Reference
2004	Pig	AM	Yes	FB	FB	[57]
2006	Rabbit/Pig	pBAM	No	-	SMC	[119]
2011	Human	hTAAM	No	-	-	[120]
2011	Human	FB Sheet	Yes	HUVEC	FB/EC	[121]
2012	Rat	PGA	Yes	FB	-	[93]
2012	Rat	AM	Yes	ADSC	-	[97]

ADSC, Adipose-derived Stem Cells; AM, Acellular Matrix; FB, Fibroblasts; hTAAM, human Tunica Albuginea Acellular Matrix; HUVEC, Human Vein Umbilical Endothelial Cells, pBAM, porcine Bladder Acellular Matrix; PGA, Polygycolic Acid; TA, Tunica Albuginea