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*Review*

# Penile Tissue Engineering: a Review of the Current Progress of Penile Reconstruction

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**Abstract:** Penile cancer, congenital abnormalities, trauma, animal bite, iatrogenic injuries, severe erectile dysfunction can lead to loss of normal penile function that needs penile reconstruction. However, success of penile reconstruction is limited by scarcity of native penile tissue and tissue complexity. Several methods of phalloplasty exist, yet none of these approaches is able to recreate a fully functional physiological penis. Applying a tissue engineering approach provides the possibility of overcoming this problem. However, the penis is a complex organ with several tissue types, each with a specific structure and function. Imitating this native architecture is difficult, making penile tissue engineering very challenging. Several cell types have been investigated for corporal regeneration and restoring adequate erectile function. Researchers have showed successful differentiation of mesenchymal stem cells (MSCs) into smooth muscle and endothelial cells after transplanting them into rat cavernosum in vivo. Their plasticity, ease of accessibility and characteristic reproducibility makes MSCs an attractive option for therapeutic regeneration of penile cavernosal tissue. We discuss the use of stem cells in penile tissue engineering, the challenges and future direction of penile reconstruction.

**Keywords:** penile reconstruction; penile tissue engineering; tissue engineering; stem cells; mesenchymal stem cells; corpus cavernosum; cavernosal tissue

**Abbreviations:** Decellularised collagen matrix (DCM); Endothelial cells (EC); Erectile dysfunction (ED); Female to male (FTM); Gender reassignment surgery (GRS); Mesenchymal stem cells (MSC);

Polyglycolic acid (PGA); poly(lactic-co-glycolic acid) (PLGA); Radial forearm free flap (RFFF); Smooth muscle cells (SMC)

## 1. Introduction

Penile cancer, congenital abnormalities, trauma, animal bite, iatrogenic injuries, severe erectile dysfunction (ED) and gender reassignment may require reconstructive surgery of the penis. There are over 600 new cases of penile cancer diagnosed in the UK annually [1,2]. Furthermore, rates of gender dysphoria have increased, perhaps due to increasing awareness, with more people seeking gender reassignment surgery (GRS). Recent reports state that rates of female to male (FTM) surgeries performed in the UK tripled between 2000 and 2010 [3]. Prevalence of ED is also reported as high as 52% with an increase to 70% in patients over 70 years old [4].

The extent of the defect dictates the means of reconstruction, and the goal of surgery is to restore or construct a penis that is aesthetically and functionally adequate. This includes the ability to void urine from the tip of the penis whilst standing, as well as full sexual functionality (erection, ejaculation and fertility) [5]. Yet, success of penile reconstruction is limited by a lack of native penile tissue [6].

A range of reconstructive techniques, such as skin grafts and flaps, can be employed to improve the aesthetic appearance of the penis [7,8]. As no other tissue in the body shares similar characteristics to penile tissue, none of the currently available surgical techniques are able to fully restore functionality. To address this issue, tissue reconstruction is combined with the use of synthetic systems (for example, prosthetic pumps), yet these are unable to restore full penile functionality [9]. Applying a tissue engineering approach, therefore, provides a means of overcoming these problems, with the prospect of fully restoring cosmesis and function. The penis is a complex organ with various tissue types, each with its own structure and function. Imitating this native architecture and associated functional physiology is difficult, making penile tissue engineering very challenging.

## 2. Anatomy and physiology

The male external genitalia is comprised of the penis, urethra and scrotum wherein lie the testicles (Figure 1). The penis consists of three tubes. The two large columns of erectile tissue, corpus cavernosa, sit side by side on the top, and are made primarily of smooth muscle cells with dense neurovasculature (Figure 2). These tubes are surrounded by tough, elastic fibrous tissue (tunica albuginea), which is anchored to the lower end of the pubic bone. The elasticity of the fibrous layer allows the penis to expand in length and girth upon arousal. The flaccid penis is soft and flexible. Upon arousal the erectile tissue becomes engorged with blood, and the rigidity of the tunica albunginea enables the blood pressure to rise above normal, making it hard and rigid. The third tube, corpus spongiosum, is smaller and lies beneath the corpus cavernosa. It contains the penile part of the urethra and forms the glans of the penis (Figure 2).

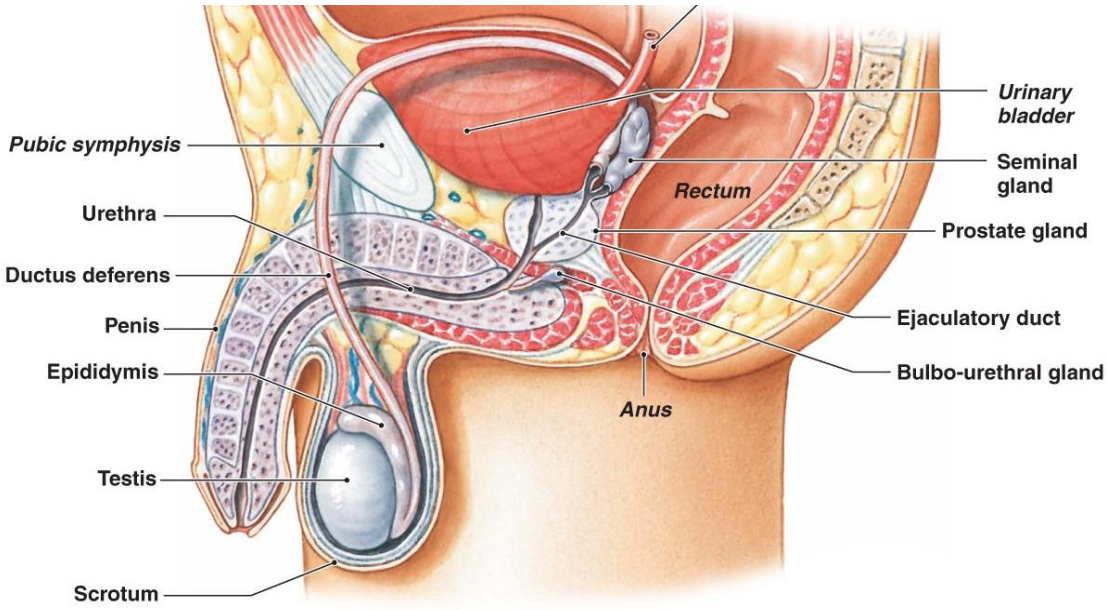


**Figure 1. External male genitalia [10].**

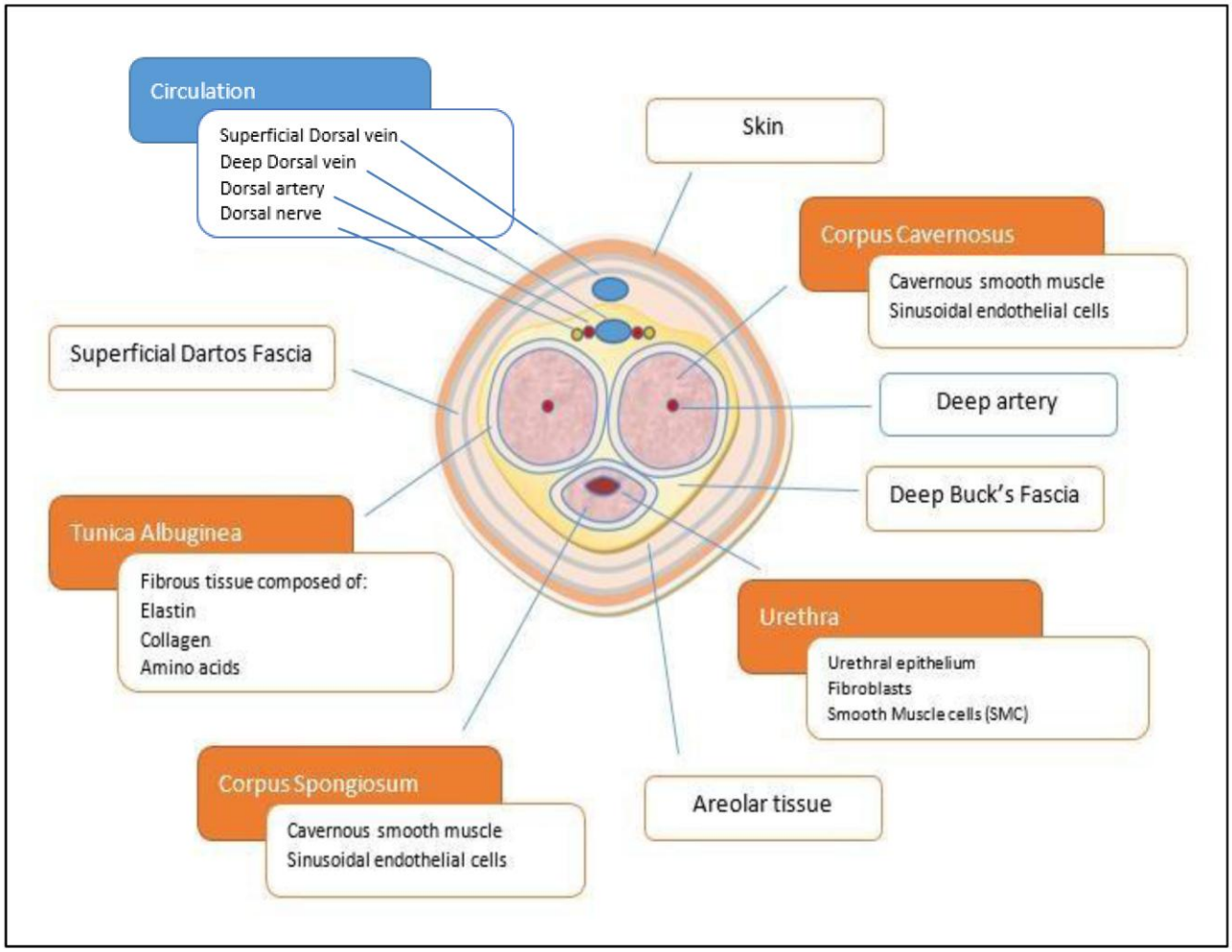


**Figure 2. Cross-section of penile tissue split into tissue components.**

## 3. Surgery

There are several indications for total phalloplasty, and scrotal reconstruction is often required in conjunction. However, this review focuses only on penile reconstruction, and where applicable, the urethra, as a large portion lies within the shaft of the penis and is crucial for voiding urine and ejaculate. The magnitude of penile reconstruction relies on the site, nature and extent of penile defect. Small defects may be closed primarily, or with simple skin grafts or flaps.

For simplicity, one may classify large penile defects into two categories: those limited to the skin and those involving the skin and inner corpora. Causes of the former may include necrotizing fasciitis, excision of benign or malignant lesions, or excessive circumcision, to name a few [9]. In such cases, it is often sufficient to use a skin graft. Where possible, hair-bearing areas should be avoided or complete hair removal sought prior to graft harvesting. Meshed and un-meshed split thickness skin grafts are often used due to ease of harvesting, decreased donor site morbidity and superior take. However, full thickness skin grafts heal with a lesser degree of contracture providing the elasticity needed to maintain adequate erection [9].

In cases of penile inadequacy the corpora, in addition to the skin, require restoration. Here maximal tissue preservation should be sought, where possible, in the first instance. Complete neo-phallus creation should be reserved as a last resort where conservative management has failed, function has not been restored, or the patient has psychological distress due to the penile defect. It is also important to determine the level of functionality desired by the patient.

Advances in microsurgical techniques and free tissue transfers have led to a decreasing use of local pedicled flaps for phalloplasty, with movement towards free flaps. Local flaps have less failure rates, decreased donor site morbidity and reduced operating time, yet their conspicuous drawbacks prevent necessary functional restoration [9,11]. Neo-phalluses created from local flaps often lack adequate tactile and erogenous sensation. This precludes the use of stiffeners and prostheses to facilitate erection, rendering the patient unable to adequately perform penetrative intercourse, or achieve orgasm. This impacts their psychosexual wellbeing. Furthermore, neo-phalluses created from local pedicled flaps are aesthetically inferior to their microsurgical free-flap counterparts. Commonly used local and free flaps for phalloplasty are listed in Table 1.

**Table 1. Pedicled and Free Flap Techniques Used for Phalloplasty.**

| *Pedicled flaps* | *Free flaps* |
|---|---|
| Groin flap | Radial artery forearm flap |
| Anterolateral thigh flap* | Radial forarm osteocutaneous flap |
| Island tensor fascia lata flap | Fibular osteocutaneous flap |
| | Lateral arm flap |
| | Latissimus dorsi flap |
| | Scapular flap |

*Reports of use as a free flap, as well.

As there is a paucity of adequate randomized controlled trials in the literature comparing outcomes of various flaps used for phalloplasty, it is difficult to identify a gold standard of treatment.

However, the most widely used technique (and certainly one that is advocated by most urologists and plastic surgeons) is the radial forearm free flap (RFFF). This method uses the radial artery as a long pedicle, which is easily harvested. The thin skin, low subcutaneous fat content, desirable blood perfusion and innervation are tantamount to the satisfactory outcomes seen in patients with RFFF phalloplasties [12]. A small flap from the forearm skin is rolled into a thin skin-lined tube, which is surrounded by a larger tubularised flap with skin on the outer surface. The inner tube eventually acts as the neo-urethra, with the outer portion serving as the penile body. This tube within a tube is transplanted to the pubic area for microsurgical anastomosis of the flap pedicle with the femoral artery and great saphenous vein, or inferior epigastric vessels [13,14]. In order to restore sensation, medial and lateral antebrachial nerves supplying the flap are preserved and anastomosed at the donor site with the ilioinguinal neve for tactile sensation and the dorsal penile (or dorsal clitoral in FTM GRS) for erogenous sensation [5]. An alternative modification to this protocol is urethra prelamination using a full thickness skin graft 6 months prior to phalloplasty [15]. The main drawback of RFFF is the significant donor site morbidity. Despite coverage by skin grafts, the appearance of the resultant defect may deter patients when choosing a phalloplasty method.

Most phalloplasties require a stiffener to facilitate adequate erection for penetrative intercourse; synthetic malleable or inflatable prostheses are often used. Recently, advances in tissue engineering have led to the development of naturally derived, even autologous, prostheses [13]. These are discussed later in this review. Free sensate osteocutaneous flaps may also be used for total phallic reconstruction. These flaps obviate the need for a penile implant for intercourse as they provide intrinsic rigidity. Yet a permanently erect penis may cause social embarrassment and psychological distress to some patients. However, patients with osteocutaneous flaps have reported sexual intercourse with ejaculation, making them a viable alternative to the RFFF [16].

## 4. Corporal tissue engineering

The corporal bodies constitute the bulk of the penis (Figure 1), and are made primarily of smooth muscle cells with surrounding connective tissue, nerves and blood vessels. The highly ordered and unique architecture give the penis its ability to become erect. Scaffold materials, therefore, need to be strong but flexible and require the ability to stretch and expand. Replicating this highly specialized structure is one of the main challenges in producing a fully functional penis and requires an engineered scaffold.

Penile rigidity is the key mechanical property that enables sexual functionality. It is a variable rigidity created by hydraulic principles acting within the corpus cavernous tissues. Penile rigidity is determined by intracavernosal pressure, penile geometry, tunica distensibility, cavernosal expandability and cavernosal bulk modulus [17]. Udelson et al. defined cavernosal bulk modulus ($\beta$) as a measure of difficulty of erectile tissue to expand with increasing intracavernosal pressure. They determined cavernosal bulk modulus of a given intracavernosal pressure, using a mathematical model, for two fixed values of cavernosal expandability (x) at 0.1 and 0.05 mmHg (Figure 3) [18]. However, a literature review highlighted insubstantial evidence regarding these biomechanical properties. This makes it difficult to select materials that mimic natural tissue function.

The pressure at which penile rigidity occurs varies for each individual. On average, rigidity occurs at pressures of 60–90 mmHg. However, in some people, rigidity may develop with magnitudes as low as 40–50 mmHg or, in others, not until pressures exceed 100–120 mmHg [18]. The soft tissue surrounding the erect corpora must be able to withstand the high pressure without buckling. Diseased, fibrotic penile tissue expands poorly and is associated with poor penile rigidity [17].

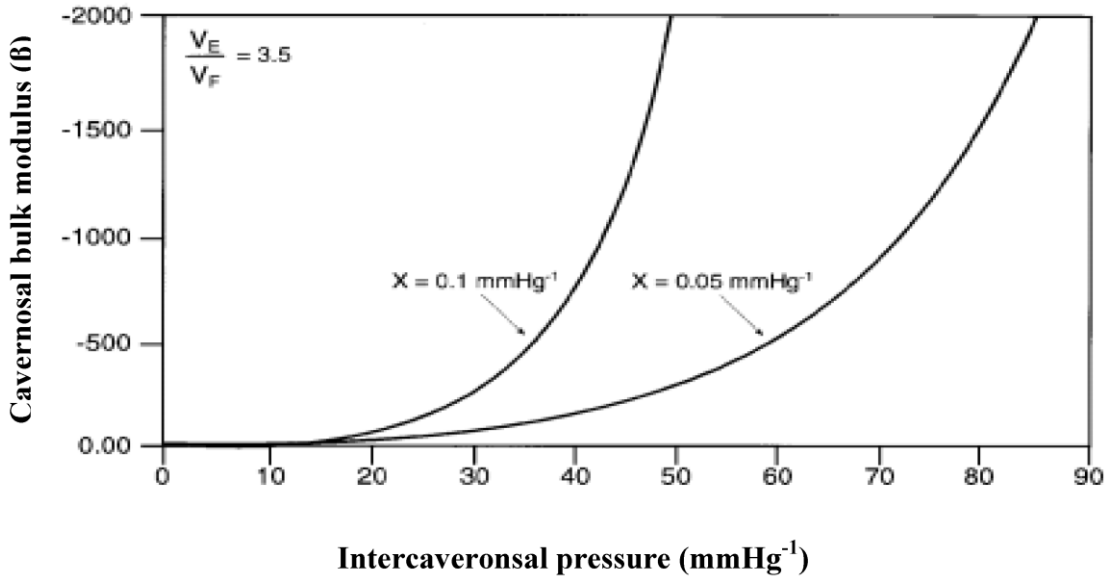


**Figure 3. Mathematical modelling of the expected cavernosal bulk modulus (β) for given intracavernosal pressure differences at two fixed values of cavernosal expandability (X) [18].**

A range of materials have been used as scaffolds in corporal tissue engineering, these include synthetic polymers, natural materials and acellular matrices. They have been seeded with several cell types and investigated for corporal regeneration, and restoration of adequate erectile function. Researches showed successful differentiation of MSCs into smooth muscle and endothelial cells after transplanting them into rat cavernosum in vivo. Their plasticity, ease of accessibility and characteristic reproducibility makes MSCs an attractive option for therapeutic regeneration of penile cavernosal tissue [18,19]

Several research groups have successfully seeded poly(lactic-co-glycolic acid) (PLGA) scaffolds with normal human corporal smooth muscle cells (SMC) and human endothelial cells (EC). They were able to form organised, vascularized cavernosal muscle tissue that showed capillary formation when implanted into athymic mice [19,20]. Although the tissue was organized, the structure varied from native corporal tissue. Furthermore, the conclusions drawn are limited as the study design does not test the functionality of the tissue and there are no reports on its ability to become erect.

To overcome the limitations of synthetic scaffolds, naturally derived scaffold materials have been studied. Acellular corporal collagen matrices derived from processed donor rabbit corpora were then seeded with human corpus cavernosal muscle cells and endothelial cells. Development of neovasularised sinusoidal spaces and organized collagen and smooth muscle was noted.

Functionality was tested, which showed that newly formed tissue had the ability to contract in response to electrical stimuli [21]. Although the cells were able to contract they were still unable to demonstrate a sustained erection.

Subsequent studies have shown successful integration of the entire cross sections of engineered corpora into animal models. Smooth muscle and endothelial cells were seeded onto collagen matrices [6,21,22]. Kwon et al. replaced a cross sectional segment of both of the corporal bodies in rabbits using autologous corpus cavernosal smooth muscle cells and endothelial cells seeded onto acellular matrices. They showed successful, sustained erections and ejaculation, which demonstrates formation of functional tissue. However, the intracaveronsal pressure was approximately half of the normal pressure possibly due to low density of smooth muscle cells [22].

Eberli et al. suggested dynamic-seeding as an option to overcome this limitation in an attempt to engineer morphologically and biochemically better corporal tissue [23]. Their laboratory is currently investigating the replacement of anterior corporal tissue using dynamic seeding techniques to engineer fully functional erectile tissue. In the first 48 hours, cells are seeded onto the matrix to allow attachment. The cell number is quantified and the same concentration of cells per volume is seeded onto each scaffold. Dynamic seeding using bioreactor systems allows simultaneous seeding and mixing of multiple cell types, a property useful for engineering corporal tissue. It also provides higher cellular content. The scientists reported cellular levels that reached 71% of the normal corporal tissue compared to 39% when using other seeding methods [23].

The most successful study to date in corporal regeneration was carried out by Chen et al. [6]. They showed successful engineering of the entire corporal component by dynamic seeding of cavernosal collagen matrices with autologous smooth muscle cells and endothelial cells in rabbits. The resulting vascular tissue was histologically similar to native penile tissue structure. Functionality was depicted by impregnation of female rabbits by male ones, suggesting successful erection and ejaculation. They also showed that dynamic seeding facilitated intracavernosal pressure that was comparable to normal erectile pressure [6]. However, they neglected to give sufficient details of how the engineered section was attached to native tissue; a key element for successful clinical application.

All of these studies (Table 2) neglected to test or discuss the importance of creating nerve structures – an integral component of functional corporal tissue and sexual functioning. They also fail to report or evaluate the aesthetic appearance of the penis. This, in addition to function, is often a key factor that patients consider when determining the success of penile reconstruction [24].

**Table 2. In vivo studies of corpus cavernosum tissue engineering applications.**

| *Study* | *Scaffold* | *Cell* | *Animal model* | *Seeding* | *Regenerative outcome* |
|---|---|---|---|---|---|
| [20] | PLGA | MSC-SMC, EC | Mouse | Yes | Histologically similar tissue produced |
| [19] | PLGA | MSC-SMC, EC | Mouse | Yes | Vascularised, histologically similar tissue |
| [22] | DCM | MSC-SMC, EC | Rabbit | Yes | Sustained erection and ejaculation |
| [21] | DCM | MSC-SMC, EC | Mouse | Yes | Contractile corporal tissue |
| [6] | DCM | MSC-SMC, EC | Rabbit | Yes | Male rabbits impregnated female ones |

## 5. Penile prosthesis

Silicone is the material of choice for the majority of penile prosthesis, however they have all the disadvantages of synthetic materials. An alternative approach is the use of a natural prosthesis, made from engineered cartilage tissue. Polyglycolic acid (PGA) polymer rods seeded with donor chondrocytes were implanted into athymic mice. The results showed the formation of rod shaped cartilaginous structures with limited inflammatory reactions. Scaffolds without cells failed to form cartilage. Histological examination showed mature cartilage formation and progressive replacement of the polymer construct with cartilage over time. Biomechanical analysis was also favourable. The rods could withstand high levels of pressure and were able to maintain penile rigidity [25]. This study was successfully replicated using autologous chondrocytes [26]. However, these materials lack functional tissue and further functional studies are need prior to application in the clinical setting. A tissue engineering approach is preferred as it overcomes these limitations.

## 6. Successes, challenges and future direction

The male phallus is a complex organ with distinct tissue and function making it challenging to recreate. Tissue engineering studies, though in their infancy, have displayed the potential of stem cells to differentiate into smooth muscle and endothelial cells, creating corporal like tissue. The use of acellular allograft scaffolds also provides a non-immunogenic, biodegradable architecture on which cosmetically accurate penile tissue can be engineered.

The current research is limited by the use of animal models. The studies discussed (Table 2) use either rodent or rabbit models. These animals have different anatomy and physiology to larger animal models and humans. Smaller animals have a higher ratio of collagen to smooth muscle cells and the intracaveronsal pressure required to produce a sustained erection in these models can be up to 50% less than in humans, limiting their translation [6,27]. Studies in larger animal models (such as primates) with greater similarities to human penile tissue are required to determine applicability in

clinical practice. Using larger animal models would allow researchers to determine whether it is possible to engineer a large corporal section with similar physiological function to human tissue. However, this also has ethical implications.

Successfully creating corporal-like erectile tissue is an achievement in the field of penile tissue engineering, yet it is not enough. In order for the tissue to be functional, it requires integration of the nervous system. A man achieves erection after physical or psychological stimulation, resulting in the corporal tissue becoming engorged with blood and growing in length and girth. Both these triggers require connections to the nervous system, something that has neither been discussed nor reported in the aforementioned studies.

Furthermore, laboratory researchers tend to focus on regeneration of individual components of penile tissue (urethra, corporal bodies or tunica). No study, to the best of our knowledge, has looked at the regeneration of a composite structure constituting all penile parts. Though research in individual components may be necessary to lay the groundwork for further investigation, it seems that significant advances have not been made over the last few years.

Naturally, a bioengineered penis cannot be tested in human clinical trials unless it a fully functional penis is first created and trialed in animal models. As a result, the translatability of research from animal to human models (and therefore, from bench to bedside) is limited.

One must also acknowledge the potential socio-political impact of the advances in penile tissue engineering and associated ethical considerations. For a biomaterial to be approved for clinical use it is essential that it meets Good Manufacturing Practice (GMP) guidelines. In addition, when using human cells for purposes of tissue engineering, is it important to consent donors for the use of their cells and explain how they will be used? Might authorised cell factories need to be used, as a result? What cohort of individuals requiring penile tissue engineering would be selected to first receive a novel engineered product and how would this be decided? Tissue engineering is a long and expensive process – one must consider how such trials would be funded. These are only a few of the factors that would need to be addressed in more detail before a fully functional tissue engineered penis can be trailed on a human participant.

## 7. Conclusion

Penile reconstruction is complex. Surgical intervention forms an important part of the process, yet psychological rehabilitation is equally vital to successful management of the patient, as penile damage can significantly impact their mental wellbeing. Corporal tissue is the most difficult to replicate in the laboratory because of its idiosyncratic properties and functions. Yet, it constitutes the main bulk of the penile body and is of particular importance in achieving and maintaining an adequate erection for intercourse. Current surgical phalloplasty methods are unable to restore this physiological need, implicating the use of implantable prostheses. The goals of tissue engineering are to, therefore, create corporal tissue capable of mimicking natural physiology. To achieve this, it is essential for urological and plastic surgeons to collaborate with tissue engineering scientists and use a holistic and multifaceted approach to creation of a fully functional neo-phallus.

## Conflict of Interest

All authors declare no conflicts of interest in this paper.

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