Tissue-Engineered Approaches for Penile Reconstruction

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Contents

Abstract

Organs in our body are not simple in composition. Penis also is composed of delicate complex tissues. Moreover penis is distinctive for its dual actions, voiding and sexual functions, and has an impact on the psychological aspect for some people. There are lots of reasons for penile reconstruction, including various penile diseases, penile traumas, and congenital penile anomalies. Other niche fields for penile reconstruction would be transsexual surgeries and penile augmentations. As other tissues, penile tissue has little substitutes and shortage of supply for reconstructive surgery. And allogenic transplantation as kidney would be impossible except for the exceptional situations because of the ethical issue. Tissue engineering would be one of the solutions for the sufficient supply of penile tissue on demand.

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This chapter deals with how the penile reconstruction using tissue engineering has been started and developed. Clinical trials in penile tissue engineering will be introduced. Also, the new approaches and investigations for better physiologic reconstruction of penis in the future including stem cell applications, cell therapy and gene therapy, new scaffolds, and 3D printing technics will be discussed.

1 Introduction

1.1 Anatomy and Physiology of Penis

Penis is the complex organ composed of various tissues. Grossly penis can be divided into two parts in structure and function. Anatomical structure of penis is well known (Drake et al. 2009; Dwyer et al. 2011) (Fig. 1). The penis contains three cylindrical structures. A pair of tissue located at the dorsum of penis is called as corpus cavernosum and these structures are responsible for penile erection. They are covered with a thick fibroelastic structure called tunica albuginea (TA). Corporal tissue is composed of trabeculae with various sizes of pores resembling sponge architecture. These sinusoidal structures are composed of connective tissue, mainly several types of collagen and elastin (Goldstein et al. 1985). Smooth muscle cells (SMCs) and endothelial cells (ECs) are intermingled with these cavernosal sinusoidal matrices. Once arterial blood flows into corpus cavernosum, erection starts. This inflow of blood is pooled in the irregular size sinuses of corpus cavernosum resulting in expanding volume of cavernosal tissue. This tissue expansion is restricted by the surrounding thick, partially elastic TA. As the arterial inflow slowing down for back pressure of corpus cavernosum, the venules penetrating TA, that bridges in and out

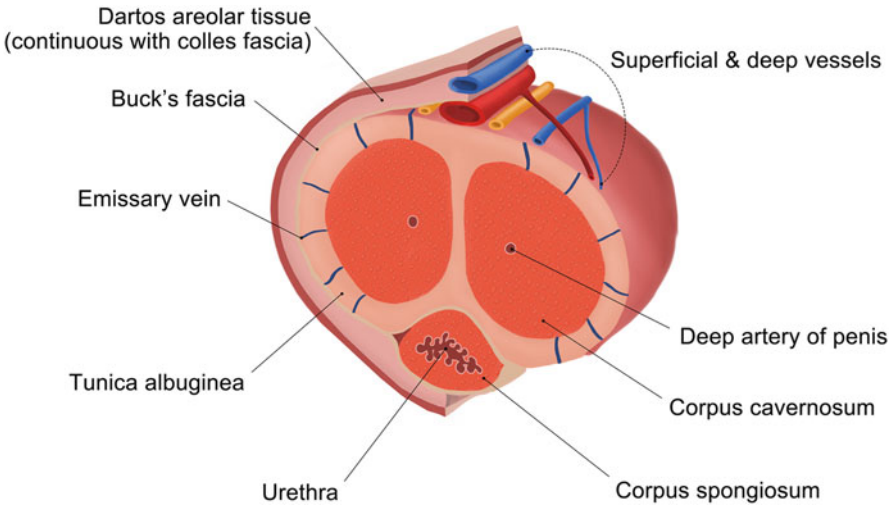

Fig. 1 Cross-sectional anatomy of penis

of corpus cavernosum, and draining blood pooled inside the corpus cavernosum, are compressed by increasing intracavernosal pressure inside the TA. This results in stasis of blood inside corpus cavernosum and maintenance of high pressured cavernosal tissue (Lue 2016). The stiffness that originates from corpus cavernosum is erection.

Another cylindrical structure is the corpus spongiosum. It is located at the ventral side of the penis and surrounds the urethra. The urethra is composed of several muscle layers and acts as a common pathway of urine and ejaculate. The corpus spongiosum is also covered with TA. Penile subcutaneous tissue is composed of Buck's fascia, Colle's fascia, and small arteries, veins, and nerves covering the TA (Awad et al. 2011; Hsieh et al. 2012).

Histology of corpus cavernosum shows that this structure is able to shrink and dilate under appropriate conditions, and sustaining erection is possible with pooling of blood in the sinusoidal structure of corpus cavernosum with the help of TA. The key mechanism of erectile process is coordination of various nerves and vessels. Aside of erectile process, ejaculatory process happens in a millisecond, also with the cooperation of autonomic nerves, sensory and motor nerves, and muscles (Lue 2016).

Though penile reconstruction includes reconstruction of urethra as well as corporal tissue, reconstruction of urethra and corpus spongiosum is dealt in another chapter of this book.

1.2 History of Tissue-Engineered Penile Reconstruction

There are instances when penile reconstruction is needed. These are pathologic conditions to deteriorate the penile functions or ruin penile cosmetic aspects. These penile diseases include Peyronie's disease (PD), urethral stricture, penile fracture, burns, infections, penile cancer, penile trauma as penetrating injuries and blunt force trauma, bites, and congenital anomalies like hypospadias, epispadias, buried penis, and so on (Lumen et al. 2015). Nowadays there is another specific demand for penile reconstruction, as penile augmentation or transsexual surgery. Damaged penis usually loses its function as erection and urination. A lot of pathologic conditions of penis could not be restored with conservative approaches, therefore depending on severity of disease, surgical reconstruction would be the appropriate solution (Williams et al. 2016).

Even though various surgical treatment modalities have been introduced to restore penis since the 1930s, the results had not fulfilled the expectations of doctors and patients. The primary goal of penile reconstruction was structural restoration. Since the Russian surgeon Nikolai Borgoras tried penile restoration using a tubed abdominal pedicled flap combined with rib cartilage in 1936 (Bogoras 1936; Goodwin and Scott 1952), clinicians have utilized various well-vascularized musculofascial tissues such as the radial forearm flap, thigh flap, free fibular flap, osteofasciocutaneous flaps, or the anterolateral thigh flap as well as autologous skin, muscle, vessels, facial tissue from various parts of body in penile reconstruction

(Felici and Felici 2006; Mutaf et al. 2006; Salgado et al. 2011). Materials, allogenic nongenital tissues including skin, muscle, and xenogenic tissues from various locations were also applied as the substitution of penile reconstruction due to deficit autologous genial tissue (Palese and Burnett 2001). Nevertheless the results of surgical procedures revealed risks of graft failure due to tissue necrosis, infection, and donor site problems (Horton and Dean 1990). Adverse immunologic reactions were another problem in non-autologous graft materials. Usually these surgical procedures have been performed in multiple stages depending on the cases and methods. The first one-staged penile reconstruction was performed in 1984 (Chang and Hwang 1984).

A novel technic called "tissue engineering" was introduced in 1988 to create the appropriate tissue using cells and biomaterials. For the purpose of properly functioning tissue or organ reconstruction, tissue engineering would be the appropriate treatment modality compared to the traditional methods in obtaining sufficient and efficient biologic substitutes (Machluf and Atala 1998). In 1999, the concept of "regenerative medicine" has been proclaimed. The terminology Regenerative Medicine include wider range of field than Tissue Engineering, including cell therapy, nuclear, or gene therapy adding to tissue engineering.

As another approach for penile reconstruction, penile transplantation has been tried three times until now. In spite of the ethical issues in 2006, penile allotransplantation was performed without serious physical postoperative complications of the transplanted penis. Instead, the result was dismal for the psychological problem of recipient (Caplan et al. 2017; Hu et al. 2006).

There are two main factors of tissue engineering: cells and biomaterials as scaffolds. Researchers have investigated for the selection and culture of proper cells, methods to harvest enough cells, inventing appropriate biocompatible scaffolds to restore specific organs.

Studies have been started for the proper cell selection and effective methods of cellular proliferation composing corpus cavernosum. Cell proliferation technic like cell culture was not familiar and not studied systematically in the clinical field until the 1980s (Atala 2012). Major cell composite of corpus cavernosum is smooth muscle cells (SMCs) and endothelial cells (ECs). Researchers have successfully set up technics to culture and harvest purified SMCs and ECs (Carson et al. 1989).

Another wing of necessary component is biocompatible biomaterials. Ideal biomaterials would support the growth of cells, act as a scaffold of aimed structure, and avoid immune reactions to the seeded cells and implanted host. Biomaterials are classified into two categories. The first one is synthetic materials. Ideal properties of synthetic biomaterials would be biocompatible, nontoxic, nonantigenic, non-teratocarcinogenic. A lot of synthetic polymers were introduced as polyglycolic acid (PGA), polylactic acid (PLA), poly (lactic-co-glycolic acid): PLGA, poly (caprolactone: PCL), and so on. These synthetic materials have advantages such as production in large scale, reproduction, and availability of mixing components as enhancing or inhibiting factors and are also capable in quality control on specific demands. On the other hand, some synthetic polymers have limitations of their own as PLGA is not elastic enough for a scaffold in contractile tissues (Boland et al. 2006).

Another category would be natural materials like acellular matrices (ACM) from native tissue and decellularized bladder submucosa or small intestinal submucosa (SIS), collagen, alginate, hyaluronic acid (HA), elastin, and fibronectin derivatives manufactured from allogenic or xenogenic sources. The merits of natural materials are lower immune reaction and natural 3D architecture in case of ACM. Collagen has been one of the most popular substances of them. It can be derived from human and animals, and has been approved for clinical application from the US Food and Drug Administration (FDA). Collagen has been adopted in the production of various textures for its properties of low immunogenicity and low inflammatory responses. Another advantage of collagen is that it is one of the raw materials relatively easy in molding to certain shapes and structures. Also collagen is one of the main component of the ACM (Williams et al. 2016). About the safety issue, FDA approved collagen and PLA, PGA, PLGA as the materials applicable to humans in 1981. This was followed by approval of HA in 2003, Poly-L-lactic acid (PLLA) in 2004, Polymethylmethacrylate (PMMA), and calcium hydroxyapatite (CaHA) in 2006. Autologous fibroblast was approved in 2011. Studies have developed to combine natural materials with synthetic polymers as hybrid biomaterial on demand. One of the examples would be mixture of hybridizing a high molecular weight Poly (ε-caprolactone) (PCL) and type I collagen. This composite biomaterial was designed for the endurable material under the environment of high flow and pressure as in physiologic vascular status. This composite biomaterial showed improved mechanical properties as longer stability and provided better condition for vascular cells compared to scaffold made from single material (Lee et al. 2008). A variety of biomaterials have been tried for penile reconstruction using tissue engineering, synthetic materials as PLGA, natural materials as ACM, collagen, etc.

2 Corpus Cavernosum

Researches for reconstructing penile tissue using tissue engineering have progressed by the following steps: (1) establishing method and process of culture and expansion of necessary cells, SMCs and ECs, (2) animal studies of cell-biomaterial complexes using cells combined with synthetic biomaterial scaffolds, (3) animal studies of cell-corporal ACM complexes, and (4) replacing entire corporal tissue with cell-corporal ACM complexes in animal for the recovery of penile function.

The first step was constructing a tissue similar to corpus cavernosum from the cell-scaffold complex. In the 1980s technics of cell culture for implantation was not familiar and infrequently performed by biologists and clinicians. Human corporal SMCs, one of the major cells composing corpus cavernosum, was isolated from human corporal tissue using explant technic, then cultured and expanded in the cell culture chambers under the sterile condition. The PGA polymer scaffolds were manufactured as porous architecture for its durability and hydrolytic properties. These cultured SMCs were combined to the biodegradable PGA polymers. The SMCs-polymer complexes have been implanted at the subcutaneous space of rats. In 1998, Kershen et al. reported the results of a study about engineered tissue in vivo

using animals. The implanted cell-polymer complexes were harvested at 7, 14, and 24 days after surgery. Histologic analysis showed retrieved SMCs-PGA complexes formed smooth muscle cell layers and showed penetrating vessels into SMCs-PGA complexes from surrounding tissue, which was necessary for cell viability and development of cells into tissue. Alpha smooth muscle actin, the smooth muscle specific marker, was detected by Western blotting and immunocytochemical staining in these neo-tissues formed from cell-polymer complexes. The PGA scaffolds degraded with time in the rats as designed. The cultured cells in vitro combined with synthetic biodegradable polymer scaffolds developed into smooth muscular tissue in vivo. This was the first study on reconstruction of corpus cavernosal tissue using cultured human corporal SMCs seeded on biodegradable PGA polymers (Kershen et al. 2002).

Oxygen and nutrients are necessary in cell survival and reconstituting tissue from cells. These elements are delivered by blood. The sufficient vasculatures would be important to promote the reconstitution of tissue or organ from individual cells and to avoid necrosis and fibrosis of implants or grafts (de Vocht et al. 2018; Novosel et al. 2011). Surgical procedures result in disconnection of vascular supplies at the site of incision or implantations from surrounding native environments. The organic implants as cells or tissue need ingrowth of vessels from surrounding tissues into implanted tissues or autologous vascular formation from implants by themselves. One of the huddles in reviving cellular implants was to build abundant vasculatures around the cell-containing implants. ECs are the basic component of vasculatures and are necessary for vascular formation and it is another major composite of corpus cavernosum together with SMCs. Investigation for coexistence of SMCs and ECs in the implant complexes was attempted. Human SMCs and ECs (ECV 304) were cultured individually, and then seeded on PGA polymer scaffolds in stepwise manner. These SMCs- and ECs-seeded PGA polymer complexes were implanted in the skin of athymic nude mice. The basic process of these methods including cell preparation and seeding, are similar to various other applications of tissue engineering (Lanza 2002; Yoo et al. 1998b). Park et al. (1999) reported the construction of vascularized corporal tissue in vivo by combining human SMCs and ECs to PGA scaffolds. The implanted cell-PGA complexes were retrieved at 1–42 days after surgery and they showed well-organized smooth muscle architecture surrounded by the accumulation of endothelium lining the porous luminal structures and plentiful of neo-vascularities around the implanted complexes from 5 days of implantation. This study revealed that ECs had facilitated the ingrowth of vasculature from the surrounding native tissue and new capillary formation by itself, resulting in assisting reconstruction of corporal tissue with abundant vasculatures. This was the first study of the possibility in combination of different types of cells on a scaffold at a time and the way to supply enough oxygen and nutrients through neo-vasculatures using cultured ECs in vivo.

Studies stepped ahead to (1) introduction of new three-dimensional matrix for penile reconstruction, as ACM, (2) improved cellular distribution and attachment technics as dynamic seeding method applying bioreactors, and (3) developing method of total replacement of penis by cell–matrix complexes in vivo.

There was a need for more physiologic, biocompatible scaffold specific for corpus cavernosum. Manufacturing scaffold similar to the native cavernosal sinusoids was an obstacle. Pores of sponge-like corporal sinusoids vary in size and are irregular in distribution. Fabricated synthetic polymer scaffolds were not suitable for the natural corpus cavernosal architecture. Corpus cavernosum is composed of elastic fibers, collagen (mainly type I and part of type III) and SMCs, ECs (Lue 2016). Three-dimensional scaffold similar to corpus cavernosum was investigated. Decellularization method is removing any cells from tissue and results in acellular backbone structures as end product. Acellular Matrix (ACM) made of allogenic tissue has the merits of same meticulous microarchitecture as native properties, less immune responses, and contains some growth factors or cytokines that help in cellular growth compared to conventional synthetic biomaterials (Hoganson et al. 2010). In previous studies, acellular matrices were processed using animal bladder and urethra by cell lysis technic, then these ACMs were applied in animal for bladder and urethral reconstructions (Atala et al. 1999). Falke et al. (2003) processed donor rabbit corporal tissues to get corporal ACM using cell decellularization technic, which was the same method used in previous bladder or urethral tissue preparations. These products had the same architecture of corporal sinusoids avoid of cells as native corpus. Acellularity of these matrices was confirmed by histology and electron microscopy. They have cultured human SCMs, ECs, and then implanted 80 of ACM-human SMCs and ECs complexes in athymic nude mice. These implants were harvested from 3 days to 8 weeks after implantation. Retrieved implants were analyzed histologically, physiologically including organ bath study, scanning electron microscopy (SEM), and by molecular analyses including Western blot, RT PCR. The results showed the presence of neo-vascularities in the sinusoidal spaces of ACM with increased organization of smooth muscle and collagen over time. Analysis showed the characteristics of human SMCs and ECs in immunohistochemical studies (alpha-actin and factor VIII, each) and Western blot, and revealed expression of muscarinic acetylcholine receptor subtype m4 mRNA on RT-PCR in 8 weeks implants. In 4 weeks implants, ACM-cells complexes showed cell-covered sinusoidal architecture. Appropriate collagen composition was found with hydroxyproline quantification. Contractility was detected in harvested ACM-cells complexes on organ bath electrical field stimulation also. This study meant human SMCs- and ECs- seeded corporal ACM complexes constructed well-vascularized corporal structures similar to the native corporal body. ACM worked as a better substitute than conventional corporal scaffolds as cell delivery vehicle in vivo (Falke et al. 2003).

Kwon et al. (2002) replaced segments of rabbit corporal body with the cells-ACM complexes made in the same processes as mentioned above. They implanted 18 of ACM-SMCs and ECs complexes along with 8 of ACMs without cell as control into the rabbit penis. Rabbit corpus cavernosum was excised segmentally in 7 mm length leaving urethra intact, then cells-ACM complexes were interposed at the excised cavernosal sites. The rabbits sacrificed after 3, 6 months of implantation. Rabbit cavernosal structure and intracavernosal pressure were evaluated before retrieving cells-ACM complexes. They showed continuous visualization of cavernosum, this meant intact patent corpus cavernosal cylindrical architecture despite of corporal

replacement on cavernosography and improved intracavernosal pressure up to 50% of normal compared to null in control group (only ACM implantation) on cavernosometry. Histological analysis showed corporal sinusoids were covered with SMCs and ECs compared to fibrosis and calcifications in ACM-only group. Levels of eNOS and nNOS of cell-ACM complex group were similar to that of normal rabbit in Western blot analysis compared to decreased nitric oxide synthase activity in ACM without cell group. The rabbits implanted with the cells–matrix complexes mated 3 month after implantation and the sperms were detected from female rabbit after mating. This result meant tissue-engineered penile implant worked more physiologically, about 50% of normal and even showed ability to achieve the sufficient erection, vaginal penetration, and ejaculation. This study showed successful tissue-engineered autologous corporal tissue in structural and functional aspects, implying potentials for applying this technic into clinical penile reconstruction.

To improve the maturation of implanted cell–matrix complex, cellular distribution in the matrix is important. The methods were investigated for improving delivery, attachment, and growth of cells in the new environment of scaffolds (Hasan et al. 2014).

In normal instance, penis erects spontaneously for several times during sleep physiologically (nocturnal tumescence) without any sexual stimulations. Nocturnal tumescence is known as preventing fibrotic degeneration of cavernosal tissue and inducing angiogenesis in corpus by regular oxygenation of corpus cavernosum. Insufficient supply of oxygen and nutrients result in fibrosis and degeneration of tissue. Abundant vasculature around and inside the reconstituting structure helps cells to mature into organized tissue. Appropriate distribution of ECs into the scaffold together with the ECs on scaffold's surface would be ideal to form vascular structures than ECs residing on surface of scaffold only. Similar condition as nocturnal tumescence was designed during cell culture and cell seeding on biomaterials in vitro with the help of bioreactor (Eberli et al. 2008). Previous results of Kwon et al. (2002)'s trial was not satisfactory as intracavernosal pressure was less than normal in reconstructed corporal tissue in rabbit. It was assumed that one of the reasons for deficit intracavernosal pressure might be insufficient number of functional cells in the implanted ACM-cells complexes (Persson et al. 1989). Previous implants showed that cell densities in engineered reconstructed tissues were lesser than cells in normal corporal sinusoids. Increasing cell densities in the implants, especially SMCs, would improve intracavernosal pressure to the physiologic level. It was postulated that applying the hydrodynamic forces on solution containing cells would be advantageous in distributing cells into the irregular, narrow spaces by the form of cells in solution. Since 1990s, there have been studies about cell seeding technics and improved results were stated in the use of hydrodynamic forces, dynamic seeding, and bioreactor system (Burg et al. 2000; Xiao et al. 1999).

So Eberli et al. (2008) tried to improve the quantity and quality of cell viability and cell attachment on biomaterials for corpus cavernosum using bioreactor system consisting of a closed glass beaker and a magnetic stirrer with hydrodynamic forces. ECs and ACMs obtained from rabbits were processed as routine. EBM2 solution

containing rabbit ECs were saturated on ACMs in static manner or dynamic method. For dynamic seeding group, ECs-ACM complexes were cultured using bioreactors stirred in 40 rpm for 48 h. Each cell-ACM complexes were implanted in athymic mice subcutaneously. These implants were harvested and analyzed after 14 and 28 days of surgery. Specimens of dynamically seeded group showed better cell viability and better cell attachment compared to that of static group. Retrieved tissues of dynamically seeded group revealed enhanced tissue organization, higher cellular density, intact cellular lining of the sinusoids, and complete coverage of the sinusoidal space on immunohistochemical, histologic evaluations. MTT assay represents overall metabolic activity of 3D tissue, not a quantifying tool for counting cell number. It measures mitochondrial activity that would imply active metabolizing cells. Dynamic seeding group showed greater metabolic activity compared to static seeding group in MTT assay. Superior in cellular density was measured with DNA assay and also showed superior in the dynamically seeded ACMs than static group (71% versus 39% compared to the superior in cellular density of normal corpus cavernosum). Results of histology and SEM revealed structural superiority in the dynamic seeding group also. These results implied dynamic seeding using bioreactor could result in enhancement of cellular distribution, cellular differentiation improving tissue restoration.

Following Eberli et al. (2008)'s trial of dynamic seeding of ECs only, Falke et al. (2003) made the cell-seeded ACMs by dynamic seeding of dual cells, as SMs and ECs, and replaced short segment of rabbit corpus cavernosum with these ACM-autologous cells complexes. After the successful results of above trials, Chen et al. (2010) replaced total corpora with engineered tissue in the rabbits applying dynamic cell seeding. The basic procedure was similar to the previous studies using cells and biomaterials (Fig. 2) SMCs and ECs were prepared from biopsied rabbit corpus tissue. They processed ACMs from donor rabbit corpus by decellularization. Then they seeded these cells on ACMs in a manner of multistep static/dynamic seeding method for the purpose of homogenous cellular distribution in ACMs and to enhance cellular attachment. These cells-ACM complexes were implanted into excised penile space of 12 rabbits. Nonsurgical rabbit group and rabbits operated without implantation group were as control and comparison groups. Evaluations were made in 1, 3, 6 months after implantation. Cavernosography and cavernosometry showed normal shape of gross corpus cavernosum and normal intracavernosal pressure in cell–matrix complex group compared to nonsurgical group. On physiologic test using organ bath studies of muscle contractors, relaxants, and electrical stimulation, they could observe the contraction of implanted ACM-cell complexes by electric stimulates or pharmaceutics and these implied that penile nerve innervated into the implanted engineered corpus. Similar to previous trial in vivo, mating, ejaculation was observed in these engineered tissue implanted rabbits and gave birth to descendants. This research showed the development of penile tissue engineering achieved the tissue construction performing similar functions as normal animal (Chen et al. 2010). Even bearing problem of allogenic corporal ACM supply (Caplan et al. 2017), this technic implied potential of reconstructing penile tissue. Also this study triggered the need for the technics to

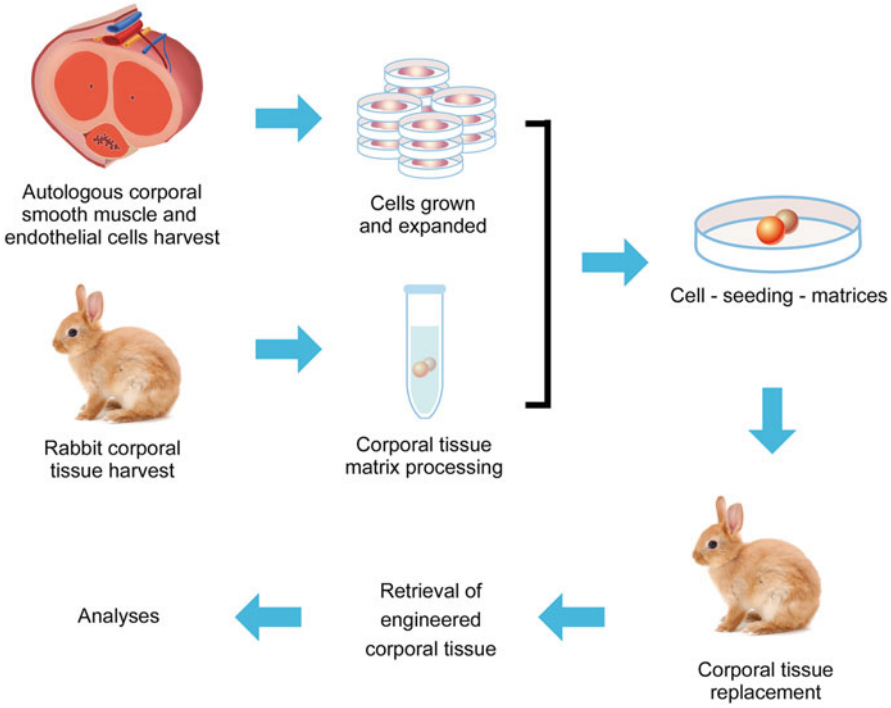

Fig. 2 Basic process of in vitro and in vivo study of cavernosal reconstruction

fabricate meticulous synthetic polymer scaffolds identical to natural corporal matrix along with ACMs.

There was a report of corporal ACM processed from human corpus cavernosum. They obtained human tissue under guidance of institutional review board approval and got informed consent from transgender patients before sex reassignment surgery. They decellularized human corpus cavernosum, then implanted human corporal ACM into the peritoneal cavity of Sprague–Dawley rats. Histologic evaluation revealed intact corporal sinusoidal structure with increasing vasculature around, ingrowth of SMCs and ECs from 1 month of implantation. The implanted ACM connected to the microcirculation of host rat and inward spread of host cells into ACM was observed. These results might imply that there was natural regenerating ability in the body, as the concept of natural bioreactor (Kajbafzadeh et al. 2017).

There have been clinical applications in several organs made by tissue engineering already. Clinical applications on bladder and urethra have been reported. Atala et al. (2006) applied tissue-engineered autologous bladder as cystoplasty clinically. They also reconstructed human urethra utilizing engineered tissue (Raya-Rivera et al. 2011).

In the long run, researchers are expecting that clinical trial of tissue-engineered penile tissue could be approved by FDA. Definitive rabbit study must be the first step for providing safety data. This study includes scaffolds preparation, cell isolation,

cell characterization, cell seeding, and characterization of seeded scaffold, implantation, and characterization in vivo. After confirming these studies, permission of human clinical trial from FDA would be expected. A Phase 1 clinical trial using autologous corporal cells was approved by the FDA and is being initiated to demonstrate safety and feasibility.

A lot of suggestions are proposed for the construction of the tissue-engineered penis closer to physiologic status. To create corporal tissue from cells-scaffold complexes efficiently, we expect the developments in several aspects, proper distribution of cells with help of bioreactors or 3D bioprinting, methods of collaborating pharmaceutical compounds, cytokines or growth factors aiding tissue maturation from seeded cells, methods of enriching vasculature into the cell-scaffold complexes, introduction of modified cells including stem cells (SCs), or applying DNA or gene therapies.

3 Penile Prosthesis

Another issue of penile reconstruction is penile prosthesis. Inserting penile prosthesis is the definitive treatment of erectile dysfunction (Levine et al. 2016). Initially, the concept of penile prosthesis was for the structural buttress in reconstructing damaged penis aside of erectile functions. Bone or cartilages as rib covered with autologous musculofascial tissue was applied to form penis-like construct for substitution of damaged penis. Following the introduction of inflatable penile prosthesis (Scott et al. 1973), a variety of penile prosthesis has developed around 1980s to treat erectile dysfunction. Currently available penile prosthesis is classified as malleable type and inflatable type. Penile prosthesis implantation: past, present, and future (Simmons and Montague 2008). Malleable penile prosthesis is semirigid and simply bendable with the spring compartment in the prosthesis. The merits of malleable type are its lower incidence of mechanical failure and it is less expensive than inflatable prosthesis. But implanted penis is not natural and it is difficult to conceal for its constant semirigid property. Complications as erosion and protrusion of implantation might occur. Inflatable prosthesis includes two-pieces, three-pieces, and positionable prosthesis. These provide better tactile sensation as normal penis and these can be deflated when not using. But mechanical dysfunction is more frequent than malleable type and is more expensive. Major composition of this conventional prosthesis is silicon. Prosthesis made of silicon is widely used but still have some drawbacks as biocompatibility, infection, erosion, mechanical problems like autoinflation, leakage, and pump malfunction (Muench 2013; Nukui et al. 1997). Yoo et al. (1998a) reported tissue-engineered penile prosthesis. They created penile prosthetic material using cartilage cells. Harvesting articular cartilage tissue from calf, chondrocytes were isolated from these cartilages, and then cells were culture-expanded. These culture-multiplied chondrocytes were seeded on rod-shaped PGA polymer scaffolds. These chondrocyte-PGA polymer complexes were implanted at the back of athymic mice along with polymer without cell implant group as control. Chondrocyte-PGA polymer complexes have formed in milky solid cartilaginous rod compared to

shrinkage of hydrolyzed polymers without cell (Yoo et al. 1998a). As a next study, cartilage was harvested from rabbit ear, isolated chondrocytes, and expanded in culture. These autologous chondrocytes were seeded on polymer rods and implanted in the rabbit corpus compared to implanting polymers without cell. One month later, implants were retrieved and cell-seeded polymers formed milky cartilage rods. On the other hand, implants of polymer without cell degraded in 2 months after surgery. The cell–polymer complex implanted rabbits could copulate and impregnate female rabbits (Yoo et al. 1999). Investigation of mechanical properties of engineered cartilage rod was taken for the possibility in clinical application. Cartilage was obtained from human ear and chondrocytes were isolated. Cultured chondrocytes were seeded on polymer rods in the same manner as the previous experiments and harvested after 2 months. Mechanical properties were evaluated in harvested implants. These cartilaginous tissues were flexible, elastic, and endurable to high degrees of compressive pressure that might have had potential for pressure durability in vaginal penetration during intercourse. These mechanical properties were comparable to silicone prosthesis used in clinical field (Kim et al. 2002). These results showed tissue-engineered cartilage tissue would have potential in application to malleable prosthesis.

In general, biomaterials originated from natural tissue might have merits of tissue friendly properties as lower immune reactions. So these studies opened the possibility of developing penile prosthesis with ideal mechanical properties and biocompatibility: as semirigid penile prosthesis made of cartilage equipped with spring components inside, bi-layered inflatable prosthesis as outer biocompatible tissue layer covering inner synthetic biomaterial as silicon regarding the virtue of the materials, or designing single layered prosthesis made of material combining natural and synthetic biomaterials. The technologies of 3D bioprinting could assist the precise design of engineered penile prosthesis.

4 Tunica Albuginea

Tunica albuginea (TA) is a relatively firm, elastic tissue covering corpus cavernosum. TA is a two-layered structure of outer longitudinal and inner circular layer with 1.5–3 mm thickness depending on its location. The main composition of TA is known as type I and type III collagen fibers nesting on elastin fibers (Wein et al. 2016). In case of penile injuries involving TA as penile fracture, old age, or Peyronie's disease (PD), malfunction of TA result in the so-called venogenic impotence. Penile fracture is the laceration of TA arising from exacerbating penile bending by external forces. It is one of the emergent conditions to be operated promptly.

PD is a pathologic condition of formation of fibrous plaque on TA. The fibrous plaque shows abundant abnormal collagen and elastin tissue (Davis Jr. 1997). The cause is uncertain. Blunt trauma as excessive bending of penis intolerable to elastic limit of TA would be suspected. It is estimated that TGF-β1 plays a major role in pathogenesis of Peyronie's plaque (Hinz 2015). The incidence of PD is estimated to

be about 1–13%. PD could develop penile deformities as curvature, penile shortening, and pain during erection and even erectile dysfunction. Spontaneous resolution of penile deformity in PD occurs only in 3.2–12% of patients. The Impact of Peyronie's Disease on the Patient: Gaps in Our Current Understanding (Goldstein et al. 2016).

Kinds of conservative medical treatments have been tried for PD: cold massage, compression, oral medications as vitamin E, potassium aminobenzoate, tamoxifen, colchicine, analgesics, anti-inflammatories, vasodilators, intralesional injections as steroid, calcium antagonists, vasodilators, interferon or collagenase enzymes (Abdel Raheem et al. 2017), botulinum toxin (Munoz-Rangel et al. 2015) vaccum device, extracorporeal shockwave therapy (ESWT), and combination of some of these (Tan et al. 2014). Results of current medical therapies have not been successful. Surgical correction is the effective treatment modality of PD (Guillot-Tantay et al. 2014). Definite treatment of PD would be elimination of fibrous plaque on TA. Surgeons have tried to correct PD using incision and plication of plaque and complete excision of plaque followed by grafting with various materials depending on size and location of plaque (Perovic and Djordjevic 2001).

Incision without removing plaque usually resulted in shortening of penile length. Patch grafts after excision of plaque has been tried for compromising shortening of penis. Several materials have been applied for a tunical graft, usually made by autologous, allogenic natural biomaterials. These were tunica vaginalis, SIS, veins allogenic pericardium, dermal graft, and muscle fasciae, depending on the defect size of the excised plaque (Chun et al. 2001; Kadioglu et al. 2007; Montorsi et al. 2003). However these materials also had some drawbacks as donor site complications, shrinkage of graft, and availability of material supplies, hence alternatives are needed. Surgeons needed efficient, biocompatible, and off-the-shelf graft materials.

Porcine vesical ACM was evaluated for graft material of TA. ACMs were obtained from porcine bladder by decellularization. Histologic analysis using SEM and RT-PCR assay about growth factors that would assist tissue regeneration were made. SEM showed fibrous collagen tissue of various sizes of pores. Vascular endothelial growth factor (VEGF) receptor, FGF-1 receptor and neuregulin mRNA were detected in ACMs by RT-PCR. These ACMs were implanted to TA of rabbits and the ACM implants were harvested after 2 months. Histologic analysis revealed grafted ACM was linked to neighboring native TA without serious inflammatory reaction. There was minimal contracture in grafted ACM and restored ACM-graft tissue on corpus cavernosum was not different from native TA histologically after 6 months of implantation. It implied the possibility of porcine vesical ACM as a graft material of TA (Joo et al. 2006).

Schultheiss et al. (2004) reported efficacy of dynamic seeding of fibroblasts in vitro compared to static seeding technic in constructing tunical collagen tissue as for graft material of TA. They biopsied porcine fascia and isolated fibroblasts. Collagen matrices were prepared using decellularization technic. Cells were seeded on these matrices. In dynamic seeding group, the fibroblasts–matrix complexes were cultured in a bioreactor under the multiaxial stress for 3 weeks. Static cultures were done in other group of cell–matrix complexes as control. Results after 7 days showed

better cell array, better cell infiltration into the matrix, and formation of multilayered tissue superior to static culture group in dynamic group. Also dynamically cultured fibroblasts produced more extracellular matrix proteins than that of static culture group.

TGF-β1 is known as enhancing Peyronie's plaque. Most of the animal models of Peyronie's disease were inducted using TGF-β1 treatment. Castiglione et al. applied stem cell (SC) therapy in PD rats. They made the rat model of PD with TGF-β1 treatment and then injected adipose tissue–derived stem cells (ADSCs) at Peyronie's plaque intralesionally at the active phase of PD along with no injection of ADSCs as control group. Cavernosometry and histologic analysis was made. Compared to sham-operated group and control group, which showed increased abnormal collagen III and elastin proteins on histologic analysis, ADSC injected rats revealed increased intracavernosal pressure and prevention of fibrotic changes of PD plaques. This was the first trial of SC injection therapy on PD animal (Castiglione et al. 2013; Shindel 2013).

Lander et al. (2016) reported the results of clinical trial using adipose stromal vascular fraction (SVF) combined with ESWT in PD patients. They evaluated the effect and safety of intralesional injection of SVF into fibrous plaque followed by ESWT in 11 PD patients. The results were decrease of plaque size, improved penile curvature, and erectile function.

5 Penile Augmentation

Penile augmentation is another issue regarding reconstruction of penis. Concept of penile surgery as augmentation is not familiar yet. This procedure is still on debate as a controversial issue, so is not a recommended medical procedure by most of the medical authorities. Uncertain indications for surgery, absence of standardization of procedures, lack of guidelines, and lack of evidence-based studies could be the reasons (Park 2016).

At present, the only positive consensus for this procedure would be in the cases of congenital problems of penis. There are congenital problems as micropenis, penile hypoplasia, or hermaphroditism (Gillies 1948; Horton et al. 1987; Johnston 1974; Snyder 1964; Woo et al. 1996). Similar situation happens in traumatic total loss of penis and genial thermal burn (Hotchkiss et al. 1956).

Deformity or decrease in length or girth of penis occurs after prostatectomy, pelvic surgeries, PD, and other penile diseases. Men who underwent radical prostatectomy and possibly radiation therapy and hormonal treatment are susceptible to penile shortening (Kabalin et al. 1990). Some of these patients feel depression, anxiety, locker room syndrome, and represent erectile dysfunction (Dillon et al. 2008; Pietropinto 1986; Wylie and Eardley 2007).

Another example would be as the penile dysmorphophobia (Spyropoulos et al. 2005). This medical problem occurs when a man is dissatisfied with his penile size despite anatomically normal developed penis. Penile augmentation is helpful in some patients psychologically. Also a lot of evidence has been found historically

that there is a tendency to relate the size of the penis to the self-esteem or potency of sexual power, the so-called phallocentrism (Cheng et al. 2007; Lever et al. 2006; Thompson et al. 1999; Wylie and Eardley 2007). Because of this myth, various methods were tried to enlarge penis for ages as traction, massage on penis, foreign body insertion, herbal medicines, and snake poison, which proved to be not only ineffective but also harmful.

Medical treatments were not able to achieve satisfactory results, and surgical methods were introduced (Nugteren et al. 2010). Development of penile augmentation technic has close relationship with penile reconstruction, PD, graft or flap procedures, and materials used in tissue engineering. Documents revealed attempts made to augment penile girth using fat graft since 1893 by Neuber. Also there have been various surgical procedures for penile lengthening procedures. Experiencing unexpected complications such as infection and graft failures, through trials and errors, technics and materials have developed (Vardi et al. 2008; Wassermann and Greenwald 1995). Various materials have been applied for penile augmentation as bone, ivory, metal, silicon, artificial oil forms of Vaseline, paraffin, silicon, collagen and fat, dermis, fascia, cartilage, pericardium, and so on. Some of them resulted in serious complications and they are not used anymore (De Siati et al. 2013; Moon et al. 2003; Park 1991; Sukop et al. 2013).

Nowadays synthetic biocompatible materials or autologous, allograft, and xenograft natural materials are used in augmentation penoplasty, as are similar to the materials used in tissue engineering (Austoni et al. 2002; Perovic and Djordjevic 2000).

Examples of synthetic biomaterials used in penile augmentation are calcium hydroxyapatite, polyacrylamide, silicone, and PLGA. HA. Silicon and PLGA are used as fillers also.

Examples of xenogenic materials are dermis, SIS, pericardium of bovine or porcine origin, which have stable structures and lower immunologic reactions on host. There are several commercial xenogenic products as porcine dermal collagen tissue, Permacol® (Covidien, Mansfield, USA). Lyoplant® (B. Braun Aesculap, Tuttlingen, Germany) is a collagen implant made from acellular bovine pericardium. MegaDerm Ultra® (L&C BIO Inc., Seongnamsi, Korea) is derived from porcine dermis. InteXen® (American Medical Systems, Minnetonka, MN, USA) is made from porcine acellular dermal matrix. Xenogenic materials have been used in various clinical fields. Acellular dermal matrix from fetal bovine dermis, SurgiMend® (TEI Biosciences Inc., Boston, USA), has been used for hernia repair. Porcine dermal ACM is applied clinically for penile girth augmentation (Alei et al. 2012). Xenogenic type I collagen was applied clinically in penile augmentation also (Kim 2013). Permacol has been applied as a graft material for TA in PD patients also.

Allogenic materials as acellular dermal matrix are processed from human source, as ACM (Alloderm: LifeCell Inc., Branchburg, USA) from human cadaver skin, MegaDerm® (L&C BIO, Korea), and SureDerm® (Hans Biomed, Korea). AlloDerm® has been applied for treatment in burn, breast reconstruction, hernia repair, and other reconstructive procedures (Gaertner et al. 2007), and this was applied as grafting material in penile girth enhancement along with other allogenic materials

(Solomon et al. 2013). Collagen products are xenogenic or allogenic. Allogenic collagen material is derived from human fibroblasts. One of the xenogenic collagen product approved by the US FDA in 2008 is Evolence® made from swine tendon. Xenogenic product showed higher allergic reaction than allogenic one but the adverse events were usually mild and uncommon.

Compared to penile lengthening that mostly depends on surgical skills and tissue rearrangement as suspensory ligament release, Z plasty, V-Y advanced plasty, or penile disassembly technic, a variety of materials are interposed in augmentation of glans and penile girth. The materials used at present include allogenic materials as Alloderm®, xenogenic materials as Lyoplants®, autologous fat, dermal fat graft, fascia, pedicled graft, and fillers made of silicon, PLGA, HA, etc. HA is a natural component of human skin, thus host immune reaction is far rare. Kwak et al. (2011) reported clinical results of penile girth enhancement using injectable hyaluronic acid gel. HA gel injection has been used to glans augmentation clinically (Kim et al. 2003).

Complications were infection, edema, ulceration, skin necrosis, absorption of implants, seroma, epidermal cyst, uneven skin, penile deformity including asymmetry or curvature, calcification, and so on. The most serious complication of penile augmentation would be infection. Solomon et al. reported that patients using allograft materials showed 42% postoperative infection rate (Plaza and Lautenschlager 2010; Solomon et al. 2013).

Female to male transsexual surgery needs neo-penis construction (Laub et al. 1989). Various pedicled flaps have been tried in phalloplasty (Bettocchi et al. 2005). Tubed groin flap with hydraulic inflation device (Puckett and Montie 1978), island tensor fascia lata flap (Santanelli and Scuderi 2000) radial artery-based forearm free flap (Garaffa et al. 2010) had been used for phalloplasty in transsexual surgery.

6 Recent Novel Technics

6.1 Cell Therapy and Stem Cell Therapy in ED

The position of the penis is easily accessible for injection therapy. In 1979, Green et al. proposed clinical application of cell therapy as cultured human cells for grafting. One of the considerations would be hemodynamic change of the cavernosal tissue. Continuous circulation results in washing the injected materials out from corporal tissue. The method of anchoring cells inside corpus cavernosum for the appropriate duration would be the task.

Various cells have been introduced to reconstruct penile tissue using tissue engineering technic for the physiologically compatible to native tissue. Mature adult cells have several drawbacks as inability to differentiate into different cell types and low proliferative capacity in culture and expansion (Atala 1998). To overcome the immunologic issues and to produce enough tissue easily, stem cells (SCs) were investigated as an available source of off-shelf tissue production.

The merits of SC are potential of "homing" differentiation as differentiating into target tissue, self-renewal, capacity of differentiating potentials to various cell types, capability of extensive proliferation, and secreting various cytokines (Chamberlain et al. 2007). Martin (1981) reported about the existence of SC in mouse embryo. At first it was thought that SCs resided only in the embryonic tissues, so researches in human SCs were difficult for ethical issue. Nevertheless Thomson et al. (1998) reported about the human embryonic stem cells (ESCs). ESCs are pluripotent that can be differentiated into all cell types of the three germinal layers through embryonic developments. ECSs are known as provoking immune reaction and having teratogenic potential. Researches about human ESCs have barrier for the ethical issue also. There are multipotent stem cells as adult SCs which differentiate confined to the same native germinal cell types. The merit of adult SCs would be nonimmune reactive and low risk of oncogenic potential. One of the variant SCs is stromal vascular fraction cells (SVFCs). SVFCs are derived from autologous adipose tissue, which contains Adipose-derived stem cells (ADSCs), endothelial progentior cells (EPCs), and various other cells (Bora and Majumdar 2017).

SCs are divided into three categories based on their differentiation capacity as totipotential, pluripotential, and multipotential SCs (Mahla 2016). Also SCs are classified by their origin. 1) ESC derived from the inner cell mass of a blastocyst. ESC is pluripotent; 2) SCs from amniotic fluid, placenta, and umbilical cord blood. They are multipotent and have partially differentiated cells and express both markers of embryonic and adult SCs (Williams et al. 2016); 3) Adult stem cell (ASC) derived from various adult tissue as neural crest SCs (NCSCs), epithelial SCs, mesenchymal SCs (MSCs) from bone marrow, muscle (MDSCs), or adipose tissue (ADSCs), urine, and so on. MSCs have paracrine effect as releasing various cytokines and growth factors. ASC is multipotent (Alwaal et al. 2015; Gimble et al. 2007; Volarevic et al. 2011). One of the SCs frequently adopted in researches is ADSCs. The source of ADSCs is adipose tissue, which is relatively convenient to obtain by subcutaneous liposuction which is a less invasive procedure compared to harvesting other adipose tissues (Zuk et al. 2001).

Novel concepts were reported about transforming multipotent ASCs into pluripotent SCs. Takahashi and Yamanaka (2006) reported about reprogrammed mouse fibroblast into induced pluripotent state, called as iPS. These cells showed immortal growth, proliferated into embryonic bodies in vitro and teratoma in vivo.

There are various sources of SCs in our body including penis (Lin et al. 2015). These SCs repair the injured tissue and keep homeostatic tissue environment (Xin et al. 2016). These innate SCs reside with supporting cells and extracellular matrix. They are connected and communicate with each other through signal molecules. This microenvironment is called SC niche (Moore and Lemischka 2006). Mobilization of innate SCs to target tissue and activation of innate SCs would be helpful in regeneration of tissue, apart from extrinsic supply of SCs as injecting cells. Several molecular factors have been known to help mobilizing SCs into blood stream for target tissue, as granulocyte colony-stimulating factor (Deng et al. 2011). Activation of p38 pathway is important in cellular differentiation of SCs (Jones et al. 2005). Chemicals as icariside II activate p38 pathway. Icariside II has been tried on

cavernosal nerve injury rat model and diabetic rat model, resulting in improved erectile function (Qiu et al. 2013; Xu et al. 2015). Investigators mentioned about the effect of ESWT in regeneration and healing process. It has been proposed that ESWT enhance activation of SCs, angiogenesis, reduce inflammatory reaction, and proliferate and differentiate cells (Aicher et al. 2006; Mittermayr et al. 2012).

There have been lots of preclinical studies applying SCs for treatment of ED and for regeneration of corporal tissue using animal models. Most of the reports showed established efficacy of SCs (Orabi et al. 2012; Soebadi et al. 2017). Song et al. (2009) reported SMCs derived from the other parts of the body could be used in penile corporal tissue regeneration. They implanted SMCs derived from the human umbilical artery-seeded ACM in athymic mice subcutaneously. The SMCs from umbilical artery, not identical to corporal SMCs, grew in tissue similar to native corpus cavernosum histologically. Laks et al. (2015) investigated the effectiveness of tissue regeneration depending on the routes of SCs delivery. They derived the MSCs from rabbit bone marrow and delivered these cells by intravenous infusion or by implantation in the form of ACM-MSCs complexes in rabbits along with no cell seeding group. Cavernosography showed that dye-filled entire corpus cavernosum of rabbits treated with intravenous infusion of MSCs and cell-ACM complexes implanted rabbits compared to filling defects of corporal tissue in control group rabbits. Cavernosometry also revealed higher intracavernosal pressure in both cell-delivered groups than control group. Implants of MSC with ACM showed organization into partial sinusoidal structure (Laks et al. 2015). In another study, MSCs derived from rabbit was seeded on ACM and then implanted at the excised corporal space of rabbits along with the control group as implants without cells. MSCs seeded ACM grew into tissues similar to natural corpus sinusoids architecture and enriched smooth muscle and increased NO synthetic activity compared to ACM without cell-implanted group in 6 months after implantation. These results meant the implanted MSCs differentiated into SMCs and ECs successfully in vivo (Ji et al. 2011).

Investigators explored the effects of SCs in treating plaque of PD. Simple SCs, or combination with interferon or gene-transfected SCs were injected into the fibrous plaque of PD in rats. These also showed changes of composition of collagen and elastin proteins, increase of NOSs and cGMP, growth factors, and improved erectile function (Levy et al. 2015; Song et al. 2008). Gokce et al. reported series of studies of SC therapy for Peyronie's plaque and ED. They have injected ADSCs alone or genetically modified ADSCs with human interferon alpha-2b intratunically in rats. TGF-β1, known as the provoking factor of fibrotic plaque on TA, was injected to these rats. Decreased collagen deposition and less fibrotic plaque formation as well as better intracavernosal pressure were noted in both ADSCs treated groups after 6 weeks. This result showed the ADSCs were effective in preventing formation of Peyronie's plaque and prevented decrease of erectile function related to PD (Gokce et al. 2015; Gokce et al. 2016). Another study was using a combination of ADSCs and SIS. Researchers prepared SIS seeded with ADSCs and implanted into rat TA. Compared to implanting SIS alone, SIS-ADSCs implants showed elevation of NOS activation, inhibition of fibrosis, improved angiogenesis, and resulted in preservation of corporal tissue and improved erectile function (Ma et al. 2012).

Castiglione et al. (2019) employed SVF injected intratunically to prevent fibrosis of PD in rat model.

Researchers used established diabetic rat model, cavernous nerve injury model, PD model, and old rats as similar to clinical ED etiologies for disease-specific investigations. ADSCs alone or combined with insulin or interferon alpha-2b, BMSCs alone or combined with extracorporeal shock wave therapy (ESWT), human urine-derived SCs c growth factors, MDSCs, have been studied on diabetic rat models (Qiu et al. 2013; Ryu et al. 2016; Sun et al. 2012; Zhou et al. 2016). ESWT has been known as stimulants for angiogenesis and endogenous SC activation (Qiu et al. 2013; Vardi et al. 2012) (Table 1). Jeon et al. (2016) reported improved erectile function treated with human ADSCs with ESWT in cavernosal nerve injury rat model. Shan et al. (2017) reported the improved erectile function with treatment of ESWT plus bone marrow MSCs in diabetes mellitus (DM) rat model. SVF from human breast adipose tissue was applied for diabetic ED rats. This xenogenic SVF resulted in increase of SMCs, ECs, neo-vascularization, eNOS, and nerve regeneration (Das et al. 2014).

For application to the nerve-damaged ED patients after radical prostatectomy or lower abdominal surgery as for colon cancer, cavernosal nerve injured models were used (Mangir and Turkeri 2017). ADSCs, BMSCs, MSCs, urine-derived SCs, and SVF alone or combined with various growth factors, PDE5I, graft, or ESWT were treated in animal models (Alwaal et al. 2015). Miyamoto et al. implanted CD133+ cells derived from human bone marrow in cavernosal nerve injury rat model. The results revealed regeneration of excised cavernosal nerve and increase of nerve-derived growth factors and cytokines, and this might be applied in ED patients after prostatectomy (Miyamoto et al. 2014). There were reports about combined transplantation of MSC and endothelial progenitor cells for restoration of injured cavernous nerve (Fang et al. 2018). Similar to ED after prostatectomy, radiation around genital area might result in ED. ADSCs were treated in radiation-induced ED rat model. The result showed increased SMCs and nNOS in corporal tissue, and improved erectile function (Qiu et al. 2012) (Fig. 3).

Intracavernosal injection of SCs, instead of intralesional application on exact location of lesion, also restored remote defected nerve function. Intracavernosal injection of ADSCs improved erectile function in rat model by modulating pelvic ganglion. This result implied recruitment of injected SCs in pelvic ganglion (Fandel et al. 2012). Another report by Matsuda et al. was that intravenous injection of bone marrow–derived MSCs improved erectile function in rat cavernosal nerve injury model (Matsuda et al. 2018).

Table 1 Summary of papers about ESWT

	Mediator	Effect
Nishida et al. (2004)	VEGF, VEGF-R	Angiogenesis
Aicher et al. (2006)	Stem cell-derived factor-1 (SDF-1)	Stem cell activation & recruitment
Ha et al. (2013)	Erk 1/2, nitric oxide synthase	Angiogenesis, immune-reaction
Weihs et al. (2014)	ATP, Erk 1/2	Cell division, stem cell activation

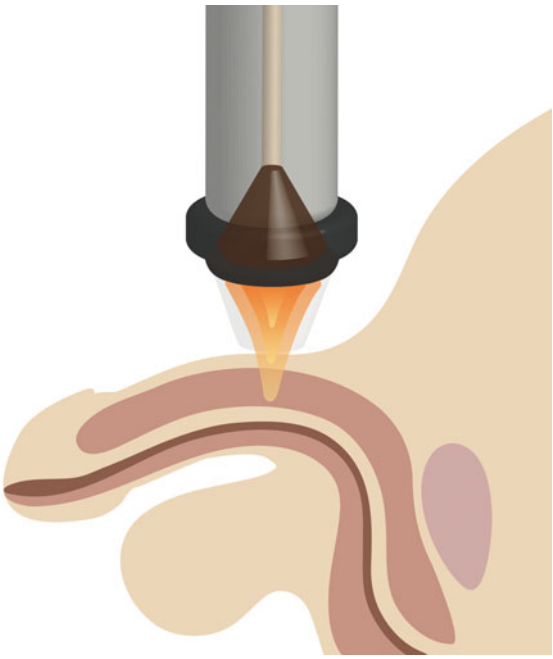

Fig. 3 Effect of ESWT. VEGF↑ → angiogenesis. Activate & recruit circulating or resident stem cells. Activate nerve cells.

Most of the studies showed improved histologic composition of corpus cavernosum; cavernosal nerve regeneration; increased cavernosal eNOS, nNOS, and vasculogenic growth factors; intracavernosal pressure; and restoration of erectile function (Albersen et al. 2010; Jeon et al. 2016; Kendirci et al. 2010; Ryu et al. 2014; Song et al. 2014). The most obvious etiologic factor of erectile dysfunction is aging. Similar studies were performed with SCs in old rats and the results were successful also (Bivalacqua et al. 2007; Liu et al. 2017).

SCs have been applied in glans reconstructing experiment. Egydio et al. (2015) processed human glans matrix by decellularization. MSCs were obtained from rats and these cells were seeded on human glans ACM in static manner. After 2 weeks in vitro culture, cells maintained their integrity and viability up to 2 weeks.

Several clinical applications of SC therapy have been already reported (Capogrosso et al. 2018). The first clinical trial might have started in 2010. Bahk et al. (2010) injected a total of 1.5 × 107 human umbilical cord blood SCs into corpus cavernosum of type 2 diabetic ED patients. No immunosuppressive measures were taken in any of the patients. Morning erections were regained in three participants within 1 month, and for all except one by the third month, and maintained for more than 6 months. Rigidity increased as a result of SCs alone, but was insufficient for penetration. With the addition of PDE5 inhibitor before coitus, two achieved penetration and experienced orgasm, and maintained for more than 6 months. Blood glucose levels decreased by 2 weeks, and medication dosages were reduced in all but one subject for 4–7 months. Glycosylated hemoglobin levels improved after

treatment for up to 3–4 months (Bahk et al. 2010). Afterward several open label, phase I clinical trials were made. Haahr et al. (2018) reported the trial of intracavernosal injection of autologous adipose-derived regenerative cells in 17 patients with erectile dysfunction following radical prostatectomy as a phase I clinical trial, and they presented 12 months follow-up data of this trial in 2018. This clinical data showed 8 out of 15 (53%) ED patients after radical prostatectomy showed improvement of erectile function in self-evaluating questionnaire, International Index of Erectile Function (IIEF) – 5 score, enough to intercourse after 12 months of SCs injection. There was no report of serious side effects (Haahr et al. 2018). Another pilot clinical trial was conducted in 2016. Autologous bone marrow–derived mononucleated cells were injected into corpus cavernosum to patients of ED undergone prostatectomy. They showed improved erectile function on IIEF symptom score and IIEF-EF domains without serious side effects. Improved erectile function was sustained until the mean follow-up point 62.1 months, aside the some decrease of IIEF scores in 62.1 months compared to the IIEF scores of 12 months after injection. Repeated treatment would be needed for maintaining improved erectile function (Yiou et al. 2017; Yiou et al. 2016). Al Demour et al. (2018) reported the open labeled phase I clinical trial of SCs for ED patients by DM. They delivered autologous bone marrow–derived MSCs (BM-MSCs) by intracavernosal injection to 4 refractory ED patients. Though the number of patients was small, the results showed improved sexual desire and sexual satisfaction in IIEF questionnaire. Also there were no tolerability and safety issues.

SC therapy was tried in PD on human also. Levy et al. (2015) attempted to treat PD using placental matrix–derived MSCs (PM-MSCs) in 2015. Seven out of 10 plaques of PD disappeared 3 months after SC injection. This study was the first human trial of SC therapy in PD patients. In succession, Levy et al. (2016) applied PM-MSCs to ED patients. Eight ED patients not treatable with PDE5I were enrolled in phase I clinical trial. Patients were injected PM-MSCs and followed up for 6 months with penile Duplex ultrasonography and IIEF symptom score. Patients showed improved IIEF symptom scores and increased penile arterial blood flow including peak systolic velocity. Lander et al. (2016) reported the results of clinical trial using adipose SVF combined with ESWT in 11 PD patients in 2016. The results were decrease of plaque size, improved penile curvature, and erectile function.

There are several chemical compounds enhancing the function of SCs. One of these medications is metformin, which has been used for treatment of DM approved by FDA. Administration of metformin improved endothelial progenitor cell function (Fatt et al. 2015). Phase I-II clinical trials are undergoing and some of them reported the results. Nitric oxide (NO) is a corner stone in erection. Topical gel containing glyceryl trinitrate (NO doner) was tested in 232 ED patients in phase II randomized trial. The effect of this compound is known as increasing penile blood flow, and 23.1% of patients reported improved IIEF-EF score with minimal side effects (Ralph et al. 2017). Other chemicals under clinical phase I-II trial for treatment of ED are arginine aspartate combined with adenosine monophosphate with 26 patients (Neuzillet et al. 2013) and mirabegron, the β-3 adrenoreceptor agonist dilating vessels in corpus cavernosum with 20 patients (Gur et al. 2016), and Botulinum

neurotoxin-A injection therapy in 24 patients with vasculogenic ED. For the treatment of premature ejaculation, serotonin-related medications as SSRIs, compounds related to oxytocin metabolism, and folic acid are used. SCs combined with these compounds would develop the efficacy of treating ED using tissue engineering.

Combining stem cells and gene therapy would be one of the promising options in improving formation of tissue-engineered architecture. Still some investigators worry about the safety issues as oncogenic possibility that should be confirmed for clinical applications (Marks et al. 2017).

6.2 Gene Therapy for ED

Erectile dysfunction (ED) is induced by abnormal relaxation of corpus cavernosum in short. Mechanism of relaxation of corpus smooth muscle are related to various factors such as endothelial cells, neurons, nitric oxide synthase, molecules like nitric oxide, cGMP, PDE5 enzyme, calcium ions and potassium channels, RhoA/Rho-kinase pathways, etc. Aging impairs the function of tissues related to erection and similar deterioration occurs with pathologic conditions like DM, hypertension, metabolic syndrome, and surgeries around low abdomen and genital area. Sildenafil, introduced in clinical field in 1998, and other phosphodiesterase 5 inhibitors (PDE5Is) showed effect in 70–80% of ED patients but remainder of ED patients did not improve with PDE5Is. Even though guidelines recommend PDE5Is as the first-line therapy for ED, successful result of PDE5Is decrease in DM-related ED patients compared to non-DM ED patients and PDE5Is are contraindicated in patients taking medication containing NO and patients with serious cardiac disease because of the risk of hypotensive crisis. Investigations of gene therapy for ED patients includes the ideas of avoiding serious side effects of PDE5Is and alternative therapeutic modalities.

Attempts of gene therapy in ED were reported in 1990s (Christ et al. 1998; Garban et al. 1997). Since then, researchers have struggled to restore damaged functions of endothelium (growth factors as VEGF, IGF-1, cGMP, modified stem cells, DNA, RNA transfection, and gene transfer), cell to cell interaction (potassium channel, calcium channel), cavernosal fibrosis (Wnt signaling pathway, Maxi-K channel), study about gene silencing or gene augmentation using various methods conjoining vectors to deliver specific genes, growth factors or electroporation in diseased animal models (Soebadi et al. 2016).

Among viral vectors, adenovirus has been accepted as superior to other viral vectors. Recently nanoparticles have been applied in delivering genes instead of the viral vectors for effectiveness and avoiding immune reactions (Gur et al. 2018) (Fig. 4). There had been several reports about the application of labeling nanoparticles to SCs (Neri et al. 2008). The position of the penis is easily accessible for injection therapy. One of the considerations would be hemodynamic change of the cavernosal tissue. Continuous circulation results in washing the injected materials out from corporal tissue. The method of anchoring cells inside corpus cavernosum for the appropriate duration would be the task. Lin et al. reported improved erectile

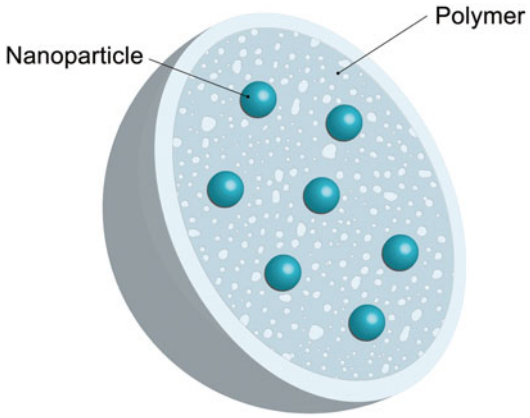

Fig. 4 Schematic figure of polymer–nanoparticle compound

function using nanoparticle with SCs in cavernosal nerve injured rat model. In order to guide the SCs to the target site and prevent the migration of injected ADSCs, they bound ADSCs with Nanoshuttle magnetic nanoparticles. Properties of ADSCs bound to nanoparticle have been maintained. These nanoparticle-ADSCs complexes were injected into corpus cavernosum of nerve injured rats, and then magnetic forces were applied for "homing" cells in one group. The nanoparticle-ADSCs injected group showed improved intracavernosal pressure, and differentiation into SMCs and ECs well. About migration of injected SCs, nanoparticle-ADSCs were staying in corpus cavernosum up to 25 days after injection (Lin et al. 2016). Zhu et al. (2017) studied another kind of nanoparticles with ADSCs. Superparamagnetic iron oxide nanoparticles (SPIONs) were labeled to ADSCs. SPIONs have been conventionally applied in MRI and investigated for application on biomedical fields (Reddy et al. 2012; Schafer et al. 2010). SPIONs labeled ADSCs maintained its characteristics in vitro. They have injected SPIONs labeled ADSCs into corpus cavernosum of diabetic rats and applied the magnetic power. Physiologic analysis showed increased intracavernosal pressure and increased SMCs and ECs compared to ADSCs without labeling SPIONs 4 weeks after injection. This study showed SPIONs did not affect viability and properties of ADSCs and external application of magnetic power was helpful in cell staying at target tissue (Zhu et al. 2017). These studies showed the possibility of application of nonorganic vehicles as nanoparticles in gene transfer for merits as immunogenic issues and cell-anchoring ability to the target tissue.

New powerful methods like electroporation, miRNA (microRNA), siRNA (small interfering RNA), and gene editing technic like CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) have been introduced in gene therapy recently. CRISPER enabled to edit target genes directly (Damian and Porteus 2013). There was a trial of reducing PDE5 enzyme by PDE5-silencer transfected siRNA. PDE5 enzyme decreased to 88.2% of control group (Lin et al. 2005). Many of these animal experiments in vivo showed favorable results as increased contents of SMCs and ECs; decreased apoptosis of cells and ECM; endothelial cell function;

normalizing ion and electrolytes channels, and improved enzyme production, growth factors, and molecules. And physiologic evaluations showed restoration or improvement of erectile function in animal models.

Several gene or molecules as VEGF, brain-derived neurotropic factor (BDNF), and vasoactive intestinal peptide (VIP) had been studied for clinical application (Shamloul and Ghanem 2013). VEGF is a well-known molecule related to angiogenesis (Burchardt et al. 1999; Dahiya et al. 1999). Several studies showed the effect of VEGF in erectile function on animal model (Park et al. 2004; Takeshita et al. 1994). Increase of smooth muscle and endothelial nitric oxide synthase (eNOS) were found after intracavernosal injection of VEGF (Park et al. 2004). To help the effect of SCs, trials of VEGF adding on SCs were attempted employing genetic engineering, for the purpose of enhancing formation of vasculatures in tissue-engineered corporal tissue by increasing the expression of VEGF in MDSC. Burchardt et al. (2005) studied about the effect of VEGF on ED therapy by DNA transfer of VEGF 165 in the rat penis. They used liposome complex of VEGF 165 expression vector instead of adenoviral vector transfected with VEGF gene. The corpus cavernosum of the rats treated with VEGF-liposome complex showed 10 folds greater VEGF concentration. MDSCs potentiated with transfection of VEGF were tested in rabbit corpus cavernosum. MDSCs were derived from gastrocnemius muscles of New Zealand white rabbits. MDSCs were transfected by the human VEGF165 lentiviral gene vector (LV-GFP-VEGF). Rabbits were implanted with non-seeded ACCM, ACCM seeded with MDSC, ACCM seeded with MDSC transfected with control vector, or ACCM seeded with MDSC expressing VEGF. The control group underwent corporal tissue excision without ACCM. MDSCs expressing higher VEGF were correlated with better distribution and growth of seeded cells on ACCM surface, higher number of nuclei, and cell adherence capacity. Also, ICP was significantly higher in this group (60% of normal), and markers for SMC and endothelium were expressed higher. Analysis including physiologic studies showed MDSC overexpressing VEGF was promoting vascular formation and cellular maturation in the engineered corpus cavernosum compared to MDSCs only (An et al. 2013). Similar work was reported by Liu et al. and the results showed improvement of erectile function accompanied by increased number of SMCs and ECs (Liu et al. 2013). Bivalacqua et al. (2007) tried to treat age-related ED using MSCs alone or combined with ex vivo modified gene with endothelial nitric oxide synthase. They modified MSCs with eNOS containing adenoviral vector and injected into corpus cavernosum of rats. The result revealed improved erectile function with increased NO pathway signals and differentiation into SMCs and ECs from MSCs.

One of the clinical trials using gene therapy for ED was reported in 2006 at first. Melman (2006) hMaxi-K gene were transferred to ED patients as a phase I clinical trial. Potassium channels act on relaxation of smooth muscle by decreasing intracellular calcium from SMCs (Christ 2002). Activation of potassium channels induces relaxation of corporal tissue resulting in erection. They have transferred naked DNA portion related to potassium channel using pVAX1 vector and injected into corpus cavernosum in 11 ED patients. Patients showed improved erectile function and improved IIEF-EF domain score up to 24 weeks after injection and

there was no serious side effect. The results could not be conclusive statistically because of the small number of patients and absence of control group, but bear the potential of gene therapy for ED patients (Melman 2006).

There are some drawbacks in introducing cell therapy or gene therapy on ED patients. The uncertainty of safety is the issue of gene therapy. Vectors like virus or even transferred genes may act in uncontrolled fashion, over express the genetic function, and result in unwanted immune problems or other side effects. Nanoparticles may play an important role because it has nonorganic property.

6.3 Bioprinting

One of the emerging technics in tissue engineering is constructing tissue using 3D bioprinters. Bioprinter is automated robotic device that enables digital biofabrication of 3D functional structures. The 3D bioprinters were designed to construct biomaterial scaffold and distribute various cells in between at a time to compose ideal tissue structures (Murphy and Atala 2014). Since Professor Ralf Mulhaupt in Freiburg University introduced commercial 3D printer, bioprinters have been evolved technically and economically on the base of 2D printing technology by Hewlett-Packard company (Huang et al. 2017). Like others commercial 3D printers, 3D bioprinters reconstruct 3D structures of biologic properties by piling up biomaterials and viable cells altogether. Apart from the digital, robotic, and automatic technical hurdles, the major problem in the use of 3D printers was the high price. More than 20 companies have been developing new 3D printers to overcome this handicap. Another issue is the absence of standard system of 3D printers. There are no FDA regulations and no FDA-approved organs made by 3D printers yet.

Basic architecture of the 3D printers is composed of five parts: robotic positioning system ruling printing axis (Cartesian type robot), controlling device, nozzle dispenser like automatic syringes, collectors for 3D printed product, and sterile cabinet for 3D printed tissue or organ.

Usually 3D bioprinters have been classified into 3 types: Inkjet 3D printers, extrusion-based 3D printers, and laser-based 3D printers (Tamay et al. 2019). Inkjet type was the first 3D bioprinter as the stereotype. In tissue engineering, frequently used inkjet types are thermal and piezoelectric types. Extrusion 3D printers have been most popular among them. It could be divided into pneumatic or mechanical by dispensing system. The advantage of laser-based type would be improved cell viability by avoiding the clogging of cells. But this type is usually the most expensive among them (Mandrycky et al. 2016). Materials used for constructing scaffold in 3D bioprinters would be biocompatible, printable, and able to help cell growth, forming supportive structure of cells. The preferable material as a bioink in 3D bioprinters has been hydrogels. Sources of hydrogel are synthetic or natural (polysaccharides as alginate, chitosan, agarose and ECM composites as collagen, fibronectin, gelatin) (Mandrycky et al. 2016) (Fig. 5). Hydrogels provide environments for different cells to adhere, communicate, and differentiate to form target

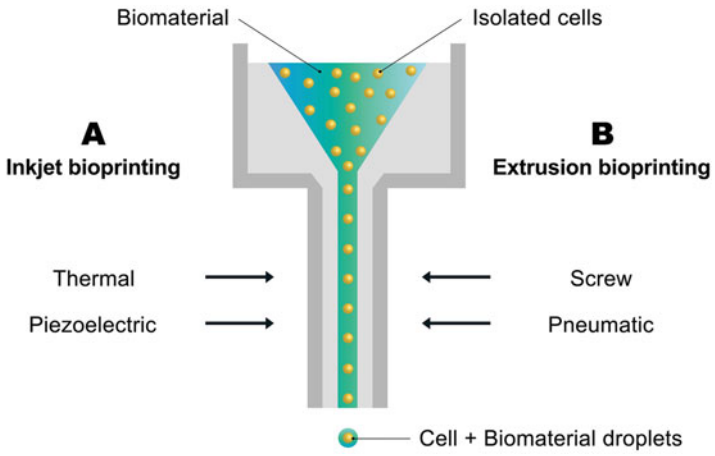

Fig. 5 Schematic figure of bioprinters

tissue as ECM. One of the drawbacks of hydrogels is low mechanical strength (Billiet et al. 2012).

Cell-biomaterial compounds would be manufactured through (A) Inkjet bioprinters with thermal or piezoelectric power, (B) Extrusion bioprinters with screw or pneumatic force.

There have been preclinical trials to reconstruct tissues using applications of 3D bioprinting. These preclinical studies included nerve, vessel, skin, bone, cartilage, and corona using various types of biomaterials and cells including SCs (Jang et al. 2017; Keriquel et al. 2017; Kim et al. 2018; Lee et al. 2017; Martínez Ávila et al. 2016; Skardal et al. 2012; Sorkio et al. 2018). But there are no reports about tissue-engineered corporal tissue using 3D bioprinting yet.

Urethral tissue was reconstructed by Zhang et al. (2017) using 3D bioprinting technology. Porous urethral scaffold was fabricated with poly (ε-caprolactone) (PCL) and Poly (lactide-co-caprolactone) (PLCL) blend using in-house designed 3D bioprinter. This 3D bioprinter, having multiple cartridges, was made in Wake Forest Institute for Regenerative Medicine (WFIRM). It is capable of printing numerous biomaterials at a time using hydrogel-based bioink. This is known as the Integrated Organ Printing (IOP) System (Kang et al. 2016). Autologous rabbit urothelial cells and SMCs were laden on hydrogel composed of fibrin, gelatin, and hyaluronic acid as bioink. Cell-laden bioinks were loaded with PCL, PLCL polymers from different nozzles. Analysis in vitro showed that this complex had acceptable mechanical properties and viable cells constructing tissue similar to the native urethra. Bioink made of fibrin hydrogel showed this material could be used in 3D bioprinting as a viable cell containing ink (Zhang et al. 2017). This 3D printer and hydrogel bioink could be utilized in producing corpus cavernosal architecture also.

Not only choosing the appropriate type of cells, but also biomaterial similar to physiologic human corporal sinusoid is necessary to create 3D bioprinted corporal tissue (Boland et al. 2006). The corporal sinusoidal trabeculae are known to be

composed of elastic fiber and type I and type III collagen fibers (Costa et al. 2006). Building trabeculae structure close to natural sinusoids with these organic materials using 3D bioprinters, ECs, and SMCs could be distributed and seeded between the fibers in exact locations meticulously. Conventional methods were seeding cells at the outer surface of ready-made biomaterials like encircling biomaterials with cells. Combining cells in 3D bioprinting enables cells piled up inside the biomaterial as cells could be distributed in the biomaterial simultaneously, then the product of 3D bioprinter would be cells-biomaterial complex, seeded cells at target location. There was report about culturing SMCs and ECs on PCL scaffolds made by 3D bioprinter for applying penile reconstruction (Oh et al. 2019). Some investigators have announced the concept of 4D bioprinting, which is different from 3D bioprinting in using programmed intelligent materials to perform predesigned functions and structures as patient-specific cell-biomaterial complex (An et al. 2016; Tibbits 2014).

Approaches have been explored to combine surgical robots with 3D printing (Da Vinci Surgical System) and in situ bioprinting (Tarassoli et al. 2018). Apart from the cells and bioink, various cytokines, DNAs, and genetic materials to enhance the growth and functions could be applied on the 3D printing technology to construct better physiologic tissues.

7 Conclusions

The technics and strategies of penile reconstruction have advanced steady. Appropriate tissue in reconstructive surgery of penis was always deficit in supply. Substituting tissues bear some problems as inadequate properties or immunogenicity. Application of tissue engineering has opened the feasible supply of needed tissues. Investigations have evolved from constructing structures of tissue to the functional performances of them. Suitable biomaterials have been chosen among the numerous materials and efficient technics for dealing cells have been established. Penile reconstruction has developed from constituting primitive cavernosal tissue to properly functioning complex tissue. There are a lot of suggestions and trials to improve the quality of reconstructed penis. Stem cells, gene therapy, chemicals and cytokines, and 3D bioprinting are the representative ones. Steady development would be followed by researches applying the combinations of these technics and resources. Tissue-engineered products have advanced their positions from the bench to the clinical applications in some organs already. Engineered penis would be the next one for the clinical field.

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