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Emerging gene and stem cell therapies for the treatment of erectile dysfunction

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Abstract

Erectile dysfunction is a prevalent condition in all parts of the world and leads to significant morbidity and distress, not just for men but for their partners. Unfortunately, very few currently available treatments ameliorate underlying causes of the disorder and “cure” the disease state. Much recent effort has been focused on the development of gene and cell based approaches to rectify the molecular and tissue defects responsible for ED in human men. Gene therapy has been investigated in animal models for well over a decade as a modality by which to restore normal function to the penis; as of this time, however, only one human trial has been published in the peer reviewed literature. Stem cell therapy has been a topic of interest in more recent years although there are currently very few published reports in animal models and none in human men. In this review, we will briefly review the current literature on gene and stem cell based therapies and briefly speculate on direction for future research.

Introduction

Erectile dysfunction (ED) is the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Pathological alteration in the anatomy of the penile vasculature or impairment of the neurovascular processes can result in ED.¹

Existing medical treatment modalities for ED are focused on short term modification of hemodynamic factors to facilitate penile engorgement. Unfortunately, only testosterone supplementation in cases of severe hypogonadism and arterial bypass in the rare case of isolated arterial injury have been definitively proven to reverse the pathological changes associated with ED and “cure” the disorder. In an effort to improve our ability to treat ED, researchers from around the world have been intensely focused on gene therapy (GT) and stem cell (SC) therapy as modalities which may reverse the underlying causes of ED and hence reduce patient reliance on erectogenic agents. The current state of the art in cell based therapies for ED was recently reviewed in an excellent manuscript by Strong et al.² In this update, we will review the literature on both GT and SC for the management of ED. We will also highlight important work that has been centered on combining gene and stem cell therapy.

Pathogenesis of ED

Aging, diabetes, hypercholesterolemia, and cavernous nerve injury are strongly associated with ED. Animal models of these conditions have been the focus of intensive research on therapies (including GT and SC therapy) which may reverse the pathological tissue changes

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underlying organic ED from these causes. An understanding of how these conditions may contribute to ED is essential to understand research in this field.

Aging is associated with elevated vasoconstrictor tone and decreased endothelium- and neurogenic-mediated relaxation of the corpora cavernosa.³ This is presumably due to an inadequate activation of neuronal nitric oxide synthase (nNOS) and subsequent defective production of nitric oxide (NO).⁴ Decreased NO bioavailability impairs vasodilation and enhances formation of reactive oxygen species (ROS) that can cause tissue damage.^{5,6}

Diabetes contributes to ED via neuropathic damage to cavernous nerves, non-enzymatic glycation of proteins, increased oxidative stress, altered angiogenesis, dyslipidemia, endothelial dysfunction, and perturbations of NOS metabolism.⁷⁻⁹

Hypercholesterolemia is thought to be related to ED due to impairment of NO mediated endothelium-dependent relaxation in the smooth muscle cells of the corpus cavernosum.¹⁰ NO has activity has also been implicated as critical for angiogenesis.^{11,12}

Cavernous nerve injury interrupts NO release from nitrergic nerve terminals in the cavernosal arteries and corporal bodies. This tends to inhibit erectile capacity. Furthermore, denervation of the corporal bodies after cavernous nerve injury has been associated with smooth muscle and collagen changes that likely exacerbate the condition of post-nerve injury erectile dysfunction

Gene Therapy for ED

Gene therapy is the technology focused on replacement and/or upregulation of genes intended to ameliorate some form of tissue defect or damage.¹³ One of the principle challenges of gene therapy is development of mechanisms to place DNA into the cells of interest. Many gene delivery systems exist, each with advantages and disadvantages (Table 1). Adenoviral vectors are easy to construct and have high transfection efficiency but carry risks such as inflammatory response, toxicity, and adverse effects or chromosomal integration, and the possibility of random expression of the transgene in almost all cell types.^{14,15} Replication defective Herpes Simplex Virus (HSV) vectors, which lack multiple essential gene functions, have been used to deliver neurotrophic factors based due to their low toxicity.¹⁶ HSV vectors have the unique ability for high-affinity uptake by nerve termini, efficient delivery of the viral DNA payload to nerve cell bodies without chromosomal integration, and a unique promoter system.¹³ Naked plasmid DNA is safe but has low transfection efficiency in most cell types.¹⁷ DNA may also be complexed to liposomal constructs for transfection.¹⁸ A non-viral Polyethylenimine (PEI) gene delivery system has also been utilized; this macromolecule system avoids the risks of viral vectors and allows for continuous expression of genes over a longer period with minimal amount of DNA injected.¹⁹ Other investigators have used *ex vivo* gene transfection in cultured cells which are then reintroduced into the host organism; this limits the potential for expression of transgenes in non-target cells.^{20,21} In addition to vector selection, tissue specific promoters and enhancers have been investigated as means to enhance treatment efficacy and minimize side effects.¹⁷

The external positioning, limited blood flow, and slow cellular turnover of the penis make it an ideal target for gene therapy.²² The risk of systemic side effects from penile administration of a gene is thought to be minimal.²³ Furthermore, the presence of gap junctions in corporal tissues allows the corporal tissue to function as a syncytium. Nonselective exchange of the numerous physiologically relevant intracellular second messengers and ions may occur between cells; therefore, gene take-up by even a few cells may have profound effects on neighboring cells.^{24,25} Given the relatively small number of

cells that need to be transfected for efficacy in management of ED, naked plasmid DNA has been the vector of choice for human studies of ED-gene therapy.²⁴

1- Enzyme replacement (Table 2)

a- NOS

Nitric oxide (NO) is a potent vasodilator that is generated by metabolism of arginine via the enzyme nitric oxide synthase (NOS).^{26,27} The release of NO activates NO-sensitive guanylyl cyclase in vascular tissues such as the penis; this increases penile tissue cGMP levels, leading to downstream molecular actions that lead to penile erection.²⁸ Decline in penile NO synthesis in cavernous nerve terminals and the penile endothelium is a very common cause of ED.^{29,30} Restoration of endogenous NO synthesis in the penis is therefore of great interest as a treatment for ED. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are the principal isoforms involved in the induction of penile erection.^{31,32} A third isoform (inducible NOS, iNOS) has a distinctive expression pattern and Ca²⁺-dependence and may also play a role in penile erection.³³

Transfer of the eNOS gene via adenoviral vector in streptozotocin (STZ) treated rats is associated with increased cavernous tissue eNOS activity and NO biosynthesis at a follow-up time point of 1–2 days post-treatment; furthermore, rats treated with the adenoviral construct had cavernous nerve stimulation mediated penile hemodynamics that were similar to what was observed in normal animals not exposed to STZ, implying restoration of erectile function in these animals.³⁰ The combination of this form of eNOS gene therapy and intravenous sildenafil in STZ-diabetic rats results in a synergistic erectile response that is greater than when either of these therapies is used alone.³⁴ Intracavernous administration of bone-marrow-derived syngeneic mesenchymal stem cells (MSC) with *ex vivo* adenovirally mediated gene transfer of eNOS in aged rats was shown to improve endothelium-derived NO biosynthesis and neurogenic-mediated erectile responses in the aged rat at 7 and 21 days post-injection, likely via differentiation into new endothelial and smooth muscle cells. Interestingly, administration of unmodified MSC led to improvements in penile hemodynamics at the 21 day time point but not at the 7 day time point;³⁵ this suggests gene the gene therapy intervention produced a more rapid improvement in penile hemodynamics.

Plasmid mediated gene transfer of iNOS has been shown to improve penile hemodynamics and enhance NOS activity in aged rats as determined by cavernous nerve electrostimulation and enhance NOS.²⁹ Transfection of the iNOS gene via plasmid vectors, adenoviral vectors, or adenovirus-transduced myoblast cells has also been explored in healthy adult rats; it was determined that myoblast mediated transfer produced a robust retention of iNOS activity in the cavernosa and that this was associated with superior erectile response to cavernous nerve stimulation; lower levels of iNOS retention were noted in animals treated with adenoviral or plasmid vectors.²⁰

b- cGMP-dependent protein kinase G1 (PKG1)

The cGMP-dependent protein kinase G1 (PKG1) is the target by which cGMP induces vasodilation in corporal tissue by actions on inositol-3-phosphate activity.³⁶ PKG1 has been shown to be down-regulated in experimentally induced diabetes in rabbits and rats.^{37,38} Adenoviral-mediated transfer of the gene that encodes for PKG1 α into the corpora cavernosa of these rats restores total PKG activity to the levels observed in control rats. These molecular changes were associated with physiologically relevant enhancement of penile hemodynamics during cavernous nerve electrostimulation.³⁸

c-HO/CO system

Heme oxygenase (HO) catalyses the rate limiting step in the oxidative degradation of heme to biliverdin and carbon monoxide (CO). CO has physiological properties similar to NO that are likely induced by CO-mediated activation of guanylate cyclase.³⁹ Upregulation of HO-1 activity by a cDNA-liposome complex transfer in aged rats resulted in increased HO enzyme activity and cGMP level up to 1 week post-treatment.⁴⁰

d-Superoxide dismutase

Elevation of serum glucose (as occurs in diabetic states) induces oxidative stress via reactive oxygen species (ROS).⁴¹ ROS react with NO, reducing its' bioavailability leading to ED.⁴²⁻⁴⁴ The enzyme extracellular superoxide dismutase reduces ROS concentration; intracavernous adenoviral transfer of the EC-SOD gene to rats with STZ induced diabetes reduces ROS, increases cavernosal cGMP, and improved erectile hemodynamics during cavernous nerve electrostimulation.⁴⁴

e- Anti- NOS: Protein Inhibitor of NOS (PIN)

The protein inhibitor of NOS (PIN) has been detected in the rat major pelvic ganglion and inhibits nNOS activity by impeding dimerization.⁴⁵ Antisense RNA and short hairpin RNA (shRNA) plasmid constructs silence PIN gene expression by inhibiting mRNA translation and/or inducing its' degradation.⁴⁶ Intracavernous injection of a plasmid encoding antisense PIN RNA or PIN shRNA reduces erectile response to cavernous nerve electrical field stimulation (EFS).⁴⁷ Both shRNA and antisense constructs against PIN have been found to improve penile hemodynamics during cavernous nerve electrostimulation in aged (20 month old) rats; shRNA was slightly more efficacious at increasing intracavernous pressure.⁴⁸

f- Anti-NOS: Anti-arginase

Increases in arginase activity may deplete the quantity of arginine available for NO biosynthesis in the penis.⁴⁹ Arginase, which may deplete arginine levels, is upregulated in the cavernous tissues of diabetic humans and animals; Inhibition of this enzyme facilitates corporal smooth muscle relaxation.^{50,51} Gene transfer of anti-sense arginase via an adenoviral vector has been demonstrated to decrease arginase I protein and mRNA in the penile tissues of aged mice. This change is associated with improved cGMP production and cavernous nerve stimulation mediated erections in vivo.⁵²

2-Growth factors and peptides: (Table 3)

Use of gene delivery techniques that enhance sustained focal release of desired trophic factors may provide a more durable means of treating neuronal and endothelial tissue damage leading to ED. Neuronal regeneration strategies have been of particular interest in animal models of cavernous nerve injury.

a-Neurotrophic growth factors

Glial cell line-derived neurotrophic factor (GDNF) promotes survival and extension of sympathetic and parasympathetic axons.⁵³ GDNF and its' receptors are expressed in penile parasympathetic and sensory neurons and are involved in the survival and regeneration of these neurons.^{54,55} GDNF via HSV vector has been shown to enhance erectile function in cavernous nerve injured rats; rats that received the HSV-GDNF treatment to the area of nerve injury had significantly improved ICP response to cavernous nerve electrostimulation at four week follow-up testing. An increase in FluroGold (FG) labeled cells in the major pelvic ganglion was detected in HSV-GDNF treated animals, suggesting that HSV-GDNF enhanced nerve regeneration and/or promoted survival.¹³

Neurturin (NTN) is a member of the glial cell line-derived neurotrophic factor (GDNF) family and has been shown to enhance survival and/or regeneration of postganglionic neurons innervating the penis in a more specific fashion than GDNF.^{56–58} After cavernous nerve injury with crush and freeze, rats treated with NTN via an HSV vector were shown to have higher mean intracavernous pressure/arterial pressure (ICP/AP) ratios relative to injured rats that received just HSV-vector or no treatment. Retrograde tracing studies revealed a significant improvement in nerve integrity in the NTN treatment group.⁵⁸

Neurotrophin 3 (NT3) is trophic for sensory and motor neurons and may be protective against mechanical and metabolic insults.⁵⁹ NT3 has been shown to induce neurite fiber outgrowth in cultured MPG.⁶⁰ NT3 via viral vector has been used to treat the cavernous tissue of rats with STZ-induced diabetes. ICP/AP was significantly higher in rats treated with cavernous injection of the NT3 compared to what was observed in control animals; furthermore, the number of nNOS positive neurons in the MPG was significantly increased in the NT3 group.⁶¹

Brain Derived Neurotrophic Factor (BDNF) has been shown to enhance nerve growth. BDNF via adenoviral gene transfer was evaluated in a rat model of cavernous nerve injury by freezing. Animals treated with BDNF gene transfer manifested superior ICP results during cavernous nerve electrostimulation and had greater staining for nNOS and NADPH.⁶² These effects of BDNF were confirmed in a rat model of hypercholesterolemia in the same laboratory; BDNF was shown to preserve neuronal content as well as erectogenic response to cavernous nerve stimulation and administration of intracavernous papaverine.⁶³

b- Insulin-like growth factor-1 (IGF-1)

IGF-I plays a key role in regeneration of (NOS)-containing nerves after cavernous neurotomy or cryo-injury in rats.^{64,65} Down-regulation of IGF-1 protein expression has been detected in penile tissues of diabetic rats.⁶⁶ Cavernosal gene transfer of IGF-1 using an adenoviral vector in STZ-induced diabetic rats was shown to increase cavernous expression of IGF-1 mRNA and protein and improve intracavernous pressure response to cavernous nerve electrostimulation relative to untreated controls.⁶⁷

c- Vascular Endothelial derived Growth Factor (VEGF)

VEGF is a potent endothelial cell mitogen that has been shown to improve erectile function in several animal models of ED, including hypercholesterolemic rabbits and diabetic rats.^{63,68} Injection of VEGF protein has been shown to prevent endothelial cell and smooth muscle cell apoptosis by the up-regulation of the anti-apoptotic protein Bcl-2.⁶⁹

A non-viral Polyethylenimine (PEI) gene delivery system has been used to transfect VEGF gene into the corpora cavernosa of STZ-induced diabetic rats; treated animals manifested greater smooth muscle content of the corporal tissue as well as improved intracavernous pressure during cavernous nerve electrostimulation relative to controls.¹⁹ Corporal injection of a VEGF gene via an adenoviral vector has also been shown to enhance angiogenesis, eNOS phosphorylation, and cGMP expression in a hypercholesterolemic rat model for ED. This effect was enhanced by concomitant transfer of the angiopoietin-1 gene (Ang-1), which counteracts VEGF-induced inflammation in endothelial cells.⁷⁰ Intracavernous pressure with cavernous nerve electrostimulation was essentially similar between non-hypercholesterolemic animals and animals that received combined Ang-1/VEGF treatment, whereas intracavernous injection of either Ang-1 or VEGF alone elicited just partial (non-significant) improvement in intracavernous pressure.⁷⁰ Intracorporal treatment with adenoviral associated VEGF has also been shown to prevent castration associated erectile

impairment in rats; however, adenoviral gene transfer was less efficacious than direct treatment with VEGF protein.⁷¹

3- Peptides (Table 4)

A number of peptides have modulatory effects on smooth muscle tone and consequently may be exploited to potentiate penile erection in ED.

a-Vasoactive intestinal polypeptide (VIP)

VIP is a potent vasodilator which acts by stimulation of adenylate cyclase; it appears to be a co-mediator of penile erection⁷²⁻⁷⁴ In an STZ-induced diabetic rat model of ED, intracorporal injection of VIP led to easily incorporation and sustained expression of the gene. Up-regulation of VIP was maintained up to 14 days and was associated with a significant increase in cavernous nerve stimulated mediated intracavernous pressure relative to untreated control animals.⁷⁵

b-Calcitonin gene related peptide (CGRP)

CGRP produces an increase in intracellular cAMP and a reduction in intracellular Ca^{2+} , and hence contributes to smooth muscle relaxation in the penis.^{76,77} Direct injection of an adenovirus encoding the prepro-CGRP gene into the penis of aged rats increases cavernosal levels of CGRP, CGRP mRNA, and cAMP. These changes are associated with higher intracavernous pressure during cavernosal nerve stimulation relative to untreated controls.⁷⁸

4-Potassium channels: (Table 4)

Potassium channels are an important final common pathway for the modulation of cellular hyperpolarization and relaxation and hence smooth muscle tone.^{1,79} Over-expression of potassium channels in corporal smooth muscle cells is presumed to increase their responsiveness to various neurotransmitters.

a- Large conductance Ca^{2+} activated potassium channel (BKCa – maxi K+)

Cellular large-conductance Ca^{2+} -activated potassium (BKCa or MaxiK+) channels are activated by NO/cGMP mediated mechanisms, leading to cellular hyperpolarization.^{80,81} Hyperpolarization of smooth muscle cell membranes prevents calcium influx and facilitates muscle relaxation and vasodilation. Suppression or knock-out of BKCa channels tends to enhance corporal smooth muscle contractions.^{82,83}

Corporal smooth muscle transfection of *hSlo* cDNA (which encodes the human smooth muscle maxi-K+ channel) was performed in 9 month old rats. *hSlo* DNA was easily incorporated into corporal smooth muscle and expression was sustained for at least 2 months in corporal smooth muscle cells; DNA incorporation was associated with enhanced intracavernous pressure during cavernous nerve electrostimulation.⁸⁴ In a follow-up study in similar rats, it was demonstrated that the efficacy of *hSlo* cDNA transfer was durable for at least 6 months and was associated with enhancement of intracavernous pressure response to cavernous nerve electrostimulation.⁸⁵ *hSlo* cDNA transfer was also shown to enhance erectile function in rats with STZ-induced diabetes up to 4 months post-treatment.⁸⁶

The first human trial of gene transfer for the treatment of ED utilized plasmid bound *hSlo* cDNA administered by corporal injection. After gene transfer of the hMaxi-K gene to six men with ED, there were no drug-related adverse events and no transfer of the plasmid to the participant's semen. In addition, there were no adverse histopathological or blood

chemistry results. Statistically significant benefit was seen in patients that received the two highest doses of *hSlo* cDNA and this was maintained out to 24 weeks.⁸⁷

b- ATP -sensitive K channels (K_{ATP})

The relaxation response of the K_{ATP} channel has been shown to be impaired in corporal tissue strips from men with diabetes; this likely plays a role in diabetes associated ED.⁸⁸ Transfer of the K_{ATP} channel gene to the penis of 18+ month old rats increases cavernosal levels of K_{ATP} channel mRNA. This was associated with enhancement of intracavernous pressure during cavernous nerve electrostimulation.⁸⁹

Stem Cell Therapy for ED (Table 5)

Stem cells (SC) are pluripotent cells that may be derived from numerous sources within the body. These cells have the capacity to divide and differentiate into many adult tissue types *in vitro*, including endothelial cells and smooth muscle. These properties make SC an appealing option for tissue regenerative therapy for ED. In many studies of SC for the treatment of human diseases cells are induced along particular developmental lines prior to reinjection; this process theoretically enhances cellular differentiation along desirable pathways.

1- Embryonic Stem Cells (ESC)

Embryonic stem cells have immense biological potential but ethical concerns have limited their utilization in research. In one study of rat ESC for ED, ESC were induced to differentiate along neural cell line by action of BDNF and administered by injection in the major pelvic ganglion or penis to cavernous nerve injured rats. Significant improvements in intracavernous pressure were detected in this model system.⁹⁰

2- Neuronally derived Stem Cells

Rat fetal brain stem cells have demonstrated the capacity to differentiate into penile smooth muscle cells; in addition, these cells have the capacity to survive when injected into the cavernous tissue for up to 6 weeks.⁹¹ Similarly, when injected into corpus cavernosa of healthy 10-week old rats, human neural crest SCs have been shown to express markers of smooth muscle and vascular endothelial cells,⁹² suggesting the potential for treating ED. Unfortunately, functional testing was not performed in either of these studies so the erection preserving capacity of these treatments is at this time unclear.

3- Mesenchymal Stem Cells (MSC)

Immortalized human fetal bone marrow MSCs have been shown to express endothelial-and smooth muscle specific antigens within the corpora 2 weeks after injection into the cavernosa of young health rats. This suggests that these MSC have the capacity to differentiate into endothelial-like and smooth muscle cells *in vivo*.⁹³ Functional testing was not performed in this study so it is unclear whether the observed effects are clinically meaningful

In a study that combined histological and functional assessment, rat skeletal muscle derived SCs implanted into the corpora cavernosa of 20 month old rats demonstrate capacity to express smooth muscle markers and significantly enhance cavernous nerve stimulation mediated increases in intracavernous pressure.⁹⁴

4- Adipose Derived Stem Cells (ADSC)

ADSC are vascular stem cells derived from perivascular areas of adipose tissue. ADSC can be harvested in large quantities with minimal subject morbidity and therefore may represent an ideal stem cell source.⁹⁵ In a pilot experiment, ADSCs have been shown to improve erectile function after bilateral cavernous crush injury in a rat model of ED. (*unpublished data*) Additional studies in our lab have supported the efficacy of ADSC for the treatment of hypercholesterolemia and diabetes-related erectile impairment. (*Garcia in press, Journal of Sexual Medicine and Huang YC, unpublished data*)

Future directions

It is abundantly clear that a great deal has been learned about the therapeutic potential of both gene and stem cell based therapy. Significant safety and efficacy concerns persist for both modalities.

Gene therapy has been a subject of interest for many years; however, the gene-therapy related death of Jesse Gelsinger in the late 90s was a serious blow to gene therapy research and has had serious ramifications on research in this field.⁹⁶ The potential for viral induced inflammation or propagation of gene transfer to non-target cells remains a problem with virally mediated transfer vectors. Non-viral vectors are available but are generally less efficient means to produce gene transfer. These significant safety concerns have contributed to slow translation of gene therapy modalities to human patients; to our knowledge there has been only one human clinical trial of gene therapy for ED.

Stem cell therapy is a newer technology and studies are relatively scant at this time. Stem cell therapies avoid some of the issues surrounding gene therapy but legitimate concerns regarding the long term fate of stem cells persist; the potential for malignant degeneration of transplanted stem cells must be elucidated before human trials can proceed. Furthermore, the ideal stem cell source has yet to be determined. Embryonic stem cells are difficult to obtain and pose serious ethical dilemmas. Non-autologous adult stem cells may be utilized but carry the risk of disease transmission or immune rejection. Autologous stem cells minimize both ethical and immunogenic concerns but carry the risk of harvest site morbidity. Identification of a stem cell source that is abundant, renewable, and may be harvested with minimum of subject morbidity will likely be very important in accelerating research in stem cell biology

Despite numerous current limitations, both technologies have the capacity to revolutionize care of the ED patient and are therefore topics of great interest to clinicians interested in sexual health. Several investigators have investigated modalities that combine both techniques; it is possible that a combination approach will be the most successful in future clinical trials. Although wide-spread clinical application of these technologies is years away, it may be speculated that either gene and/or stem cell therapy may someday play a very significant role in enhancing sexual health for men and their partners.

Conclusions

Gene and stem cell therapy have the potential to treat the underlying causes of erectile dysfunction; this would be a paradigm shift from currently available treatment modalities. Further research will be required to more accurately and precisely elucidate the efficacy of these modalities. More importantly, the long-term safety of both GT and SC therapy must be thoroughly investigated before large scale human studies can be considered.

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Table 1

Gene therapy vectors used for treatment of ED

Vector	Advantages	Disadvantages
Naked Plasmid DNA	Low risk of immunogenicity or chromosomal integration	Low transfection efficiency in most cells
Adenovirus	Easy to construct, high efficiency transfection	Risk of immunogenicity, chromosomal integration/disruption, toxicity, etc
Herpes Simplex Virus (HSV)	High transfection efficiency in neuronal cells, minimal risk of chromosomal integration, unique promoter system	Risk of immunogenicity, chromosomal integration/disruption, toxicity, etc
Mesenchymal cells with <i>ex vivo</i> transfection	Low risk of immunogenicity or chromosomal integration	Potential for malignant transformation of transplanted cells
cDNA liposome	Low risk of immunogenicity or chromosomal integration	Lower transfection efficiency in most cells
Polyethylenimine	Non-viral, allows continuous expression of genes over prolonged time	Lower transfection efficiency in most cells

Table 2

Gene Therapy for replacement of enzymatic activity

Enzyme	Studies	Vector	Model System	Time Course	Functional results	Molecular results	Miscellaneous
Studies upregulating gene expression							
eNOS	Bivalacqua 2003	Adenovirus	STZ-induced diabetes in rats	1–2 days	Enhancement in ICP and AUC during cavernous nerve electrostimulation relative to controls	Increased expression of eNOS protein, activity, and NO synthesis	Follow-up study showed synergistic effect of this treatment when combined with sildenafil (Bivalacqua 2004)
	Bivalacqua 2007	Syngeneic mesenchymal stem cells (MSC) with or without <i>ex vivo</i> transfer of eNOS	Aged (25 months) rats	7 and 21 days	Enhancement in ICP and AUC during cavernous nerve electrostimulation relative to controls	Improved endothelium derived NO synthesis, eNOS protein, and cGMP levels	Speculate that differentiation into new endothelial cells was underlying mechanism
iNOS	Garban 1997	Plasmid	5, 20, and 30 month old rats	4, 10 and 21 days	Enhancement in ICP and AUC during cavernous nerve electrostimulation at 10 days post treatment in the 20 month old group	Increased NOS activity and expression of iNOS in the corporal tissues	
	Chancellor 2003	Plasmid, Adenovirus, and myoblasts with <i>ex vivo</i> transduction	Healthy adult rats	2, 4, and 7 days	Enhancement in ICP during cavernous nerve electrostimulation relative to control and myoblast alone treated animals	Greater delivery of iNOS to corpus cavernosum with myoblast therapy relative to plasmid and adenovirus	
Protein Kinase G1	Bivalacqua 2007	Adenovirus	STZ-induced diabetes in rats	2 days	Enhancement in ICP and AUC during cavernous nerve electrostimulation relative to controls	Increased expression of PKG1- α to normal levels	
Heme Oxygenase	Abdel-Aziz 2009	cDNA-liposome	Old (2 years) rats	0.5, 1, 2, 7 and 14 days	None	Significantly increased activity of heme oxygenase and cGMP up to one week post treatment with decline to baseline by week two	HO gene activity upregulated out to 2 weeks
Superoxide Dismutase	Bivalacqua 2005	Adenovirus	STZ-induced diabetes in rats	2 days	Enhancement in ICP and AUC during cavernous nerve electrostimulation relative to controls	Decreased super oxide anion and increased SOD and cGMP activity	
Studies downregulating gene expression							
Protein Inhibitor of NOS	MaGee 2007	Antisense or short hairpin PIN RNA plasmids	20 month old rats	1 month	Enhancement in ICP during cavernous nerve electrostimulation relative to antisense RNA and controls; more robust effect noted in response to shRNA than antisense RNA	Suppression of PIN protein concentration in all treated animals. Elevated cGMP was detected in antisense mRNA treated animals (shRNA animals not tested for cGMP)	PIN mRNA suppressed by shRNA but not antisense RNA

Enzyme	Studies	Vector	Model System	Time Course	Functional results	Molecular results	Miscellaneous
Arginase	Bivalacqua 2007	Adenovirus	22–26 month old mice vs. 4–8 month old mice	1 month	Enhancement in ICP and AUC during cavernous nerve electrostimulation relative to controls	Decrease in arginase protein and mRNA, increase in cGMP level	

Table 3

Gene Therapy for upregulation of trophic factors

Factor	Studies	Vector	Model System	Time Course	Functional results	Molecular results	Miscellaneous
Glial Cell Line derived Neurotrophic Factor	Kato 2007	HSV	Cavernous nerve injured (via crush and freeze) rats	2 and 4 weeks	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	60% of MPG neurons manifest vector uptake with persistence of signal at 4 weeks	Retrograde tracing with fluorogold indicates higher content in major pelvic ganglion, c/w enhanced nerve survival or regeneration
Neurturin	Kato 2009	HSV	Cavernous nerve injured rats	4 weeks	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	70% of MPG neurons positive for green fluorescent protein marker positive for nNOS	Retrograde tracing with fluorogold indicates higher content in major pelvic ganglion, c/w enhanced nerve survival or regeneration
Neurotrophin 3	Bennett 2005	HSV	STZ-induced diabetes	4 weeks	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	More nNOS + neurons in MPG of treated animals	
Brain Derived Neurotrophic Factor	Bakircioglu 2001	Adenovirus	Cavernous nerve injured rats (by freezing)	4 and 8 weeks	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Greater density of NADPH and nNOS staining in treated animals	Increased neuronal irregularities in control animals relative to treated animals
	Gholami 2003	Adenovirus	Hypercholesterolemic Rats	4 months	Enhancement in ICP during cavernous nerve electrostimulation and papaverine installation relative to controls	Preservation of myelinated and non-myelinated neurons compared to untreated hypercholesterolemic animals	Significantly higher smooth muscle content in BDNF treated hypercholesterolemic animals, similar endothelial content
Insulin-like Growth factor 1	Pu 2007	Adenovirus	STZ-induced diabetes	1-2 days	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Increase in cavernous expression of IGF-1 mRNA and protein	
Vascular Endothelial Growth Factor	Dall'Era 2008	Polyethylenimine	STZ-induced diabetes	3 weeks	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Improved smooth muscle content in treated animals per histochemistry	VEGF construct expressed at mRNA level out to three weeks
	Rogers 2003	Adenovirus	Castrated rats with evidence of penile veno-occlusive erectile impairment	Not stated	Lower infusion rate and erectile drop rate during dynamic infusion cavernosometry after	Improved smooth muscle and endothelial tissue appearance on electron microscopy	Higher intracavernous flow and more rapid loss of intracavernous pressure in animals treated with VEGF gene vs. those that

Factor	Studies	Vector	Model System	Time Course	Functional results	Molecular results	Miscellaneous
					papaverine administration in VEGF treated animals relative to controls; ICP higher in VEGF-gene treated animals but difference not significant relative to controls		received direct injection of VEGF or testosterone replacement therapy
	Ryu 2006	Adenovirus	Hypercholesterolemic Rats	7, 14, and 56 days	Enhancement in ICP during cavernous nerve electrostimulation relative to controls at 14 and 56 days when combined with angiopoietin1 treatment; difference not significant relative to controls as monotherapy	Increased NOS activity, endothelial content, and cGMP expression at all time points. Ang and VEGF protein levels declined to baseline by 14 days post treatment	Synergistic effect when combined with gene transfer of Angiopoietin-1
Angiopoietin 1	Ryu 2006	Adenovirus	Hypercholesterolemic Rats	7, 14, and 56 days	Enhancement in ICP during cavernous nerve electrostimulation relative to controls at 14 and 56 days when combined with VEGF treatment; difference not significant relative to controls as monotherapy	Increased NOS activity, endothelial content, and cGMP expression at all time points. Ang and VEGF protein levels declined to baseline by 14 days post treatment	Synergistic effect when combined with gene transfer of VEGF

Table 4

Gene Therapy, miscellaneous

Factor	Studies	Vector	Model System	Time Course	Functional results	Molecular results	Miscellaneous
Vascoactive Intestinal Peptide	Shen 2005	cDNA	STZ-induced diabetes	1, 3, 7, and 14 days	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Sustained elevated expression of VIP up to 14 days in treated animals	No expression in other tissues
Calcitonin Gene Related Peptide	Bivalacqua 2001	Adenovirus	Aged rats (60 weeks)	5 days	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Increased cavernosal levels of CGRP mRNA and protein and cAMP	
Large conductance Ca-activated potassium channels (AKA Maxi-K)	Christ 1998	Plasmid	Aged (9+ months) rats	1, 2, 3 and 4 months	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Enhanced expression of gene product out to 2 months	Easily incorporated with sustained improvement in intracavernous pressure in 2 of 2 animals out to 4 months
	Melman 2003	Plasmid	Aged (9+ months) rats	1, 2, 3, 4, 5, 6 months	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Enhanced expression of gene product out to 6 months	No tissue defects or evidence of inflammatory changes on histology
	Christ 2004	Plasmid	STZ-induced diabetes	1, 2, 3 and 4 months	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Enhanced expression of gene product out to 4 months	Relaxant response to iberoitoxin indicates incorporation of MaxiK channels into functional membrane of corporal cells
	Melman 2006	Plasmid	Eleven men with ED	24 weeks	Significant improvement in mean IIEF-EF domain scores in the two highest dose therapy groups starting two weeks post treatment, maintained to 24 weeks	No gene transfer to semen	No adverse events clearly related to therapy
ATP-sensitive K channels	So 2007	Naked DNA	Aged (18+ months) rats	Immediate post-injection	Enhancement in ICP during cavernous nerve electrostimulation relative to controls	Increased levels of KATP channel mRNA	

Table 5

Stem Cell Therapy

Study	Type of Stem Cell	Animal Model	Method of Introduction	Time Course	Functional Effect	Molecular Effect
Bochinski D et al. 2004	Rat embryonic stem cells with neuronal cell induction	Cavernous Nerve Crush	Intracavernous and major pelvic ganglion injection	3 months	Enhancement in ICP during cavernous nerve electrostimulation in both treated animal groups relative to controls	No significant difference in NADPH positivity between treatment and control animals but nerve tissue qualitatively more normal in treated animals relative to controls
Song Y et al. 2009	Rat embryonal brain stem cells with smooth muscle induction	6 month old rats	intracavernous injection	2, 4, and 6 weeks	No functional testing	Transplanted cells manifested cell markers for smooth muscle
Song YS et al. 2008	Human neural crest SCs	10 week old rats	intracavernous injection	2 weeks	No functional testing	Transplanted cells manifested cell markers for smooth muscle and endothelium
Song et al. 2007	immortalized human fetal MSCs	10 week old rats	intracavernous injection	2 weeks	No functional testing	Transplanted cells manifested cell markers for smooth muscle and endothelium
Nolazco et al., 2008	Skeletal muscle derived SCs	20 month old rats	intracavernous injection	2 and 4 weeks	Enhancement in ICP during cavernous nerve electrostimulation	Transplanted cells manifested cell markers for smooth muscle
Kim Y et al., 2006	Skeletal muscle cells	Cavernous Nerve Crush	intracavernous injection	2 and 4 weeks	Enhancement in ICP during cavernous nerve electrostimulation	
Garcia MM et al, in press 2009	Adipose Derived Stem Cells	Type II diabetic rats	intracavernous injection	3 weeks	Enhancement in ICP during cavernous nerve electrostimulation	Enhanced positivity for nNOS and smooth muscle, decreased TUNEL staining in treated animals
Huang YC et al, unpublished data under consideration for publication	Adipose Derived Stem Cells	Hypercholesterolemic rats	intracavernous injection	1 month	Enhancement in ICP during cavernous nerve electrostimulation	Enhanced positivity for nNOS, smooth muscle, and endothelial markers in treated animals