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CELL BASED THERAPIES IN LOWER URINARY TRACT DISORDERS

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Abbreviations:

SMC – Smooth muscle cell; UC – urothelial cell, BMSC – bone marrow stem cell; DM – diabetes mellitus; MDSC – muscle-derived stem cell; ADSC – adipose-derived stem cell; MTT – myoblast transfer therapy; AFMSC – amniotic fluid derived stem cell. Hematopoietic stem/progenitor cell - HSPC

Running header: Cell-based therapies in LUT disorders.

Abstract:

Cell based therapy for the bladder has its beginnings in the 90s with the successful isolation and culture of bladder smooth muscle cells. Since then several attempts have been made to artificially implant native cell-types and stem cell-derived cells into damaged bladders in the form of single-cell injectables or as grafts seeded onto artificial extra cellular matrix. We critically examined in literature, the types of cells and their probable role as an alternative to non-drug based, non-bowel based graft replacement therapy in disorders of the urinary bladder. The limitations and plausible improvements to these novel therapies have also been discussed keeping in mind an ideal therapy that could suit most bladder abnormalities arising out of varied number of disorders. In conclusion, muscle-derived cell types have consistently proven to be a promising therapy to emerge in the coming decade. However, tissue-engineered constructs have yet to prove their success in pre-clinical and long-term clinical setting.

Keywords: Cell transplantation, bladder, literature review, tissue engineering, urology

Introduction:

Lower Urinary Tract (LUT) can be affected by various diseases and disorders arising out of multiple factors, intrinsic or extrinsic to the human body, eg: peripheral neuropathy as in diabetes or alcohol abuse, post surgical central neuropathy, spinal cord lesions, multiple sclerosis and many more.

Traditional drug based therapies have involved the use of cholinergics, anti-adrenergics, anti-muscarinics and more. Surgical interventions, including electrical modulation and stimulation have proven to be of importance in cases of urinary incontinence, uro-genital obstruction and neurogenic bladders of various origins.

An estimated 400 million people in the world suffer from various bladder diseases (24). Many disorders and injuries affect the smooth muscles or the uroepithelial layers of the urinary bladder. Tissue replacement surgeries have been conducted to compensate for the damaged tissue. Many replacements from skin, intestine, polylactic glycolic acid (PLGA), silicone, collagen have been widely used in this regard. Bulking agents such as collagen, fat and teflon have been employed as injectables at the bladder neck for cases of sphincter-urethral dysfunction (8,28,39). In this literature review, we reviewed cell based injectable therapies and tissue-engineered graft based therapies that have been attempted since its inception to current developments covering over two decades.

Cell-based therapy for the bladder could not have been a reality today without the successful isolation and culture of bladder smooth muscle cells, first by Baskin et al (4). Several attempts to artificially implant cells into damaged bladders in the form of single-cell injectables have shown some promising results. Tissue grafts seeded onto artificial Extra Cellular Matrix (ECM) have been pushed as experimental therapies into clinics. We conducted a literature review taking in few landmark publications under various topics described below and critically analyzed the cell types and their probable role as an alternative to non-drug based, non-bowel based graft replacement therapy. For sake

of convenience, we have divided the topic into cell-based injectables and tissue engineered reconstructive grafts with further sub-divisions detailing the different sources of cells. A Table (Table 1) summarizing the key developments, outcomes and critical analysis has been outlined to give a brief idea about the contents of this review.

Cell based injectables:

Smooth muscle cells and its precursor cells:

Every attempt made at regenerating a defective bladder muscular function invariably aims at developing a technique to replace native smooth muscles with a non-diseased healthy population of smooth muscle cells (SMCs) capable of fusing into host tissue. Muscle cell precursor cells and muscle cells have been thought to improve various types of urinary incontinence. This has been experimented in various pre-clinical and clinical models over the past two decades.

Studies concerning the transplantation of smooth muscle cells from autologous and allogenic sources are very few in number. Interestingly, smooth muscle replacement therapies have only been carried out in cardiac and urological tissues in the form of single-cell injections or as tissue-engineered grafts. The idea of smooth muscle cell replacement therapy through cell transplantation was first introduced in cardiac tissues affected by myocardial infarction that resulted in myocardial scars(31). In the urological tissues, we found only one (our) study (17) that deals with direct use of autologous transplantation of SMCs in diabetes rat model. Later, various cell types with lineage leading to an SMC have been experimented. Even though the resultant cell type for any transplantation is an SMC, direct SMC transplantation is a neglected area of study. The answer to this might lie in the lower survival rates of transplanted SMCs and the non-availability of unaffected SMCs from any patient in need of such a therapy.

Myoblasts are precursor cells that mature to form SMCs. Taking a clue from the Myoblast Transfer Therapy (MTT) proposed during late 80s as a cure for Duchenne Muscular Dystrophy which involved skeletal myoblast cells (26), Chancellor et al attempted to transplant similar skeletal myoblasts from immortalized MDX cell lines into the urinary bladder and descending bladder neck (9). They reported formation of multinucleated Myotubes between transplanted cells and also with native cells. Defective adenoviral vectors when used to transfect myoblast cells, expressed the required protein during the formation of myotubes. This property of myoblasts makes them an effective vehicle to transfect muscle cells and continuously express required proteins for an extended period of time. An innovative 2-step treatment from another clinical trial (5) involving myoblast injections along with electrical stimulation showed promising results. Although critical analysis of results suggested that improvement in Quality of Life assessment could be due to mechanism similar to bulking agent, there is no evidence to suggest muscle cells act as bulk when transplanted into the urinary tract.

This strategy was persistently applied over many years to develop transplantation of muscle-derived stem cells that exhibited better survival rates than myoblast cells. Successful clinical results demonstrated safety of patients and absence of any major adverse events (6). Patients also saw an improvement in quality of life after being relieved from symptoms of stress urinary incontinence (SUI). This is the first study to demonstrate a dose-related improvement of stress leaks in patients of both the gender over a 12-month period. Further clinical studies in Europe with 8 (37) subjects have reported the procedure of implantation to be well tolerated with no adverse events. They pointed to decrease in loss of urine followed by insignificant change in parameters in female patients., These female subjects failed to show significant improvement in tested parameters such as rate of urine flow and bladder pressure during micturition. Even though the reasons for failure in them are not mentioned, we presume that it has to do with greater sphincter control provided by extended urethra of male anatomical structures.

In conclusion, even though mixed results are available with various groups undertaking muscle-derived stem cell transplantation in clinical settings, this methodology of using muscle progenitor and muscle-derived stem cells has proven to be successful in reaching the final stages of patient care.

Non-muscle cells and stem cells:

The hunt for ideal replacement of diseased cells started off with targeting smooth muscle replacement therapy. Smooth muscle cells, muscle-derived cells, myoblasts and various precursor cells were found to be the right candidate. Gradually, this success was found to be short-lived because of lack of healthy source of cells. For example, in cases of incontinence, spinal cord injury or diabetes, SMCs get irreversibly damaged due to hypertrophy or were found to lack the endogenous population of fully functional cells for complete regeneration. This led to renewed efforts to find non-urinary, non-muscle cell type capable of differentiating into a SMC and thereby pave way for regeneration (30,32). Here, we discuss the use of various types of stem cells from different origins and their scope of success in bladder environment.

Mesenchymal stem cells:

Stem cells have proven to exist in every organ of the human body. Even though their presence has been confirmed with successful isolation from various sources, this cell type has never been successfully isolated from the bladder, though there are some suitable candidates that fit the idea of a progenitor smooth muscle cell. Adult stem cell transplantation has been explored as replacement for pathologically defective smooth muscle cells. Initially, stem cell transplantation studies in myocardial tissues were found to be successful in improving cardiac function. Owing to the similarities in smooth muscle architecture and functions of contraction, pathological bladder models were also transplanted with stem cells in the belief that they would be capable of neurogenic and myogenic differentiation.

Most of such attempts have produced positive results and are explained in detail under separate headings.

While most researchers consider bone marrow mesenchymal stem cells (BMSCs) as an ideal candidate (38), sources from adipose (ADSCs) and muscle derived stem cells (MDSCs) have also found to be of immense differentiation potential and easily available through very minimal surgical intervention. BMSCs have exhibited vast differentiation potential into urothelium and SMC lineages (15). In vitro myogenic differentiation of human bone marrow-derived mesenchymal stem cells were evaluated as a potential treatment for urethral sphincter muscle repair. BMSCs were transplanted into bladder tissue and have also been demonstrated to directly home to the region of damaged bladder when injected intravenously (40). While mechanism behind homing of MSCs remain unclear, it would suffice to mention that such data have been rare even though they hold immense potential in view of clinical benefits associated with non-surgical, preferred route of transplantation. Transplantation into bladder tissue has also led to various inventions in reconstruction surgery (12). BMSCs show a fibroblastic morphology similar to SMCs and readily differentiate into SMCs.

In addition to BMSCs, a similar type of MSC derived from amniotic fluid (AFMSC) have shown that in models of cryo-injured bladders, both BMSCs and AFMSCs fail to show greater morphogenesis and integration into native smooth muscle bundles (10). Upon quantitative analysis, large population of transplanted cells remained undifferentiated probably owing to reduced availability of growth factors such as hepatocyte growth factors and TGF-β. These factors related to low differentiation in-vivo prompted researchers to either process the BMSCs to de-differentiate in-vitro or adopt stem cells from other sources. Use of AFMSCs in an interesting approach to ameliorate bladder dysfunction in animal model of Parkinson disease was first detailed by Soler et al (36). Even though the cells were not directly transplanted into the urinary tract, the urodynamic improvement was observed as a result of paracrine effect at the site of injury (brain). AFMSCs remain an untapped and promising source for

direct cell grafting and indirect regeneration of LUT in neurogenic models via unclear pathways. Their clinical benefits however, are beginning to ascertain the usefulness of this niche.

Muscle derived stem cells (MDSCs) offer higher survival and integration rates when compared to muscle cells and muscle precursor myoblast cells (5). Interestingly, MDSCs are the only cell types that were found to develop myotubes with native host smooth muscle cells. This unique property has so far made it an ideal candidate for bladder neck and mid-urethral repair. MDSCs are the only cell types to successfully make it to the stage of single-cell injectable therapy in clinical trial. Encouraging results of clinical trials have begun to emerge from a mixture of procedures using MDSCs transplantation and traditional SUI techniques such as vaginal tapes and slings in cases of stress urinary incontinence and prostatectomy related sphincter damage (7,16,18).

Adipose derived stem cells (ADSCs):

Bladder smooth muscle cells are derived from bladder mesenchyme. Hence, multipotent cells from the mesenchyme are well poised to develop into myogenic lineage. Adipose tissue derived stem cells were found to be capable of multi-lineage differentiation. These cells have specifically been tested in models of SUI and have shown myogenic differentiation in-vivo (19,23,42). The issue with this cell type is the presence of mixed cell types in culture. Some researchers used the whole component of lipoaspirate cells without further selection while most researchers have been successful in isolating adipose derived stem cells (ADSCs) in transplants to bladder neck and mid urethra. Further standardization of this technique is necessary to ascertain specific cell types and destiny of transplanted cells. While there have been consistent reports of SMC differentiation over longer periods post-transplantation, natural neurogenic differentiation of ADSCs and one report of such differentiation in-vivo raise questions about the safety of these source of cells in regenerative urologic treatment (23). Also, the bulky nature

of adipose tissue cells makes them natural alternatives to artificial bulking agents such as collagen and polytetrafluoroethylene. Their role as bulking agents must clearly be negated while assessing cell integration and functional improvement in diseased animal and clinical studies.

Adipose tissue has been a useful source of mesenchymal stem cells because of vestigial nature and ease of access to this tissue. Their ability to differentiate into SMC phenotype via TGF-β pathway has made them a preferred choice in various diseases of the LUT (22,32). While mode of transplantation and delivery remain important factors influencing the success of any cell transplantation, Zhang H et al report amelioration of bladder dysfunction in 40% of test animals of diabetes mellitus where the cells were introduced into the intravenous route, and direct homing of ADSC were observed into affected bladder probably with the expression of certain homing factors. Direct transplantation of these cells into the bladder tissue resulted in an improvement in voiding function in 60% of treated animals (42).

ADSCs also showed equally appreciable improvements resulting in lower micturition frequency combined with higher voided volume in hyperlipidemic model of overactive bladder (19). Lipoaspirate cells have hence been proven to have greater potential, but further studies and its clinical implementation have not been established. It is also unclear if use of ADSCs sourced from metabolically compromised cases of diabetes or hyperlipidemia would be of any use considering effect of these disorders on defective signaling and growth pathways.

Tissue engineered reconstructive grafts:

Autologous and allogenic cells from various sources used as single cell injectables for bladder regeneration have been discussed in the previous segment. But the biggest breakthroughs in regenerative medicine in urology, as described in detail below, have been from using these cells as an alternate material to mimic the native host tissue and finally act as the best replacement for defective tissue. Tissue engineering of urological tissues such as urethra, bladder wall and ureters have provided

short term success in clinical setup which would be impossible to achieve through traditional conservative management options such as drugs and surgical interventions. Cell types native to the bladder have today been successfully implanted as graft tissue and have shown the way to replace enterocystoplasty using bowel tissues. Several groups have attempted, for over two decades, to design such a biological analogue tissue for grafting into urinary bladder (11,29).

Cells from the urinary tract for tissue-engineered grafts:

The credit for creating the first autologous tissue engineered bladder goes to Atala and his team currently at the Wakeforest Institute for Regenerative Medicine.

Prior to the early 90s, natural and artificial materials were explored to replace intestinal bowel tissues which were being used in enterocystoplasty procedures. Sero-muscular tissues, omentum flaps, Teflon and other synthetic meshes were being used. These turned out to be lithogenic, mucous-producing and immunogenic substances. Inventions in cosmetic surgery to treat burnt patients led to discovery of cell-seeded tissue engineered tissues capable of supporting natural regeneration of damaged skin. This discovery was applied into finding ideal replacement for urological tissues.

Initially, degradable (resorbable) polymers of polyglycolic acid were used as lattice and coated with urothelial cells (UC) grown in serum-free media (3). Sustained research led to invention of a completely de-novo reconstructed urinary bladder successfully transplanted in canine models of cystectomy. Later, the first reported clinical trials in patients with myelomeningocele announced an era of re-engineered tissues that could support any ailment arising of urological structures (2). These bladders used the scaffold of collagen-PGA polymer and were seeded with urothelial and smooth muscle cells in layers. Patients post-operatively demonstrated a dramatic increase in bladder compliance, bladder capacity and increased leak point pressures. Renal and bladder functions were preserved and showed normal recovery during the 5-year follow up. A recently concluded similar

clinical trial involving spina bifida patients over a 36 month follow-up suggest unsuccessful outcome with serious adverse effect including bladder rupture during later stages and bowel obstruction (20). Although two more multi-centric clinical trials involving pediatric spina bifida cases and adult spinal cord injury patients have been initiated by Atala et al (41), results of these studies are yet to be published but the subsequent licensing of this technology with a private company (Tengion Inc) has provided very less information regarding the progress made in this field ever since.

Critical evaluation of the above mentioned approach indicates several points that need to be answered in subsequent publications and scientific communications. 1) Long term results of fully functional neo-bladders need to be assessed to find problems that have previously been associated with enterocystoplasty. 2) Reinnervation of entire bladder seems to be a problem that has never been addressed. Although pre-clinical studies in large canine models with no neurogenic bladder condition and pathologically normal bladders, showed normal reinnervation capable of supporting regeneration, such an effect in clinical case studies need to be assessed. Especially when pathological bladders have been used as a source for procuring cells and grafting 3/4th of neo-bladders with 1/4th from patient's native tissue. Whole bladder substitutions have always remained doubtful. 3) Effect of neo-bladder constructs on other cases of end-stage bladder disease such as bladder cancer, congenital bladder defects and neurogenic bladders arising out of various pathological diseases. Apart from these medical queries, technical problems involving high costs and challenging human expertise make it improbable to implement it in regular clinics in the near future.

Moving ahead from engineered neo-bladders, it became clear that source of cells from pathological bladders have remained irreversible changed in their signaling and growth pathways. Hence, the ultimate goal of regenerative medicine would be to use cells from allogenic source or from autologous non-urinary organs. As discussed in the previous topic dealing with introduction of stem cells, these cell types were the alternative source of cells differentiating into native smooth muscle and urothelial

cells. Pluripotent and multipotent stem cells from hair follicles, adipose tissue, bone marrow, muscle biopsies, wharton jelly and amniotic sac were tested. Myogenic and uroepithelial differentiation could be achieved with cells from mesenchymal stem cells (1) and embryonic stem cells (25).

Cells from non-urinary tract tissues for tissue engineering:

Apart from the multipotent MSCs used as cell based injectables in bladder regeneration, pluripotent stem cells have been used in various other cardio-vascular, gastro-intestinal and neurological dysfunction. Smooth muscle cells derived from pluripotent hair follicle stem cells were first reported by Liu et al (21) which were channelized to bladder research by Drewa T et al (14). Under specific microenvironment related to urinary smooth muscle cells and urothelial cells, pluripotent stem cells derived from hair-follicles were cultured on bladder acellular matrix and anastomosed to defective bladders of rats.

Interestingly, the pluripotent nature of hair follicle stem cells (HFSCs) allows us to use only a single cell type that serves as progenitor to various types of lineages in the urinary bladder. Myogenic, urothelial, neuronal and vascular cells seem to originate from the HFSC. The same property of pluripotency is also a disadvantage. Controlled differentiation into the above mentioned lineages is a matter of concern. Long term effects of these induced differentiation in-vitro and in-vivo need to be observed. Also a matter of concern is the use of exogenous agents to induce lineage specific differentiation. Most transplant procedures in cell therapy have always preferred autologous trophic-factor driven in-vivo differentiation, whereas this is the only case where induced differentiation of cells have been experimented (13). The choice of biomaterials play an important part in assisting the regeneration of augmented cell types. Some of the materials proved detrimental to the growth of native tissue over the transplanted ones. Revascularizing the tissue became an inevitable strategy to lengthen the survival of graft tissue. One of the latest materials to have shown a greater degree of success are PGA and electrospun nanofibers. Many studies have begun to show that these materials allow greater

movement of growth factors and available nutrition from native to host tissue and hold a promising future in resolving the challenges in tissue engineering. One such study (35) describes the use of these nano scale fibers seeded with MSCs which showed formation of muscle bundles and partial regeneration of bladder in partially cystectomized rats..

MSCs from the bone marrow have potential to differentiate into smooth muscle and urothelial lineages. MSCs subjected to co-culture and conditioned medium environments, readily differentiate into required cell type (38). This principle was used in an in-vivo transplantation of induced MSCs cultured on a nano-fibrous poly L-lactic acid scaffold. The in-vitro induced differentiation, as well as the in-vivo studies showed expression of smooth muscle and urothelial markers such as α-SMA, myosin, uroplakins and pancytokeratins. However, no functional studies were performed on the animal models undergoing augmentation procedure. Also, the question regarding the permanent nature of SMC and UC de-differentiation or further differentiation into unknown phenotypes remain a matter of further studies.

MSCs have also been used in experimental augumented cystoplasty procedures wherein they were seeded onto small intestinal submucosa (SIS) and grafted into bladder (34). Improvements in bladder capacity and compliance values in non-human primate animal models provided excellent proof of better performance of cell-seeded grafts against acellular mimetic tissues. A more recent development in the same technique from Sharma et al (33) goes further to address the crucial lacunas of reinnervation and revascularization in tissue engineered constructs. With the BMSCs seeded onto an elastomeric scaffold that mimics the native bladder tissue, the group introduces a combinational approach of using CD34+ hematopoietic stem progenitor cells (HSPCs). These secondary treatments showed enhanced vascularization and innervation of triple-layered in-vivo graft tissue. Such combination of treatments show promising future for tissue engineered urological tissues.

An encompassing analysis of 131 cases of clinical outcomes of tissue-engineered bladders (27) detailing the success and critical analysis of drawbacks, states that the core issues affecting the tissue engineering approaches remain unsolved. Innervation and vascularization strategies by omental flaps, endothelial cells along with Schwann cell seeding strategies have been analyzed. An observation is also made regarding the use of healthy animal models in testing such new strategies and the lack of testing in diseased models whose impaired regeneration capability might prove to be an overlooked matter of utmost importance. On the whole, they provide an insight into an optimistic future for tissue engineering of urinary tract in the days to come.

Conclusion:

A consensus of sorts seems to be emerging between the use of cell injectables and artificially reconstructed bladders and its replacements. Injectable autologous cells capable of pluripotency and multipotency are becoming a natural way of replacing and regenerating some of the damaged cell functions of bladders and LUT tissue. However, artificial tissues and tissue replacements have had their fair share of problems yet to be overcome.

In the coming decade, native cell-types as well as specialized stem cells isolated from vestigial parts of the human body with very little effort may become a better choice for the above-mentioned disorders. Similar views from our previous work (17) as well as the continuing works from various scientists leading this effort seem to converge at the same point. It is now clear that the bladder and lower organs undoubtedly posses an ability to regenerate after cell grafting because of mechanisms, either due to trophic bystander effect or due to active cell replacement phenomena. Further research on the mechanisms, as well as long-term safety in clinical setting needs to be ascertained. Certainly, an unaffected, non-urinary cell type with clear potential to differentiate into urinary tract cell types would be an ideal choice.

Tissue replacements and artificially de-novo constructed tissues have shown a lot of promise in the past. Unfortunately, insufficient data regarding mechanism of action responsible for the regeneration of cellular structures along with reinnervation, combined with lack of long-term safety data seem to suggest a bleak future for this strategy until proven otherwise.

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Table 1: A Table summarizes the landmark developments describing the approach, outcome and analysis of various attempts

Cell-based Injectables			
Approach	**Authors (references)**	**Outcome**	**Analysis**
Myoblast Transfer Therapy	Chancellor et al (9) Blaganje and Lukanovic (5)	Myoblasts showed potential for clinical use in multiple trials. Whereas, in case of gene therapy approach using myoblasts, expression of required protein via myotubes were found in native tissue. Study limited to preclinical setup.	A gene therapy approach that has not been implemented in clinical setting due to ethical considerations involving pseudo-viral vectors. It i used only as a tool to understand basic mechanisms underlying incontinence.
Muscle-derived stem cell transplantation	Carr et al. (6) Surcell et al. (37) Hoshi et al. (18)	Clinical trials in Stress Urinary Incontinence patients resulted in safely tolerated dose-related improvement of clinical conditions	Mixed results of efficacy-oriented studies. Studies longer than 12 months are needed to assess mode of action and clinical benefit.

at regenerating the lower urinary tract.