14

Aspects of Urological Tissue Engineering

Arun K. Sharma[1,2,3] and Dorota I. Rozkiewicz[3,4]
[1]*Children's Memorial Hospital, Chicago Division of Pediatric Urology*
[2]*Northwestern University Feinberg School of Medicine, Department of Urology*
[3]*Northwestern University, Institute for BioNanotechnology in Medicine (IBNAM)*
[4]*Northwestern University, Department of Chemistry*
USA

1. Introduction

Factors affecting the homeostasis of the urological system stem from a multitude of different sources that include developmental miscues to aberrant signaling pathways leading to malignant transformations. In order to create functional tissues that can act as surrogate tissues while simultaneously mimicking endogenous anatomical and physiological functions, strategies utilizing regenerative medicine based methodologies must be employed. The urological system is comprised of the kidneys and their corresponding ureters; the bladder and sphincter as well as aspects of the genitourinary system. The complex architectural makeup and intricate physiological processes of the urological system at the gross and microscopic levels make tissue engineering attempts to recapitulate these tissues a very difficult and arduous process. Although great strides have been made in the field of urological tissue engineering over the last two decades, the clinical needs of patients suffering from urological defects have largely gone unfulfilled. However, the advent of novel approaches that span multiple disciplines including the material and clinical sciences as well as the constantly evolving field of stem cell biology have curtailed these issues to some extent. This Chapter will delve into strategies that are currently utilized for urological based tissue engineering while also providing alternative tactics to prevailing approaches.

2. Conditions affecting the urological system

2.1 The kidney

A simplistic overview of nephrogenesis proceeds as three sequential yet overlapping phases during human embryogenesis. During the third to fourth week of development, primitive cell populations derived from intermediate mesoderm converge upon the cervical region of the embryo to form one of two transitory structures termed the pronephros. (McCrory, 1974) These converging cell groups form rudimentary excretory units or nephrotomes that join with the pronephric duct. Although the pronephros is non-functional in mammals, it serves as a precursor structure for the slightly more defined mesonephros. (McCrory, 1974) The progressive development of the definitive kidney continues as the pronephros regresses and the development of mesenophric ducts again derived from intermediate mesoderm becomes

apparent. (Pelliniemi, 1983) The mesonephros is composed of two distinct structures, the mesenophric ducts and the mesonephric tubules. The mesonephric tubules acquire a bed of capillaries and are analogous in physiological function as well as anatomic structure to adult nephrons in the definitive kidney. These elementary nephrons include the glomerulus, renal corpuscles, and Bowman's capsule. The mesonephric ducts (Wolffian ducts) eventually give rise to the epididymis, the vas deferens, and the seminal vesicles in males and as vestigial organs in females. (McCrory, 1974; Hannema, 2006; Welsh, 2006) An outpocketing from the mesonephric duct arises during the fifth to six week of development to form the ureteric bud which becomes the decisive ureter as well as the collecting system found within the kidney including major and minor calyces, and the renal pelvis within the final stage of kidney formation, the metanephros. (McCrory, 1974; Fischer, 2001) The physiological aspects of kidney function are numerous in nature and non-exclusively include hormone production and regulation, homeostatic balance, pH and blood pressure control, waste removal and nutrient re-absorption. It is therefore imperative that the various physiological aspects of the kidney remain under proper control and maintenance. The introduction of insult in the forms of either trauma or aberrant developmental events can signify a poor outcome for the patient.

Congenital abnormalities of the kidney and urinary tract (CAKUT) occur in 1 out of 500 newborns which constitutes approximately 30% of all prenatal anomalies. (Toka, 2010) These include a plethora of conditions including horse-shoe kidney, ureter malformation/abnormalities, renal hypoplasia, renal agenesis, polycystic kidney disease, and Wilms' tumor formation and can arise at varying stages of the developmental process. (Toka, 2010; Stahl, 2006; Gimpel, 2010; Acien, 2010) Depending upon the severity of the malformation and organs affected, various strategies can been utilized to treat patients afflicted with CAKUT that include surgical and conservative management. However, even with treatment, kidney and urinary tract functions are typically far from ideal. Certain signaling pathways have been elucidated but only scratch the surface of this complex disorder. (Stahl, 2006; Nakanishi, 2003)

Aside from congenital defects affecting the kidney, acute and chronic conditions also predispose patients to poor clinical outcomes. Acute renal failure occurs with rapid onset and can result in loss of kidney function with eventual renal failure. (Bellomo, 2004; de Mendonca, 2000) As there are myriad circumstances that may cause acute kidney failure, it is a life threatening situation requiring great urgency in order to alleviate the underlying cause. Chronic conditions affecting the kidney are also in great number, most notably those involved with chronic hypertension and diabetes mellitus. The destruction of the kidney due to consistently high blood pressure or continual exposure to copious amounts of glucose results in several scenarios in which to manage the disease. Diabetes is the most common cause of kidney failure within the United States. Data from the 2011 National Diabetes Fact Sheet provided by the American Diabetes Association estimates that there are approximately 26 million people living with diabetes within the United States representing approximately 8% of the population. (http://www.diabetes.org/diabetes-basic/diabetes-statistics) Kidney failure as a result of diabetes accounted for 44% of new cases in 2008 and approximately 50,000 people began treatment for end stage kidney disease. Lastly, approximately 200,000 people were on either dialysis or underwent kidney transplantation as a result of chronic diabetes within the United States. A total of $348 billion was spent in 2007 to address diabetes related issues. This stagnating level of evidence suggests a great

medical need to overcome current shortcomings involved with kidney disease as tissue engineering and nanofabrication strategies for the kidney become more prevalent.

Kidney transplants and dialysis are the only current means to treat kidney failure. Attempts to recapitulate the kidney involve the transplantation of fetal kidneys, the integration of new nephrons into the kidney, and the use of embryonic stem cells. Fetal kidney transplantation was thought to be a possible means in which to identify tissue for replacement therapies since it was believed that fetal tissue did not present the high levels of major histocompatibility complexes (MHC) I and II as well as other immunostimulatory antigens. Studies performed by Dekel el al demonstrate the use of fetal human kidneys that were transplanted beneath the renal capsule of immune deficient rats. (Dekel, 1997a) Data suggests that fetal tissue displayed growth and development for as long as 4 months post-transplantation without tissue rejection that may have been caused by populations of T-cells previously residing in the fetal tissue. Analogous adult tissue underwent graft rejection upon stimulus with allogeneic injections of peripheral blood mononuclear cells (PMBC). (Dekel, 1997b, 1997c, 2000) Nephron transplantation became an alternative means in which to rejuvenate a faltering kidney. Woolf et al transplanted sections of metanephroi from E13-E16 mice into the kidney cortex of newborn outbred mice. (Woolf, 1991) Relevant kidney micro-architecture formed over time but complete incorporation of transplanted metanephroi was not found in the collecting ducts. A similar study using an analogous system (but not identical) failed to produce glomeruli. (Rogers, 1998) The lack of positive data with regard to kidney replacement methodologies leaves alternatives such as xenotransplantation with embryonic porcine kidneys. This tissue is not pluripotent but rather precursor tissue that has been naturally differentiated along kidney specific pathways. (Hammerman, 2011)

Lastly, highly compelling work by Roy and colleagues appears to be very promising in that they have created a bio-artificial implantable device that may take the place of damaged or deteriorating kidneys. Preliminary data suggests that this microfluidic bioreactor can mimic the physiological conditions found in the kidney with regard to fluid flow rate in the presence of kidney epithelial cells. (Ferrell, 2010) Furthermore, Roy et al has determined that nanofabricated silicon membranes used in this device demonstrate low levels of complement activation, low platelet adhesion, and low blood coagulability. (Muthusubramaniam, 2011) Studies are currently under way to test the feasibility of this device in animal models.

2.2 The ureter

Conditions which may warrant the replacement of a new ureter include a myriad of congenital afflictions including congenital atresia, stricture, bifid ureters as well as ureteral cancer and trauma. Arising from the renal pelvis of each kidney, the ureter descends and crosses into the urinary bladder. Damage to the valve found at the distal end of each ureter can result in vesicoureteral refux with eventually kidney damage if not treated. Within this scenario, damage can also manifest itself within the ureters as evidenced by dilated ureters caused by increased internal pressures. Tissue transplant studies employed to replace defective ureters have been promising. Armatys et al report the use of ileum as a ureteral replacement for reconstruction purposes. (Armatys, 2009) A retrospective study of 91 patients who underwent ureteral reconstruction using grafted ileum revealed that only 3.3% and 6.6% of patients endured long-term anastomotic stricture and fistula, respectively, and

had attained normal kidney function. Other groups have utilized vestigial tissue such as the appendix as well as buccal tissue with favorable results. (Dagash, 2008) Ureters constructed from synthetic materials have been used in the past to bypass the use of autolgous tissue sources. Desgrandchamps et al published two reports in which ureters constructed from a silicone polytetrafluoroethylene bonded tube was introduced into the pyelocaliceal renal graft cavities and placed subcutaneously into the suprapubic area where it was eventually guided into the bladder within human patients. (Desgrandchamps, 1995, 1998) A mean 2.5 year follow-up demonstrated that all tubes were patent and displayed no evidence of encrustation or obstruction. More recently, xenogenic accellular collagen membranes have also been used with limited success. (Koziak, 2004) The aforementioned studies provide evidence that various tissues derived and artificial matrices can be used for ureteral reconstruction. In order to further improve on the design and physiological function of the ureter, smart scaffolding material possessing antimicrobial attributes or capable of growth factor release, for example, may be incorporated into this setting such as those found in self assembling nanomolecules.

2.3 The urinary bladder

The urinary bladder is a hollow, musculocutaneous organ whose primary function is to expel waste products in the form of urine from the body. Damage or insult to this organ leads to bladder dysfunction which can have dire clinical consequences on upper and lower urological tracts including renal insufficiency with eventual renal failure. Patients suffering from severe bladder dysfunction caused by developmental defect, often resort to surgical means to stabilize poor bladder function. The various forms of spina bifida present the clinician and basic scientist alike with a battery of issues stemming from gross anatomical restructuring to the fine aspects of physiological function. The typical procedure to alleviate problems associated with the neurogenic bladder includes bladder augmentation cystoplasty. What is considered the "gold standard" procedure for patients suffering from end stage neurogenic bladder, a portion of the pathological, native bladder undergoes cystectomy. (Zini, 2004; Husmann, 2004) A detubularized portion of the bowel is then affixed to the cystectomized area of neurogenic bladder and hence serves as a neo-bladder in order to increase capacity. As the bladder is considered functional, it still lacks key attributes of a normally functioning bladder including sensory and contractile/expansion responses requiring lifetime self-catheterization. The reconstructed bladder also possess inherent complications including electrolyte imbalances as an absorptive tissue has been used to replace an excretory tissue; the high potential of infection; excess mucus formation; pronounced stone formation; spontaneous perforation, and the increased likelihood of malignant transformation. (Bankhead, 2000) Although the augmentation cystoplasty procedure is considered standard care for this situation, it is still a stopgap measure.

The biomedical applications of regenerated tissue took its roots from the pioneering work of Langer and colleagues. (Vacanti, 1988; Langer, 1993) Within these studies, it was determined that donor derived cells could be seeded upon synthetic, biodegradable scaffold materials and subsequently manipulated in vitro for eventual in vivo applications. Applying these basic principles of cell biology and engineering, Atala and colleagues embarked upon a mission to recapitulate functional urological tissue with the goal of clinical applicability. Initial studies utilizing rabbit derived urological tissue subsequently seeded upon polyglycolic acid (PGA) meshes and implanted into the tissue within and adjacent to the

peritoneal cavity of athymic mice yielded poignant results. (Atala, 1992) Rabbit derived urothelium was able to proliferate and populate the PGA scaffold to an approximate 3 cell thickness while retaining key urothelial markers after 30 days in vivo. This initial study set the stage for several other studies that continued to evaluate the roles of specific cell types combined with synthetic scaffolds for bladder regeneration. (Atala, 1993, 1998) Concurrently, studies by Knapp et al and others were evaluating the use of small intestinal submucosa (SIS) as an alternative biological scaffold for bladder tissue engineering. (Knapp, 1994; Kropp, 1995) The results from these studies seemed to suggest that SIS was a suitable biological material for the bladder augmentation process. However, in two separate studies by Ashley et al, unseeded SIS would be found to be highly pro-inflammatory. (Ashley, 2009, 2010) The seminal work that laid the foundation for future bladder regenerative studies came from Oberpenning et al (Oberpenning, 1999) Within this study, autologous sources of canine urothelial and smooth muscle cells were harvested then seeded onto polymers and subsequently expanded ex vivo. Completely cystectomized canines were then transplanted with this new bladder construct and observed for approximately a year. Very compelling data suggested that the engineered bladders demonstrated a normal micro-architecture, mechanical properties and the ability to store and express urine. This study set the foundation for a human clinical trial utilizing neurogenic urothelial and smooth muscle cells from spina bifida patients with myelomeningocele as a model system with the hopes that the bladder milieu could be recreated in human. (Atala, 2006) Following bladder biopsies, these two cell populations were isolated and expanded ex vivo and subsequently seeded upon either a collagen/PGA hybrid scaffolds or collagen alone scaffolds. These composite scaffolds were anastomosed to the native tissue following an initial cruciate incision to the native bladder. An approximate mean 4 year follow-up revealed no statistically improvement in collagen only based scaffolds for a number of different urodynamic functions including capacity, leak point pressure, and compliance. PGA/collagen scaffolds encased in omentum appeared to display a very modest increase in bladder capacity. The approach taken by Atala et al was bold and extremely novel and provided highly relevant data for this specific patient population. However, the data presented within the work failed to acknowledge that utilizing a pathological source of cells at the onset of this work could possibly result in abnormal cellular behavior or possible reformation of a diseased bladder state. Characterization of bladder smooth muscle cells derived from patients with myelomeningocele indicates that cultured bladder smooth muscle cells possess different characteristics than their normal counterparts. (Lin, 2004; Beqaj, 2005) Finally, the aforementioned augmented bladders continued to be non-contractile thus requiring the patients to presumably self catheterize for the remainder of their lifespan. Although groundbreaking in nature, much work needs to be performed in order to fully realize the goal of creating a functional bladder.

2.4 The urinary sphincters

The urinary sphincters are composed of a collection of muscles whose primary function is to prevent the premature leakage of urine from the bladder. (Feki, 2007; Delancey, 2004) The internal urethral sphincter is located at the proximal portion of the urethra and is a continuation of the detrusor muscle consisting of smooth muscle cells. The external urethral sphincter is comprised of skeletal muscle and is situated inferior to the prostate in males and at the bladder's distal inferior end in females. (Koyanagi, 1980) Disruption or dysregulation

of the sphincter system can lead to incontinence that encompass various forms including stress urinary incontinence, structural incontinence, urge and overflow incontinence as well as mixed incontinence. (Botlero, 2011; Siegler, 2004; Brubaker, 2011) The economic costs of urinary incontinence are quite staggering and account for greater than $20 billion dollars per year within the United States. (Wilson, 2001) Attempts to alleviate the dysfunction associated with incontinence have been manifested in various tissue engineering attempts to rejuvenate or replace poorly functioning sphincters. Yiou et al tracked the fate of muscle precursor cells that were implanted into a rat model of striated urethral sphincter injury. (Yiou, 2003) Muscle precursor cells were able to adapt to their environment and form myotubules in which acetylcholine receptors were present. Urodynamic studies demonstrated a 41% restoration of sphincter function at approximately one month post muscle precursor cell implantation. A second study similarly utilized muscle derived progenitor cells in a denervated female rat urethra model. (Cannon, 2003) Two weeks following cell injection, urethral muscle strips were analyzed under electrical field stimulation. Injected muscle derived progenitor cells greatly improved the amplitude associated with fast-twitch muscle contractions at a rate of approximately 87% as compared to control animals. The aforementioned studies and those that have incorporated the use of synthetic bulking agents such as poly(lactic-co-glycolic acid) (PLGA) microspheres have demonstrated alternative means in which to overcome obstacles associated with urinary incontinence. (Berjukow, 2004; Oh, 2006) The foundation that these works have created has resulted in over 50 clinical trials related to urinary incontinence. (http://clinicaltrials.gov) The field continues to evolve at a rapid pace partially driven by a need to create a solution to this problem as well as the potential for high financial benefits.

2.5 The urethra

Urethral narrowing is the most common affliction affecting the urethra. Physical trauma or insult by bacteria or virus can result in an abundance of scar tissue caused the local invasion of inflammatory cells as the body attempts to resolve the situation at hand resulting in stricture. Clinical presentation includes painful urination, decreased urinary output, dark colored urine, pelvic pain, and incontinence among some of the symptoms. (Ghoniem, 1994; Barbagli, 2007; Mangera, 2010) Depending upon the anatomical location and the severity of the stricture, different techniques have been utilized to remedy stricture exacerbation. Urethroplasty has been successfully utilized as a treatment option to repair the stricture. One method is to excise the stricture from the urethra and then rejoin the opposing ends. A second approach requires tissue grafting from typically autologous sources of buccal mucosa, amniotic membrane, or saphenous vein tissue. (Mangera, 2010; Goel, 2011, Levine, 2007, Shaeer, 2006) Complications associated with this type of surgery include fistula formation, infection, and stricture reformation. (Olajide, 2010) Tissue engineering approaches using synthetic and biologic matrices have also been investigated as alternative approaches to urethral repair. Derivatives of PLGA have been created to mimic buccal mucosa with the added benefit of possessing superior mechanical and elastic properties. (Selim, 2011) Other biomaterials such as SIS, bladder acellular matrix (BAM), and acellular corpus spongiosum matrix (ACSM) have also been utilized in studies with favorable results. (Feng, 2010; Powers, 2010) Since host inflammatory responses appear to be a major culprit behind stricture formation and its persistence, means to address this issue should be

explored. One practical solution may be the localized delivery of anti-inflammatory agents into the affected area to prevent further stricture formation. This may be accomplished via a number of delivery vehicles including hydrogel releasing anti-inflammatory agents as well as self-assembling nanomolecules capable of down-regulating the cellular inflammatory response through surface expression of anti-inflammatory epitopes. Current research is focusing upon the creation of peptide amphiphiles that may be capable of such feats. Combined with the anti-inflammatory characteristics of mesenchymal stem cells, this one-two punch to either attenuate inflammation or act in a prophylactic manner may help with stricture development and other biologically relevant other pro-inflammatory situations.

3. Stem cells and potential applications in urological regenerative medicine

Stem cells can be described in several different embodiments such as those that are derived from embryonic sources or somatic (adult) in nature. The differential capabilities of these cells can further be sub-categorized as being either totipotent, pluripotent, or multipotent. Totipotent stem cells are a result of a fusion of sperm and egg that have undergone limited divisions during early embryological development in mammalian systems. (Mitalipov, 2009; Seydoux, 2006) At this early stage of development, totipotent stem cells have the potential to become any type of cell in the body which also includes extraembryonic tissue. (Mitalipov, 2009; Seydoux, 2006) As a zygote undergoes further differentiation, totipotent stem cells lose their ability to create whole organisms and become more lineage restricted, hence becoming pluripotent. Pluripotent stem cells can give rise to tissues of endoderm, ectoderm, and mesoderm origin, but not extraembryonic tissue and can be derived from both embryonic and somatic cells. (Pittenger, 1999, Thomson, 1998; Takahashi, 2007; Ko, 2009) Multipotent cells are greatly restricted in their ability to generate specific differentiated cell types and subsequently tissue. These cells retain the ability to self-renew and divide into daughter progeny, but these characteristics are greatly limited. A highly characterized population of cells that fits this definition is mesenchymal stem cells that have been described to differentiate into adipocytes, osteocytes, chondrocytes, and others. (Pittenger, 1999, Phadnis, 2011)

3.1 Mesenchymal stem cells

The hematopoietic system and its constituents have been extensively characterized at multiple levels via decades of research and clinical experiences. The hematopoietic system found within the bone marrow of long bones in mammals consists of a hierarchy of cells originating from a primitive hematopoietic stem cell. The hematopoietic stem cell is capable of giving rise to all blood derived cells including those of lymphoid and myeloid origin. (Akashi, 2000) Also residing in the bone marrow are non-hematopoietic multipotent mesenchymal stem cells capable of variable cellular differentiation as previously described. (Takahashi, 2007; Ko, 2009; Phadnis, 2011) The frequency of which bone marrow derived mesenchymal stem cells are found within long bones is approximately 0.001% to 0.1% of nucleated cells which include adipocytes, macrophages, osteoblasts, and reticular connective tissue. (Pittenger, 1999) Therefore, fibroblast cells that reside in the bone marrow are not synonymous with mesenchymal stem cells. The plasticity demonstrated by mesenchymal cells can be utilized in a variety of regenerative medicine settings, especially those targeting urologic defects.

In a series of studies utilizing bone marrow derived mesenchymal stem cells from normal human, adult donors, Sharma et al demonstrate the phenotypic and physiological similarities between bladder smooth muscle cells and adult mesenchymal stem cells. (Sharma, 2009) The mesenchymal stem cells utilized throughout this study as well as subsequent studies were an epitope defined population of highly purified mesenchymal stem cells. This study further demonstrated that both populations of cells can respond to agonist stimulation with statistically indistinguishable contractile responses in vitro while possessing similar contractile machinery. A second study by Sharma and colleagues utilizes adult mesenchymal stem cells in a nude rat bladder augmentation model. (Sharma, 2010a) Within the context of this study, bladder regeneration occurred more potently with mesenchymal stem cells than normal human bladder smooth muscle cells and controls. Scaffolds seeded with mesenchymal stem cells expressed high levels of bladder smooth muscle markers along with typical muscle architecture in the form of muscle fascicles. These scaffolds also demonstrated an approximate 1:1 muscle to collagen ratio at the termination of the experiment which was vastly different from bladder smooth muscle cell seeded and unseeded controls. A third and more recent study by Sharma et al again demonstrate the utility of mesenchymal stem cells in a bladder regenerative setting except in this instance, autologous sources of epitope defined mesenchymal stem cells were utilized to establish a novel bladder augmentation model in a non-human primate. (Sharma, 2010b) Data from this study provides evidence that mesenchymal stem cell seeded scaffolds performed exceptionally as defined by physiological measurements and tissue phenotyping studies with a battery of proliferative and smooth muscle markers compared to controls. More importantly, this newly described augmentation model demonstrates potential clinical feasibility as this non-human primate is highly analogous to humans both anatomically and physiologically. Hence the use of autologous sources of non-pathological cells can be successfully utilized in a bladder regenerative setting.

Although the aforementioned studies provided strong evidence for bladder regeneration in hemicystectomized animals, future endeavors will require a great deal of scientific prowess to regenerate a fully functional bladder. Hence, the natural course of action would be the continuance of these studies. One aspect in conjunction with regenerating tissue is to create an environment that can provide a robust blood supply to developing tissue. Genetically modified mesenchymal stem cells carrying pro-angiogenic growth factors under the control of inducible promoters may aid in this function. A second strategy involves the use of nanomolecules capable of delivering much needed growth factors to tissues undergoing regeneration. Rajangam et al demonstrate robust in vivo angiogenesis via the delivery of heparin binding nanostructures. (Rajangam, 2006) The cross-pollination of stem cell biology with nanotechnology derived materials can potentially provide reliable and reproducible results when applied to urologic tissue regeneration which is the basis for future studies.

There are several tissue sources in which mesenchymal stem cells can be identified and isolated. Two of those sources are found within adipose tissue as well as amniotic fluid which bathes the fetus in utero. These cell populations have been shown to function within urological settings. Dyslipidemia has been associated with a variety of poorly compliant physiological conditions including erectile dysfunction. (Huang, 2010) Damage occurs to the cavernous endothelium and associated nerves through mechanisms that are not quite fully understood. In order to facilitate recovery, adipose derived mesenchymal stem cells

have been utilized to augment repair in the penis with the understanding that various factors secreted from mesenchymal stem cells promote re-growth of endothelium and improved nerve conduction. Within the context of this study, adipose derived mesenchymal stem cells were injected into the corpus cavernosum of dyslipidemic rats. Following a period of recovery, erectile function was assessed and mean intracavernous pressure/arterial pressures ratios were superior to control samples. Increased levels of nitric oxide synthase and alpha smooth muscle actin were also observed. (Huang, 2010) This study provides preliminary evidence that adipose derived mesenchymal stem cells can be used to ameliorate the urologic complications that arise in urologic settings. In order to obtain continual exposure of these factors in the afflicted areas, seeding these cells upon implantable nanofabricated materials may allow for immediate regeneration accompanied by long term maintenance. Nanofabricated materials can recreate specific cell niches in order to potentially enhance the regenerative effect and this topic will be discussed in greater detail within subsequent sections. (Murtuza, 2009)

Adipose derived mesenchymal stem cells have also been utilized in bladder regenerative settings and provide a means to obtain a multipotent cell source through adipose tissue aspirates. (Jack, 2009) Adipose derived mesenchymal stem cells maintain an approximate 3% of all nucleated cells within a sample of aspirate. Jack et al seeded adipose derived mesenchymal stem cells upon PLGA scaffolds and implanted them in 50% cystectomized athymic nude rats. 12 weeks post-transplantation, physiological measurements were assessed as well histological and immunofluorescent analyses, and organ bath studies. All data derived from this study indicate that the adipose derived mesenchymal stem cells perform similarly or better than control samples in almost every biological aspect. The novelty of this study obviously involves the use of this unique population cells. Future studies involving potentially sub-total cystectomized large animal models will hopefully provide a great deal of physiological information that may be applied to the human condition.

The recently described characterization of amniotic fluid mesenchymal stem cells offers another potential alternative cell source that may be applied to urologic tissue engineering. Initially described by In 't Anker et al, 2ml of second trimester amniotic fluid was obtained through transcervical isolation. (In't Anker, 2003) Subsequent cell populations were cultured in vitro and coerced to undergo terminal differentiation where they demonstrated differentiation into adipocytes and osteocytes. Transplantation studies also provided evidence that these cells could be used in an allogeneic setting, which suggests that these cells may possess a greater immune-privileged status than their adult counterparts. The application of amniotic fluid derived mesenchymal stem cells in a bladder injury model convincingly demonstrates the wound healing potential of these cells. (De Coppi, 2007) The direct injection of amniotic fluid derived mesenchymal stem cells into the cryo-injured bladder resulted in markedly reduced levels of smooth muscle hypertrophy but also demonstrated a limited level of smooth muscle cell regeneration which may have been attributed to inadequate numbers of injected cells since a dose response curve was not performed. Although both experimental procedures describe this source of cells as potent in function, the means in which they are acquired are highly invasive and pose great risk to fetus and mother. It is estimated that 1 in 300 women experience fetal loss, infection, cramping/vaginal bleeding or other complications during or following amniocentesis. (Simpson, 2007) The isolation and subsequent banking of these cells may allow a convenient means for future therapies in both autologous and allogeneic settings.

3.2 Endothelial progenitor cells

The lack of a properly vascularized graft is the bane of many tissue engineering studies regardless of the tissue attempting to be regenerated. Inadequate or improper vascularization leads to poor tissue development or graft failure of implanted cell/scaffold composites. This is evident towards the center of grafted composites as these areas are typically inaccessible to nutrient and gas exchange potentially resulting in a necrotic core. A manner in which to avoid the aforementioned scenarios would be to utilize autologous sources of endothelial progenitor cells. These cells could promote vascular growth even at the most cell dense levels of the graft since the endothelial progenitor cells can simultaneously be seeded along with other bladder cell types thereby encouraging vascular growth. Endothelial progenitors can typically be isolated from either the bone marrow or rather innocuously from the peripheral blood as circulating endothelial progenitor cells.

3.2.1 Bone marrow derived endothelial progenitor cells

It was initially speculated that cells that were destined to become constituents of blood vessels as a result of vascular growth were present throughout embryonic and early stages of fetal development. This was later revealed to be somewhat inaccurate with the advent of specific antibodies utilized to phenotype specific cell populations. The $CD34^+$ hematopoietic stem cell has been greatly characterized over the last several decades and it has been demonstrated that subset populations derived from bone marrow sources have the capacity to either form functional vasculature in or contribute to its remodeling vivo. (Abu El-Asrar, 2009; Ruger, 2008; Baum, 1992; Takahashi, 1999) $CD34^+/CD133^+$ cells derived from the bone marrow have provided evidence that this population can give rise to highly purified endothelial cells. (Quirici, 2001) Sharma et al describe the in vivo potential of this cell population as it was seeded within a chicken chorioallantoic membrane model. (Sharma, 2009) Post experimentation analyses of this work clearly demonstrate the angiogenic potential of bone marrow derived $CD34^+/CD133^+$ cells as human specific vasculature was formed utilizing this model. Other derivatives of this cell population including $CD34^+/CD133^+/VEGFR2^+$, $CD34^+/CD133^+/VEGFR3^+$, $CD34^+/VEGFR2^+$, and $CD133^+/VEGFR2^+$ cells maintain similar properties. (Salven, 2003; Luppi, 2010) However some may argue that the CD34 epitope is not required to describe an endothelial progenitor/precursor cell, although those groups appear to be in the minority. (Friedrich, 2006) In all cases, the frequency of bone marrow derived endothelial progenitor cells is quite low, hence other alternatives cell sources should be examined for potential regenerative medicine purposes.

3.2.2 Circulating endothelial progenitor cells

The seminal discovery that endothelial progenitor cells were persistent throughout the vascular system gave a tremendous boost to a number of tissue engineering approaches. Approximately 11 years ago, Asahara et al describes a simplistic and non-invasive means to isolate circulating endothelial progenitor cells. (Asahara, 1997) By utilizing $CD34^+$ cells isolated from peripheral blood, the study demonstrates that this population of cells was able to differentiate into endothelial cells in vitro while concurrently incorporating into sites of active angiogenesis in an animal model of ischemia. Data goes onto describe the concept that circulating endothelial progenitor cells can home to areas of pre-existing angiogenesis and contribute to the angiogenic process. Since this revelation, many studies have been performed that confirm the concept of circulating endothelial progenitor cells. (Dubois, 2011;

Baker, 2011) One recent example demonstrates the construction of a tri-leaf heart valve seeded with circulating endothelial progenitor cells. (Sales, 2010) Data revealed that endothelial progenitor cells were able to colonize the heart valve and provide both interstitial and endothelial functions to the evolving valve. (Sales, 2010) This again demonstrates the potential utility of endothelial progenitor cells. The use of endothelial progenitor cells is pivotal for tissue regeneration and cannot be understated. The goal of utilizing endothelial progenitor cells for tissue regeneration is definitely not simplistic in the sense by just applying a population of endothelial progenitor cells to a graft and assuming angiogenic events. The shear number of molecules that are involved in the angiogenic pathway need to be carefully balanced in order to prevent aberrant results. This can be demonstrated as an over-abundance of localized vascularization leading to excessive bleeding and subsequent pathological issues. The strengths of using circulating endothelial cells are that they can be isolated in large numbers and more importantly, they can be isolated in an autologous fashion. The latter avoids a battery of immunological events and immunosuppressive therapies that may have to be endured for the lifetime of the patient. Unfortunately, works describing the use endothelial progenitor cells in urological tissue engineering is very limited and should be further investigated as this resource is being squandered.

3.3 Neural stem/progenitor cells

The greatest issue involved with urological based tissue engineering strategies (in this author's opinion) is the recapitulation of functional nervous tissue. Although attempts have been made to provide neural functionality to urologic organs such as the bladder utilizing nerve re-routing techniques, these results have been met with skepticism and concern. (Xiao, 2005; Kurzrock, 2010; Park, 2010) Thus, alternate avenues should be evaluated to create functional neural circuitry for urologic organs such as the use of stem cells. The initial isolation and identification of neural stem cells occurred in 1992 by Reynolds and Weiss. (Reynolds & Weiss, 1992) Neural stem cells were isolated from the striatum of the mouse brain and under specific culture conditions coerced to differentiate into neurons and astrocytes. Further testing demonstrated morphological features that were indicative of functional neural cells. This seminal work provided compelling evidence that argued against the long established belief that brain derived tissue could not undergo neurogenesis after birth or damaging insult. Subsequent in vivo studies exploited the use of neural stem and more differentiated neural progenitor cells in a variety of in vivo settings as cells were also being isolated from peripheral nervous systems. (Gage, 2000) One study in particular describes the transplantation of neural progenitor cells into the cortical region of the rat brain that underwent lesion induction. (Prajerova, 2010) Electrophysiological assessments of brain slices revealed the generation of action potentials created by the neural progenitor cells that were statistically greater than control samples. Evidence was also provided in which transplanted neural progenitor cells exhibited expression of differentiated neural markers by immuno-staining. (Prajerova, 2010)

As of this writing, attempts to utilize bona fide neural stem or progenitor cells have not come to fruition in a urological regenerative medicine setting. One study does report the use of neuronal-glial precursor cells which facilitated the mild improvement in bladder physiological function. These included various urodynamic parameters such as mean voiding pressure, capacity, bladder pressure, and mean contraction amplitude. These values were marginally better than control animals. (Temeltas, 2009) It is quite apparent that a

considerable amount of research is required to address the major concerns regarding urologic tissue neuro-regeneration. Cultivating cross-discipline approaches may provide a suitable means to an end.

3.4 Embryonic stem cells

The seemingly limitless therapeutic potential of human embryonic stem cells has commanded the attention of the scientific community since its initial discovery. (Thomson, 1998) Derived from the inner cell mass of the developing blastocyst, pluripotent embryonic stem cells can be isolated and possess several unique features. These are typified by high nuclei to cytoplasm ratios, very pronounced nucleoli, the capability of indefinite cellular propagation, and most significantly, the ability to differentiate into cell types composed of all three germ layers. (Thomson, 1998) The isolation and characterization of embryonic stem cells laid the foundation for literally thousands of studies that now spans multiple disciplines and aspects of regenerative medicine. (Birket, 2011; Nistor, 2005; Farzaneh, 2010) The use of embryonic stem cells is slowly making its way into the realm of urological tissue engineering. One study describes an in vitro culture environment in which conditioned media from primitive ureteric bud cells were used to support the differentiation of murine mesoderm cells initially derived embryonic stem cells. (Ren, 2010) The mesoderm cells were primed with activin and retinoic acid and subsequently forcibly differentiated into cells of renal lineage with the aid of the conditioned media. The strong inductive effect of the secreted molecules from the ureteric bud culture provides a means to elucidate potential mechanisms involved with mesoderm-renal cell lineage differentiation. A second, more recent study sheds light upon some of the molecules involved in the differentiation of murine embryonic cells into urothelium. (Mauney, 2010) As previously utilized in the aforementioned study by Ren et al, all trans-retinoic acid had the profound ability to induce pluripotent embryonic stem cells in to urothelial cells through a GATA4 and GATA6 signaling pathway. Stimulation of the embryonic stem cells with all trans-retinoic acid caused a prominent increase in several uroplakin genes including UP1A, UP1B, UP2, and UP3B with a simultaneous down-regulation in the pluripotency factor OCT-4 compared to control cell populations. Concurrently, transcription factors GATA4 and GATA6 were also greatly up-regulated. GATA4/6 null embryonic stem cell lines demonstrated a marked reduction in uroplakin expression when undergoing similar treatments as compared to wild type controls. This data suggests a partial mechanism with regard to the inductive effect of all trans-retinoic acid and its ability to drive embryonic stem cells into urothelial cells. Several other studies have also utilized embryonic stem cells or embryoid body derived cells to generate bladder tissue to varying capacities. (Thomas, 2008; Kinebuchi, 2008; Lakshmanan, 2005) Unfortunately, a lack of follow-up studies has seemed to put a damper on this potentially ground breaking field of research.

The utility of embryonic stem cells is obviously not without debate. Ethical and moral dilemmas have significant impact on political policy and are of grave concern for future studies utilizing embryonic stem cells that use federal funds to support research. 2001 saw restrictions placed upon the use and creation of embryonic stem cell lines, a ban that was later rescinded in 2009. The recent political atmosphere continues to be in a state of flux witnessed most recently by a recent court injunction that stopped the use of federal dollars to be used for embryonic stem cell research, thus effecting thousands of researchers. Although the injunction was temporarily stayed upon an appeal, it could set a damaging

precedent for the future. Privatized monies as found within the California Institute of Regenerative Medicine currently finance studies with embryonic stem cells with other states in pursuit. (http://cirm.ca.gov/for-researchers/researchfunding) Nevertheless, several clinical trials set forth by privately held companies such as Geron and Advanced Cell Technology are currently underway. (Strauss, 2010; http://www.advancedcell.com/news-and-media/press-release) The controversies surrounded embryonic stem cell research in part fueled the scientific community to pursue other avenues of research that would lead to the identification of an alternative source of pluripotent stem cells.

3.5 Induced pluripotent stem cells

Preliminary observations involving studies with somatic cell nuclear transfer into recipient oocytes resulted in cell populations that displayed features that were highly analogous to embryonic stem cells in morphology and function. (McGrath, 1983; Campbell, 1996) Via successive rounds of a highly selective molecular screening process, four genes were determined to be essential in the de-differentiation of terminally differentiated cells back to a pluripotent, embryonic-like state. Takahashi et al produced the first generation of induced pluripotent stem cells from human adult fibroblasts with the over-expression of four transcription factors including Sox2, Oct3/4, c-Myc, and Klf4. (Takahashi, 2007) These cells possessed embryonic stem cell-like qualities as they could differentiate into cells of endoderm, mesoderm, and ectoderm origin; were able to form teratomas in vivo; and proliferate indefinitely without losing their pluripotency. A subsequent study from Yu et al utilized a similar procedure derived from the Takahashi study but determined that a slightly different cocktail of genes (or proteins) could induce the same effect. (Yu, 2007) OCT4, SOX2, NANOG, and LIN28 were identified as pluripotency factors that had the same effect as the aforementioned gene set. Since a slightly different set of genes was utilized in the second study, it would be easy to speculate that different combinations of genes or yet to be identified factors that are tissue specific may be utilized to create pluripotent cell lines. This groundbreaking work led to a number of other studies focusing on specific cell type and disease states. (Moretti, 2010; Ebert, 2009) However, with each great discovery come certain drawbacks. It has been recently demonstrated that induced pluripotent stem cells outwardly function similarly to embryonic stem cells, but the gene expression signatures possessed by each cell type are quite different. (Chin, 2009) This is most likely a result of differential promoter binding by the reprogramming factors. Other studies also demonstrate the detrimental effects that coerced cellular reprogramming may induce upon target cell populations. (Hussein, 2011; Lister, 2011) The use of induced pluripotent stem cells in urological settings (excluding spinal and nerve injury scenarios) is still in its infancy with a paltry number of studies. (Morizane, 2009) As more data emerges with regards to the molecular machinery involved with the reprogramming process (along with potential consequences), perhaps these data will be applied to future urological regenerative medicine based studies.

4. Polymeric materials and their potential applications in urologic regenerative medicine

The choice of scaffold material is of paramount significance in determining the outcome of implanted cell/scaffold composites. There are a wide variety of scaffold materials that vary

greatly in composition, texture, mechanical and surface characteristics that could potentially be applied in a urological regenerative medicine setting. A number of these materials have been previously discussed in great detail such as bladder acellular matrix, small intestinal submucosa, varying embodiments of poly(lactic-co-glycolic acid) and polyglycolic acid, and collagen. Similarly, other chemically synthesized nano- and macro- based structures are also pivotal in creating hospitable environments for tissue growth and development. The focus of the forthcoming section will concentrate on relatively newly described elastic based materials that may be utilized for urological tissue engineering along with bioactive peptides and nanomolecules.

4.1 Synthetic elastomeric substrates for urological tissue engineering

4.1.1 Poly(diol citrates)

The mechanical properties that contribute to the overall composition of various urologic organs in part define its ultimate structure and function, specifically with regard to the urinary bladder. The urinary bladder is a very dynamic organ as demonstrated by its wide elastic properties further exemplified by its ability to undergo repeated contractile/expansion cycles without permanent deformation. Characterization studies of human urinary bladder tissue describe the elastic modulus to be within the low-hundreds of kPa (kilopascal) (Dahms, 1998). Commonly used scaffolds for urinary bladder tissue engineering such as non-woven polyglycolic acid and poly(lactic-co-glycolic acid), and collagen typically exhibit elastic moduli in the giga- and megaPa range. These features predispose this materials to possess poor elongation characteristics of <10% hence making them unsuitable for functional urinary bladder replacement strategies. (Middleton, 2000) It has also been demonstrated that degradation byproducts of these polymers contribute to a localized decrease in pH which can have a negative impact on cell function, including decreased collagen synthesis and impaired cellular differentiation capacity. (Kohn, 2002) Cyclical mechanical forces have also been demonstrated necessary for bladder smooth muscle growth and development while static and non-contractile environments are less likely to produce robust contractile responses. (Heise, 2009) POC [poly(1,8-octanediol-co-citrate)] is a relatively newly described member of a family of diols which is a synthetic polymer that has a wide range of applications including those for very elastic tissues such blood vessels, orthopedic applications, and perivascular wraps. (Yang, 2005; Qiu, 2006; Serrano, 2011) The elastic potential of this polymer is dependent upon several polymerization properties including the length and temperature of scaffold crosslinking. This allows for scaffold customization with regard to elastic potential. Sharma et al demonstrate the use of POC in a bladder augmentation model using bone marrow derived mesenchymal stem cells. (Sharma, 2010) Data from this study suggests the synthesis of a POC film that has elastic properties approaching that of a native human bladder and the ability of the POC scaffolds to undergo elongation up to 137% of their initial length. In vivo data suggests a high regenerative potential when utilizing POC/cell seeded composites as demonstrated by superior muscle to collagen ratios 10 weeks post-implantation (as compared to controls). The non-toxic, degradative by-products of POC also appear to limit the level of stone formation with the context of this study, which may aid future bladder regenerative studies. Lastly, data also suggests that POC can be chemically modified to release pro-angiogenic growth factors over-time that contributes to robust levels of angiogenesis in vivo. (Sharma, unpublished data)

4.1.2 Elastomeric poly(ester urethane)urea

A second well described elastomeric compound, poly(ester urethane)urea (PEUU), can also be configured into several different forms including those consistent with blood vessels while retaining elastic properties. (Stankus, 2007) By concurrent electrospraying and electrospinning of vascular smooth muscle cells and PEUU, respectively, a tubular conduit containing the cells was formed with uniform cellular distribution in a radial and circumferential manner. Further testing revealed the ability of the material to remain sutured, while maintaining compliancy that was similar to native arteries. Modification of PEUU also demonstrates the ability to control gene expression utilizing a cleverly created gene induction system. (Baraniak, 2011) Further modifications also allow for the release of bioactive molecules of insulin-like growth factor-1 and hepatocyte growth factor. (Nelson, 2011) Although this material has not been utilized in a urological regenerative setting to date, the implications of this material are obvious as this material could be structured for urinary bladder regenerative studies or the creation of synthetic ureters. An overly simplistic study design would include the seeding of porous scaffolds with relevant bladder cell types as previously described by Baraniak et al accompanied by the appropriate functional testing and immunohistochemical analyses. The added benefit of utilizing PEUU is its ability to become modified so that relevant genes such as those found within urologic epithelial stem cells may contribute to regeneration, for example. (Pascal, 2007) It is hopeful that studies involving PEUU and urological tissue engineering will be seen in the not too distant future.

4.2 Bioactive peptides and peptide amphiphiles for regeneration and wound healing

Polymeric materials which are enhanced with bioactive peptides show perhaps the greatest degree of promise for application in tissue regeneration scaffolds. Short peptide sequences originating from the cell binding regions of extracellular matrix (ECM) proteins can be incorporated into a graft material to obtain biospecific adhesion of cells. RGD (arginine-glycine-aspartic acid), the most commonly used peptide in cell adhesion studies, originating from fibronectin, collagen, fibrinogen and other ECM proteins, enhances adhesion and spreading of most cell types (fibroblasts, endothelial, and smooth muscle cells) and interacts with several different integrin receptors. (Humphries, 1990; Massia, 1991) It has been shown in the past that migration of smooth muscle cells and fibroblasts is biphasically dependant on the concentration of RGD attached to the migratory surface with intermediate loadings of RGD displaying maximal mobility. (Olbrich, 1996; Mann 2002) Polymers containing RGD sequence have been used in several wound healing in vivo and in vitro applications. For instance, incorporation of RGD peptide into PEG hydrogels partially eliminated inflammatory reaction of a scaffold implanted subcutaneously in immunocompetent in c57bl/6 mice resulting in a reduced presence of macrophages. (Lynn, 2011) Hydrogels containing RGD peptide were able to promote healing of ulcers, partial thickness burn wounds, and encourage ingrowth of glial tissue in the rat. PHSRN (proline-histidine-serine-arginine-glutamate), a cell binding peptide sequence has been found to act synergistically with RGD sequence for cell adhesion. (van der Veen, 2010; Davis, 2001) When applied individually it can stimulate invasion of ECM by keratinocytes and fibroblasts in vitro and it can enhance the re-epithelialization and concentration of dermal wounds in healing-impaired diabetic mice. (Livant, 2000) Other peptides sequences such as laminin-derived YIGSR (tyrosine-isoleucine-glycine-serine-arginine), LRE (leucine-arginine-glutamic acid) or

IKVAV (isoleucine-lysine-valine-alanine-valine) have potential application in nerve regeneration. (Fittkau, 2005; Pittier, 2005) YIGSR, unlike RGD and PHSRN, does not interact with the 67 kDa laminin binding protein. (Graf, 1987) In addition, YIGSR promotes the adhesion and spreading of many cell types including endothelial cells, fibroblasts, and smooth muscle cells. (Massia, 2011) It was found that PEG surfaces functionalized with cell binding peptide RGD and YIGSR, significantly increased microvascular endothelial cell migration rates relative to RGD alone. (Fittkau, 2005)

Another group demonstrated that degradation of endothelial ECMs with bacterial collagenase releases proangiogenic peptide fragments which can stimulate epithelial responses to injury and wound healing in vivo. (Demidova-Rice, 2011) It has been shown that sequences such as Col4-1, Col4-2, Fibr2, Fibr3, Ten1, Ten2, and Comb1 significantly increase the rate of sprout formation, which enhances cell-cell interactions and improves endothelial motility. Comb1 and Ten2 peptides contain the GXXPG sequence, which was previously reported to enhance endothelial migration and tube formation on collagen type I in vitro; both induce morphogenesis in a Matrigel-based assay. Comb1, as a larger combinatorial peptide with more complex tertiary structure, and two GXXPG sequences also stimulates cellular proliferation. The aforementioned sequences can also stimulate endothelial response to injury in complex environments where the cells remain viable for a prolonged time (up to one week). The 3D model of injury repair which was applied in that research consisted of two layers of Matrigel collagen mixture and a layer of endothelial cells sandwiched in between. Full thickness injury was created by aspiration, and the defect was filled with matrices containing serum, proangiogenic growth factors, or peptides. It was demonstrated that in addition to identification of promising proangiogenic or antiangiogenic therapeutics, the 3D model of injury repair can be employed to study the stability of wound healing compounds and to evaluate drug delivery systems. As described in this Chapter bioactive peptides play an important role in a wound healing process and tissue regeneration. Fabrication of scaffolds with a high specificity for the adhesion of a certain type of cells might require the use of a combination of bioactive peptides such as RGD, IKVAV, Ten 2, Comb 1, and biomimetic synthetic polymers, depending on the in vivo application.

Peptide amphiphiles (PA) are small molecules that contain a peptide sequence and a hydrophobic segment such as an alkyl tail. (Silva, 2004; Rajangam, 2006) Hydrophobic collapse of these filament-forming molecules under strong screening conditions leads to the display of biological signals on their surfaces in high density (on the order of 10^{15} signals per cm^2). (Cui, 2010) In vivo and in vitro studies have shown that certain PA molecules, bearing bioactive epitopes promote regeneration of spinal cord axons, angiogenesis, bone regeneration, cartilage repair, and selective differentiation of neural progenitor cells into neurons. (Sargeant, 2008; Webber, 2010; Silva, 2004) Previously, PAs were used to coat PGA microfiber scaffold for the improved attachment of smooth muscle cells. (Harrington, 2006; Behanna, 2005) This PGA scaffold was submerged in a suspension of smooth muscle cells in media and modified with PA gel mixed with growth factors as a top layer for the entrapment of cells. The opposite face of the scaffold could them be modified using a second component, which in this case was a mixture of PGA and urothelial cells, since these cells could potentially send necessary signals to smooth muscle cells during regeneration. (Beqaj, 2005; Imamura, 2007) When the human bladder smooth muscle cells and urothelial cells where embedded in a PA-scaffold with a growth factor (bFGF) and incubated in vivo in a

subcutaneous nude rat model, it was found that the human bladder smooth muscle cells were retained and composed the majority of the scaffold cellular content. It was also found that the system which uses a combination of PGA, PA, growth factors and cells demonstrated higher levels of phenotypic alpha-smooth muscle actin than control scaffolds made of PGA and cells only. PAs offer a great potential in regenerative medicine due to their nanoscale filamentous assembly resembling the one in ECM, the ability to incorporate any bioactive epitopes into their structure, and propensity to form gels.

4.3 Nano- and microstructured surfaces for bladder regeneration

Nano- and microstructured surfaces offer great potential for a range of biomedical applications from biochips, bioarrays and biosensors to functional membranes and cell adhesion substrates. It is much easier to create nano- or micrometer patterns on surfaces such as metal, glass or silicon than on polymeric surfaces since the surface composition and flatness is much better defined. (Qin, 2010; Jackman; 1998) The techniques that are usually employed for nano- and microstructuring of surfaces include nanoimprint lithography (NiL), microcontact printing, shadow-mask lithography, photolithography, chemical etching processes, robotic spotting techniques, and more. Unfortunately only very few of these techniques are suitable for the surface modification and structuring of (bio)polymers commonly used as the bladder regeneration materials such as PGA, PLGA, poly(L-lactic acid) (PLLA), poly(ε-caprolactone) (PCL), and poly(carbonate-urethane) (PCU). (Miller, 2004; Pattison, 2006) Nanoscale roughness is believed to improve the adhesion of proteins due to its higher surface area and surface energy. Surfaces functionalized with protein such as fibronectin, laminin, collagen I or IV, ECM-type of protein and surfaces with nanometer roughness visibly improve bladder cell adhesion and interactions with the substrate. This phenomena is important in bladder regeneration since many implanted polymeric membranes need to be relatively quickly and efficiently replaced by healthy host tissue. Tissue integration into the biomaterial must occur; therefore resident cells need to adhere appropriately to the substrate in order for subsequent cellular functions to occur at the implant site. Nanoscale roughness of biopolymers or synthetic biodegradable polymers used in bladder regeneration research can be achieved by numerous techniques including surface functionalization with nanometer-size fibers such as PAs, electrospinning, chemical etching, nano- and micromolding and more. (Harrington, 2006; Doshi, 1995; Thapa, 2003) The aforementioned techniques may be applied as potential methods for the fabrication of improved scaffolds for bladder regeneration.

Alternative means to achieve nanostructured membranes for bladder regeneration research utilizes micro or nanomolding. PLGA or PU polymers can be easily cast into molds with pre-fabricated nanometer features made of poly(dimenthylsiloxane) (PDMS). (Thapa, 2003) The procedure relies on pouring a solution of a polymer (for example PLGA or PU) onto a PDMS master mold, curing the polymer until is solid and releasing the polymer from the mold simply by peeling the film off from the mold. The minimum size of the features that could be achieved is defined by the master mold. This process is commonly used in the fabrication of microcontact printing stamps for soft lithography purposes. In this process the mold is usually fabricated in silicon through photolithography or e-beam lithography and the polymer which is used for stamps is usually PDMS. (Xia, 1998) PLGA and PU nanostructured polymers showed advantage in ovine bladder smooth muscle cells adhesion over polymers with larger features.

An interesting method for the bladder surface nanostructuring is the application of nanoparticles to introduce nanoscale roughness or improve certain properties such as permeability of the scaffold. One example was recently introduced where hyaluronic acid (HA) modified PLGA nanoparticles (HA PLGA NPs) were applied for the modification of SIS as a scaffold for bladder regeneration to alter its permeability and simultaneously deliver pro-regenerative compounds. (Roth, 2010) When SIS scaffolds were modified with PLGA NPs of the size in the range of 200-500 nm the scaffold showed a decrease in permeability to urea when compared with unmodified scaffolds. In addition, PLGA modified scaffolds demonstrated significantly higher adhesion of endothelial cells in comparison to unmodified scaffolds. Introduction of pro-regenerative compounds into the SIS scaffold via modification of PLGA NPs with HA (which has been recognized for its ability to promote scarless wound healing), improved the SIS scaffold in vivo performance. HA was attached through electrostatic interaction to the positively charged PLGA NPs which were previously modified with polyethylene imine to produce cationic NPs. The attachment of particles to the SIS scaffold was conducted by incubation and absorption of particles from solution. The modified SIS membrane was investigated for angiogenesis and permeability studies. It was found that HA-NP SIS demonstrated a statistically higher number of blood vessels when compared with unmodified. In addition, HA-NP SIS significantly decreased or even eliminated the propensity to form calcifications, decreased urea permeability and improved full-thickness bladder regeneration.

Electrospinning is one of the most commonly used techniques for the (nano)fiber formation. In this process, nanofibers can be formed through an electrically charged jet of polymer solution or polymer melt. The simplest setup for electrospinning is composed of a high voltage power supply, a polymer solution reservoir (e.g. a syringe with a small diameter needle), and two electrodes (one connected to the needle and the other to the surface of the collecting mat or other substrate). The polymer supply is connected with a power supply through an electrode clipped to the metal needle for electrospinning. Flow control pump can be used to precisely control the flow rate of the polymer solution which is spun onto a substrate. The fiber-collecting surface must be conductive in order to attract the electrospun polymer and can be either stationary or rotating plate for collecting nonwoven or aligned fibers, respectively.

Electrospinning permits fabrication of biodegradable elastomers into matrices that can resemble the scale and mechanical behavior of the native extracellular matrix. There is a great selection of polymers available for the electrospinning of nanofibrous scaffolds used in tissue engineering applications. Moreover, this process can be easily scaled up making the transformation from small-scale laboratory research into large-batch processes feasible. (Greiner, 2008; Zhong, 2010) In addition to being used to fabricate nonwoven mats for wound dressings, there is currently much interest in making scaffolds for bladder tissue engineering. (Roth, 2010; Gelain, 2008) Baker et al used a 3D electrospun nanoscaffold of polystyrene to determine whether the phenotype of cells isolated from the stroma of human ureter specimens could be modulated by growing cells within such scaffold. (Baker, 2008) Non-aligned scaffolds made out of fibers with mean diameter of 200 nm were used in that study. Unfortunately the analysis of SM-MHC protein expression in cultures grown in 3D revealed that those cultures failed to undergo differentiation. It was explained that nanostructured polystyrene scaffolds have a high propensity to adsorb serum which maintained stromal cell cultures in a synthetic non-differentiated phenotype. In this case,

the approach of growing cell cultures in a serum-free media on the 3D polystyrene scaffold did not bring positive results. It was found that despite of its promising advantages of electrospun scaffold, stromal cells did not differentiate primarily due to the high propensity of the scaffold to the absorption of proteins from the serum. However, serum-free media reduced the absorption of proteins but cells still showed a greatly reduced plating efficiency and cultures failed to survive.

Findings describing the interactions between human bladder urothelial cells (TEU-2) and a scaffold composite consisting of an electrospun fibrous matrix made of PCU and PLLA fused to a thin film for potential use in urothelial tissue regeneration. (Kundu, 2011) Their results suggested that when electrospun scaffolds alone (without the thin film) were used, their fibrous surface structure while allowing good cellular adhesion, inhibited the proliferation of urothelial cells, whereas cells seeded onto thin films with non-fibrous surfaces proliferated quickly. In addition, it was found that cells on electrospun fibers demonstrated numerous cellular extensions along the matrix fibers, rather than the substantial cell-cell integrations. It was explained that this finding may suggest that the fibrous nature of the electrospun materials do not facilitate the formation of an intact urothelium. The addition of thin films onto electrospun scaffolds, enhanced urothelial cell proliferation, surface coverage, cell-cell interactions, and multilayering in vitro. Electrospun scaffolds alone offer highly porous structures and deep cellular penetration, however they are unlikely to provide a sufficient barrier to water and solute transport when not embedded in a biomimetic synthetic polymer. Furthermore, the three-dimensional structure of such scaffolds may affect cell proliferation, differentiation or adhesion.

5. Conclusion

The task to create functional organs as a replacement therapy is indeed quite daunting. Tissue engineering studies during the late 1980s and early 1990s laid the groundwork for the current windfall of clinical applications that have been deployed for organ replacement. The continual evolution of materials science influenced materials combined with discoveries in the field of stem cell biology provides hope that goals of regenerative medicine can be achieved. Although there have been many significant advances with regard to urologic tissue engineering, the field is still in its infancy. There is still a great need to pursue basic and clinical science studies in order to benefit those afflicted with urological trauma or disease.

6. References

Abu El-Asrar A., Struyf S., Verbeke H., Van Damme J., Geboes K. (2009) Circulating bone-marrow-derived endothelial precursor cells contribute to neovascularization in diabetic epiretinal membranes. *Acta Ophthalmol.* [Epub ahead of print]

Acién P., Acién M. (2010) Unilateral renal agenesis and female genital tract pathologies. *Acta Obstet Gynecol Scand*. 89(11):1424-1431.

Akashi K., Traver D., Miyamoto T., Weissman I. (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature.* 404(6774):193-197.

Armatys S., Mellon M., Beck S., Koch M., Foster R., Bihrle R. (2009) Use of ileum as ureteral replacement in urological reconstruction. *J Urol.* 181(1):177-181.

Asahara T., Murohara T., Sullivan A., Silver M., van der Zee R., Li T., Witzenbichler B., Schatteman G., Isner J. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 275(5302):964-967.

Ashley R., Palmer B., Schultz A., Woodson B., Roth C., Routh J., Fung K., Frimberger D., Lin H., Kropp B. (2009) Leukocyte inflammatory response in a rat urinary bladder regeneration model using porcine small intestinal submucosa scaffold. *Tissue Eng Part A*. 15(11):3241-3246.

Ashley R., Roth C., Palmer B., Kibar Y., Routh J., Fung K., Frimberger D., Lin H., Kropp B. (2010) Regional variations in small intestinal submucosa evoke differences in inflammation with subsequent impact on tissue regeneration in the rat bladder augmentation model. *BJU Int.* 105(10):1462-1468.

Atala A. (1998) Autologous cell transplantation for urologic reconstruction. *J Urol.* 159(1):2-3.

Atala A., Bauer S., Soker S., Yoo J., Retik A. (2006) Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet.* 367(9518):1241-1246.

Atala A., Freeman M., Vacanti J., Shepard J., Retik A. (1993) Implantation in vivo and retrieval of artificial structures consisting of rabbit and human urothelium and human bladder muscle. *J Urol*. 150(2 Pt 2):608-612.

Atala A., Vacanti J., Peters C., Mandell J., Retik A., Freeman M. (1992) Formation of urothelial structures in vivo from dissociated cells attached to biodegradable polymer scaffolds in vitro. *J Urol.* 148(2 Pt 2):658-662.

Baker J., Zhang L., Imadojemu S., Sharpe A., Patil S., Moore J., Mohler E., Von Feldt J. (2011) Circulating endothelial progenitor cells are reduced in SLE in the absence of coronary artery calcification. *Rheumatol Int.* [Epub ahead of print]

Baker S., Southgate J. (2008) Towards control of smooth muscle cell differentiation in synthetic 3D scaffolds. Biomaterials. 29(23):3357-3366.

Bankhead, R., Kropp, B., Cheng, E. (2000) Evaluation and treatment of children with neurogenic bladders. *J Child Neurol.* 15(3):141-149.

Barbagli G., Lazzeri M. (2007) Surgical treatment of anterior urethral stricture diseases: brief overview. *Int Braz J Urol.* 33(4):461-469.

Baraniak P., Nelson D., Leeson C., Katakam A., Friz J., Cress D., Hong Y., Guan J., Wagner WR. (2011) Spatial control of gene expression within a scaffold by localized inducer release. *Biomaterials.* 32(11):3062-3071.

Baum C., Weissman I., Tsukamoto A., Buckle A., Peault B. (1992) Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A*. 89(7):2804-2808.

Behanna H., Donners J., Gordon A., Stupp S. (2005) Coassembly of amphiphiles with opposite peptide polarities into nanofibers. *J Am Chem Soc.* 127(4):1193-1200.

Bellomo R., Ronco C., Kellum J., Mehta R., Palevsky P. (2004) Acute Dialysis Quality Initiative Workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care.* 8(4):R204-R212.

Beqaj S., Donovan J., Liu D., Harrington D., Alpert S., Cheng E. (2005) Role of basic fibroblast growth factor in the neuropathic bladder phenotype. *J Urol.* 174(4 Pt 2):1699-1703.

Berjukow S., Margreiter E., Marksteiner R., Strasser H., Bartsch G., Hering S. (2004) Membrane properties of single muscle cells of the rhabdosphincter of the male urethra. *Prostate.* 58(3):238-247.

Birket M., Orr A., Gerencser A., Madden D., Vitelli C., Swistowski A., Brand M., Zeng X. (2011) A reduction in ATP demand and mitochondrial activity with neural differentiation of human embryonic stem cells. *J Cell Sci.* 124(Pt 3):348-358.

Botlero R., Davis S., Urquhart D., Bell R. (2011) Incidence and resolution rates of different types of urinary incontinence in women: findings from a cohort study. *J Urol.* [Epub ahead of print]

Brubaker L., Lukacz E., Burgio K., Zimmern P., Norton P., Leng W., Johnson H., Kraus S., Stoddard A. (2011) Mixed incontinence: comparing definitions in non-surgical patients. *Neurourol Urodyn.* 30(1):47-51.

Campbell K., McWhir J., Ritchie W., Wilmut I. (1996) Sheep cloned by nuclear transfer from a cultured cell line. *Nature.* 380(6569):64-66.

Cannon T., Lee J., Somogyi G., Pruchnic R., Smith C., Huard J., Chancellor M. (2003)Improved sphincter contractility after allogenic muscle-derived progenitor cell injection into the denervated rat urethra. *Urology.* 62(5):958-963.

Chin M., Mason M., Xie W., Volinia S., Singer M., Peterson C., Ambartsumyan G., Aimiuwu O., Richter L., Zhang J., Khvorostov I., Ott V., Grunstein M., Lavon N., Benvenisty N., Croce C., Clark A., Baxter T., Pyle A., Teitell M., Pelegrini M., Plath K., Lowry W. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 5(1):111-123.

Cui H., Webber M., Stupp S. (2010) Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials. *Biopolymers.* 94(1):1-18.

Dagash H., Sen S., Chacko J., Karl S., Ghosh D., Parag P., Mackinnon A. (2008) The appendix as ureteral substitute: a report of 10 cases. *J Pediatr Urol.* 4(1):14-19.

Dahms S., Piechota H., Dahiya R., Lue T., Tanagho E. (1998) Composition and biomechanical properties of the bladder acellular matrix graft: comparative analysis in rat, pig and human. *Br J Urol.* 82(3):411-419.

Davis S., Eaglstein W., Cazzaniga A., Mertz P. (2001) An octyl-2-cyanoacrylate formulation speeds healing of partial-thickness wounds. *Dermatol Surg.* 27(9):783-788.

De Coppi P., Callegari A., Chiavegato A., Gasparotto L., Piccoli M., Taiani J., Pozzobon M., Boldrin L., Okabe M., Cozzi E., Atala A., Gamba P., Sartore S. (2007) Amniotic fluid and bone marrow derived mesenchymal stem cells can be converted to smooth muscle cells in the cryo-injured rat bladder and prevent compensatory hypertrophy of surviving smooth muscle cells. *J Urol*. 177(1):369-376.

Dekel B., Burakova T., Ben-Hur H., Marcus H., Oren R., Laufer J., Reisner Y. (1997) Engraftment of human kidney tissue in rat radiation chimera: II. Human fetal kidneys display reduced immunogenicity to adoptively transferred human peripheral blood mononuclear cells and exhibit rapid growth and development. *Transplantation*. 64(11):1550-1558.

Dekel B., Burakova T., Marcus H., Shezen E., Polack S., Canaan A., Passwell J., Reisner Y. (1997) Engraftment of human kidney tissue in rat radiation chimera: I. A new model of human kidney allograft rejection. *Transplantation.* 64(11):1541-1550.

Dekel B., Burakova T., Shezen E., Marcus H., Canaan A., Reisner Y. (1997) Human renal allograft rejection in the SCID/rat radiation chimera. *Transplant Proc.* 29(4):2255-2256.

Dekel B., Marcus H., Herzel B., Böcher W., Passwell J., Reisner Y. (2000) In vivo modulation of the allogeneic immune response by human fetal kidneys: the role of cytokines, chemokines, and cytolytic effector molecules. *Transplantation.* 69(7):1470-1478.

Delancey J., Ashton-Miller J. (2004) Pathophysiology of adult urinary incontinence. *Gastroenterology.* 126(1 Suppl 1):S23-S32.

de Mendonça A., Vincent J., Suter P., Moreno R., Dearden N., Antonelli M., Takala J., Sprung C., Cantraine F. (2000) Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive Care Med*. 26(7):915-921.

Demidova-Rice T., Geevarghese A., Herman I. (2011) Bioactive peptides derived from vascular endothelial cell extracellular matrices promote microvascular morphogenesis and wound healing in vitro. *Wound Repair Regen*.19(1):59-70.

Desgrandchamps F., Cussenot O., Meria P., Cortesse A., Teillac P., Le Duc A. (1995)Subcutaneous urinary diversions for palliative treatment of pelvic malignancies. *J Urol.* 154(2 Pt 1):367-370.

Desgrandchamps F., Paulhac P., Fornairon S., De Kerviller E., Duboust A., Teillac P., Le Duc A. (1998) Artificial ureteral replacement for ureteral necrosis after renal transplantation: report of 3 cases. *J Urol*. 159(6):1830-1832.

Doshi J., Reneker D. (1995) Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*. 35:151-160.

Dubois S., Stempak D., Wu B., Mokhtari R., Nayar R., Janeway K., Goldsby R., Grier H., Baruchel S. (2011) Circulating endothelial cells and circulating endothelial precursor cells in patients with osteosarcoma. *Pediatr Blood Cancer*. [Epub ahead of print]

Ebert A., Yu J., Rose F., Mattis V., Lorson C., Thomson J., Svendsen CN. (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature.* 457(7227):277-280.

Farzaneh Z., Pournasr B., Ebrahimi M., Aghdami N., Baharvand H. (2010) Enhanced functions of human embryonic stem cell-derived hepatocyte-like cells on three-dimensional nanofibrillar surfaces. *Stem Cell Rev.* 6(4):601-610.

Feki A., Faltin D., Lei T., Dubuisson J., Jacob S., Irion O. (2007) Sphincter incontinence: is regenerative medicine the best alternative to restore urinary or anal sphincter function? *Int J Biochem Cell Biol.* 39(4):678-684.

Feng C., Xu Y., Fu Q., Zhu W., Cui L., Chen J. (2010) Evaluation of the biocompatibility and mechanical properties of naturally derived and synthetic scaffolds for urethral reconstruction. *J Biomed Mater Res A.* 94(1):317-325.

Ferrell N., Desai R., Fleischman A., Roy S., Humes H., Fissell W. (2010) A microfluidic bioreactor with integrated transepithelial electrical resistance (TEER) measurement electrodes for evaluation of renal epithelial cells. *Biotechnol Bioeng*. 107(4):707-716.

Fischer E., Verpont M., Garrett-Sinha L., Ronco P., Rossert J. (2001) Klf6 is a zinc finger protein expressed in a cell-specific manner during kidney development. *J Am Soc Nephrol.* 12(4):726-735.

Fittkau M., Zilla P., Bezuidenhout D., Lutolf M., Human P., Hubbell J., Davies N. (2005) The selective modulation of endothelial cell mobility on RGD peptide containing surfaces by YIGSR peptides. *Biomaterials.* 26(2):167-174.

Friedrich E., Walenta K., Scharlau J., Nickenig G., Werner N. (2006) CD34-/CD133+/VEGFR-2+ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. *Circ Res*. 98(3):e20-e25.

Gage F. (2000) Mammalian neural stem cells. *Science* 287:1433-1438.

Gelain F. (2008) Novel opportunities and challenges offered by nanobiomaterials in tissue engineering. *Int J Nanomedicine*. 3(4):415-424.

Ghoniem G., Lapeyrolerie J., Sood O., Thomas R. (1994) Tulane experience with management of urinary incontinence after placement of an artificial urinary sphincter. *World J Urol.* 12(6):333-336.

Gimpel C., Masioniene L., Djakovic N., Schenk J., Haberkorn U., Tönshoff B., Schaefer F. (2010) Complications and long-term outcome of primary obstructive megaureter in childhood. *Pediatr Nephrol.* 25(9):1679-1686.

Goel A., Goel A., Jain A. (2011) Buccal mucosal graft urethroplasty for penile stricture: only dorsal or combined dorsal and ventral graft placement? *Urology.* [Epub ahead of print]

Graf J., Ogle R., Robey F., Sasaki M., Martin G., Yamada Y., Kleinman H. (1987) A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. *Biochemistry.* 26(22):6896-6900.

Greiner A., Wendorff J. Self-Assembled Nanomaterials I: Nanofibers, Vol. 219, 2008:107-171.

Hammerman M. (2011) Xenotransplantation of embryonic pig kidney or pancreas to replace the function of mature organs. *J Transplant.* 2011:501749.

Hannema S., Print C., Charnock-Jones D., Coleman N, Hughes I. (2006). Changes in gene expression during Wolffian duct development. *Horm Res.* 65(4):200-209.

Harrington D., Cheng E., Guler M., Lee L., Donovan J., Claussen R., Stupp S. (2006) Branched peptide-amphiphiles as self-assembling coatings for tissue engineering scaffolds. *J Biomed Mater Res A*. 78(1):157-167.

Heise R., Ivanova J., Parekh A., Sacks M. (2009) Generating elastin-rich small intestinal submucosa-based smooth muscle constructs utilizing exogenous growth factors and cyclic mechanical stimulation. *Tissue Eng Part A*. 15(12):3951-3960.

http://clinicaltrials.gov (utilizing search terms “urinary” and “incontinence”)

http://www.advancedcell.com/news-and-media/press-releases

http://www.cirm.ca.gov/for-researchers/researchfunding

http://www.diabetes.org/diabetes-basics/diabetes-statistics

Huang Y., Ning H., Shindel A., Fandel T., Lin G., Harraz A., Lue T., Lin C. (2010) The effect of intracavernous injection of adipose tissue-derived stem cells on hyperlipidemia-associated erectile dysfunction in a rat model. *J Sex Med.* 7(4 Pt 1):1391-1400.

Humphries M. (1990) The molecular basis and specificity of integrin-ligand interactions. *J. Cell Sci*. 97:585-592.

Husmann D., Snodgrass W., Koyle M., Furness P., Kropp B., Cheng E., Kaplan W., Kramer S. (2004) Ureterocystoplasty: indications for a successful augmentation. *J Urol.* 171(1):376-380.

Hussein S., Batada N., Vuoristo S., Ching R., Autio R., Närvä E., Ng S., Sourour M., Hämäläinen R., Olsson C., Lundin K., Mikkola M., Trokovic R., Peitz M., Brüstle O.,

Bazett-Jones D., Alitalo K., Lahesmaa R., Nagy A., Otonkoski T. (2011) Copy number variation and selection during reprogramming to pluripotency. *Nature.* 471(7336):58-62.

Imamura M., Kanematsu A., Yamamoto S., Kimura Y., Kanatani I., Ito N., Tabata Y., Ogawa O. (2007) Basic fibroblast growth factor modulates proliferation and collagen expression in urinary bladder smooth muscle cells. *Am J Physiol Renal Physiol.* 293(4):F1007-F1017.

In 't Anker P., Scherjon S., Kleijburg-van der Keur C., Noort W., Claas F., Willemze R., Fibbe W., Kanhai H. (2003) Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood.* 102(4):1548-1549.

Jack G., Zhang R., Lee M., Xu Y., Wu B., Rodríguez L. (2009) Urinary bladder smooth muscle engineered from adipose stem cells and a three dimensional synthetic composite. *Biomaterials*. 30(19):3259-3270.

Jackman R., Brittain S., Adams A., Prentiss M., Whitesides G. (1998) Design and fabrication of topologically complex, three-dimensional microstructures. *Science.* 280(5372):2089-2091.

Kinebuchi Y., Johkura K., Sasaki K., Imamura T., Mimura Y., Nishizawa O. (2008) Direct induction of layered tissues from mouse embryonic stem cells: potential for differentiation into urinary tract tissue. *Cell Tissue Res.* 331(3):605-615.

Knapp P., Lingeman J., Siegel Y., Badylak S., Demeter R. (1994) Biocompatibility of small-intestinal submucosa in urinary tract as augmentation cystoplasty graft and injectable suspension. *J Endourol*. 8(2):125-130.

Ko K., Tapia N., Wu G., Kim J., Bravo M., Sasse P., Glaser T., Ruau D., Han D., Greber B., Hausdörfer K., Sebastiano V., Stehling M., Fleischmann B., Brüstle O., Zenke M., Schöler H. (2009) Induction of pluripotency in adult unipotent germline stem cells. *Cell Stem Cell.* 5(1):87-96.

Kohn D., Sarmadi., Helman J., Krebsbach P. (2002) Effects of pH on human bone marrow stromal cells in vitro: implications for tissue engineering of bone. *J Biomed Mater Res*. 60(2):292-299.

Koyanagi T. (1980) Studies on the sphincteric system located distally in the urethra: the external urethral sphincter revisited. *J Urol*. 124(3):400-406.

Koziak A., Kania P., Marcheluk A., Dmowski T., Szcześniewski R., Dorobek A. (2004)Reconstruction of long ureteral obstructions using xenogenic acellular collagen membranes. *Ann Transplant.* 9(4):18-120.

Kropp B., Eppley B., Prevel C., Rippy M., Harruff R., Badylak S., Adams M., Rink R., Keating M. (1995) Experimental assessment of small intestinal submucosa as a bladder wall substitute. *Urology*. 46(3):396-400.

Kundu A., Gelman J., Tyson D. (2011) Composite thin film and electrospun biomaterials for urologic tissue reconstruction. *Biotechnol Bioeng*. 108(1):207-215.

Kurzrock E. (2010) Editorial comment. *J Urol.* 184(2):707; discussion 708.

Lakshmanan Y., Frimberger D., Gearhart J., Gearhart J. (2005) Human embryoid body-derived stem cells in co-culture with bladder smooth muscle and urothelium. *Urology.* 65(4):821-826.

Langer R., Vacanti J., Vacanti C., Atala A., Freed L., Vunjak-Novakovic G. (1995) Tissue engineering: biomedical applications. *Tissue Eng*. 1(2):151-161.

Levine L., Strom K., Lux M. (2007) Buccal mucosa graft urethroplasty for anterior urethral stricture repair: evaluation of the impact of stricture location and lichen sclerosus on surgical outcome. *J Urol.* 178(5):2011-2015.

Lin H., Cowan R., Moore P., Zhang Y., Yang Q., Peterson J., Tomasek J., Kropp B., Cheng E. (2004) Characterization of neuropathic bladder smooth muscle cells in culture. *J Urol.* 171(3):1348-1352.

Lister R., Pelizzola M., Kida Y., Hawkins R., Nery J., Hon G., Antosiewicz-Bourget J., O'Malley R., Castanon R., Klugman S., Downes M., Yu R., Stewart R., Ren B., Thomson J., Evans R., Ecker J. (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature.* 471(7336):68-73.

Livant D., Brabec R., Kurachi K, Allen D., Wu Y., Haaseth R., Andrews P., Ethier S., Markwart S. (2000) The PHSRN sequence induces extracellular matrix invasion and accelerates wound healing in obese diabetic mice. *J Clin Invest.* 105(11):1537-1545.

Luppi P., Powers R., Verma V., Edmunds L., Plymire D., Hubel C. (2010) Maternal circulating CD34+VEGFR-2+ and CD133+VEGFR-2 + progenitor cells increase during normal pregnancy but are reduced in women with preeclampsia. *Reprod Sci.* 17(7):643-652.

Lynn A., Blakney A., Kyriakides T., Bryant S. (2011) Temporal progression of the host response to implanted poly(ethylene glycol)-based hydrogels. *J Biomed Mater Res A.* 96(4):621-631.

Mangera A., Chapple C. (2010) Management of anterior urethral stricture: an evidence-based approach. *Curr Opin Uro*l. 20(6):453-458.

Mann B., West J. (2002) Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. *J Biomed Mater Res.* 60(1):86-93.

Massia S., Hubbell J. (1991) An RGD spacing of 440 nm is sufficient for integrin alpha V beta 3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation. *J Cell Biol.* 114(5):1089-1100.

Massia S., Rao S., Hubbell J. (1993) Covalently immobilized laminin peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) supports cell spreading and co-localization of the 67-kilodalton laminin receptor with alpha-actinin and vinculin. *J Biol Chem.* 268(11):8053-8059.

Mauney J., Ramachandran A., Yu R., Daley G., Adam R., Estrada C. (2010) All-trans retinoic acid directs urothelial specification of murine embryonic stem cells via GATA4/6 signaling mechanisms. *PLoS One.* 5(7):e11513.

McCrory WW. (1974) The normal embryologic development of the kidney: a basis for understanding structural abnormalities. *Birth Defects Orig Artic Ser.* 10(4):3-11.

McGrath J., Solter D. (1983) Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. *Science.* 220(4603):1300-1302.

Middleton JC., Tipton AJ. (2000) Synthetic biodegradable polymers as orthopedic devices. *Biomaterials.* 21(23):2335-2346.

Miller D., Thapa A., Haberstroh K., Webster T. (2004) Endothelial and vascular smooth muscle cell function on poly(lactic-co-glycolic acid) with nano-structured surface features. *Biomaterials.* 25(1):53-61.

Mitalipov S., Wolf D. (2009) Totipotency, pluripotency and nuclear reprogramming. *Adv Biochem Eng Biotechnol.* 114:185-199.

Moretti A., Bellin M., Welling A., Jung C., Lam J., Bott-Flügel L., Dorn T., Goedel A., Höhnke C., Hofmann F., Seyfarth M., Sinnecker D., Schömig A., Laugwitz K. (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med.* [Epub ahead of print]

Morizane R., Monkawa T., Itoh H. (2009) Differentiation of murine embryonic stem and induced pluripotent stem cells to renal lineage in vitro. *Biochem Biophys Res Commun*. 390(4):1334-1339.

Mundy A., Andrich D. (2011) Urethral strictures. *BJU Int*. 107(1):6-26.

Murtuza B., Nichol J., Khademhosseini A. (2009) Micro- and nanoscale control of the cardiac stem cell niche for tissue fabrication. *Tissue Eng Part B Rev.* 15(4):443-454.

Muthusubramaniam L., Lowe R., Fissell W., Li L., Marchant R., Desai T., Roy S. (2011)Hemocompatibility of silicon-based substrates for biomedical implant applications. *Ann Biomed Eng*. [Epub ahead of print]

Nakanishi K., Yoshikawa N. (2003) Genetic disorders of human congenital anomalies of the kidney and urinary tract (CAKUT). *Pediatr Int*. 45(5):610-616.

Nelson D., Baraniak P., Ma Z., Guan J., Mason N., Wagner W. (2011) Controlled release of IGF-1 and HGF from a biodegradable polyurethane scaffold. *Pharm Res*. [Epub ahead of print]

Nistor G., Totoiu M., Haque N., Carpenter M., Keirstead H. (2005) Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia*. 49(3):385-396.

Oberpenning F., Meng J., Yoo J., Atala A. (1999) De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol.* 17(2):149-55.

Oh S., Lee J., Ghil S., Lee S., Yuk S., Lee J. (2006) PCL microparticle-dispersed PLGA solution as a potential injectable urethral bulking agent. *Biomaterials*. 27(9):1936-1944.

Olajide A., Salako A., Aremu A., Eziyi A., Olajide F., Banjo O. (2010) Complications of transverse distal penile island flap: urethroplasty of complex anterior urethral stricture. *Urol J.* 7(3):178-182.

Olbrich K., Andersen T., Blumenstock F., Bizios R. (1996) Surfaces modified with covalently-immobilized adhesive peptides affect fibroblast population motility. *Biomaterials.* 17(8):759-764.

Oottamasathien S., Wang Y., Williams K., Franco O., Wills M., Thomas J., Saba K., Sharif-Afshar A., Makari J., Bhowmick N., DeMarco R., Hipkens S., Magnuson M., Brock J., Hayward S., Pope J., Matusik R. (2007) Directed differentiation of embryonic stem cells into bladder tissue. *Dev Biol.* 304(2):556-566.

Park J. (2010) Editorial comment. *J Urol.* 184(2):708; discussion 708.

Pascal L., Deutsch E., Campbell D., Korb M., True L., Liu A. (2007) The urologic epithelial stem cell database (UESC) - a web tool for cell type-specific gene expression and immunohistochemistry images of the prostate and bladder. *BMC Urol*.7:19.

Pattison M., Webster T., Haberstroh K. (2006) Select bladder smooth muscle cell functions were enhanced on three-dimensional, nano-structured poly(ether urethane) scaffolds. *J Biomater Sci Polym Ed*. 17(11):1317-1332.

Pelliniemi L., Kellokumpu-Lehtinen P., Hoffer A. (1983) Glycogen accumulations in differentiating mesonephric ducts and tubuli in male human embryos. *Anat Embryol* 168(3):445-453.

Phadnis S., Joglekar M., Dalvi M., Muthyala S., Nair P., Ghaskadbi S., Bhonde R., Hardikar A. (2011) Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo. *Cytotherapy.* 13(3):279-293.

Pittenger M., Mackay A., Beck S., Jaiswal R., Douglas R., Mosca J., Moorman M., Simonetti D., Craig S., Marshak D. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science.* 284(5411):143-147.

Pittier R., Sauthier F., Hubbell J., Hall H. (2005) Neurite extension and in vitro myelination within three-dimensional modified fibrin matrices. *J Neurobiol.* 63(1):1-14.

Powers M., Campbell B., Weisse C. (2010) Porcine small intestinal submucosa augmentation urethroplasty and balloon dilatation of a urethral stricture secondary to inadvertent prostatectomy in a dog. *J Am Anim Hosp Assoc.* 46(5):358- 365.

Prajerova I., Honsa P., Chvatal A., Anderova M. (2010) Neural stem/progenitor cells derived from the embryonic dorsal telencephalon of D6/GFP mice differentiate primarily into neurons after transplantation into a cortical lesion. *Cell Mol Neurobiol.* 30(2):199-218.

Qin D., Xia Y., Whitesides G. (2010) Soft lithography for micro- and nanoscale patterning. *Nat Protoc.* 5(3):491-502.

Qiu H., Yang J., Kodali P., Koh J., Ameer G. (2006) A citric acid-based hydroxyapatite composite for orthopedic implants. *Biomaterials.* 27(34):5845-5854.

Quirici N., Soligo D., Caneva L., Servida F., Bossolasco P., Deliliers G. (2001) Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol*. 115(1):186-194.

Rajangam K., Behanna H., Hui M., Han X., Hulvat J., Lomasney J., Stupp S. (2006) Heparin binding nanostructures to promote growth of blood vessels. *Nano Lett.* 6(9):2086-2090.

Ren X., Zhang J., Gong X., Niu X., Zhang X., Chen P., Zhang X.(2010) Differentiation of murine embryonic stem cells toward renal lineages by conditioned medium from ureteric bud cells in vitro. *Acta Biochim Biophys Sin (Shanghai)*. 42(7):464-471.

Reynolds B., Weiss S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*. 255(5052):1707-1710.

Rogers S., Lowell J., Hammerman N., Hammerman M. (1998) Transplantation of developing metanephroi into adult rats. *Kidney Int.* 54(1):27-37.

Roth C., Donovan B., Tonkin J., Klein J., Frimberger D., Kropp B. (2010) Endoscopic injection of submucosal bulking agents for the management of incontinent catheterizable channels. *J Pediatr Urol.* 5(4):265-268.

Rüger B., Breuss J., Hollemann D., Yanagida G., Fischer M., Mosberger I., Chott A., Lang I., Davis P., Höcker P., Dettke M. (2008) Vascular morphogenesis by adult bone marrow progenitor cells in three-dimensional fibrin matrices. *Differentiation.* 76(7):772-783.

Sales V., Mettler B., Engelmayr G., Aikawa E., Bischoff J., Martin D., Exarhopoulos A., Moses M., Schoen F., Sacks M., Mayer J. (2010) Endothelial progenitor cells as a sole source for ex vivo seeding of tissue-engineered heart valves. *Tissue Eng Part A.* 16(1):257-267.

Sargeant T., Oppenheimer S., Dunand D., Stupp S. (2008) Titanium foam-bioactive nanofiber hybrids for bone regeneration. *J Tissue Eng Regen Med.* 2(8):455-462.

Selim M., Bullock A., Blackwood K., Chapple C., MacNeil S. (2011) Developing biodegradable scaffolds for tissue engineering of the urethra. *BJU Int.* 107(2):296-302.

Serrano M., Vavra A., Jen M., Hogg M., Murar J., Martinez J., Keefer L., Ameer G., Kibbe M. (2011) Poly(diol-co-citrate)s as novel elastomeric perivascular wraps for the reduction of neointimal hyperplasia. *Macromol Biosci.* [Epub ahead of print]

Seydoux G., Braun R. (2006) Pathway to totipotency: lessons from germ cells. *Cell.* 127(5):891-904.

Shaeer O., El-Sadat A. (2006) Urethral substitution using vein graft for hypospadias repair. *J Pediatr Urol.* 2(5):518-521.

Sharma A., Bury M., Marks A., Fuller N., Meisner J., Tapaskar N., Halliday L., Matoka D., Cheng E. (2010) A non-human primate model for urinary bladder regeneration utilizing autologous sources of bone marrow derived mesenchymal stem cells. *Stem Cells.* [Epub ahead of print]

Sharma A., Fuller N., Sullivan R., Fulton N., Hota P., Harrington D., Villano J., Hagerty J., Cheng E. (2009) Defined populations of bone marrow derived mesenchymal stem and endothelial progenitor cells for bladder regeneration. *J Urol*. 182(4 Suppl):1898-1905.

Sharma A., Hota P., Matoka D., Fuller N., Jandali D., Thaker H., Ameer G., Cheng E. (2010) Urinary bladder smooth muscle regeneration utilizing bone marrow derived mesenchymal stem cell seeded elastomeric poly(1,8-octanediol-co-citrate) based thin films. *Biomaterials.* 31(24):6207-6217.

Siegler E., Reidenberg M. (2004) Treatment of urinary incontinence with anticholinergics in patients taking cholinesterase inhibitors for dementia. *Clin Pharmacol Ther.* 75(5):484-488.

Simpson J., Otaño L. (2007) Prenatal genetic diagnosis. In: Gabbe SG, Niebyl JR, Simpson JL, eds. Obstetrics:Normal and Problem Pregnancies. 5th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; chap 7.

Silva G., Czeisler C., Niece K., Beniash E., Harrington D., Kessler J., Stupp S. (2004) Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science.* 303(5662):1352-1355.

Stahl D., Koul H., Chacko J., Mingin G. (2006) Congenital anomalies of the kidney and urinary tract (CAKUT): a current review of cell signaling processes in ureteral development. *J Pediatr Urol.* 2(1):2-9.

Stankus J., Soletti L., Fujimoto K., Hong Y., Vorp D., Wagner W. (2007) Fabrication of cell microintegrated blood vessel constructs through electrohydrodynamic atomization. *Biomaterials.* 28(17):2738-2746.

Strauss S. (2010) Geron trial resumes, but standards for stem cell trials remain elusive. *Nat Biotechnol.* 28(10):989-990.

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K., Yamanaka S. (2007)Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 131(5):861-872.

Takahashi T., Kalka C., Masuda H., Chen D., Silver M., Kearney M., Magner M., Isner J., Asahara T. (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med.* 5(4):434-438.

Temeltas G., Dagci T., Kurt F., Evren V., Tuglu I. (2009) Bladder function recovery in rats with traumatic spinal cord injury after transplantation of neuronal-glial restricted precursors or bone marrow stromal cells. *J Urol*. 181(6):2774-2779.

Thapa A., Miller D., Webster T., Haberstroh K. (2003) Nano-structured polymers enhance bladder smooth muscle cell function. *Biomaterials.* 24(17):2915-2926.

Thapa A., Webster T., Haberstroh K. (2003) Polymers with nano-dimensional surface features enhance bladder smooth muscle cell adhesion. *J Biomed Mater Res A.* 67(4):1374-1383.

Thomas J., Oottamasathien S., Makari J., Honea L., Sharif-Afshar A., Wang Y., Adams C., Wills M., Bhowmick N., Adams M., Brock J., Hayward S., Matusik R., Pope J. (2008) Temporal-spatial protein expression in bladder tissue derived from embryonic stem cells. *J Urol.* 180(4 Suppl):1784-1789.

Thomson J., Itskovitz-Eldor J., Shapiro S., Waknitz M., Swiergiel J., Marshall V., Jones J. (1998) Embryonic stem cell lines derived from human blastocysts. *Science.* 282(5391):1145-1147.

Toka H., Toka O., Hariri A., Nguyen H. (2010) Congenital anomalies of kidney and urinary tract. *Semin Nephrol*. 30(4):374-386.

Vacanti J., Morse M., Saltzman W., Domb A., Perez-Atayde A., Langer R. (1988) Selective cell transplantation using bioabsorbable artificial polymers as matrices. *J Pediatr Surg*. 23(1 Pt 2):3-9.

van der Veen V., van der Wal M., van Leeuwen M., Ulrich MM., Middelkoop E. (2010) Biological background of dermal substitutes. *Burns.* 36(3):305-321.

Webber M., Kessler J., Stupp S. (2010) Emerging peptide nanomedicine to regenerate tissues and organs. *J Intern Med.* 267(1):71-88.

Welsh M., Saunders P., Marchetti N., Sharpe R. (2006) Androgen-dependent mechanisms of Wolffian duct development and their perturbation by flutamide. *Endocrinology.* 147(10):4820-4830.

Wilson L., Brown J., Shin G., Luc K., Subak L. (2001) Annual direct cost of urinary incontinence. *Obstet Gynecol* 98:398-406.

Woolf A., Hornbruch A., Fine L. (1991) Integration of new embryonic nephrons into the kidney. *Am J Kidney Dis.* 17(6):611-614.

Xia Y., Whitesides G. (1998) Soft Lithography. In. *Annu. Rev. Mater. Sci*. 28:153–184.

Xiao C., Du M., Li B., Liu Z., Chen M., Chen Z., Cheng P., Xue X., Shapiro E., Lepor H. (2005) An artificial somatic-autonomic reflex pathway procedure for bladder control in children with spina bifida. *J Urol.* 173(6):2112-2116.

Yang J., Motlagh D., Webb A., Ameer G. (2005) Novel biphasic elastomeric scaffold for small-diameter blood vessel tissue engineering. *Tissue Eng.* 11(11-12):1876-1886.

Yiou R., Yoo J., Atala A. (2003) Restoration of functional motor units in a rat model of sphincter injury by muscle precursor cell autografts. *Transplantation.* 76(7):1053-1060.

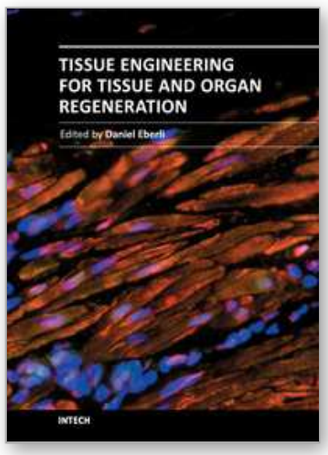
Yu J., Vodyanik M., Smuga-Otto K., Antosiewicz-Bourget J., Frane J., Tian S., Nie J., Jonsdottir G., Ruotti V., Stewart R., Slukvin I., Thomson J. (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 318(5858):1917-1920.

Zhang Q., Jiang J., Han P., Yuan Q., Zhang J., Zhang X., Xu Y., Cao H., Meng Q., Chen L., Tian T., Wang X., Li P., Hescheler J., Ji G., Ma Y. (2010) Direct differentiation of

atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. *Cell Res*. [Epub ahead of print]

Zhong S., Zhang Y., Lim C. (2010) Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology 2:510-525.

Zini L., Yiou R., Lecoeur C., Biserte J., Abbou C., Chopin D. (2004) Tissue engineering in urology. *Ann Urol.* 38(6):266-274.



Tissue Engineering for Tissue and Organ Regeneration

Edited by Prof. Daniel Eberli

ISBN 978-953-307-688-1
Hard cover, 454 pages
Publisher InTech
Published online 17, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen