Moderated Poster Session 1: Basic Science/Physiology/Research Monday, June 28, 1505-1605

MP-01.01

Development of a Novel Method for Rapid *in vitro* Stent Encrustation

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Introduction and Objective: Encrustation of urologic devices can increase patient morbidity thereby limiting their clinical use. Alteration of stent materials and coatings has been attempted to limit encrustation. Encrustation is associated with organic salt deposits. Our objective was to develop a urologic device encrustation model that would mimic the *in vivo* process better than the ones that currently rely on either the actions of enzymes or bacteria. The ideal model would be: 1) rapid, 2) sterile, and 3) reproducible, using chemical solutions.

Methods: Four different stent types were tested using this model (Sof-Flex® [Cook Urological], Optima® [Bard Urological], Percuflex Plus® and Triumph®[both Boston Scientific Corp.], with each stent used in duplicate. Stent segments (1.5cm) were suspended in 500 mL artificial urine (AU) anchored to pipette tips positioned in the buoyant midsection of a pipette tip box. A modified version of Brooks and Keevil's AU was used containing 7.5mM $CaCl_2$. This media was replaced daily to better represent *in vivo* conditions and maintain sterility. Stent pieces were incubated at 37°C with 5.0 mM ammonium oxalate solution added constantly at a rate of 0.4 mL/min. After 7 days, the encrustation was photographed, physically removed and weighed. The mass of encrustation was compared amongst the different stent types. The results were the average of six independent experiments.

Results: The slow, continuous addition of ammonium oxalate to AU promoted perpetual precipitation and deposition of crystals on all exposed surfaces. After 7 days, all device segments harboured visible surface encrustation. Encrustation was found to be the greatest on the Triumph® devices ($8.20 \pm 0.33 mg/cm^2$) followed by Sof-Flex (6.00 ± 0.38), Percuflex Plus (5.45 ± 0.56) and Optima (4.38 ± 0.86). These differences were found to be statistically significant ($p = 0.004$).

Conclusions: This novel method can rapidly yield *in vitro* urologic device encrustation in the magnitude of milligrams/cm^2. The protocol can be modified to hasten or slow the encrustation rate. One further benefit is the ability to test multiple stents simultaneously. This is the first method to produce non-urease-based encrustation in such a short period, using a setting of sterile artificial urine that is reproducible and closely mimics physiologic conditions. This model may be used in the future to advance stent design with the goal of improving clinical outcomes.

MP-01.02

Uropathogenic E. Coli Infection Provokes Epigenetic Downregulation of CDKN2A in Urothelial Cells

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Introduction: Host cell and bacterial factors determine severity and duration of infections. To allow for bacteria pathogenicity and persistence, bacteria have developed mechanisms that modify expression of host genes involved in cell cycle progression, apoptosis, differentiation and the immune response. Infection of the urinary tract with uropathogenic E. coli (UPEC) is highly prevalent, and can incite significant morbidity and mortality. Although it is known that epigenetic mechanisms, such as DNA methylation, allow downregulation of host genes by bacteria, the pathogen factors responsible for this effect are generally unknown, and completely unknown for UPEC.

Methods: Persistent infection of urothelial cells with FimH[+] UPEC results in DNMT1 upregulation and CDKN2A hypermethylation which is associated with CDKN2A downregulation and increased cell proliferation. Infection with FimH[-] E.coli had no effect on DNMT1 expression, CDKN2A methylation or UC growth.

Results/Conclusions: These results demonstrate that FimH adhesin plays an important role in the epigenetic reprogramming of host cell gene expression by E.coli, raising the possibility that CDKN2A methylation status may vary further with UTI recurrence. UTI and UTI recurrence can seriously compromise patients with dilated urinary tract pathologies, immunosupression, or other preexisting medical conditions. However the inability to predict which of these patients are at such risk for infection or recurrence often forces pre-emptive surgical correction of dilating uropathies and widespread indiscriminate antibiotic use. Identification of uropathogeic bacteria-induced gene methylation may constitute a biological marker for UTI recurrence, providing a valuable diagnostic tool for refining medical and surgical therapy for such patients, thereby curbing antibiotic use and reducing the incidence of prophylactic surgery.

MP-01.03

Aberrant Androgen Receptor Signaling due to Activated Fer Kinase in Prostate Cancer

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Introduction and Objectives: Androgen receptor (AR) signaling is implicated in diverse stages of prostate cancer (PCa), from local to metastatic disease and even after endocrine therapy. Underlying mechanisms are not fully understood but aberrant AR signaling appears to contribute to progression. Earlier results from our lab and recent reports suggest a new form of AR activation *via* tyrosine (Y) phosphorylation in response to diverse growth-promoting factors. Our aim was to investigate whether the Fer kinase, which is overexpressed in PCa, controls AR activation in an environment containing interleukin-6 (IL-6) or androgens.

Methods: LNCaP cells were transfected with siRNA (*fer* and *AR*) or *fer* cDNA, wild-type and double mutant. Growth was monitored by MTT following exposure to IL-6 or synthetic androgen Methyltrienolone (R1881). PSA mRNA levels were measured by real-time PCR. Proteins (Fer, AR, STAT3) and their extent of Y-phosphorylation were analyzed by immuno -fluorescence, -precipitation & Western blotting. Pull-down assays were performed using the Fer-SH2 domain. The Fer kinase domain was used to phosphorylate recombinant AR *in vitro*.

Results: In line with observations on IL6 mediating pSTAT3 crosstalk with AR signaling, we found that IL6 controls Fer/AR and Fer/pSTAT3 complexes together with their nuclear translocation and activation by Y-phosphorylation. Interestingly, R1881 stimulates the Y-phosphorylation and nuclear accumulation of both Fer and AR but not of STAT3. Both AR and pSTAT3 were found in Fer-SH2 pulldowns, inferring the coexistence of multi-molecular Fer complexes in PCa cells. In functional assays, downregulation of *fer* and *AR* revealed a strict requirement of Fer and only partial of AR for IL-6 growth response, whereas the converse was observed for R1881. PSA levels were reduced when Fer was silenced and more so

in the IL6 *vs.* R1881 context. Similarly the AR Y-phosphorylation was favored in presence of IL6. AR was also a direct Fer substrate.
Conclusions: Fer overexpression together with its ability to activate AR and control its nuclear translocation in response to IL-6 and low levels of androgens may explain PCa cell growth and PSA expression after endocrine therapy, thereby favoring progression of the disease.

MP-01.04
Delivery of Small Interference RNAs as a Strategy for Treatment of Urological Diseases
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Introduction and Objective: siRNA specifically and efficiently degrade target mRNAs. The therapeutic potential of siRNAs to treat urological disease is limited by their short half lives and their short dwell time within the bladder. To increase the half-life of siRNAs within the bladder, we have loaded siRNA into nanoparticles and have complexed the nanoparticles with peptides that bind to urothelium.
Methods: We have incorporated fluorescein labeled siRNAs into antennapedia (AP)-poly(lactide-co-glycolide) PLGA using spermadine as a complexing reagent to increase encapsulation efficiency. AP is a targeting peptide that was conjugated to PLGA to enhance intracellular delivery. Using siRNA-AP-PLGA nanoparticles, we tested release of siRNA into artificial urine, the uptake of fluorescence into T-24 bladder cancer cells using FACS cell sorting, and the downregulation of target mRNA and protein in T-24 cells and in human ureteral urothelium using real time PCR, ELISA, and western blots.
Results: We have complexed vascular endothelial growth factor (VEGF) siRNA and survivin siRNA, survivin being an inhibitor of apoptosis, into PLGA nanoparticles and characterized their physical and chemical properties and their ability to downregulate target protein and mRNA. VEGF siRNA and survivin siRNA are released into artificial urine from fluorescein siRNA AP-PLGA nanoparticles for 10 days in amounts sufficient to downregulate target protein and mRNA. Similar concentrations of siRNAs complexed in lipids, are not detected after 24 hrs. Fluorescence is enhanced several fold in T-24 cells treated with fluorescein labeled siRNA–AP-PLGA for 3 and 4 days, indicating the uptake of siRNAs into cells. To confirm siRNA uptake, we measured downregulation of survivin levels. In T-24 cells treated with survivin siRNA AP-PLGA (1 mg/mL) for three days, survivin mRNA is decreased by 88% and there is a large decrease in survivin protein compared to cells treated with control AP-PLGA. In human urothelium treated with VEGF siRNA AP-PLGA for 5 days, VEGF levels were reduced 63%, (240 + 9 pg VEGF/mL) compared to urothelium treated with control AP-PLGA (655 + 39 pg VEGF/mL).
Conclusions: Encapsulation of VEGF and survivin siRNAs into AP-PLGA nanoparticles produced sustained siRNA release in sufficient amounts to downregulate target protein and mRNA in both cancer cells and normal urothelium.

MP-01.05
Intermittent Caloric Restriction Facilitates Erectile Recovery after Bilateral Cavernous Nerve Crush Injury in the Rat
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Introduction and Objective: Dietary restriction increases longevity may have potential benefits for injury-response and disease. Preclinical data has shown to improved outcomes from cervical spinal cord injury with candidate mechanisms including trkB, the receptor for brain-derived nerve growth factor (BDNF) which is a key cavernous nerve (CN) neuromodulator. The purpose of this study was to determine whether every-other-day-fasting (EODF), a form of intermittent caloric restriction, conferred an erectile recovery advantage following CN crush injury in the rat, focusing specifically on CN biology.
Methods: Forty-four 3-month old Sprague-Dawley male rats were separated into a control (sham, n = 8) group and cohorts consisting of 9 animals divided into crush-injury only (non-treated, no EODF), and treatment arms of EODF started 2 weeks prior to injury, EODF at day of injury, and EODF initiated 2 weeks post-CN injury. The change in intracavernous pressure (ICP) standardized to mean arterial pressure (MAP) at 5 months was measured, with this extended in-treatment phase chosen in order to aid in washing out of any short term fasting effects between groups. Tukey-Kramer test was used for post-hoc analysis and statistical significance was set at $p < 0.5$. The proximal corpora was cryosectioned and stained with primary antibodies against the catecholamine synthesis marker tyrosine hydroxylase, neuronal NO synthase (nNOS), and vesicular acetylcholine transporter (VaChT).
Results: The mean maximal increase in ICP/MAP (with standard deviation) for control animals was 0.673 (.09) versus the crush-control cohort (no caloric modification) ICP/MAP change of 0.17 (0.06). Rats treated with EODF started two weeks prior to injury demonstrated significant ($p < 0.05$) improvement in erectile function with ICP/MAP ratio of 0.37 (0.07) Fasting started day of injury demonstrated less robust recovery, with 0.28 (.05) compared to the "pre-treated" group. The final group, starting EODF two weeks post injury demonstrated results statistically similar to no caloric restriction, with a ICP/MAP ratio of .168 (0.06). The two primary neurobiological endpoints in this study, retrograde axonal transport of fluorogold to the major pelvic ganglion and cavernous body determination of nNOS and VaChT were significantly reduced in crush injury, and 2 week post-injury EODF, compared to EODF initiated 2 weeks prior to/and day of injury.
Conclusions: This is the first study to demonstrate endogenous stress-response neuromodulation in a model of radical prostatectomy induced CN injury. Intermittent caloric restriction in the form of EODF confers a recovery advantage for CN function post injury as measured by ICP/MAP and intact parasympathic neurons.

5-STAR
MP-01.06
Adipose-Derived Stem Cells for the Reconstruction of a Human Vesical Equivalent
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Introduction and Objective: For several years, fibroblast cells have been primarily used for tissue engineering but adipose-derived stem/stromal cells (ASCs) show promising potential due to their facility to obtain, their capacity to differentiate and their ability to secrete mediators. Our group previously reported on the production of a bioengineered vesical equivalent using dermal fibroblasts without exogenous matrix. The aim of this study was therefore to evaluate the possibility of engineering an autologous vesical equivalent with human ASCs in order to validate if our model can benefit from the attributes of the ASCs.
Material and Methods: ASCs were obtained from lipoaspirated adipose tissue and fibroblasts were extracted from a dermal biopsy. These human cells were cultured with serum and ascorbic acid to stimulate the formation of extracellular matrix and obtain cell sheets. Cells were cultured with constant media motion (Gyrotwister™, Woodbridge, NJ) during three weeks and then three cell sheets of ASCs or fibroblasts were superimposed. After 4 days of maturation allowing cell sheet fusion, human urothelial cells were seeded on top of the construction and matured at the air/liquid interface. The vesical equivalents were characterized by histology, immunofluorescence as well as mechanical and suture resistance tests.
Results: Complete vesical equivalents were obtained with ASCs or fibroblasts. The histology clearly showed that cell sheets forming the ASC vesical equivalents featured a strong cohesion between cell sheets and were 1.8-fold thicker than the fibroblast vesical equivalents. Immunolabelings

of the mature constructions showed the presence of cytokeratin 8\18, a differentiation marker for urothelial cell; and collagen 1 and 3, which are the major components of the extracellular matrix. The ASC vesical equivalents were easy to manipulate resistant enough to suture, therefore allowing the 3D reconstruction of a bladder shaped tissue engineered substitute.
Conclusions: Human vesical equivalents were successfully produced using either dermal fibroblats or ASCs, without the use of exogenous scaffolding components. The ASC vesical equivalents could sustain suturing without tearing. Considering their accessibility, abundance and increased matrix production ACSs therefore represent a great cell source to further optimize our innovative model for vesical reconstruction.

MP-01.07
Laparoscopic Donor Nephrectomy in the Presence of Multiple Renal Arteries
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Introduction and Objective: We reviewed our data of laparoscopic donor nephrectomies with multiple renal arteries. We evaluated the donor and recipient outcomes on these donors.
Methods: All donor nephrectomies performed at our centre from 2004 to 2008 were reviewed retrospectively. Results were compared between laparoscopic donor nephrectomy kidneys with multiple arteries and those with a single vessel.
Results: Out of 171 donor nephrectomies, 21 (12%) were performed for kidneys with multiple renal arteries. Of the 150 (88%) donor nephrectomies in the single vessel group all were performed laparoscopically. In the multiple artery group 9 (43%) underwent an open procedure while 12 (57%) underwent the procedure laparoscopically. The results were evaluated for laparoscopic donor nephrectomy kidneys with multiple and single renal artery. The warm ischemia time was longer in multiple artery group but was not statistically significant (4.25mins ± 0.87 vs. 4.12 mins ± 0.95). Regarding the transplant recipients the vascular anastomosis time was similar in both groups (30 mins ± 4.6 vs. 29.5 mins ±3.7). The operative blood loss in the transplant recipients was significantly more in the multiple artery group as compared to the single artery group (339 ml ±292 and 130.7 mL ± 44.8; $p = 0.03$). The recipient renal function was similar for both the groups at postoperative day 7, 1 month and at 1 year.
Conclusions: Our data supports that the laparoscopic approach to donor nephrectomy in the presence of multiple renal arteries can be safely performed with adequate laparoscopic experience.

Table 1. MP-01.07.Recipient and donor outcome in laparoscopic donors with single and multiple renal arteries

		Single artery (n = 150)	Multiple artery (n = 12)	*p* value
Donor outcome	Warm ischemia time (mins)	4.12	4.25	0.65
Recipient outcome	Mean blood loss (mL)	130.73	339.58	0.03
	Mean creatinine 1 week (µmol/L)	112.76	118.83	0.52
	Mean creatinine 1 month (µmol/L)	114.22	110.33	0.51
	Mean creatinine 1 year (µmol/L)	117.11	123.83	0.48

MP-01.08
Long-Term Function and Clinical Outcome of Pediatric Kidneys Transplanted to Adults: A Single-Centre Experience
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Introduction and Objective: There is an increasing discrepancy between supply and demand in renal transplantation. Utilization of pediatric deceased donor kidneys has been introduced to address this problematic. In this study, we reviewed cases of en bloc and single pediatric kidneys transplanted to adults and evaluated allograft long-term function and survival.
Methods: From April 1990 through February 2009, 60 adult recipients received deceased donor renal transplants from donors aged 10 years or less in a single centre. Eleven cases were performed with en bloc kidneys (EBK) and 49 cases were done with single separated allograft (SK). Recipient and donor demographic data, operative parameters, complications, long-term renal function, graft and patient survival were analyzed.
Results: Donor age and donor weight were lower in the EBK group: 4.4 vs. 7.1 years ($p < 0.001$) and 16.1 vs. 27.6 kg ($p < 0.0001$) respectively. Recipient characteristics were comparable on all points. Cold ischemia time was shorter in the EBK group (16.1 vs. 21.6 hours, $p = 0.02$). Regarding complications, acute rejections were more frequent in the SK group (57% vs. 18%, $p = 0.04$). Thrombosis rate and urologic complications were similar between the two groups. However, we found a trend for a higher transplant renal artery stenosis rate in SK group (26% vs. 9%). No allograft was lost following TRAS or related treatment complication. Both groups presented a good long-term renal function but eGFR was higher at all time points for the EBK group (75±19 vs. 60±17 mL/min/1.73m^2 at 1 year and 83±20 vs. 63±18 mL/min/1.73m^2 at 5 years). Graft survival was 100 vs. 96% at one year, 100 vs. 87% at 5 years and 100 vs. 81% at 10 years for the EBK compared to the SK group respectively (log rank test, $p = 0.17$). Finally, patient survival was similar between the groups (1-year, 5-year and 10-year patient survival was respectively 100 vs. 98%, 100 vs. 91% and 100 vs. 85%).
Conclusion: Pediatric donor kidney transplant is a viable option to address shortage of organs. EBK transplant allowed utilization of small donors with excellent outcomes. Despite a higher rate of rejection and TRAS, SK transplant achieved good function and survival with the potential advantage of giving access to transplantation to more listed-patients. This article did not assess the optimal selection criteria that should be used to decide between EBK and SK. Future studies should try answering this important question.