Regenerative Medicine and Tissue Engineering in Urology: A Brief Overview

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Department of Urological Sciences Grand Rounds
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Objectives

1. Define regenerative medicine
2. Outline the basic concepts of tissue engineering
3. Review current concepts of regenerative medicine in bladder augmentation
4. Briefly review other potential areas of regenerative medicine in Urology
5. Outline roadblocks to regenerative medicine application
6. Discuss the future of regenerative medicine in Urology
A proposed definition of regenerative medicine

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JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function from any cause (ie. Congenital defects, disease, trauma and ageing)
A proposed definition of regenerative medicine

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Interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function from any cause (ie. Congenital defects, disease, trauma and ageing)

Combines converging technological approaches, both existing and newly emerging, moving it beyond traditional transplantation and replacement therapies

A proposed definition of regenerative medicine

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Approaches often aim to stimulate and support the body’s own self-healing capacity
A proposed definition of regenerative medicine

Approaches often aim to stimulate and support the body’s own self-healing capacity

Approaches may include use of soluble molecules, gene therapy, stem and progenitor cell therapy, tissue engineering and the reprogramming of cell and tissue types.

Regenerative Medicine in Urology

Organ transplantation
Tissue for reconstructive procedures
Novel therapies for chronic illness

Dr. Anthony Atala, Wake Forest Institute for Regenerative Medicine
Cell based therapy
Biomaterial based therapy
Combined (tissue engineering)

Cell based therapy

Transplant cells to replace or repair diseased tissue

Wezel et al. BJU. 2011
Cell based therapy

- Clinical applications
  - Urology (?SUI)
  - Hematology (malignancy)
  - Cardiovascular (heart, PVD)
  - Ophthalmology (retina)
  - Neurologic (huntington’s, parkinson’s, spinal cord injury)
  - Musculoskeletal

- Challenges
  - Rejection
  - Host integration
  - Targeting cells to proper location
  - Malignancy

Biomaterial based therapy

- In vivo tissue repair by in-growth of host cells into an acellular matrix
  - Decellularized biomaterials (SIS, BSM)
  - Organic polymers (ie. collagen, alginate)
  - Synthetic (ie. PGA, PLGA)
  - Composite (ie. collagen and synthetic)

- Ideal biomaterial
  - Biocompatibility
  - Bioresorbability
  - Minimally immunogenic
  - Integration into local environment
Biomaterial based therapy

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Pathology</th>
<th>ECM</th>
<th>Urological Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Renal tumour</td>
<td>Acellular SIS</td>
<td>Hernietosis in nephrectomy sparing surgery for renal tumors^{15,17}</td>
</tr>
<tr>
<td>Bladder</td>
<td>Leiomyosarcoma</td>
<td>Acellular SIS</td>
<td>Urinary bladder augmentation/reconstruction^{22}</td>
</tr>
<tr>
<td></td>
<td>Neoplastic urinary incontinence</td>
<td>Cell seeded iBM + synthetic scaffold</td>
<td>Bladder neck sling^{22}</td>
</tr>
<tr>
<td></td>
<td>Stress urinary incontinence</td>
<td>Acellular SIS</td>
<td>Commercially available as a pubovaginal sling</td>
</tr>
<tr>
<td>Urethra</td>
<td>Stricture ≤3 cm</td>
<td>Acellular iBM</td>
<td>Onlay graft post urethral stricture^{15,17}</td>
</tr>
<tr>
<td></td>
<td>Stricture &gt;3 cm</td>
<td>Tubularized cell seeded iBM</td>
<td>Maintaining penile post resection of larger urethral defects (e.g. &gt;3 cm)^{22}</td>
</tr>
</tbody>
</table>


Challenges
- Breakdown
- Fibrosis
- Rejection

Tissue Engineering

- Process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage or congenital defects.

US National Institute of Health 2006

Components

![Diagram of tissue engineering components](image)
Tissue Engineering - Cells

- Obtained via biopsy and expanded in culture

- Types
  - Autologous
    - Best option – no rejection
  - Allogenic
  - Heterologous

Tissue Engineering - Scaffolds

- 3D matrix for cells to adhere, proliferate, migrate and differentiate so that functional tissue can form in vitro

Atala. Fertility and Sterility. 2012
Cell adhesion molecules (ie. Cadherins, selectins, integrins)

Bioactive factors can be loaded along with cells onto scaffolds to help regulate cellular function


Bladder Augmentation

Bladder
Pediatric Neurogenic Bladder

- Most common cause in children = abnormal development of the spinal canal (90%)

- Other causes
  - Sacral agenesis, imperforate anus, cerebral palsy, traumatic spinal cord injuries, neurologic disease
Pediatric Neurogenic Bladder

- Characteristics
  - High filling pressures
  - Poor compliance/capacity
  - Overactive
  - Incontinence

- Treatment goal
  - Preserve renal function
  - Prevent UTIs
  - Preserve continence

Evolution of management

- Diversion
- CIC 1972
- CIC & augmentation 1980’s
- CIC & augmentation & Mitrofanoff 1980+
- CIC & medical management 1999+
  - Botox
  - Anticholinergics (oral, topical, intravesical)

Augmentation Cystoplasty

History
- Enterocystoplasty (Tutkowski and Mikulicz 1899)
- Gastrocystoplasty (Leong and Ong 1972)
- Ureterocystoplasty (Eckstein and Martn 1973)
- Autoaugmentation (Cartwright and Snow 1989)
- Seromuscular enterocystoplasty (Dewan and Stefañek 1995)
- Other → peritoneum, omentum, fascial free grafts, lyophilized human dura, bovine pericardium, fresh placental membranes, preserved bladder grafts, synthetic materials


Enterocystoplasty Complications

- Competing interests
  - Intestines absorb
  - Urothelium impermeable

- Mucous production
- Bacterial colonization
- Electrolyte imbalances
- Metabolic acidosis
- Somatic growth retardation
- Vitamin B12 deficiency
- Bladder stones
- Malignancy

Augmentation Cystoplasty

- Ideal material
  - Easily available
  - Allow large areas to be potentially used
  - Be easily mobilized without jeopardizing blood supply
  - Allow regrowth of urothelium
  - Have sufficient pliability to assume the shape of the bladder
  - Have an inherent functioning bladder layer
  - Not absorb electrolytes or nitrogenous wastes of the urine
  - Not produce mucous or irritating substances


Tissue Engineered Bladder Augment

- Avoid complications associated with enterocystoplasty

- Acellular vs cellular approach
Acellular Biomaterials

- Theory → scaffold recruits cells for new tissue formation
- SIS and BSM widely explored
- Results → non-seeded scaffolds fail to show full regeneration of the bladder wall.
- Reason for failure → extensive scarring within graft, early exposure of scaffold and newly implanted cells to urine

Orabi et al. The Scientific World Journal. 2013

Cellular Approach

Orabi et al. The Scientific World Journal. 2013
Acellular vs Cellular

- Oberpenning et al. Nature Biotechnology. 1999
- Canine model
- 3 groups
  - Subtotal cystectomy
  - Subtotal cystectomy with nonseeded scaffold
  - Subtotal cystectomy with seeded scaffold
- Results
  - Seeded – 95% original capacity, trilayer histology
  - Non-seeded – 46% original capacity, minor cell ingrowth and fibrosis
  - None – 22% original capacity

Tissue-engineered autologous bladders for patients needing cystoplasty

- First clinical trial of an engineered organ being implanted in human
- 9 pts with myelomeningocele (2 lost to f/up)
  - Poorly compliant bladders
  - Frequent urinary leakage
  - Failed pharmacotherapeutic interventions
  - On CIC
  - Mean age 11 yrs
Engineered human bladder tissues
- Autologous bladder biopsy 7-8 weeks pre cystoplasty
- Urothelial and muscle cells isolated then cultured
- Cells expanded in vitro
- Cells attached to biodegradable 3-D matrix
  - First 4 pts – collagen
  - Last 3 pts – composite collagen and polyglycolic acid
Mean f/up 46 months

No metabolic complications, renal function preserved, no mucous production, no stones
Conclusion

- Composite scaffold with omental wrap best results
- Important step in evaluating transfer of tissue engineering technology to the clinical setting
- However, improvements in capacity not analogous to those achieved by gold standard

Autologous Cell Seeded Biodegradable Scaffold for Augmentation Cystoplasty: Phase II Study in Children and Adolescents with Spina Bifida

Autologous seeded biodegradable scaffold (Tengion) for bladder augmentation
- Phase II prospective study
- Children with neurogenic bladder due to spina bifida
  - High bladder pressures > 40mmHg
  - Failed maximum antimuscarinic medication
- Primary and secondary outcomes at 12 and 36 months
  - Change compliance, capacity and safety
Autologous Cell Seeded Biodegradable Scaffold for Augmentation Cystoplasty: Phase II Study in Children and Adolescents with Spina Bifida

David B. Joseph,* Joseph G. Borer, Roger E. De Filippo,† Steve J. Hodges‡ and Gordon A. McLorie

THE JOURNAL OF UROLOGY® May 2014

Results

❖ Compliance mildly improved in 5/10 at 36 months
❖ No change capacity
❖ Adverse events occurred in all patients
  ✷ Serious AE occurred in 4 patients (bowel obstruction
     and/or bladder rupture)

Conclusion

❖ No improvement in compliance or capacity
❖ Serious adverse events surpassed acceptable safety
   standard

Urologic tissue engineering

❖ Lower Urinary Tract
  ✷ Bladder
  ✷ Urethra
  ✷ Urethral sphincter
❖ Upper Urinary Tract
  ✷ Kidney
❖ Reproductive Tract
  ✷ ED
  ✷ Vagina
Lower Urinary Tract

Bladder

- Bladder cancer
  - Cystectomy mainstay of therapy for high risk disease
  - Reconstruction options
    - Incontinent ileal conduit
    - Continent cutaneous diversion (ie. Indiana pouch)
    - Orthotopic neobladder diversion (ie. Studor neobladder)
- Complications
  - Largely due to bowel in GU tract
Replace ileal conduit with lab grown conduit

Successful porcine cystectomy model using lab grown conduit (NUC)  
Basu 2009

- Phase 1 trial
- Goal 10 patients
- Autologous SMCs procured from fat biopsy propagated ex-vivo x 3-4 weeks then seeded onto synthetic (PGA/PLGA) mesh scaffold
- Primary outcome ➔ functionality, structural integrity, conduit patency
- Estimated study completion - 2017
Urethra

Biomaterial based therapy
Tissue engineering

Urethra – Biomaterial based therapy

- Acellular collagen matrix in onlay fashion for creation of a neourethra in……

  - 4 boys with prev failed hypospadias repair
  - 3 pts success (mean f/up 22 months)

  - 28 pts with anterior urethral stricture
  - 24 pts success (mean f/up 37 months)

- Complications → scarring, fistula
Urethra – Tissue Engineering

Tissue-engineered autologous urethras for patients who need reconstruction: an observational study

Atlantida Royo-Rivers, Diego R Esquillano, James J Yoo, Esther Lopez-Bouglen, Shoy Soker, Anthony Atala

- 5 boys with urethral strictures
- Autologous cells → seeded onto tubularized scaffold

Results:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Primary diagnosis</th>
<th>Previous urethrosatopy</th>
<th>Defect site</th>
<th>Defect length (cm)</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motor vehicle accident</td>
<td>No</td>
<td>Membranous urethra</td>
<td>5</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>Straddle trauma</td>
<td>Buccal mucosa</td>
<td>Membranous urethra</td>
<td>6</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td>Motor vehicle accident</td>
<td>No</td>
<td>Membranous urethra</td>
<td>4</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>Motor vehicle accident</td>
<td>Foreskin</td>
<td>Membranous urethra</td>
<td>4</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>Straddle trauma</td>
<td>No</td>
<td>Membranous urethra</td>
<td>5</td>
<td>36</td>
</tr>
</tbody>
</table>
Urethra – Tissue Engineering

Tissue-engineered autologous urethras for patients who need reconstruction: an observational study

Atlantida Royo-Rivera, Diego R. Esquivel, James J. Yoo, Esther Lopez-Baughen, Shuy Soker, Anthony Atala

Results:

![Graph showing follow-up and flow rate over time for different patients.]

![Images showing different stages of urethral reconstruction.]
Urethra – Tissue Engineering

Tissue-engineered autologous urethras for patients who need reconstruction: an observational study

Atlantida Reyes-Rivera, Diego R. Esquiliano, James J. Yoo, Esther Lopez-Baugher, Shay Soker, Anthony Atala

Conclusion

- Tissue engineered urethras can be used for stricture repair with good results at 6 year f/up.

Urethra – Tissue Engineering

Autologous in vitro cultured urothelium in hypospadias repair

M. Fossum a,b,*, J. Svensson a, G. Kratz c, A. Nordenskjöld a,b


- 6 pts (age 14 – 44 months)
- Cells harvested by catheterization and bladder lavage
- Lab culture → seeded onto allogeneic acellular dermis
- Results → 3 pts developed complications (fistula and stricture)
Urethral Sphincter

- SUI common problem
  - Pelvic floor weakness, post-partum damage, post-prostatectomy incontinence, idiopathic, epispadias

- Current treatment – conservative and surgical
  - Conservative – pelvic floor exercises
  - Surgical – slings, bulking agents, artificial sphincters

- Stem cell injection into external urethral sphincter
  - Bent et al. Neurourol Urodyn. 2001
    - Ear derived autologous chondrocytes for periurethral injection into 32 women
    - >80% symptomatic improvement at 12 months

    - Autologous muscle-derived cells for intra and periurethral injections in 8 women
    - 1 yr f/up in 5 women
    - 1 dry, 4 improved

- Benefit may be due to passive bulking effect rather than regeneration of contractile sphincteric tissue
Upper Urinary Tract

Kidney

- Clinical problem
  - Increasing prevalence of CKD and ESRD

- Current treatments
  - Dialysis – costly, significant morbidity and mortality
  - Renal transplantation – limited by organ shortage and toxicity of immunosuppression
Regenerative Medicine Approaches

- Cell therapies to regenerate renal function at an early stage of CKD
  - Tengion – Phase 1 trial currently recruiting
  - Neo-Kidney Augment (NKA)
    - Made from expanded autologous, homologous, selected renal cells obtained via kidney biopsy then implanted back into the kidney
    - Goal to delay loss of renal function

Efficacy and Safety of Renal Tubule Cell Therapy for Acute Renal Failure

- Phase II multi-center, randomized, controlled, open-label trial
- Renal tubule assist device
  - Conventional hemofilter lined by monolayers of renal cells.
  - Belief that renal cells will perform absorptive, metabolic, endocrine and immunologic functions
- 58 pts with ARF requiring CRRT
- 2:1 randomization (40 pts RAD, 18 CRRT)
- Primary endpoint → all cause mortality
- Results
  - Improved survival
  - More rapid recovery of kidney function

![Graph showing survival comparison between RAD and CRRT](image-url)
Reproductive Tract

ED

- Clinical problem
  - High prevalence of ED in older males

- Current treatments
  - Pharmacological – PDE5, injections, vacuum assist, etc.
  - Surgical - prosthesis

- Current research
  - Reconstruction of corporal smooth muscle
  - Engineered penile prostheses
  - Stem cell implantation

Vagina

Tissue-engineered autologous vaginal organs in patients: a pilot cohort study

Adolfo M. Reyna-Rivera, Diego Esquivel-Reyna, Piero Fierro-Pétrenea, Esther López-Baygshed, Pedro Valencia, Ricardo Ordorica-Flores, Shay Soker, James Yoo, Anthony Atala

- Engineered vaginal organs implanted into 4 pts with vaginal aplasia from MRKH syndrome
- Age 13-18
- Vulvar biopsy
- Autologous tissue cultured, expanded and seeded onto scaffold
- Implanted via perineal approach

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at surgery (years)</th>
<th>Presentation</th>
<th>MRKH syndrome or vaginal aplasia</th>
<th>Uterine anomaly</th>
<th>Renal anomaly</th>
<th>Other malformations</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>17</td>
<td>Previous failed vaginoplasty</td>
<td>Type II</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>102</td>
</tr>
<tr>
<td>Patient 2</td>
<td>13</td>
<td>Abdominal mass</td>
<td>Type I</td>
<td>Hypoplastic uterus</td>
<td>None</td>
<td>None</td>
<td>91</td>
</tr>
<tr>
<td>Patient 3</td>
<td>16</td>
<td>Amennorhoea</td>
<td>Type II</td>
<td>Absent uterus</td>
<td>Right renal agenesis</td>
<td>Anorectal</td>
<td>cervicothoracic somite dysplasia</td>
</tr>
<tr>
<td>Patient 4</td>
<td>18</td>
<td>Amennorhoea</td>
<td>Type I</td>
<td>Absent uterus</td>
<td>None</td>
<td>None</td>
<td>62</td>
</tr>
</tbody>
</table>

MRKH-Mayer-Rokitansky-Küster-Hauser
Tissue-engineered autologous vaginal organs in patients: a pilot cohort study

- No long term complications
- Histologically and functionally normal
- Successful to date
Roadblocks

- Science
  - Neovascularization of implants and ingrowth of nerves in vivo.
  - Complexity of organs

- Ethics
  - Guidelines re: stem cell therapy
  - Randomized controlled trials in pediatric population
    - Complex, unpredictable nature of human tissue engineered products
    - Lack of a RCT to compare new treatment to gold standard
    - Pt's young age and possible long term effects
    - Informed consent
  
- Regulatory groups (ie. FDA)


Future

- ? organ creation
- Bioprinting

- Main goals underlying the most current research in regenerative medicine
  - Determination of the optimum scaffold for cell seeding
  - Best source of stem cells
  - Optimal way to differentiate and define stem cells
  - Promotion of neovascularization and nerve regeneration into the implant
Conclusion

- Regenerative medicine is an exciting, interdisciplinary field that has the potential to revolutionize medicine.

- Despite extensive research, regenerative medicine techniques have still not made a meaningful transition into urologic practice.

- Main role at the current time seems to be in cases when native tissue is not available or has failed.

Thank You

Dr. Afshar and Dr. Black