Tissue Engineering Potential of Urothelial Cells From Diseased Bladders

Ramnath Subramaniam, Jennifer Hinley,* Jens Stahlschmidt and Jennifer Southgate*,†

From the Departments of Pediatric Urology (RS) and Pathology (JSt), Leeds Teaching Hospitals NHS Trust, Leeds and Jack Birch Unit of Molecular Carcinogenesis, Department of Biology, University of York, York (JH, JSo), United Kingdom

Abbreviations and Acronyms

CK = cytokeratin

KSFMc = keratinocyte serum-free medium (complete)

NHU = normal human urothelial

TER = transepithelial electrical resistance

UPK = uroplakin

UTI = urinary tract infection

v/v = volume per volume

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Study received research ethics committee approval.

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† Correspondence: Jack Birch Unit of Molecular Carcinogenesis, Department of Biology, University of York, York YO10 5DD, United Kingdom (telephone: 44-190-432-8705; FAX 44-190-432-8704; e-mail: js35@york.ac.uk).

Purpose: We examined the suitability of urothelium from patients with abnormal bladders for use in surgical reconstruction using a tissue engineering approach that would require autologous urothelium to be expanded by propagation in cell culture.

Materials and Methods: Resection specimens from 8 children (median age 9.8 years) with abnormal bladders (neuropathic in 4, posterior urethral valves in 2, epispadias in 1, nonneurogenic in 1) were collected with informed parental consent during planned urological procedures. Six patients had recurrent urinary tract infections and 7 underwent frequent intermittent catheterization. A representative sample was immunohistologically processed to assess urothelial proliferation and differentiation status, and the remaining 7 cases were processed for urothelial cell culture. Five normal adult urothelial samples were included as controls.

Results: Immunohistological assessment indicated that 3 of 8 samples lacked urothelial differentiation associated expression of UPK3a or CK20. Four of 7 samples resulted in successful primary culture, with 1 sample lost to underlying infection and 2 not surviving in culture. All 4 cultures grew beyond passage 3 before senescence but all showed reduced proliferation capacity and a compromised ability to form a barrier urothelium compared to controls.

Conclusions: While normal human urothelium is highly regenerative and derived cells are highly proliferative in culture, our results with urothelium from abnormal pediatric bladders indicate a reduced capacity for proliferation and differentiation in vitro. This finding may indicate a need to identify alternative cell sources for engineered bladder reconstruction.

Key Words: cell culture techniques; cell proliferation; tissue engineering; urinary bladder, neurogenic; urothelium

RECONSTRUCTIVE surgery is frequently required in congenital abnormalities of the bladder and its innervation to prevent renal failure and manage urinary incontinence. Enterocystoplasty is a major advance in this respect, providing a compliant urinary reservoir that protects the upper tract and frequently transforming quality of life. However, the use of intestinal segments for bladder augmentation or substitution is associated with well documented problems of mucus production, chronic infection, stone formation and potential malignancy.[1] These problems are primarily due to the fact that bowel epithelium has not evolved for prolonged contact with urine.

The search for alternative approaches to augment or replace the diseased bladder has included tissue engineering, where natural or syn-

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Table 1. *Clinical characteristics*

Pt No.—Age—Sex	Catheterization	Clinical Findings	Urodynamics (pressure)	Recurrent UTIs*
1—7 —M	Yes	Posterior urethral valves	High	Yes
2—5 —M	Yes	Posterior urethral valves	High	Yes
3—15 —M	Yes	Neurogenic	Low	Yes
4—5 —M	Yes	Bladder dysfunction	Low	Yes
5—8 —F	Yes	Neurogenic	Low	No
6—15 —F	No (complete bladder neck incompetence)	Epispadias	Low	No
7—12 —F	Yes	Neurogenic	Low	Yes
8—12 —M	Yes	Neurogenic	Low	Yes

* Patients with recurrent UTIs received prophylactic antibiotics at surgery.

thetic biomaterials are combined with autologous cells in vitro to generate a full-thickness bladder wall construct for implantation.[2] This approach is highly ambitious, and we favor instead a refinement of enterocystoplasty, where the epithelium of the intestinal segment is substituted with autologous urothelium propagated in vitro. The latter approach is attractive since, by using a ready vascularized host smooth muscle tissue to form the wall of the neobladder, only the urothelium itself needs to be engineered (reviewed).[3]

Advances in cell culture techniques have made it possible to cultivate NHU cells in vitro in terms of generating an adequate quantity of cells and in their capacity to form a differentiated urothelium with barrier properties akin to normal urothelium.[4,5] We have found no major differences in cell cultures established from different regions of the urinary tract, or from adult or pediatric sources.[4,6] Adapting these techniques to porcine urothelium has formed the basis of a successful surgical model of composite cystoplasty.[7–9]

Our cell culture techniques have been developed using normal human urothelium and have not been tested in urothelial cells harvested from patients with diseased bladders who are candidates for composite cystoplasty. This is an important step that is clearly needed for translation of composite cystoplasty to the clinic. We investigated the in vitro properties of urothelial cells harvested from pediatric patients with benign diseased bladders.

MATERIALS AND METHODS

Patient Samples

Nontrigonal bladder samples were obtained at surgery with appropriate parental consent and research ethics committee approval. Eight samples were harvested from children who demonstrated abnormal bladder dynamics and were suitable candidates for bladder replacement (table 1). Six of the children had recurrent UTIs, and 7 underwent clean intermittent catheterization or had an indwelling cystostomy button.

Surgical samples (1 cm^2) were collected aseptically in Hanks' balanced salt solution containing 10 mM HEPES pH 7.6 and 20 KIU/ml aprotinin (Trasylol®) and containing 1% (v/v) penicillin and streptomycin (Invitrogen Corp, Paisley, United Kingdom). A small representative sample was fixed and processed for immunohistological evaluation, and the remaining tissue was used to establish urothelial cell cultures. Experiments were performed against control normal bladder and ureteral urothelium from adults with no known urinary tract abnormality.

Histological and Immunohistological Assessment

Following standard fixation in 10% buffered formalin and processing into paraffin wax, 5 μM sections of the bladder biopsies were assessed by hematoxylin and eosin and by combined alcian blue/van Gieson staining to determine the presence of mucins. For immunohistochemical analysis 5 μM dewaxed sections were blocked for endogenous peroxidase, subjected to antigen retrieval by microwave boiling in 10 mM citric acid buffer (pH 6.0) for 10 minutes and treated to block free avidin/biotin (Vector Laboratories) and secondary antibody binding sites (10% rabbit or goat serum) before incubation with primary antibody for 16 hours (table 2),

Table 2. *Primary antibodies*

Specificity	Antibody	Host	Concentration for Immunohistochemical Analysis	Concentration for Immunofluorescence Analysis	Manufacturer
CK13	1C7	Mouse	—	12.5 μg/ml	Abcam
CK14	LL002	Mouse	—	200 μg/ml	Serotec
CK18	CY90	Mouse	—	1:1,000 dilution	Sigma
CK20	Ks20.8	Mouse	5 μg/ml	—	Novocastra
UPK3a	AU1	Mouse	1:40 dilution	—	Progen, Heidelberg
Ki67	MM1	Mouse	0.5 μg/ml	—	Novocastra
Claudin3	Z23.JM	Rabbit	—	2.2 μg/ml	Zymed (Invitrogen)
ZO1	ZO1-1A12	Mouse	—	1.25 μg/ml	Zymed (Invitrogen)