European Journal of Cancer (2013) xxx, xxx–xxx

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journal homepage: www.ejcancer.com

A novel precision-engineered microfiltration device for capture and characterisation of bladder cancer cells in urine

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KEYWORDS
Bladder cancer
Nanotechnology
Urine cytology
Screening
Surveillance

Abstract ***Background:*** Sensitivity of standard urine cytology for detecting urothelial carcinoma of the bladder (UCB) is low, attributable largely to its inability to process entire samples, paucicellularity and presence of background cells.
Objective: Evaluate performance and practical applicability of a novel portable microfiltration device for capture, enumeration and characterisation of exfoliated tumour cells in urine, and compare it with standard urine cytology for UCB detection.
Methods: A total of 54 urine and bladder wash samples from patients undergoing surveillance for UCB were prospectively evaluated by standard and microfilter-based urine cytology. Head-to-head comparison of quality and performance metrics, and cost effectiveness was conducted for both methodologies.

☆ DOI of original article: 10.1016/j.ejca.2013.06.007.
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http://dx.doi.org/10.1016/j.ejca.2013.04.033

Results: Five samples were paucicellular by standard cytology; no samples processed by microfilter cytology were paucicellular. Standard cytology had 33.3% more samples with background cells that limited evaluation ($p < 0.001$). Microfilter cytology was more concordant ($\kappa = 50.4\%$) than standard cytology ($\kappa = 33.5\%$) with true UCB diagnosis. Sensitivity, specificity and accuracy were higher for microfilter cytology compared to standard cytology (53.3%/100%/79.2% versus 40%/95.8%/69.9%, respectively). Microfilter-captured cells were amenable to downstream on-chip molecular analyses. A 40 ml sample was processed in under 4 min by microfilter cytology compared to 5.5 min by standard cytology. Median microfilter cytology processing and set-up costs were approximately 63% cheaper and 80 times lower than standard cytology, respectively.

Conclusions: The microfiltration device represents a novel non-invasive UCB detection system that is economical, rapid, versatile and has potentially better quality and performance metrics than routine urine cytology, the current standard-of-care.

1. Introduction

Urothelial carcinoma of the bladder (UCB), a common malignancy, accounts for one of the highest management costs per patient of any cancer.[1,2] Non-invasive tumours, the most common UCB subtype, are treated with bladder-sparing surgeries and are at high risk for recurrence.[3] Guidelines therefore demand frequent, long-term follow-up by cytology and cystoscopy.[4,5] Non-invasive UCB detection techniques including standard urine cytology and adjunct molecular tests have been unable to substitute or reduce the need for cystoscopy, leading to steady rise in its use with increasing financial burden on the healthcare system.[6] Standard cytology is limited by its sensitivity, often due to paucicellularity and presence of confounding background non-urothelial cells.[7] An alternate sensitive, rapid and cost-effective UCB detection and surveillance assay would therefore be beneficial.

This prospective, proof-of-concept study describes the development of a novel microfilter-based device for capture, on-chip enumeration and characterisation of exfoliated tumour cells from urine of subjects undergoing screening or surveillance for UCB. The principle of cell-size-based capture is used to trap and enrich larger tumour cells on a small surface area, while smaller background-blood cells pass through the filter. The goal was to determine whether microfilter-based cytology was at least comparable, if not superior, to standard urine cytology with respect to performance metrics, processing time and costs.[8]

2. Materials and methods

2.1. Microfiltration device fabrication

1 cm × 1 cm transparent parylene microfilter membranes of 10 μm thickness were precision-engineered by photolithography as described previously.[9] After iterative testing to optimise filtration efficacy and cytological evaluation, a 6 mm × 6 mm filtration area with 90,000 evenly distributed circular pores of 7.5 μm diameter was constructed; centre-to-centre distance between adjacent pores was 20 μm (Fig. 1). Each microfilter was sandwiched between two polydimethylsiloxane slabs with a central area cut out to accommodate it, placed within an acrylic housing device and clamped at opposite ends to create a leak-proof cassette. 20 ml syringe containing the sample was attached to the upper acrylic jig's luer lock, and filtrate flowed into a beaker from an outlet at the cassette's bottom.

2.2. Cell line

T24 bladder cancer cells were propagated in McCoy's 5A medium supplemented with 50 U/ml of penicillin and streptomycin, and 10% foetal bovine serum.

2.3. Sample acquisition and processing

Voided urine and bladder wash samples from patients undergoing surveillance for UCB were collected prospectively under an Institutional Review Board-approved protocol. Bladder washings were also collected from confirmed UCB patients to better estimate sensitivity and potential qualitative differences between both cytology methodologies. Voided urine was also collected from normal donors with no evidence of UCB. Informed consent was obtained from all subjects. Cystoscopy-guided biopsy results were considered confirmatory for UCB diagnosis.

To optimise a fixation protocol that maintained cellular morphology while passing urine through the microfilter, varying concentrations and fixation times for ethanol, the standard fixative for urine cytology, were tested on normal urine spiked with T24 cells. 25% ethanol was identified as most optimal, and was also ideal for standard cytology.

For processing, 40 ml sample was diluted 1:1 in phosphate buffered saline and ethanol, and incubated on rotator at room temperature for 20 min. Samples were then equally divided and processed by both methodolo-

Please cite this article in press as: Birkhahn M. et al., A novel precision-engineered microfiltration device for capture and characterisation of bladder cancer cells in urine, *Eur J Cancer* (2013), http://dx.doi.org/10.1016/j.ejca.2013.04.033