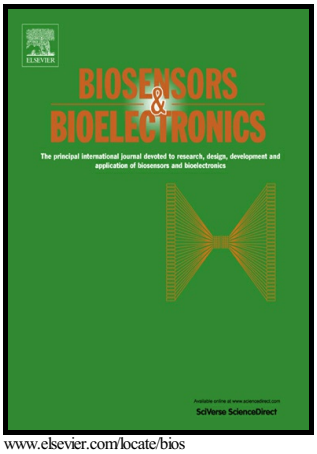

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A microfluidic *multiwell chip* for enzyme-free detection of mRNA from few cells

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Abstract

Isogenic cell populations possess heterogeneous gene expression patterns. Most methods for mRNA expression analysis start with the reverse transcription of mRNA into cDNA, a process that can introduce strong signal variations not related to the actual mRNA levels. Miniaturized lab-on-a-chip systems offer properties – e.g. low sample dilution, low contamination – that enable new reaction schemes for molecular analyses. To enable transcription-free mRNA expression analysis of few single cells, a one-step cell lysis, target labelling and hybridisation approach as well as a corresponding passive *multiwell chip* with a volume of 25.5 nL/well were developed. The method enabled the parallel analysis of up to 96 samples and 6 target genes per sample. Preceding light microscopy of the living cells allowed correlating mRNA levels and cell number. As a proof-of-principle, the pancreatic cancer cell line Panc-1 was investigated for expression heterogeneity of a reference gene plus 5 genes reported to be overexpressed in cancer stem cells (CSCs). A good correlation ($r(51)=0.739$, $p<0.001$; $rs(51)=0.744$, $p<0.001$) between the cell number per well and the number of detected reference gene mRNA confirmed the proper function of the device. Moreover, a heterogeneous expression of the CSC-associated target genes was found which matched well with reports on the presence of CSCs in the Panc-1 cell line.

Keywords: ultra-sensitive detection, mRNA expression, microarray, cellular heterogeneity, lab-on-a-chip

1. Introduction

Cell populations are inherently heterogeneous, which can lead for instance to significant differences in disease development and treatment (Altschuler and Wu, 2010). Heterogeneity in eukaryotic cells is introduced to a major part at the transcriptional level (Blake et al., 2003). A reason for this is that gene expression occurs in transcriptional bursts (Dar et al., 2012; Suter et al., 2011) which can lead to large variations in the total number of mRNA molecules per cell (Raj et al., 2006). The investigation of cellular heterogeneity requires methods sensitive enough to analyse one (best case) to few cells.

The majority of methods for mRNA expression analysis in single cells include enzymatic reverse transcription (RT) of mRNA into cDNA and subsequent amplification of the target molecules (Haselgrübler et al., 2014) (e.g., RT quantitative PCR (RT-qPCR) (Ståhlberg and Bengtsson, 2010), RNA-sequencing (Saliba et al., 2014)). The reason for this is that mRNA is often present in very low copy numbers per cell (Marinov et al., 2014). However, there are numerous reports on highly