Miniaturized PCR systems for cancer diagnosis

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Abstract
PCR retains a pivotal role in making accessible marker nucleic acid sequences for ready analysis in cancer diagnosis. For certain cancers such as acute lymphoblastic leukaemia, the application of quantitative procedures to assess and subsequently direct therapy has given rise to the slowly maturing field of MRD (minimal residual disease) management. Although excellent protocols exist for performing these analyses, akin to all PCR procedures the limit of detection can vary markedly between laboratories. The present paper is an overview that describes how the analytical field relating to miniaturization is likely to identify the missing link that integrates sample processing with downstream PCR, analysis and eventual therapy. Miniaturized devices are suited to the multi-parallelized handling of defined numbers of cells, and PCR-based microfluidic procedures have become reasonably established. The integration of sample processing and PCR in microfluidic devices is beginning to offer reproducible quantitative data that relate the number of biomarker nucleic acids to the defined analysed cell or cells for meaningful clinical assessment. The application of MRD may, through integrated miniaturized PCR, become more reliable and routine with additional applications in defining disease threshold levels for other cancer types. These enabling integrated platforms may facilitate biomarker measurements to predict the response and outcome, which are also of current interest for personalized medical care.

Background
Cancer biology continues to rely extensively on the comparison of diseased and healthy tissues, with statistically proven differences revealing disease-associated pathways. This approach, although relatively simplistic, has been remarkably successful, enables association of many types of biomarker with disease presence and provides the means to develop less cytotoxic, enhanced pharmaceutical agents to pertinent targets. The cancer cell possesses certain characteristics that distinguish it from the equivalent normal cell. Importantly too, there are often different subcategories of cancer cells, which have their own distinguishing features, and these are often revealed by characterization at the molecular level. For successful patient care, the avoidance of both over- and under-treatment of the cancer has presented the oncology specialist with the greatest challenge. Most clinical practitioners rely on visual screening of patients for indications of cancer onset and cancer. However, this form of monitoring has major limitations in that visual cancer-related identifiers are required and most typically the progression of cancer cannot be readily distinguished. For example, early treatment of pre-malignant or oral cancer lesions is believed to greatly reduce the morbidity associated with late disease treatment and to improve overall patient survival. Therefore, perhaps not surprisingly, enormous effort has been expended on measurement of disease-related markers. Particularly for nucleic acids, the provision of meaningful quantitative assessment to determine cancer pathogenesis can be established and effective therapy derived.

Measurement of cancer by using PCR
The identification of disease thresholds that correlate to disease treatment is a topical area. The treatment of certain leukaemias such as ALL (acute lymphoblastic leukaemia) has made significant advances in moving towards the quantitative assessment of leukaemic cells within the peripheral blood of patients in a tool designed to measure submicroscopic refractory cancer disease, which is more typically referred to as MRD (minimal residual disease). Most MRD calculations are based on PCR-based applications to profit from the sensitivity offered by the geometric branching tree amplification process. Although the PCR process ‘can’ be quantitative [QPCR (quantitative PCR)], the reality is that, for MRD, a more qualitative strategy is adopted where the presence of a PCR product from the fusion transcript indicates a sufficient presence of leukaemic cell subpopulation to warrant treatment. Indeed, this is a rare example of where a molecular analysis directs treatment [1]. There are some problems with the current application of MRD. Standard protocols are utilized by laboratories participating in MRD studies; however, MRD laboratories may not always derive similarly analogous limits of detection, which typically can be expected to be in the range of 1 leukaemic cell detected in 10000−100 000 normal nucleated cells.

Quantification of biomarkers in cancer
Therefore, with MRD representing one of the few molecular tests employed to specify timing of therapy, and with
the MRD application being largely qualitative, will true quantification of transcript ever gain a significant role in stratifying disease state and intensity or type of treatment? Or, argued slightly tangentially, will oncology (medicine) ever become a discipline with a solid quantitative basis? This is not to say that meaningful statistics currently evade the clinical sciences, but methodological rigor and prediction, customary in engineering, may not be achieved in cancer therapy without an enhanced tool for quantitative measurement [2]. Despite being in its relative infancy, systems biology promises to bridge this gap by holistically modelling organism pathway responses to changes in environment to permit the prediction of outcome of disease or after intervention via therapy. In many contexts, the challenge for translational medicine is, ultimately, to define personalized medicine risk groups with tailored treatment regimes. Outwardly, quantification is mandatory to determine whether this objective is plausible, although a new approach to clinical assessment with access to disease-related patient databases and vertical patient records is required to make sense of quantitative numbers [1].

Difficulties associated with cancer measurement in clinical samples

Medical care based on quantitative molecular marker assessments does not typify the usual clinical diagnostic environment. Currently, the closest approximation to this situation relates to the measurement of biomarkers in biological fluids where sampling rarely induces inaccurate analyte assessment. The situation with clinical solid tissue specimens is, however, rather different where the three-dimensional architecture of the tissue can often lead to imprecise measurement of biomarkers caused by non-representative sampling when tissues are observed directly or to the averaging effect even if the sampling is accurate but cells are lysed [1]. Quantification of disease-related molecular markers is often found only in the more specialist laboratories where the high skill set available affords an ‘aggregation of marginal gains’ (a quote borrowed from the successful British Olympic Cycling Team in Beijing), which is not readily achievable by the more general routine laboratory.

What is the point of accurate, precise and reproducible molecular biomarker assessments being available on a routine basis? Some physicians may argue from their experience that quantification of molecular biomarkers is not essential. For those who wish to realize the prediction of disease outcome and applications of personalized medication, the quest for routine meaningful molecular quantification in fluids and tissues is mandatory. Interestingly, in the case of MRD for ALL, trials are in progress to monitor disease using FACS. This is intriguing for straightforward yet important reasons, because for the analysis of cellular matter, the cell itself becomes the denominator to which results are referred. At present, the description of units depicting the quantity of biomarker presence is complex or even impossible to convey. Given the high potential for simplification of units when reporting the extent of nucleic acid marker presence in tissues, for example as numbers of molecules per cell, this approach could readily provide a basis for ubiquitous molecular biomarker quantification.

Use of miniaturization for the PCR quantification of disease-associated nucleic acids

Extending the use of FACS by way of lab-on-a-chip, also termed μTAS (micro total analytical system), integrated miniaturization further promises to enable cancer-cell-containing material to be exactly and exquisitely analysed by employing homogeneous extraction and analysis procedures, thus obviating the need for extensive state-of-the-art laboratory facilities with sophisticated equipment, and elaborate and time-consuming processing by skilled personnel.

This study describes the development of μTAS devices for biomarker identification of cells within peripheral blood and is adaptable to solid tumours. Results are shown for how miniaturized devices are used to handle clinical samples, lyse samples, perform PCR and record data.

There are a large number of papers describing the use of miniaturization for PCR, but for present-day macrofluidic PCR-based analyses, probably the PCR component is the least problematic facet of these bioanalytical processes. Indeed, there is little reason to venture into the expensive and complex world of de novo microfabrication and laminar flow to emulate a PCR process that currently works perfectly well in the macrofluidic world. Furthermore, most laboratories already possess an in vitro gene amplification capacity throughput exceeding the demands of their most demanding PCR applications.

The aim of μTAS PCR is to handle and manoeuvre real clinical samples within the confines of usually portable miniaturized platforms, for the enhanced analysis of informative nucleic acid sequences [3,4]. The main benefit offered by μTAS over the current procedure is that any treatment or change influencing the sample before the measurement of label can be tightly regulated and accounted for [4]. This is because sample losses can be eliminated by avoiding conventional extractions and manipulations [5,6]. Instead, the homogeneous bioassay analysis formats are well suited to μTAS, which will prove critical for the quantitative assessment of the analyte biomarker [1]. Therefore μTAS-based PCR with incumbent homogeneous μTAS assay formats resembles an inflection point in enabling ready quantitative assessment of the true biological context concealed within clinical samples.

Cells as the units for quantification in microfluidic devices

There are two basic clinical sample types: fluid and solid. For most of the nucleic acid-based assessments of cancers, the important component within all clinical samples is the cell. Therefore, as noted previously, the difference between fluids and tissues is that the latter is often associated with a three-dimensional structure where cell–cell proximity has importance. However, the common denominator is the cell.
This is fortuitous for μTAS given that the dimensions of channels resemble those of cancer cells (cells are often 6–20 μm in diameter), which enables cells to be analysed discretely and in single file [1]. A number of variations of cell analysis will be described and studies utilizing two-phase flow to generate droplets will be discussed [7]. The generation of data comprising a plethora of single cells that could constitute a whole population is novel. However sampling vast numbers of cells may be needless, and indeed the analysis of cells in defined groups may be equally informative if the nucleic acid marker remains discernible. An important factor is to determine the required sensitivity of the assay. For example, for the Philadelphia-positive leukaemic cell, some 2000 copies of the fusion transcript can be present per cell. Therefore, when lysed, detection of the contents of one such cell in a high background of other cells is readily achievable. The requirements for each analysis type are likely to be varied, and this in turn will require highly bespoke μTAS devices, and it is therefore unlikely that one device will accommodate all applications.

**Future directions of PCR in personalized health management and cancer detection**

At present, with limited quantitative information available at the resolution promised by μTAS, various detailed analyses will be needed to determine whether the vision of prediction and measured treatment of cancer at the personalized level can be accomplished by QPCR. PCR-positive-related thresholds for cancer disease will need to be identified that are clinically meaningful and not related to or imposed on by technical constraints. After years of development and know-how, the pharmaceutical sector offers excellent agents to combat cancer. Many experts argue strongly that there is no need to change products on offer as they work rather well. Others offer a spectrum of stances related to the future value offered by personalized medication, including the inherent attributes of μTAS (incorporating PCR) for cancer diagnostics and patient management. Many companies are also investing in the treatment of specific subgroups of patients, also moving away from cytotoxic agents and towards druggable targets. The molecular characterization of patients has recently further benefited from assessing the rate of drug metabolism. Through miniaturization of QPCR, access to routine and reproducible quantification of nucleic acid biomarkers promises to promote a move to personalized cancer care.

**References**


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