Rapid Detection of Multiple Myeloma using a microfluidic platform

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ABSTRACT

Diagnosis platforms incorporating microfluidic chips enable sensitive, rapid and accurate genetic analysis at low cost that could facilitate customized therapies tailored to match the vulnerabilities of each individual cancer clone. Multiple Myeloma (MM) is characterized by a distinct immunoglobulin gene rearrangement, and by IgH translocations that enable unequivocal identification of the MM clone. Here, microfluidic chip based approaches for genetic amplification via PCR and chip-based electrophoretic detection is demonstrated. Further development of the microfluidic platform could facilitate monitoring of response to therapy, detection of residual cancer cells that lead to relapse and potential prognostic capabilities in myeloma.

Keywords: Microfluidics, polymerase chain reaction (PCR), capillary electrophoresis (CE), multiple myeloma (MM).

1 INTRODUCTION

We are developing microfluidic platforms that offer rapid, inexpensive and sensitive detection of molecular characteristics of cancer and other diseases. Disposable inexpensive and reusable microfluidic chips are being developed to meet clinical needs for real-time testing. This study utilizes disposable polymer-based microchips. Multiple Myeloma (MM) is an incurable cancer of the immune system localized to the blood and bone marrow, with a median survival rate of 3-4 years post-diagnosis. For each patient, the malignant clone is characterized by a unique immunoglobulin gene rearrangement termed the clonotypic IgH VDJ [1] and often by IgH translocations, that enable unequivocal identification of the MM clone [2]. These molecular signatures, which remain constant throughout the course of disease, identify all cells that are part of the MM clone, independent of changes in morphology, differentiation markers or other genetic changes that arise as disease progresses. Furthermore, the detection of genomic IgH VDJ provides a quantitative measure of tumor burden because each cancer cell has only one copy of the rearranged IgH gene. This facilitates monitoring of response to therapy and early warning for relapse. Currently this type of testing is complex and extremely expensive for routine clinical monitoring. Implementation, on microfluidic chips, of testing for unique molecular signatures and quantitative real-time monitoring of disease burden on a routine basis, would thus improve patient care while reducing the costs of testing. In this study, a microchip-based approach is demonstrated using two types of molecular signature as "proof-of-concept" for use in MM diagnosis and monitoring. The intent is to port to chips numerous other clinically valuable molecular tests that inform prognosis and/or treatment decisions, in the context of multiparameter molecular testing within the clinic. Procedures developed for testing in MM are readily applicable to other types of cancers and diseases, with only minor changes in reagents and testing protocols [3].

1.1 Monitoring clonotypic signatures

One informative method for monitoring MM evaluates the extent to which the cells with the clonotypic IgH VDJ dominate the normal immune system [1]. The normal immune system is comprised of many individual clones, characterized by extensive diversity of clonal signatures. When MM is progressing, the MM clone inhibits the normal immune system to the extent that the MM clone becomes dominant and the polyclonal population of immune cells becomes undetectable. On molecular analysis, this results in a profile with one dominant signature. When MM is in "remission" the normal diversity returns and many signatures are detected by PCR, indicating that the normal immune system has undergone some degree of restoration.

1.2 Chromosomal translocations

MM is characterized by extensive and complex chromosomal abnormalities. Recurrent translocations involving the immunoglobin heavy chain gene on chromosome 14 and a partner chromosome are found in 70-80% of myeloma patients [2]. Patients having the t(4;14) translocation have reduced survival and respond poorly to conventional chemotherapy. Clinical monitoring for the t(4;14) translocation would enable more informed treatment decisions. This translocation can be detected by an RT-PCR assay for hybrid transcripts created by the translocation [2].

PCR/RT-PCR based detection approaches lend themselves to the use of an automated microfluidic-based platform. Heterogeneity of MM necessitates a multi-parameter analysis for different signatures and subsequent linkage analysis. Using the microfluidic platform, analysis of ex-vivo cancer cells is performed in an automated, rapid, low-volume (μL and sub-μL) regime, leading to accelerated thermal kinetics and faster testing of MM patents, risk stratification of patients with associated genetic abnormalities, and the design of customized therapy targeted to the characteristics of each MM clone.

2 MATERIALS AND METHODS

2.1 Microchip fabrication and chip-based PCR

PCR is performed in a hybrid polymer/glass microchip comprising of wells and channels moulded in poly(dimethyl)siloxane (PDMS) and fabricated using the soft-lithography replica molding approach [4]. The chips are comprised of a 1.2 mm thick layer of molded PDMS (Sylgard 184, Dow Corning, NC, USA) and a 1.1 mm thick borofloat glass (Paragon Optical Company, PA, USA) substrate. The glass and PDMS are irreversibly bonded after an oxygen plasma exposure of the mating surfaces. Further details are reported in [5].

After informed consent, bone marrow samples were obtained at diagnosis or relapse from patients with multiple myeloma (from the Cross Cancer Institute, Edmonton). Bone marrow was processed as previously described [6], using ficoll hypaque density gradient centrifugation. All samples were confirmed as having the t(4;14) translocation by detection of hybrid IgH-MMSET transcripts as previously described [2, 7].

The on-chip PCR protocol was as follows: All PCR reactions were prepared in a total volume of 25 μl. The PCR mixture included 2.5 μl 10X PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM final concentration of KCl), 0.5 μl MgCl2 (2mM), 1ul dNTP mix (0.4 mM), 1.5 μl of each of the forward and reverse primers (0.2 μM), and 0.5 μl Platinum Taq (0.5 U) (Invitrogen Life Technology), 2 μl cDNA template, 2.5 μl of BSA (1 mg/ml), and 12.5 μl of double distilled water. Chip thermal cycling conditions for the Peltier system were 94 °C for 5 minutes, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 10 minutes, subsequently stored at 4 °C.

2.2 Microchip thermal cycler and fluid handling

Thermal cycling for PCR was performed in a custom-made instrument used in conjunction with the fluidic handling system. A dual-Peltier within a custom-built physical housing performs rapid thermal cycling by the control of current flow into the system using a micro-controller. Rapid temperature transitions result with both during heating (5-6 °C/s) and cooling (3-4 °C/s) along with stable hold temperatures (± 0.50 °C of the set point). More details can be found in [8].

To eliminate the influence on electrokinetic fluid handling of variations in physical parameters of bodily fluids, a servo-motor based diaphragm pumping and pinch-off valving method was developed. This approach accurately controls and manipulates fluids, and is suitable for immobilization of reaction mixtures at elevated temperatures [9].

Both the fluidics and the thermal system are reusable. Both are external to the assay, eliminating contact with the PCR mixture and thus minimizing potential cross-contamination, thereby allowing for efficient and effective inter-run cleaning. This ensures a single system can be used for multiple runs on multiple chips.

2.3 Microchip capillary electrophoresis (CE)

Fragment analysis for PCR product detection and sizing is performed within the cross-channel CE section of the microchip using a modified procedure employed for the glass-based microfluidic chips [10]. Fragment analysis (CE) of the amplified PCR mix is performed using a microfluidic tool kit (μTK, Micralyne, Edmonton, Canada). The μTK provides the optical detection and high voltages needed to perform CE with confocal laser-induced fluorescence (LIF) detection. The LIF system uses excitation at 532 nm and detection at 578 nm. PCR product was analyzed using on-chip capillary electrophoresis (CE) within the glass chips. GeneScan® polymer (Applied Biosystems, Foster City, CA) polymer was used as the sieving matrix in the CE chip. Sizing was performed by simultaneously loading 0.3 μl of a DNA ladder GeneScan® 500 TAMRA (Applied Biosystems, Foster City, CA) in the CE chip along with the PCR product to be analyzed. Further details could be found in [11].

To perform "gold standard" verification fragment analysis was performed on the ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) with POP4 polymer (ABI) for size verification. 1 μl of the PCR product was mixed with 12 μl of HiDi formamide (ABI) and 0.2 μl of GS500 and after denaturing for 4 minutes at 96 °C and snap cooled on ice for 5 minutes were run on the ABI 3100 with 15 kV voltage.

PCR and CE functionality on a microchip provides proof-of-concept for rapid, large-scale and inexpensive MM molecular signature screening on integrated microfluidic chips.

3 RESULTS AND DISCUSSION

We successfully performed on-chip PCR with ~2 µl of template/PCR mix. To detect these molecular signatures, PCR was performed in the central enclosed chamber of a 3-well PCR chip using fluorescent-tagged primers. After PCR amplification using fluorescenated primers, product was detected by fragment analysis on a glass capillary electrophoresis (CE) chip using 50-250 pl of the amplified product performed in ~2 minutes and verification done using ABI 3100 (Fig. 1). Surface adsorption of the PCR mixture components leads to inhibition in amplification partially due to the large surface-to-volume ratio typical in microchips.

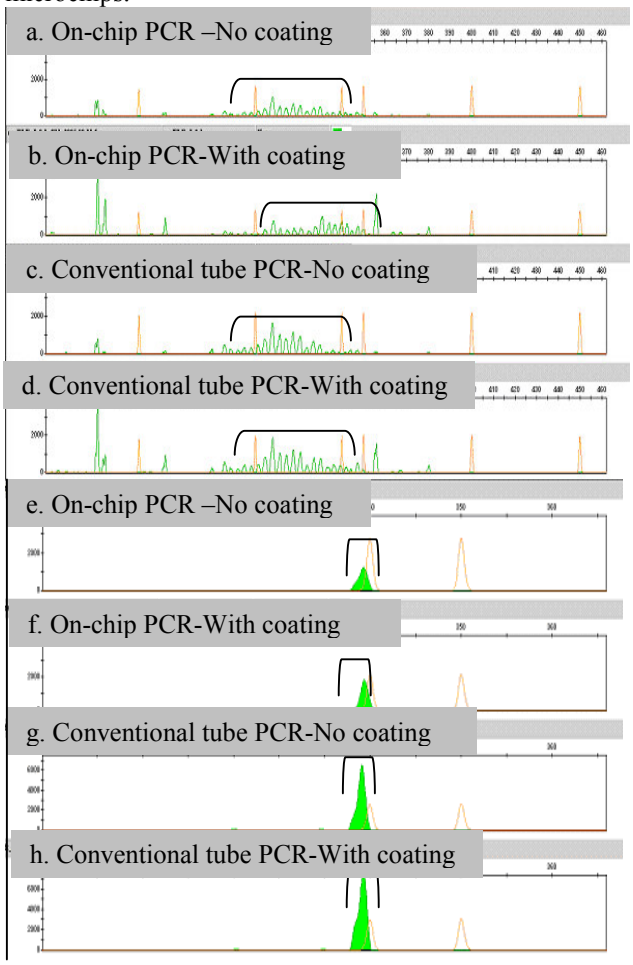

Figure 1: 2 µl PCR amplifications performed on-chip (a, b; e, f) and subsequent analysis performed on the ABI 3100 (c, d; g, h) genetic analyzer. A family of peaks (a-d) indicates polyclonal IgH and a single peak (e-h) identifies monoclonal IgH: a & e) represents the electropherograms when no surface coating was applied to the PCR chamber, b & f) PCR using nucleic acid from patient cells when the inner surface of PDMS and glass has been passivated with BSA to minimize adsorption of PCR components, c &g) standard tube-based PCR reactions, d & h) dynamic surface coating using BSA in the tube-based PCR reactions.

However, with the addition of BSA (that preferentially adsorbs to the surfaces) this effect is successfully overcome as shown in Figure 1. For a MM patient using the CE chip, both genomic DNA (data not shown) and IgH VDJ transcripts amplified from individual cells were detectable on-chip with 50 pl of product, which accounts for 0.001% of the amplified product from one individual MM cell or from groups of MM cells. IgH VDJ clonotypic transcripts are clearly resolved using CE (Fig. 2).

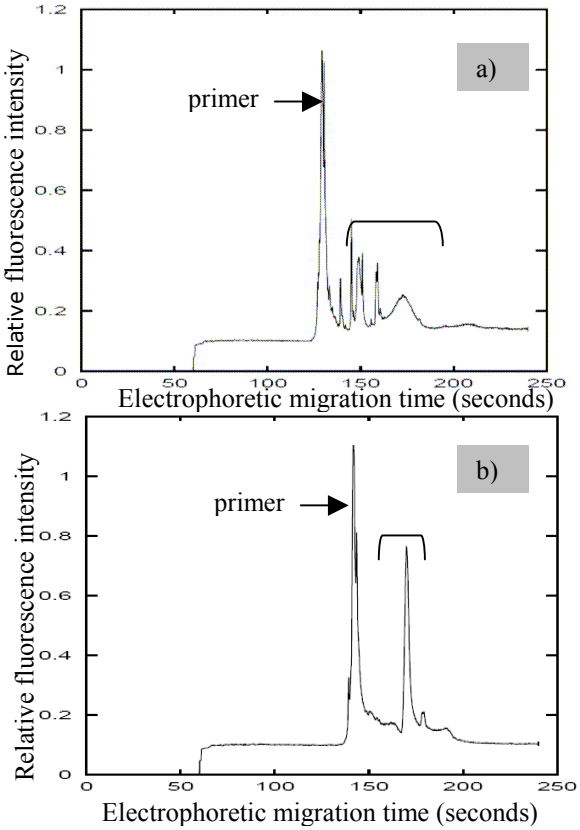

Figure 2: On-chip PCR performed on cDNA from patients analyzed on the microfluidic tool kit (µTK) using a glass-based CE chip: a) multiple PCR product peaks identify a polyclonal IgH VDJ signature, i.e. MM clone not present or in remission, b) a single spike of PCR product indicates the dominant MM clone.

Analysis of PCR product from IgH VDJ transcripts on the chip produces a distinct signature (Fig. 2) that indicates the difference between a patient whose malignant clone was dominant, thus showing a single peak, and from a patient whose malignancy was in remission, showing a variety of peaks. Figure 2(b) has two prominent peaks, representing the PCR primer and the product while Figure 2(a) consists of many distinct peaks followed by a hump comprising a family of unresolved individual peaks (when compared with the ABI 3100 run in Figure 1(b)). However, the current chip resolution is sufficient to clearly demarcate the clonotypic signatures.

T(4;14) is also readily detectable using on-chip PCR using cDNA from the bone marrow plasma cells and detection using CE in the µTK (Fig. 3). When compared to analysis on the ABI 3100, the gold standard technology, the chip provided comparable sensitivity for detecting fluorescent product [10]. The electropherogram in Figure 3 of the on-chip PCR product for a 275 bp DNA fragment (patient specific primers designed, and hence varying fragment size for each patient) indicates a t(4,14) translocation in a patient, and thus used as a diagnostic for MM.

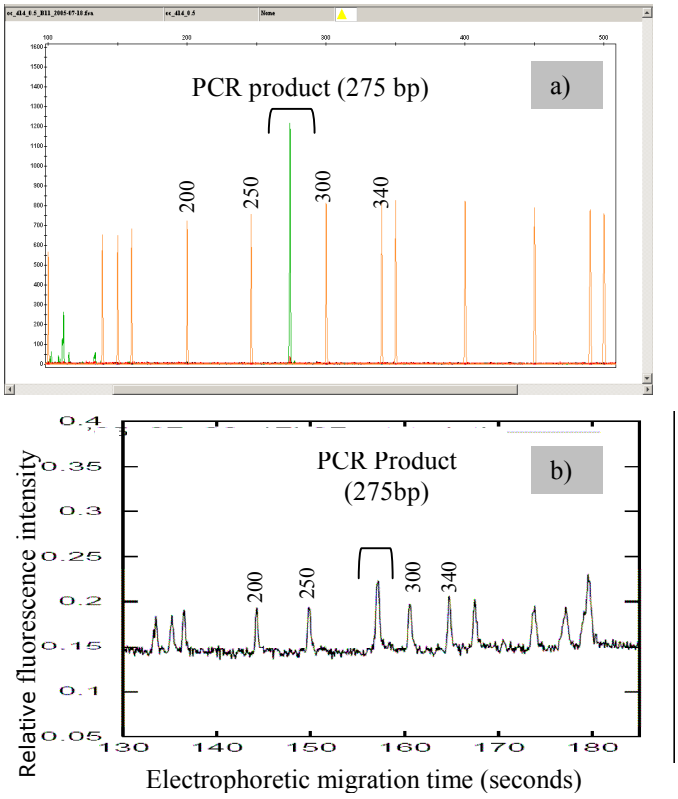

Figure 3: Successful on-chip PCR was run on the µTK using a CE glass chip at 1:10 dilutions, indicating the presence of t(4;14) translocation by a 275 bp peak: a) verification of chip-based PCR product on the ABI 3100 run and b) is the electropherogram by running the on-chip PCR product on a glass CE chip along with GS500 size standard for product sizing and verification.

4 CONCLUSION

Microfluidic platforms developed here employ novel technology suitable for large scale genetic screening of MM patients, enabling predictions of risk and stratification for treatment, based on the genetic signature of each cancer. The inexpensive and multifunctional characterization tool with PCR/ RT-PCR along with detection capabilities (CE) would enable widespread pre-screening of patients, providing more precise diagnosis. Real-time detection of complex genetic abnormalities will allow sensitive detection of emerging aggressive variants as disease progresses. This will enable custom tailored therapies that target the genetic vulnerabilities of the malignant clone in each individual patient. The microfluidic chips described here hold great promise for use as a point-of-care monitoring tool for cancer patients.

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