Improved Diagnosis and Monitoring of Cancer Using Portable Microfluidics Platforms

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Abstract: Microfluidics chips offer the promise of fast and accurate diagnostic and monitoring tools that are inexpensive and automated. Tests that otherwise require expensive instrumentation and consume valuable reagents can be implemented on microfluidic chips for a fraction of the cost with dramatic improvement in speed. Minaturization offers multiple benefits, enabling multiple tests on one chip, requiring tiny tissue samples and fast reaction times for picoliter volumes. More complex microfluidics chips will be able to carry out sample processing followed by cell or molecule fractionation, molecular analysis and readout of the test result. Integrated platforms incorporating microfluidics offer portable testing devices for "point of concern" testing in health care facilities, in remote areas or in the home. These devices are likely to increase access to sophisticated testing within the health system while decreasing costs and improving the quality of care.

Expected front line innovations using microsystems are likely to provide demonstrable economic benefits to health care systems in developed and undeveloped countries, benefits to industry, and perhaps most importantly, improvements in the quality and accessibility of sophisticated health care diagnosis and monitoring. By optimizing medical interventions through more precise and faster medical testing, high quality health care becomes more affordable, providing significant economic benefits while maintaining social values. Automating technically complex tests using platforms that incorporate microfluidics and molecular manipulations has a high probability of improving health care delivery by lowering the costs of analysis, and increasing the availability of testing, enabling risk-adapted tailoring of treatment customized for each patient, earlier detection of disease and the potential for preventive strategies.

Microfluidic devices are the result of applying microelectronic fabrication technologies to produce, instead of microconductor networks in silicon and metal, microchannel networks in glass. Within these microchannels, reagents can be manipulated by applying electric fields and results detected by optical means. Microsystems provide high resolution molecular separations, and can combine multiple functions on a single chip (e.g. cell selection and extensive genetic analysis). Microsystems lend themselves to the analysis of individual cells which may lead to a greatly improved understanding of many diseases, particularly cancer where the cells comprising a tumor mass are often quite heterogeneous.

There is an enormous disconnect between the generation of new biomedical knowledge, particularly genomics and proteomics, and the ability to usefully apply it in the community. Disease profiling offers the promise of more effective treatment but clinical laboratories lack the required expertise to perform such testing and health care systems throughout the developed world cannot afford it. In the undeveloped world, these issues are not even being

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considered. The potential ability of automated chip platforms to carry out many of the tasks normally performed by technologists and/or by multiple large and expensive pieces of equipment is enormous. Microsystems with nanoscale molecular manipulations will enable more specific disease classification based on predicted response to treatment, thereby allowing more directed clinical decision making for appropriate patient management. Cost-effective pre-screening strategies that could monitor patients over time would enable identification of high risk genetic profiles as soon as they arise. The development of a suitable integrated platform(s) that is versatile, reliable, multifunctional and socially acceptable, requires intensive interactions among multiple sectors of the scientific community, the social sciences, government and the general public.

Microsystems are predicted to perform all of the manipulations of a large laboratory housed in what will eventually be a hand held device. The central component is a glass and/or inexpensive elastomeric polymer material polydiemethysiloxane (PDMS). The slide that incorporates etched wells and channels for manipulating cells and molecules, sometimes called a "lab on a chip". It will be sturdy enough to be used anywhere and simple enough that it can be used by unskilled operators. It will make possible personalized health care, tailored to the needs of each patient and readily available, even in the home. Our first priority is to establish these devices for use in cancer diagnosis and monitoring. Cancer masses include many types of genetic variants, some of which are highly aggressive. New technology is needed to readily and cheaply distinguish subtypes or aggressive variants that ultimately kill patients. Current tests are too difficult and too expensive to be preformed in routine clinical laboratories. Never-the-less, there are significant benefits to the patients and the health care system if such testing were widespread in all health care centres. Some therapies are very expensive for the health care system, but we do not have the means to screen for those patients most likely to benefit. Patients are likely to benefit from customized treatment strategies based on molecular profiles. With novel microsystems platforms, a device the size of two fingers could replace most of a medical diagnostics laboratory, interpretation of test results would be automated and standardized so the same results could be obtained in any health centre. The results would be available in minutes, not days or weeks. Microsystem platforms may ultimately enable pre-screening for rapid early detection of disease, rapid identification of therapy-resistant cells or infectious agents, effective monitoring of response to therapy, and optimal use of expensive therapeutics. For air, water and food, "in the field" testing for biological agents would enable rapid and accurate identification of infectious agents or biological pollutants. Handheld microsystems will also facilitate forensic analysis on site and screening for bioterrorist agents to preserve public safety. In agriculture, screening for diseases such as "mad cow" disease could be possible for every animal and monitoring of genetically modified crops would be readily performed in the field. We intend for these devices to be inexpensive and cost-effective, making them feasible for use in rural areas, wilderness areas, underdeveloped world countries or even outer space.

Cancer is a highly diverse family of diseases. A significant clinical problem is that the characteristics of one form of cancer may be radically different from those of another form, or even of another phase of development of the same cancer. A non-

invasive, inexpensive and multifunctional characterisation tool would enable widespread pre-screening, providing better diagnosis, and more effective monitoring. With the large volume of genetic information so far accumulated, it becomes increasingly important to consider the logistics of performing these tests.

Microsystems are capable of testing cell populations from blood, bone marrow, or other tissues for indications of cancer-related anomalies, and performing large numbers of tests for genetic sequences of interest. The end result would be to assemble a detailed cancer signature for each patient. Microsystems hosting nanoscience molecular manipulations to detect cancer signatures could enable noninvasive early detection of rare aggressive variants, detection of residual tumor cells, or metastatic cells at distant sites following the removal and treatment of an existing tumor. They will facilitate targeted intervention delivery, and monitoring of intervention, as well as analysis of tumor heterogeneity with the possibility for real time assessment and tailoring of therapy for each patient.

Understanding the risk factors for developing any type of cancer, including myeloma, will provide insight into strategies that may increase the length and quality of life for afflicted individuals. Identification of the genetic signature for each cancer is likely to enable predictions of risk and stratification of treatment. The capabilities of PCR and RT-PCR have accelerated progress in the measurement of molecular and genetic changes in patients, thus increasing identification of the genetic signatures of many types of cancers. The products obtained from RT-PCR are measured by gel-based analysis that frequently fails to detect low-level expressed genes or genes with shorter lifespans. This means that only genes with relatively abundant expression are readily detectable. Capillary electrophoresis is a more sensitive method that allows successful detection of low-level gene expression, but it is time-consuming, expensive, and difficult to implement for routine clinical testing at, for example, every clinic visit of every patient. Integrated and automated microfluidic chips (MFCs,) offer a fast, inexpensive and sensitive to detect molecular and genetic changes in patients when using, for example, diagnostic/monitoring tests that include RT-PCR, capillary electrophoresis, and/or fluorescent staining. MFCs that include sample processing capability can be operated using minute amounts of tissue without the need for specialized operators. Automated and standardized MFC platforms offer many advantages over existing macroscale systems: rapidity, compactness, reproducibility, and decreased sample volumes. Here, we describe novel microsystems for fast, accurate and real time genetic screening of multiple myeloma (MM) patients. In MM, multiplex analysis of the clonotypic IgH VDJ signature and of accompanying genetic abnormalities enables genetic profiling and monitoring throughout treatment. This means the impact of therapy can be rapidly ascertained and custom tailored to target newly arising cancer clones in each patient. Comparative analysis confirms analysis of RT-PCR products on-chip is as sensitive, sometimes considerably more so, than conventional analysis of PCR products using capillary electrophoresis on DNA genetic analyzer. On-chip PCR is robust and less susceptible to contamination than is conventional electrophoresis. On-chip sample processing, and the ability to detect product with few cycles of PCR, indicates that quantitative PCR is feasible. The practical aspects and limitations of a

microfluidics approach were tested by amplifying hyaluronan synthase 1 (HAS1) and its novel splice variants in MM patients and in cell lines. HAS1 and its novel variants appear to be the first described markers of circulating MM B cells with clinical predictive value, whose expression correlates with reduced survival/poor outcome. They belong to a group of short-lived genes that are difficult to amplify by conventional gel-based analysis. On-chip analysis enabled detection of HAS1 and its novel variants PCR products after 15 cycles of PCR. The same product is undetectable with capillary electrophoresis performed on a 3100 genetic analyzer even when concentrating 25 ul of PCR reaction. We successfully performed on-chip PCR using an automated intelligent valving system and amplified HAS1 and its novel splice variants in 2 ul of total PCR reaction. Currently we are evaluating sensitivity of PCR and CE chips, and will integrate these functions once optimized. This characterization of the microfluidic methods will determine how many molecules of RNA and/or DNA will be required to identify expression patterns of low level expressed, short-lived disease related genes. Currently we are also developing more sophisticated MC, which will allow us to perform RT-PCR, PCR product and SNP (single nucleotide polymorphism) analysis on MCF chips using intact blood or bone marrow cells. This approach further eliminates sample contamination issues and minimizes the time and sample volume for analysis. This work forecasts high-throughput automated devices able to analyze genetic information using minimal amounts of genetic material in minutes, inexpensively. Once fully integrated systems capable of seamless sample processing, selecting cells of interest and performing genetic analyses of individual cells or groups of cells are available for clinical use, we anticipate that they will contribute significantly at the time of diagnosis and facilitate monitoring genetic characteristics of the malignant clone at every subsequent clinic visit. Real time detection of complex genetic abnormalities in a given cancer clone is likely to detect aggressive variants as they arise and thus enable the development of therapeutic options tailored to the genetic signature of a cancer in each individual patient, at diagnosis and as cancer progresses.

To date much of the application of genetic technologies has been limited, for reasons of cost and accessibility, to relatively simple diseases that can be described by a few genetic changes. It is expected however, that most diseases are polygenic, i.e. involving many genetic changes. At present our health care system is greatly restricted in its use of genetic methods. We believe that cancer provides an excellent example of a larger class of ills besetting humanity that are polygenic in nature. Once applied to cancer, we believe that our MFC technologies will aid in understanding, treating and preventing a variety of diseases on a much wider scale than is currently possible.

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