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Integration of Electro-Optical Mechanical Systems and Medicine: Where are we and Where can we go?

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Abstract

The marriage of microfabricated materials with microbiological systems will allow advances in medicine to proceed at an unprecedented pace. Biomedical research is placing new demands on speed and limits of detection to assay body tissues and fluids. Emerging microfabricated chip technologies from the engineering community offer researchers novel types of analysis of human samples. In guiding these developments, the ability to swiftly and accurately gain useful information for identification and establish a diagnosis, is of utmost importance. Current examples of such technology include DNA amplification and analysis, and fluorescent cell analysis by flow cytometry. Potential applications include the development of rapid techniques for examining large number of cells in tissue or in blood. These could serve as screening tools for the detection and quantification of abnormal cell types; for example malignant or HIV infected cells. Micro/Nanofabrication methods will make these devices compact, providing access of this technology to point of care providers; in a clinic, ambulance, or on a battlefield. Currently, these tools are in the construction phase. Upon delivery to researchers, validation of these instruments will lead to clinical demand that requires approval from the Food and Drug Administration. This paper will outline criteria that successful devices must satisfy.

Keywords: Biomedical, Analysis, DNA, Flow cytometry, microelectromechanical systems, Microchip, DNA amplification, Fluorescent Activated Cell Sorting, Diagnosis, Medicine

1. Introduction

As we approach a new millennium, the advent of a new era in medicine is being forecast. Innovative microtechnology from the laboratory bench is moving to the patient's bedside to provide an unprecedented level of quality care. This technology integrates materials, physical, chemical and biological science with engineering disciplines to create microdevices for point of care delivery with improved sensitivity, specificity, speed, and low cost. These improvements are dramatically demonstrated by the well known scientific advance in the characterization of DNA by the Polymerase Chain Reaction (PCR). This technique allows amplification of DNA, from as little as one cell, such that the sequence of base units can be determined. All of this can now be accomplished on a small hand held chip. The impact of these kind of devices on medicine are far-reaching, from laboratory research for developing new drugs to point of care emergency medical care.

DISCLAIMER

2. Emergency medical care scenario

The following scenario may be routine to those in the medical profession, but for those individuals unfortunate enough to experience a life threatening injury, it becomes very serious. The scene goes something like this: the sirens become louder as you lay on the ground, and soon you see someone approaching you. The figure is an Emergency Medical Technician who leans over your critically ill body. A chain of events is triggered. First, the EMT assesses your body's vital signs, looking for spontaneous respirations, then feeling for a pulse. If a pulse is present, he attempts to measure your blood pressure by placing a mechanical device around your arm. Next, to protect you from further injury your cervical spine or any other body parts that may be injured are immobilized. Now, a vein in your arm is found so an intravenous catheter can be positioned and life sustaining fluids can rush into your circulatory system. Drugs may then be injected through this i.v. to maintain blood pressure and other vital body parameters. Observation of the blood pressure is crucial because without a minimal blood pressure, vital organs such as the brain and kidneys will sustain irreversible damage.

Quickly, you are placed into the ambulance for the ride of your life to the closest emergency room. Once inside the trauma room, nurses and technicians cut away your clothing to examine the extent of the trauma and to continue further treatment. Again, veins and arteries are penetrated by needles to obtain aliquots of blood that will be tested to answer questions such as: How much blood has been lost? Is there any damage to the heart or liver? What is your blood type? Are there any drugs in your body? Then EKG's, x-rays, a CAT scan, or other diagnostic tests are obtained to locate the injury and determine the extent of damage. After gathering the data, a final assessment is rendered as to the severity of the injuries. The physician walks over and tells you that no serious injuries can be found but, an overnight hospitalization is necessary for more complete observation. If all goes well, you will be back home with your family tomorrow.

These situations occur daily. Response time and point of care delivery are critical. The first several minutes following any injury are the most important. Survival is greatest when therapy can be given in a rapid fashion, thus allowing the least amount of damage to be sustained to the body. This applies to almost any injury, whether from a motor vehicle accident or a battle injury during conflict. Rapid identification of vital human parameters directly on site is critical. To assist in identifying parameters such as blood volume or glucose level, an instrument that is portable, accurate, reliable and easy to use is needed. The purpose of this paper is to discuss novel devices that involve microfabrication and their potential role in medicine and basic scientific research.

3. Blood Analysis

To help understand recent advances achieved through the use of microfabricated systems, let us recall the protocol for normal human blood analysis. First, the patient must undergo a needle puncture of a vein to obtain the blood sample. A minor discomfort, provided the patient has good venous access and the sample can be obtained with one stick. This yields approximately 5 to 8 milliliters of blood. If the purpose is to examine blood cells, a device is put

into the tube and the blood is aspirated into an instrument that can quantify the types of cells present. To analyze the serum cholesterol, the tube must be spun in a centrifuge. This drives the cellular components of blood to bottom of the tube and the serum can then be obtained in the supernatant. An aliquot of serum is placed into an automated instrument and a cholesterol determination will be made through a chemical reaction. This entire process is very inefficient because it is labor intensive and only one result can be obtained for each aliquoted sample .

Microelectromechanical (MEMS) systems offer strategic advantages over traditional biochemical analysis (Table 1). The ease in sample acquisition dramatically reduces both time and invasiveness with a simple prick of a finger providing a sufficient sample for multiple studies. Sample allocation is not needed and multiple analysis can be derived from one specimen. These improvements bring about results in minutes as opposed to hours, save patient's the discomfort of a phlebotomy, reduce reagent use and ultimately save money.

Table 1.

Estimated time required to obtain sample results from clinical analysis.

Traditional Laboratory	***Time***	***MEMS***	***Time***
1. Sample Acquisition • apply tourniquet • find venous access • insert needle • obtain sample in large tube (ml) • transport to lab	minutes to hours	• finger stick • collect in microtube(µl) • point of care service	one minute
2. Sample Preparation • separation of cells from serum • opening tube and obtaining sample • aliquoting sample • loading automated instrument	one hour	• analysis of whole blood • no processing needed • load analysis chip	minutes
3. Data Acquisition • sample analysis • data display	minutes	• sample analysis • data display	minutes

A good example is the I-stat blood analysis device implemented in the clinical laboratory several years ago.[1] It consists of a small cartridge, about the size of a domino. The amount of blood needed for analysis is reduced to a few drops and can be obtained by pricking your finger with a lancet. The blood is collected within a capillary tube that is then placed into an inlet in the cartridge where it flows into a small channel by capillary flow. The cartridge is placed into a hand held portable clinical analyzer where a test cycle is initiated. This test cycle calibrates the device, correcting for barometric pressure and at the same time heating the analytical sensors to body temperature. Once properly calibrated, the blood sample flows into a sensing chamber where bioelectrical signals are measured by potentiometry, amperometry, or conductivity. The results are then calculated and displayed within a few minutes. Presently, this instrument can

determine several blood parameters such as blood glucose, sodium, potassium, and hematocrit (quantification of red blood cells).

4. DNA analysis and molecular research

This process of analyzing a clinical sample has direct application to the basic science laboratory. Whether the laboratory analyzes chemicals, DNA, or cell growth and function (for important cells such as immune or malignant cells), significant man power is expended to obtain a result. Therefore, any application that can reduce the amount of human intervention, reagent use, diminish cost, and allow a high throughput with parallelization is highly desirable. Examples of such technology exist in the research laboratory and many more are on the horizon.

Microelectromechanical systems have incredible potential applications to the basic science laboratory. A great deal of attention with this technology is focused in several areas: DNA analysis, cellular investigation of human blood cells, and biochemical determinations. Studies of human blood cells spotlight specific cellular characteristics, identification of cell type, size or shape and properties of cells following perturbation. Devices that focus on DNA analysis strive to achieve in a micro-fraction of time, sample and reagent volume to what is now considered routine in today's molecular biology laboratory.

Individuals are pursuing DNA analysis in many fashions. Southern et al. reports on the binding properties of nucleic acids to short sequences of DNA (oligonucleotides).[2] DNA has unique properties because each nucleic acid will bind to a single complementary nucleic acid. This allows short sequences of nucleotides to be synthesized in a specific sequence that is complementary to a portion of a gene. Therefore, this oligonucleotide serves as a very specific probe for a gene. Modifications of single nucleotides within an oligonucleotide probe assist in studying the binding behavior of nucleic acids and to find the presence of mutations within a gene. Southern et al. uses arrays on oligonucleotide probes attached to glass plates which offers enormous advantages over conventional gel electrophoresis.

Another example of this new technology is the Genechip from Affymetrix (Santa Clara, CA).[3] The goal of this chip thus far has been to detect small quantities of HIV-1 virus, or other known DNA sequences. This device uses microlithographic techniques allowing an array of polycarbonate reactors to perform DNA amplification. Standard laboratory gel electrophoresis shows that this microchamber technique correctly amplifies DNA at a fraction of the previously used volumes. This approach to identifying nucleic acids has direct applications to the human genome project in assisting to rapidly identify genetic mutations.

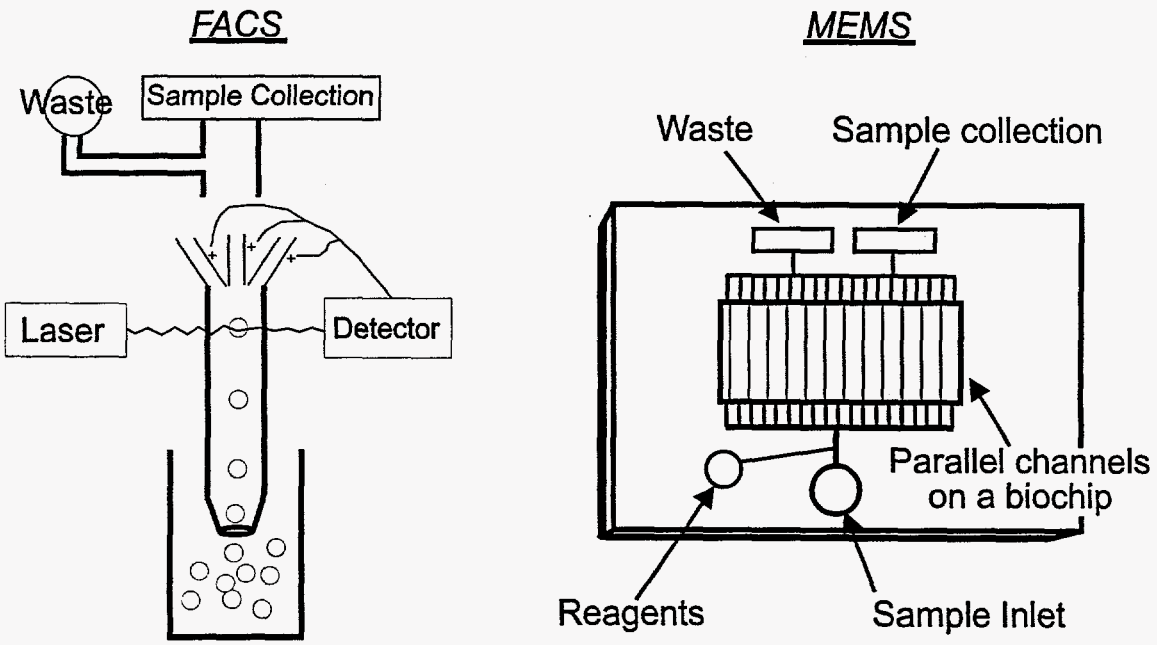
5. Cell Biology

MEMS devices have tremendous potential in cell biology. Because cells have unique structural, chemical and electrical properties, they serve as ideal objects to study. Conventionally, cells are visually observed by the microscope, biochemically and metabolically by substances they produce, and immunologically by their reaction to other cells or soluble mediators. Let me discuss a potential use of MEMS and cell analysis by flow cytometry.

Cells have surface proteins that identify the cell type and therefore its function. Through the use of flourescently labeled antibodies, these surface proteins can be tagged and detected by Fluorescent Activated Cell Sorting (FACS). An example of the clinical application with FACS analysis is the enumeration of $CD4^+$ T cells in an individual that is infected with the HIV virus. Whole blood is incubated with flourescently labeled antibodies that will bind specifically to the CD4 molecule on a T lymphocyte. These cells are then identified by flowing in single file through laser light (Figure 1). The laser light excites the fluorescent molecule on the antibody that is attached to the cell causing an event to be recorded. In this fashion, cells can be quantified. Furthermore, cells bearing the tagged antibody can be sorted and collected to near 100% purity. Sorting cells is a very powerful technique to obtain pure population for study in vitro or in the case of a bone marrow transplant, these cells may be infused into a patient.

Figure 1.

Comparing a Fluorescence-Activated Cell Sorting (FACS) system to a MEMS device.



	FACS	MEMS
Overall Dimensions	18 x 30"	1 x 1"

Requirements

FACS	MEMS
- ml sample volume	- µl sample volume
- capillary flow involving valves	- flow by electrophoretic fields
- large volume of reagents	- microvolumes of reagents
- single cell analysis	- multiple cell analysis by parallelization
- analysis consists of:	- analysis consists of:
. size and shape of cell	. high contrast imaging
. granularily of cell	. extra and intracellular information
. 5 color fluorescence	. spectral analysis
- large volume of waste	- small volumes of waste
- large overall dimensions of instrument	- hand held unit

Sandia Laboratories are developing a semiconductor microlaser to study cell structure.[4] This biocavity laser (a MEMS device) has the capability to function in a similar fashion to FACS. Using a semiconductor mirror into which microchannels have been etched, cells can be potentially flowed, analyzed and sorted. A novel advantage offered by this technology is the ability to analyze cells without sample preparation, such as affixing a monoclonal antibody to the cell. Moreover, a light spectra can be obtained for individual cells, thereby serving as a signature for each cell type. Using both images of cells and their coherent light spectra offers many advantages over conventional cellular imaging techniques. Further details regarding this device instrument are described by P. L. Gourley in this conference proceedings.

Microfabricated lattices are useful to fractionate and sample white blood cells.[5] In an attempt to recreate the human microcirculatory system, using microlithographic techniques, an array designed of channels mimicking the physical dimensions of capillaries was created. White blood cell migration in the array was then studied. Carlson et al. found that lymphocytes are better able to penetrate the array of microchannels compared to granulocytes and monocytes, which have larger nuclei. Findings such as this may have important implications in the understanding of inflammation because they provide clues about cell migration. In inflammatory states, such as the joint of an individual with Rheumatoid Arthritis, a dense infiltrate of lymphocytes can be found within the diseased joint tissue. Therapies that alter molecules involved in cellular migration may be aided by devices such as this.

6. Chemical analysis on a chip

Micro-optical systems are well suited to perform chemical analysis, i.e., analysis on a chip. Several examples of this capability now exist and many more are on the horizon.[6,7,8] These units utilize electric fields to flow samples through small channels etched in glass or silicon substrates by micromachining techniques. Numerous advantages can be gained by these devices. Because the sample migrates by electrophoresis, chromatographic separation of individual components within the sample can be observed. Valving can be achieved, without the use of mechanical valves, by altering electrophoretic fields, thereby allowing mixing of various reagents with the sample at specified times. This application is extremely important because it allows samples to incubate with different reagents for various time periods before the final detection phase. Several assay systems have used this technique. They involve immunoassays to detect the concentration of labeled antibodies bound to antigen, serum drug level determinations, and chemical identification and detection of DNA fragments following restriction enzyme digestion.

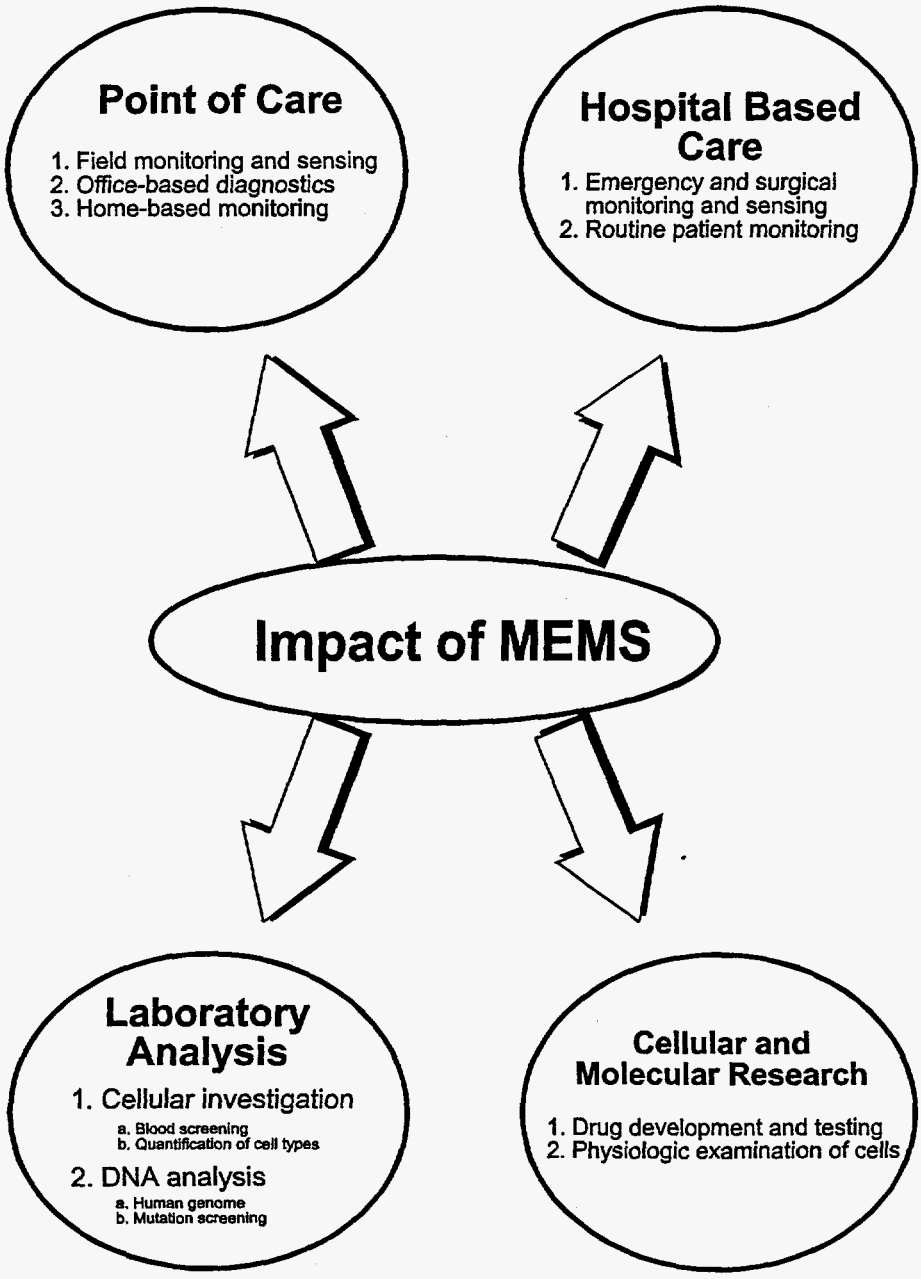
An example of how this technology is applied to medicine entails the detection of serum drug levels. Patients taking medications to control seizure disorders or cardiac arrhythmias frequently need to have their blood levels of the medication determined to avoid toxicity. Harrison and Chiem describe a technique for determining Theophylline levels (Theophylline is a drug used in respiratory disorders such as emphysema).[8] Using a competitive inhibition assay with labeled versus unlabeled Theophylline, they determined drug levels using their "on-chip" design. This assay technique can detect as little as 5 ng/ml of the drug. (Normal Theophylline

levels range between 10 to 20 µl/ml.) The degree of precision offered is within 5%.

7. Summary and conclusions

A driving engine to speed the development of bioMEMS devices is the crisis in federally funded health care. The cost effectiveness of our nations health care delivery must be radically upgraded to avoid a decline in quality of care. Microelectromechanical systems are well positioned to spearhead such changes (Figure 2). Multiple advantages are offered: low cost, high sensitivity and specificity, a compact size allowing point of care delivery, high throughput, dependability and ease of operation. For the laboratory scientist these tools provide the ability to challenge the limits of sensitivity, making the former undetectable substance readily detectable.

Figure2.



We can hope to change the opening scenario to the following. The Emergency Medical Technician kneels beside you and straps a small box to your chest. Immediately she views your heart rhythm and blood pressure. The device makes a small puncture into your skin and detects vital body laboratory parameters. In real time, the physician in the Emergency Department views the data and initiates therapy over the telephone. It is these first few crucial minutes that can offer the critically sick patient the best hope of survival. We clearly stand on the brink of a new age in health care delivery.

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