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Micromachining: A new direction for clinical analyzers

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Abstract: Conventional analytical reactions are performed in test tubes, but in the future, reactions may be routinely performed in minute chambers micromachined in small silicon or glass chips. Disposable micromachined devices are : easily designed, low cost, small, portable, easy and fast to operate, use micro volumes of sample and reagents, are adaptable for simultaneous multiple-assays, and provide system integration. We have constructed micromachined devices for semen analysis, in vitro fertilization (IVF), PCR, and immunoassay. The key structural element of the sperm chips (silicon-glass and glass-glass) is a tortuous channel for assessment of sperm motility. IVF chips consist of a semen application chamber and an egg nesting chamber separated by a tortuous sperm selection channel. Chips were evaluated for IVF using the mouse model, and fertilization and growth from 2 cell to the blastocyst stage was successfully demonstrated. Silicon-glass chips containing 40-80 um deep micro-chambers were effective for PCR reactions. Excellent thermal conductivity of the silicon, plus the high surface/volume ratio (>20 mm^2/uL) provides efficient heat transfer. This PCR chip (volume, approx 10 uL) was successfully used to amplify a range of targets (e.g., bacteriophage lambda, *Campylobacter jejuni*).

INTRODUCTION

An emerging trend is the construction of analytical devices using micromachining techniques (1, 2). This form of miniaturization is being aggressively pursued for a number of reasons including, increased design flexibility, ease of manufacture, rapid design cycles, low sample volume, low reagent consumption, faster response times, multiple simultaneous testing using different types of analytical methods, and hand-held portable analyzers. However, one of the most compelling reasons, is the prospect of total analytical system (TAS) integration in which all of the steps in an analytical process (sample preparation, pretreatment, analytical reactions, detection and read-out) are combined on a single analytical device.

Precise and controlled removal of material at a um scale can be accomplished using a range of micromachining techniques (photolithography, e-beam) and in a variety of materials (silicon, glass). Conventional wet etching of silicon is the most commonly used and extensively developed technology, but there is a growing use of micromachined glass (soda glass and Pyrex) because of its transparency and insulating properties. In conventional wet etching, feature sizes of approximately 1 um are achievable and other techniques allow fabrication of structures with smaller feature sizes (e.g., 0.1 um, x-ray and e-beam; <1 nm, scanning tunneling microscope) (3).

Devices can be a single chip capped with a cover, or in the form of a sandwich of interconnected chips, in which each layer of the sandwich performs a particular analytical task (4). The range of analytically useful devices fabricated on a chip is extensive and includes valves, filters, diaphragms, pumps, motors, lasers, light emitting diodes, optical filters, heaters, and ion selective electrodes. These components permit design and construction of miniaturized versions of conventional analytical devices, and many examples have already been built, such as a mass spectrometer, liquid and gas-liquid chromatographs, glucose analyzer, flow injection analyzer, capillary zone electrophoresis analyzer. Microchip devices for clinical analysis are still in an experimental phase, except for electrolyte analyzers such as the i-STAT (5). A related area of microfabrication, that is growing rapidly, is genosensors (6). These are arrays of nucleic acid hybridization sites created by immobilizing polynucleotides on glass or porous silicon substrates. Genosensors are being mainly applied to DNA sequencing, but other applications include screening for genetic disease caused by a series of different mutations, such as cystic fibrosis, and detection of infectious agents (eg HIV). We have been mainly concerned with microfluidics (7) and the adaptation of clinical analyses to chip formats. This article reviews the application of microchips in immunoassay, semen analysis, in vitro fertilization and polynucleotide amplification.

IMMUNOASSAYS

Immunoassay is an important analytical technique, and there are several examples of microchip immunoassay devices. These are currently not very sophisticated and use simple channels, chambers, or pits as the reaction vessel. Glass-capped silicon microchannels (150 um wide x 40 um deep) have been used for microagglutination immunoassays for qualitative ABO typing using microchannels filled with anti-A or anti-B antiserum, and for IgG using anti-IgG coated 4.55 um diameter fluorescent beads. Also, a fluorescent bead latex-agglutination immunoassay for alpha-fetoprotein has been accomplished in glass-capped microchannels (0.4 uL; 10 mm long, 80 um deep, 500 um wide) etched in Pyrex glass (detection limit 10 pg/mL) (8).

Silicon pits (4 uL volume, 260 nL in active area) that incorporate a pH sensitive light addressable potentiometric sensor (LAPS) can be used in combination with a biotinylated capture membrane for immunological assays. The silicon pit-pH sensor detects urease conjugates bound specifically to analyte captured onto the membrane via action of the urease label on urea substrate (0.5 uL) contained in the silicon pit. Assays for of mouse IgG (linear over the range 25 - 5000 pg), and contaminants of recombinant protein products (eg, *E. coli* protein, protein A), hormones (eg, hCG), and infectious agents (eg, *Yersinia pestis*) have been devised using this microchip technology (9).

SEMEN ANALYSIS

Conventional sperm testing uses a Makler chamber, hemocytometer, or a simple glass microscope slide. A microchannel provides a unique and advantageous environment for studying a motile cell such as a spermatozoa (10, 11). Tortuous channels etched in silicon or glass provide a means of assessing the motility of sperm in a semen sample. The channel confines the sperm such that they swim along the channel away from the application port (Fig. 1). Assessment of the motility of a semen sample is aided by a numerical scale etched alongside the channel. Correlation of data obtained using the sperm chips and obtained using conventional procedures is excellent as shown in Table 2. The time taken for sperm to swim to the end of the channels in the type of chips shown in Fig. 1 is reproducible, and correlates with motility and progression data. Other semen tests can be adapted to a microchip format. Sperm antibodies present in a semen sample can be detected by simply filling a microchannel with anti-human IgG, IgA or IgM coated beads (4.55 um diameter). Sperm penetration into cervical mucus and into hyaluronic acid can be assessed using microchannels prefilled with these test substances. The potency of spermicides can be determined using a series of channels prefilled with spermicide solutions and connected to a central chamber containing a fresh semen sample.

TABLE 2. Testing of semen samples using sperm chips

Specimen	Motility %	Sperm count 10×10^6	Progression	Time minutes
Sperm chip S7 (duplicate test channels)				
1	76	71	3, 3+	6, 6
2	70	66	3, 3+	6.3, 6.4
3	65	78	3	7.1, 7.14
4	60	50	2, 2+	10, 10
5	50	21	1+, 2	12.1, 12.5
Sperm chip S8 (quadruplicate test channels)				
1	76	71	3, 3+	6.23, 6.27, 6.4, 6.4
2	70	66	3, 3+	6, 6.1, 6.3, 6.3
3	60	60	2, 2+	10, 10, 10, 10
4	50	21	1+, 2, 2+	9.4, 10, 11.33, 10

IN VITRO FERTILIZATION

A microchip has been designed for performing *in vitro* fertilization. It consists of two microchambers connected by a tortuous microchannel (12) (Fig. 2). Eggs are placed in one of the chambers (egg nesting chamber) and a washed semen sample

placed in the other chamber (semen application port). The sperm swim along the channel and are selected based on their ability to negotiate the channel. The fastest swimming sperm (highest fertilizing potential) are the first to arrive at the egg nesting chamber and have the highest probability of fertilizing the eggs. The IVF chips have been tested in a mouse model for the human and fertilization of the eggs and development to the blastocyst stage demonstrated (Fig. 3). In preliminary studies, fertilization rates up to 46% were achieved and this compares favorably with the 65-80% fertilization rates in conventional procedures using tissue culture dishes.

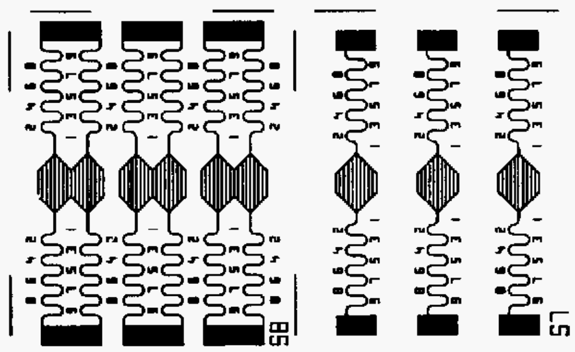

Fig. 1 Design of glass-glass microchips (S7 and S8) for assessing sperm motility. (semen is added to the central chamber and sperm swim into the duplicate (S7) or quadruplicate (S8) channels that link the central chamber to the two outer chambers. A scale (1-9) etched into the glass next to the channel aids assessment of the distance that individual sperm have swum along the channel).

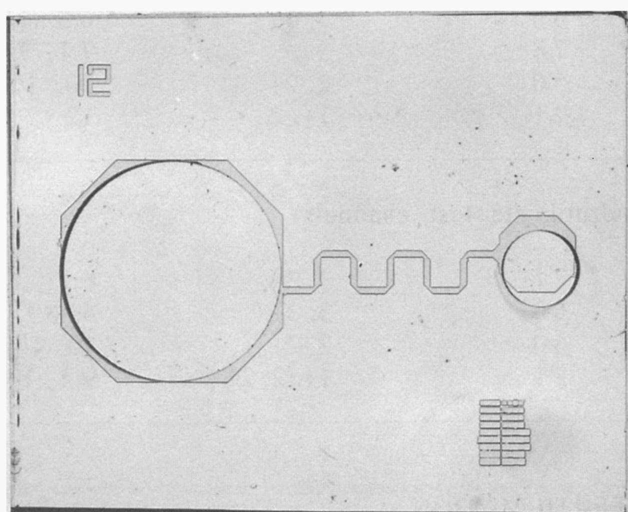

Fig. 2 A glass-glass microchip for in vitro fertilization
[reproduced courtesy of the American Association for Clinical Chemistry from *Clinical Chemistry* **41,** 1358 (1995)].

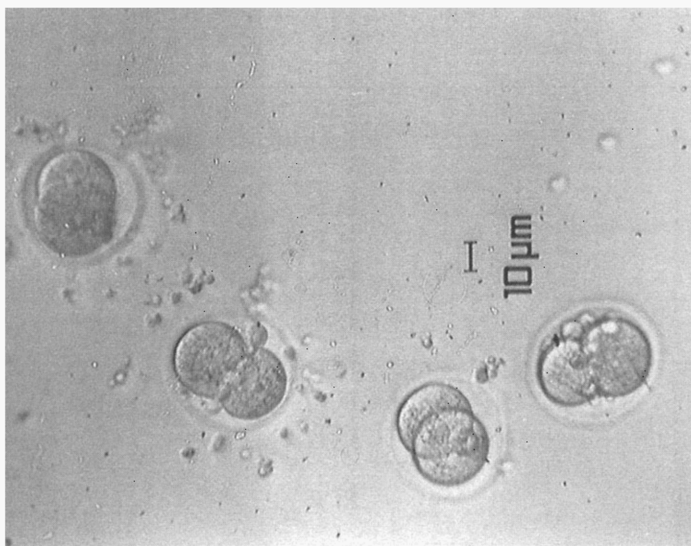

Fig. 3 Mouse eggs fertilized in an IVF microchip. Embryo are shown at 2 cell stage.

POLYNUCLEOTIDE AMPLIFICATION

Silicon has excellent thermal conductivity and is thus an ideal material for fabricating a reaction vessel for an analytical procedure that involves repetitive heating and cooling, such as the polymerase chain reaction (PCR). Microchips for performing PCR reactions have been constructed from silicon and capped with glass (13, 14). The design of the chip is a chamber (40 - 80 um deep) connected via short channels to a hole for filling and a hole for emptying the chip. The volume of the chip is 10 uL and it has a surface to volume ratio of >20 mm^2/uL. At this high surface to volume ratio the physical and chemical properties of the surface become important. We have shown an adverse influence of native silicon and silicon nitride on the PCR reaction. However, oxidation of the surface to produce a silicon dioxide layer passivates the surface and renders it compatible with PCR (15). The PCR microchips have been used for amplifying a variety of targets ranging in size from 50 bp (*Lac I* gene) to 1.4 kb (*Campylobacter jejuni*) (Table 1). The repetitive cycle of heating and cooling is accomplished by means of a computer-controlled Peltier device. Ligase chain reactions (LCR) have also been successfully performed in the microchips.

CONCLUSIONS

Microchip-based assays are proving viable alternatives to conventional assays performed in test tubes or cuvettes. Miniaturization of analytical reactions has a number of benefits (eg, reduced reagent consumption, smaller sample volumes), but one of the most important is the ability to integrate a series of analytical steps (eg, sample preparation, analytical reaction, detection) on a single microchip. Currently, the most active clinical application is the adaptation of polynucleotide amplification reactions to a microchip format. This is part of a concerted effort to produce a hand-held microchip-based PCR analyzer that integrates sample preparation, amplification, and detection of amplified target.

TABLE 1. Microchip polynucleotide amplification reactions

Target	size	amplification technique	reference
Lac I gene	50 bp	LCR	-
HIV DNA	100 bp	PCR	(13)
HIV DNA	142 bp	PCR	(15)
Human genomic DNA (Delta 508)	125 bp	PCR	(15)
Chlamydia DNA	207 bp	PCR	(15)
Lambda phage DNA	500 bp	PCR	(14)
Campylobacter jejuni flagellin DNA	1.4 kb	PCR	(15)
Beta-globin DNA	-	PCR	(16)

REFERENCES

1. A. Manz *et al. Trends Anal. Chem.* **10**, 144 (1991).
2. L. J. Kricka *et al. J. Int. Fed. Clin. Chem.* **6**, 54 (1994).
3. K. E. Petersen. *Proc. IEEE* **70**, 420 (1982).
4. H. Mensinger *et al. Micro Total Analysis Systems*, p. 237, Kluwer Academic Publishers, Dordrecht (1995).
5. K. A. Erickson and P. Wilding. *Clin. Chem.* **39**, 283 (1993).
6. K. L. Beattie *et al. Clin. Chem.* **41**, 700 (1995).
7. P. Wilding *et al. Clin. Chem.* **40**, 1 (1994).
8. M. I. Song *et al. Anal. Chem.* **66**, 778 (1994).
9. J. C. Owicki *et al., Annu. Rev. Biophys. Biomol. Struct.* **23**, 87 (1994).
10. L. J. Kricka *et al. Clin. Chem.* **39**, 1944 (1993).
11. L. J. Kricka and P. Wilding. *US Patent* 5,296,375 (1994).
12. L. J. Kricka *et al. Clin. Chem.* **41**, 1358 (1995).
13. M. A. Northrup *et al. IEEE International Conference on Solid-State Sensors and Actuators: Transducers '93*, p. 924 IEEE, New York (1993).
14. P. Wilding *et al. Clin. Chem.* **40**, 1815 (1994).
15. P. Wilding *et al. Clin. Chem.* **41**, 1367 (1995).
16. M. A. Northrup *et al. Micro Total Analysis Systems*, p.139, Kluwer Academic Publishers, Dordrecht (1995).