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Micromachined analytical devices: microchips for semen testing[1]

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Abstract

Micromachined devices (microchips) have been designed and tested for a range of clinically important assays. In this study we compare sperm motility determined using disposable glass microchips and a conventional Makler chamber. The 17 × 14 mm glass microchips contained three etched test structures each comprising either duplicate or quadruplicate analytical microchannels. Semen samples with sperm counts ranging from 21 to 78 million sperm per ml and forward progression scores of from 1 + to 3 + were evaluated and swimming times ranging from 360 s (3,3 + progression) to 770 s (1 + ,2 forward progression) observed in the microchips. Motility determined by the time taken for sperm to swim to the end of a microchannel (100 μm wide × 40 μm deep × 10 mm long) in the microchip correlated with forward progression of the sperm determined by the conventional Makler chamber method. This study demonstrates the feasibility of microchips for sperm motility testing and suggests that this technique would be applicable to the study of other types of motile cells. © 1997 Elsevier Science B.V.

Keywords: Micromachining; Glass microchips; Sperm motility; Semen testing

1. Introduction

Silicon-glass and glass microchips have been fabricated for a variety of applications, including the polymerase chain reaction (PCR), ligase chain reaction (LCR), immunoassay, capillary electrophoresis and DNA sequencing. This new form of miniaturized analyzer has a number of benefits which include the use of a small volume of sample, integration of a series of analytical steps, and rapid and reliable analysis [1–4]. Microchips can be fabricated in a range of materials (glass, silicon, quartz) by a number of different techniques including wet etching, reactive ion etching, laser ablation, embossing, micropatterning and metastable atom lithography [5–8]. Table 1 surveys the current range of microchip-based analytical techniques [9–25].

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Microstructures provide a versatile environment for studying and manipulating motile cells such as sperm. We have already reported on the use of silicon-glass microchips to study sperm motility, test semen for sperm antibodies, evaluate sperm penetration into cervical mucus and hyaluronic acid and to assess spermicidal activity of a number of different compounds [2]. A reflected light microscope was used to monitor progress or reaction of individual sperm in the microchips. We have now produced a series of transparent glass microchips for potential use in semen analysis [4]. Transparent glass microchips can be used with conventional transmission light microscopes and hence could find general application in laboratories. In this paper we report the performance of the new glass microchips in semen testing.

2. Materials and methods

2.1. Microfabrication

Microstructures comprising interlinking channels and chambers were etched on 17 × 14 mm pieces of 600 μm thick soda-lime glass (Corning Glass, Corning, NY) by the Alberta Microelectronic Centre (Edmonton, Alberta, Canada). Holes were drilled through the glass (INSACO, Quakertown, PA) to allow fluid access into the etched microstructures. Etched pieces of glass were sealed with identical sized drilled glass covers using a thermal bonding technique that involves a series of heating cycles in a furnace.

2.2. Sperm chips

These consist of a central semen application port containing directors (100 μm wide × 40 μm deep grooves) to guide sperm from the sample application port into two or four channels (100 μm wide × 40 μm deep × 10 mm long) towards the collecting chambers (1 mm × 3 mm × 40 μm deep). The 600-μm glass cover for the two channel sperm chip (Fig. 1, design S7) has 2 mm diameter holes over both the semen application port and the two collecting chambers, and the cover for the four channel sperm chip (Fig. 1, design S8) has a 3 mm diameter hole located over the larger semen application port and 2 mm diameter holes over the two collecting chambers. The two and four channel designs permit simultaneous multiple testing of the same sample. In addition the channels are curved to minimize the trapping of sperm in corners that was previously observed in straight-walled channels in silicon (Fig. 1).

2.3. Semen testing

Semen was obtained from normal healthy donors who participated anonymously in a research program and from patients undergoing semen analysis. A raw sample (0.5–1 μl) was loaded directly into the semen application port of the sperm chip that was prefilled with modified human tubal fluid medium (buffered with HEPES) [26] (*m*-HTF, Irvine Scientific, CA) containing 0.5% bovine serum albumin (BSA, ICN Biomedicals, Ohio). The time taken for the first sperm to reach the very end of each of the channels (the point at which the channel enters the collecting chamber) was recorded (2 channels for the S7 and 4 channels for the S8 microchip). Motility, forward progression and the concentration of the sperm in the semen sample was determined using a Makler chamber. All experiments were performed at ambient temperature.

Table 1
Microchip-based analytical techniques

Analytical technique	Reference
Blood gas analysis	[9]
Capillary zone electrophoresis	[10–12]
DNA restriction fragment analysis	[13]
Filtration	[14,15]
Flow injection	[16]
Gas-liquid chromatography	[17]
Immunoassay	[18,19]
Liquid chromatography	[20]
Ligase chain reaction (LCR)	[21]
Mass spectrometry	[22]
Polymerase chain reaction (PCR)	[3,23,24]
Reverse transcriptase PCR (RT-PCR)	[25]

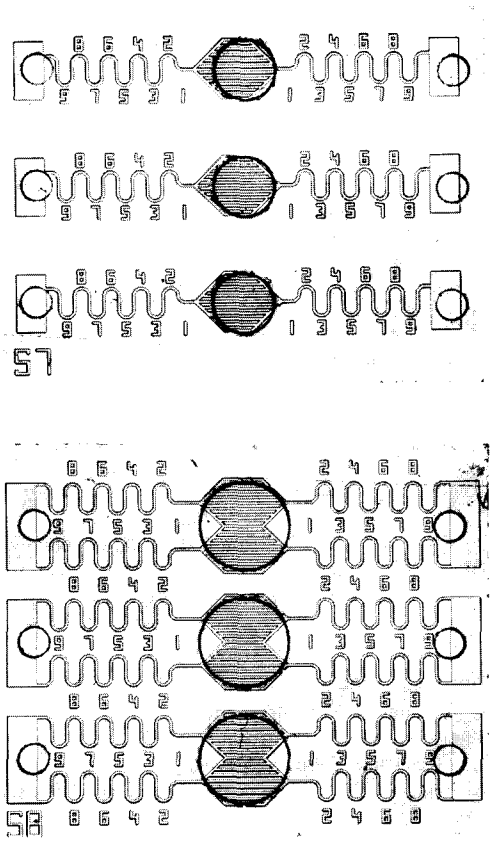

Fig. 1. Two-channel (design S7) and four-channel (design S8) glass microchips for sperm analysis (parallel grooves on the floor of the central sperm inlet port direct sperm into the channels which terminate in the collecting chambers. A numerical scale (1–9) next to the channel facilitates inspection of sperm in the channel). The chips were fabricated from two pieces of 600-μm thick glass.

3. Results

The new sperm testing microchips fabricated entirely from glass were effective for analyzing human sperm motility. The time taken for spermatozoa from raw semen samples to reach the end of the channels in the different microchips (two or four channels) correlated with the forward progression scores (Table 2). For example, sperm from poor semen samples with forward progression of 1, 1+ or 2 took the longest time to reach the end of the channel (660–770 s), whereas sperm from samples with progression scores of 3, or 3+ only took between 360 and 480 s. The results (duplicate and quadruplicate) in the same spermchip were in good agreement, e.g. for sperm sample MAR-S8 with a progression of 3, the sperm traversed the two pairs of channels in 420 s and 430 s in the four channel microchip (Table 2). Some sperm were observed to swim back towards the semen loading chamber but the number was low and had a minimal impact on the experimental results. Likewise, immotile spermatozoa or other components of the semen sample remained in the application port.

4. Discussion

We have investigated the feasibility of miniaturizing sperm testing using disposable glass microchips. The preliminary data for human sperm testing is encouraging, and these microchip devices have potential applicability in the testing of spermicides and in andrology laboratories and IVF clinics. The new glass sperm chips proved to be a fast, simple and reliable tools for sperm motility analysis. Motility is an important characteristic of spermatozoa that must be measured objectively and accurately. It is determined conventionally by using a microscope slide, a Makler chamber or by computer-aided sperm analysis (CASA) [27]. Estimation of sperm motility using these techniques can be subjective because the sperm motion is in all directions. Microchips allow motility to be determined in a single direction along the channel. The width and depth of the channel were chosen to restrict the motion of the sperm in one direction, without impeding the flagellar motion of the sperm's tail [28]. Only a few sperm returned back to the semen application port; however, this did not significantly affect the results. There was a good correlation between the time that the sperm took to migrate to the end of the channel and assessment of forward progression by a conventional method (Table 1). Unlike conventional methods, use of the microchip facilitated simultaneous replicate analysis (duplicate or quadruplicate analysis) and hence the reliability of the results was improved over methods that rely on single or serial measurements.

Table 2
Analysis of sperm using glass microchips and conventional methods

Donor	Conventional methods			Glass microchip S7[a]			
	Motility%	Count 10^6 sperm per ml	Progress	Channel 1	Channel 2		
MSP	76	71	3,3+	360	360		
MDP-S8	76	71	3,3+	383	387		
R125	70	66	3,3+	390	400		
ACI	69	60	2+,3	480	480		
MAR	60	50	2, 2+	600	600		
AC	50	21	1+,2,2+	570	636		
AC	50	21	1+,2,	730	770		
				Glass microchip S8[a]			
				Channel 1	Channel 2	Channel 3	Channel
R125-S8	70	66	3,3+	360	370	390	390
MAR	65	78	3	430	434	400	400
MAR-S8	65	78	3	420	420	430	430
ACI-S8	60	60	2,2+	600	600	600	600
AC-S8	50	21	1+,2,2+	580	600	693	600

[a]Swimming time (s).

5. Conclusion

Our studies have demonstrated the utility and potential of microchips in semen analysis. The glass microchips could be used to develop a standardized procedure for determining sperm motility. Dynamic motility distributions could also be determined by monitoring the progress of sperm along the length of the channel.

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