Applications of a Microfabricated Device for Evaluating Sperm Function

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Mesoscale structures (µm dimensions, nL–pL volumes) have been designed and fabricated in silicon for use in various analytical tasks. We studied sperm motility and performed sperm selection in channels (80 µm wide × 20 µm deep), branching structures (40 µm wide × 20 µm deep, eight bifurcations), and channels containing barriers (7 µm feature size). Sperm–cervical mucus and sperm–hyaluronic acid interactions were assessed by using appropriate microchannel-chamber structures filled with either cervical mucus or hyaluronic acid. Simultaneous assessment of the potency of different spermicides (e.g., nonoxynol-9, C13G) and spermicide concentrations was achieved with structures comprising chambers containing spermicide connected via channels to a central chamber into which semen was introduced. Semen was also tested for the presence of sperm-specific antibodies by using microchannels filled with human anti-IgG antibody-coated microbeads.

Indexing Terms: fertility/infertility studies · immunoassay · spermicide · microscopy

Semen analysis is a key component in the evaluation of male infertility. Various tests are performed on a semen sample, including determination of motility, morphology, viability, interaction with cervical mucus, and the presence of anti-sperm antibodies (1). Motility is assessed conventionally either by estimating the relative percentage of motile sperm through use of a hemocytometer, a Makler Counting Chamber (2), or computer-assisted semen analysis (1, 3). Motility of sperm, especially linear motility, is a very important measurement. One difficulty with the existing motility techniques is that sperm motion must be determined in several directions in a chamber or on a slide. We reasoned that constraining sperm in a channel would simplify assessment of motility because one would need to determine the movement of sperm in only one direction along the channel. We have investigated, therefore, the application of microchannels fabricated in silicon by conventional lithographic techniques (Figure 1A) for determining sperm motility and assessing other aspects of sperm function.

Materials and Methods

Microfabrication. Silicon microchannels were fabricated in 400-µm-thick <1,1,0> silicon by SGS Thompson (Montgomeryville, PA) by using masks produced by Align-Rite (Santa Clara, CA). The etched pieces of silicon were sealed with 0.625 × 0.375 × 0.062 in. (~16 × 9.5 × 1.6 mm) pieces of polished Pyrex glass (Mooney Precision Glass, Huntington, WV) by diffusive bonding to produce the silicon-glass chips (4).

Semen samples. Fresh unwashed semen samples were obtained from research semen donors or patients attending the Hospital of the University of Pennsylvania.

Spermicides. Nonoxynol-9 and C31G were gifts from Biosyn (Philadelphia, PA).

Microscopic examination of sperm. Sperm in channels and chambers were observed through a microscope (Aristom; Wild Leitz, Heerbrugg, Switzerland), equipped with a black and white TV camera (Dage-MTI, Michigan City, IN), a videocassette recorder (AG-7300; Panasonic, Secaucus, NJ), a video monitor (PVM-122; Sony, Teaneck, NJ), a photographic camera (MPS 46/52; Wild Leitz), and a video printer (UP-811; Sony).

Motility. Microchannels (80 µm wide × 20 µm deep × 10 mm long) in a glass-silicon chip (Figure 1B) were filled with HEPES-buffered human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) containing 5 g/L bovine serum albumin (Cohn Fraction V; Sigma Chemical Co., St. Louis, MO) (HTF-BSA) (5). A sample of semen (<2 µL) was placed on a glass microscope slide and the chip was placed on top of the semen sample such that the entrance to the channel was positioned on the semen sample. The progress of individual spermatozoa into the channel and along its length to the exit hole was monitored with the microscope and recorded with the TV camera and video recorder.

Sperm motility in branching channels (Figure 1C) filled with HTF-BSA medium was also determined, by examining the distance the sperm traveled along the network of channels (40 µm wide × 20 µm deep).

The bidirectional motility of a sperm sample was also examined. Channels (60 and 80 µm wide × 20 µm deep) and branching channels were filled with HTF-BSA medium, and semen was introduced simultaneously via the holes at each end of the channel.

An inclined channel experiment was performed on a sperm sample to mimic the conventional swim-up test (1). A channel (60 µm wide × 20 µm deep) was filled with HTF-BSA medium, and a sample of sperm was applied to the inlet hole. The inlet and outlet holes were sealed with adhesive tape. The chip was inclined at 45° for different periods, after which the progression of the sperm up the channel was determined visually.

The progress of sperm through a barrier in a 500-µm-wide channel filled with HTF-BSA medium was also assessed visually. The barrier spanned the width of the 20-µm-deep channel and comprised two opposing paral-

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Morphological examination of motile sperm. A chip comprising two chambers (5.2 mm long × 750 µm wide × 400 µm deep), each linked at each end to an entry hole by a channel (3.25 mm long × 100 µm wide × 20 µm deep) (shown schematically in Figure 1A) was used to study the morphology of motile sperm. The channels were filled with HTF-BSA solution and a semen sample was applied to the central chamber. The chip was placed in a moist environment for 10 min. The surface medium, containing sperm that had migrated from the central chamber, was removed from the holes at each end of the chip and placed on a glass microscope slide (previously cleaned with ethanol). The slide was dried at 40 °C and stained with Wright Giemsa stain (Curtin Matheson Scientific, Houston, TX), and then examined microscopically. Morphology was evaluated according to World Health Organization (WHO) criteria (1).

Simultaneous evaluation of spermicides. The chip shown in Figure 1A was used for the simultaneous testing of the spermicidal activity of nonoxynol-9 and C31G, an antimicrobial surfactant (6). Channel 1 was filled with HTF-BSA medium and served as a control. The other channels (2, 3, and 4) were filled with solutions of nonoxynol-9 or C31G in HTF-BSA medium at 0.05, 0.125, and 0.5 mL/L, respectively. A sample of semen (~1.2 µL) was placed in each chamber, and the progress of sperm into the adjoining channels was monitored by using the microscope. The number of sperm observed in the channels was determined by reviewing the videotape recording of the experiment.

Cervical mucus test. The interaction of a sperm sample with cervical mucus was tested by using the chip shown in Figure 1A. This test evaluates the overall quality of sperm (migration, penetration) and is a function of the various characteristics of the mucus (viscosity, fibrosity, cellularity) (2). The four channels were filled with a cervical mucus sample (collected at about day 14 of the patient’s menstrual cycle). After filling the central chambers with HTF-BSA medium, we then added a sample of semen (1.2 µL) to each chamber. Interaction of sperm with the mucus was assessed visually.

Hyaluronic acid test. Hyaluronic acid has been used as a substitute for human cervical mucus to assess the mucus penetration potential of a semen sample (7). The structure, molecular mass, and viscosity of hyaluronic acid are similar to those of glycoproteins present in human cervical mucus. The test was conducted in the chip shown in Figure 1A. Channel 1 was filled with HTF-BSA medium and served as a control. The other channels were filled with 5.0, 2.5, and 1.3 g/L solutions of hyaluronic acid (Sigma, St. Louis, MO) in HTF-BSA (channels 2, 3, and 4, respectively). A semen sample (~1.2 µL) was placed in each of the central chambers and the interaction of sperm with the different hyaluronic acid solutions was assessed visually.

Immunobead test. We tested for the presence of IgG antibodies, using microbeads coated with an antibody to human IgG (Immunobeads; Bio-Rad, Richmond, CA). A
microchannel (250 μm wide × 20 μm deep × 10 mm long) in a glass-silicon chip was filled with a sample of the immunobeads diluted to 1 g/L in HTF-BSA solution, and a semen sample (~1.2 μL) was applied to the channel entry. The agglutination of the beads was assessed visually. As a control, we also performed the experiment on a glass microscope slide, using larger volumes of the immunobead reagent and semen sample.

Results

Motility. Sperm migrated effectively in all of the different sizes of silicon channels tested (Figure 2). All semen samples showed an inherent variation in percent motility and grade of progression (I). We observed that highly motile sperm were able to travel further along the channels and eventually reached the exit. Sperm were observed traversing the entire length of the channel and could be seen accumulating in the exit hole. Migration of sperm was demonstrated in straight channels that were 40, 100, or 120 μm wide.

In two-way migration studies, sperm were observed migrating towards the center of the channel and eventually passing as they migrated towards the hole at the opposite end of the channel. In the branching channel structure, sperm were observed migrating through the tortuous pathway (a total of nine right-angle turns from the entry to the center of the channel). We repeated this experiment by using a 20-μm-deep fractal channel that was reduced in width at each bifurcation (40, 30, 25, 20, and 10 μm) and then increased in width (20, 25, 30, and 40 μm). Again, sperm migrated to the center of the branching network. In an inclined channel, the sperm migrate efficiently up the inclined channel and after a few minutes could be observed in the exit hole at the top of the channel. Sperm also successfully negotiated a barrier of two parallel rows of opposed posts with 7-μm gaps between the two rows and individual posts.

Spermicide test. The interlinking channels and chamber structures provided an efficient and effective means for simultaneous assessment of spermicides. The number of sperm observed in the channels decreased in the following order: channel 1 (control) > 2 (spermicide concentration, 0.05 mL/L) > 3 (0.125 mL/L) > 4 (0.5 mL/L). Most sperm were seen in the control channel, whereas none was seen in channel 4, which contained nonoxynol-9 or C31G at the optimum concentration for spermicidal action.

Sperm morphology. The sperm that had migrated from the central chamber to the end of the channel and into the exit hole had a normal morphological appearance, based on Wright Giemsa staining and WHO morphology criteria (I).

Cervical mucus test. Sperm did not migrate through the cervical mucus collected from an infertile woman at day 14 of the menstrual cycle. Those that penetrated did so only a short distance and then ceased to move. The poor penetration by sperm into this mucus was presumably because it was hostile to sperm.

Hyaluronic acid test. Sperm did not migrate into the channel that contained 5 g/L hyaluronic acid, but the extent of migration increased as the concentration of hyaluronic acid in the channel decreased.

Immunobead test. Agglutination of sperm by the immunobeads because of the presence of antibodies in the sperm sample was readily observed in the channel (Figure 3). As a control, the experiment was also performed on a glass microscope slide, using larger volumes of the immunobead reagent and semen sample; this test was also positive (agglutination observed).

Discussion

The silicon microchannel devices were effective in the analysis of a wide range of sperm functions. The materials used to construct the devices (silicon and glass) had
no adverse effect on the sperm. Assessing motility in a single direction along the length of a channel was simpler than the multidirectional monitoring required in a Makler chamber or a hemocytometer. The width and depth of the channels were such that the flagellar motion of the sperm (swimming) was not hindered. We envisage that sperm motility testing could be standardized by setting a scale next to the channel so that migration distances could be determined accurately. Such a device and its associated scale could be mass-produced and provide an international standard for motility testing.

The value of the new devices is that very small amounts of semen can be used for rapid evaluation of several measures of sperm function within a relatively short time. This enables the same sample to be used subsequently in procedures of assisted reproductive technology (e.g., in vitro fertilization) or artificial insemination. The devices are also suitable for simultaneous analysis of sperm and simultaneous testing of the interaction of sperm with candidate spermicides. The advantage of the simultaneous assay format is that the same fresh semen sample can be used for testing several characteristics rather than using an aging sample in the conventional serial testing formats.

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