that were TUNEL-positive (PE-positive/FITC-positive) under 1% and 20% oxygen were 14% (range, 4.3–38%) and 20% (range, 9.5–57%), respectively (Fig. 1B). Thus, the percentage of NRBCs undergoing apoptosis was significantly greater under 20% oxygen than under 1% oxygen ($P < 0.05$).

The present results suggest that, because the maternal circulation is higher in oxygen concentration than fetal circulation, the oxygen environment of maternal circulation induces apoptotic changes in fetal NRBCs transferred to maternal circulation, leading to clearance of NRBCs from the maternal circulation. The fact that approximately one-half of the NRBCs in our previous study showed apoptotic change indicates that fetomaternal cell traffic is more common than we had expected. It may also explain the difference between amounts of fetal cells and cell-free fetal DNA detectable in maternal plasma. It is generally assumed that some portion of the cell-free fetal DNA in maternal plasma originates from fetal cells in maternal circulation.

The identification of NRBCs is relatively easy by common methods of morphologic observation (5), but such methods do not yield sufficiently precise quantitative results. Therefore, we used two-color FCM to identify NRBCs and assess apoptotic changes in NRBCs. However, initially, we were not able to obtain exact numbers of NRBCs and apoptotic cells by FCM analysis. In a previous study using FACS (6, 7), we isolated NRBCs by staining with anti-y-hemoglobin antibody and identified NRBCs by morphology and staining. Because the purity of NRBCs separated by FACS analysis with this antibody is reasonably stable and reproducible (6, 7), we used the same antibody for staining in the present study. The positive controls (TUNEL-positive cells and cord blood) and negative controls (TUNEL-negative cells and mononuclear cells from nonpregnant adults) that we used in the present study helped to ensure the reliability of TUNEL staining results. Thus, the present methods used to quantify NRBCs and evaluate apoptosis in NRBCs appear to be reliable.

We conclude that the high oxygen concentration in maternal circulation induces apoptotic changes in fetal NRBCs transferred into maternal blood. We speculate that this phenomenon may be important to maintain the pregnancy because persistence of fetal cells in maternal circulation would stimulate the maternal immune system.

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References

Microchips: An All-Language Literature Survey Including Books and Patents, Larry J. Kricka†1 and Paolo Fortina2 (1 Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, 3400 Spruce St., Philadelphia, PA 19104; 2 The Children’s Hospital of Philadelphia, University of Pennsylvania School of Medicine, 310-C Abramson Pediatric Research Center, 34th St. and Civic Center Blvd., Philadelphia, PA 19104; † author for correspondence: fax 215-662-7529, e-mail kricka@mail.med.upenn.edu; † current address: Christ’s College, Cambridge CB2 3BU, England)

We now present the third, and final, part of our series of all-language literature surveys on microanalytical devices. It categorizes and lists books, book chapters, reviews, editorials, papers, abstracts, and patents on the topic of analytical microchips that have been published up to the middle of 2001. It is intended to serve as a convenient entry point into the microchip literature for those wishing to gain an insight into the scope and diversity of this important and rapidly expanding branch of science. The database has been compiled from searches of OVID Medline, INSPEC, BIOSIS, PubMed, various patent databases, and the personal databases of members of the IFCC Working Group on Nanotechnology. The listing of references for each of the 18 categories and the combined database can be found at Clinical Chemistry Online (http://www.clinchem.org/content/vol48/issue9/). Previous surveys focused on microarrays (1) and nanotechnology (2), and the databases can be found at www.clinchem.org/cgi/content/full/47/8/1479/DC1/12 and http://www.clinchem.org/content/vol48/issue4/, respectively.

An analytical microchip is a miniature analyzer that has at least one micrometer-sized component [e.g., microchannel, microchamber, or microfilter; for recent reviews of this topic, see Refs (3–9)]. This type of device is made from various materials, including silicon, glass, plastic, or combinations of glass and silicon, using techniques adapted from the microelectronics industry (e.g., photolithography) and the plastic fabrication industry (e.g., embossing, electroforming, and molding). Components that
make up a microchip are bonded together by anodic bonding (glass to silicon), thermal bonding (glass to glass), and solvent bonding (plastic to plastic) processes. Surface chemistry effects are a specific concern in devices with very high surface-to-volume ratios, such as microchips, and there is increased attention to surface properties (e.g., functionalization and texturing) and treatments (e.g., thin films). Most analytical microchips have been produced in silicon or glass, but an important trend is to make these devices out of plastic [e.g., poly(dimethylsiloxane), polymethylmethacrylate, polyimide, and polycarbonate] and so exploit the vast range of materials and low-cost, high-volume manufacturing techniques available for polymers.

Microchips have been designed for a range of chemical and biological analyses based on chromatography, electrophoresis, immunosassay, and nucleic acid target and probe amplification (e.g., PCR and the ligase chain reaction). They have also been very effective for cell isolation and selection by use of microfiltration or electrical fields, and the popular Coulter counter and flow cytometer have been successfully miniaturized into a chip format (10, 11). Microchip components are also gaining popularity for sample application in electrospray mass spectrometric methods (12).

An important advantage of the microchip approach to analysis is integration of successive steps in an analytical process (e.g., sample preparation, analytical reaction, and detection) on a single microchip or a multilevel microchip to produce a miniaturized total analytical system or a lab-on-a-chip (13). The scope of micromachined components available for incorporation into a lab-on-a-chip is diverse and includes pumps, valves, fluid channels and chambers, thermal control systems, sieves, and filters.

Key to the development of this new type of analyzer has been refinements of microfabrication techniques, development of convenient microchip-user interfaces, and a better understanding of microfluidics. Different types of microfabricated on-chip valves and pumps have been designed, and flow within chips can also be controlled by electrokinesis. An advantage of the latter option is that there are no moving parts, only the electrodes that are used to control flow. A particular concern for microchips has been the efficiency of mixing within submicroliter chambers and channels. New modeling software allows simulation of flow within microchips, and hence design optimization, before microchip fabrication (14).

Rapid progress is being made in the commercialization of microchips, particularly microchips for capillary electrophoretic separation of DNA and proteins as well as DNA analysis [see Ref. (15) for a compilation of companies active in analytical microchips]. Already there are indications that microchip analyzers are replacing existing analyzers and techniques (e.g., microchip capillary electrophoresis replacing conventional gel electrophoresis for DNA sizing), and further commercialization will hasten this process.

We divided the microchip literature into four major categories. Documents in each category are listed in chronologic order and in alphabetic order of first author topic areas and subdivided these into a series of 18 subcategories. The major categories and keywords in analytical microchips are listed in Table 1.

### Table 1. Major categories and keywords in analytical microchips.

<table>
<thead>
<tr>
<th>I. Analytical microchips</th>
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<tbody>
<tr>
<td>General (33 references)</td>
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<tr>
<td>Fabrication (97 references)</td>
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<tr>
<td>Batch manufacturing, casting, continuous manufacturing, electrophoresis, embossing, lithography, MEMs*, molding, packaging, patterning, solvent bonding, surfave functionalization, surface texturing, thin films, tooling</td>
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<tr>
<td>Integration (38 references)</td>
</tr>
<tr>
<td>Lab-on-a-chip, multilevel, μTAS</td>
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<tr>
<td>Plastic microchips (34 references)</td>
</tr>
<tr>
<td>Embossing, imprinting, polycarbonate, poly(dimethylsiloxane), photoresist, polyimide, polymethylmethacrylate</td>
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<td>Simulation and modeling (42 references)</td>
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<td>Computer models</td>
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<td>Thermal control devices (11 references)</td>
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<td>Cooling, heat exchanger, heat sink</td>
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<td>II. Microfluidics (142 references)</td>
</tr>
<tr>
<td>Capillary, convection, dielectrophoresis, electrokinetic, flow injection, microneedles, fluidic networks</td>
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<td>Mixing (14 references)</td>
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<tr>
<td>Chaotic, continuous, passive</td>
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<tr>
<td>Valves (23 references)</td>
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<tr>
<td>Ball valve, bidirectional, check valve</td>
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<tr>
<td>Pumps (15 references)</td>
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<tr>
<td>Acousto-, electrohydrodynamic, electroosmotic, self-priming</td>
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<tr>
<td>III. Applications</td>
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<tr>
<td>Amplification reactions (53 references)</td>
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<tr>
<td>Capillary electrophoresis, cartridge, genotyping, integration, ligase chain reaction, PCR, real-time analysis, sample preparation</td>
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<tr>
<td>Cell analysis (51 references)</td>
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<tr>
<td>Coulter counter, cytometer, dielectrophoresis, filters, guidance, manipulation, selection, separation, sorting</td>
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<tr>
<td>Chromatography (15 references)</td>
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<td>Electrochromatography, gas chromatography, immunochromatography, liquid chromatography</td>
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<td>DNA analysis (28 references)</td>
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<tr>
<td>Hybridization, sequencing, sizing</td>
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<tr>
<td>Electrophoresis (80 references)</td>
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<tr>
<td>Capillary electrophoresis, dielectrophoresis, electrochemical detection, electroporation, injectors, laser-induced fluorescence, electrospray ionization</td>
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<td>Immunoassay (34 references)</td>
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<tr>
<td>Capillary electrophoresis, chemiluminescence detection, immunochromatography, electrochemical, FRET, multichannel</td>
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<tr>
<td>Mass spectrometry (23 references)</td>
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<tr>
<td>Electrospray, MALDI, peptide and protein analysis</td>
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<tr>
<td>Protein analysis (7 references)</td>
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<tr>
<td>Mass spectrometry, proteomics</td>
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<tr>
<td>IV. Patents on microchips (276 references)</td>
</tr>
<tr>
<td>Devices, methods, processes</td>
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</tbody>
</table>

* MEMS, microelectromechanical system; μTAS, miniaturized total analytical system; FRET, fluorescence resonance energy transfer; MALDI, matrix-assisted laser desorption/ionization.
within each year. Table 1 provides a list of key words for each category to provide a more detailed view of the scope of each of these sections. The key words reflect the most important topics within each category. In the interest of simplicity, citations have been assigned to just one category. A more detailed and comprehensive listing of references for particular topics can be obtained by searching the online database (including title, keywords, and abstracts) using the appropriate keyword or keyword combinations. We have provided the total database and the database for the different categories for the convenience of the user [available through Clinical Chemistry Online (http://www.clinchem.org/content/vol48/issue9/)]. Please note that in many cases we have relied on the abstraction service for the citation details. The Internet is also a rich source of information on microchips, and the reader is directed to the excellent DNA Microarray (Genome Chip) web site (15), which lists many aspects of microchips science and business.

This compilation is based in part on a survey undertaken by the IFCC Working Group on Nanotechnology, chaired by Dr. Larry J. Kricka. Members of the Working Group are listed in the data supplement that accompanies this Technical Brief at Clinical Chemistry Online (http://www.clinchem.org/content/vol48/issue9/).

References


Evaluation and Performance Characteristics of the STA-R Coagulation Analyzer, Michele M. Flanders,1 Ronda Crist,1 Sekineh Safapour,1 and George M. Rodgers2,3 (1 ARUP Laboratories, Salt Lake City, UT 84108, 2 Departments of Medicine and Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; 3 address correspondence to this author at: Division of Hematology, University of Utah Health Sciences Center, 30 North 1900 East, Salt Lake City, UT 84132-2408; fax 801-585-5469, e-mail george.rodders@hsc.utah.edu)

Recently, increasing coagulation test volume and tight personnel budgets have increased interest in automated coagulation analyzers (1). The first coagulation instrument, the Fibrometer (Becton Dickinson), used a moving electrode to detect the clot. This brought some degree of standardization to the reading of the clotting endpoint, but this method was labor-intensive. The next phase in instrumentation was semiautomation. These instruments had storage and delivery capabilities for reagents, but manual pipetting was required for the samples. Examples of semiautomated instruments include the Coa-g-A-Mate X-2 (General Diagnostics) (2) and early models of the MLA (Medical Laboratory Automation). The current generation of coagulation instrumentation is fully automated. Their capabilities include primary tube sampling, automatic rerun and dilution capabilities, and clotting, chromogenic, and immunologic methodologies (3). Examples of this type of instrumentation include the MDA (bioMerieux), STA-R (Diagnostica Stago), AMAX (Sigma Diagnostics), BCS (Dade Behring), and the Sysmex CA-6000 (Dade International) (4).

We summarize our technical evaluation of the STA-R. The evaluation addressed several issues, including ease of operation, methodologies available, reagent and patient sample on-board capabilities, ability to perform automatic dilutions, and validation of performance.

The STA-R has robotic capabilities and uses clotting, chromogenic, and immunologic assays. It is an open system with room for 220 patient tubes and 75 positions for reagents. Approximately 360 prothrombin time (PT) and partial thromboplastin time (PTT) tests can be run per hour. The analyzer makes automatic dilutions, reruns samples, and processes STAT samples without interrupting current testing. The system uses Windows NT software and is interfaced.

Maintenance includes a daily assessment of the condensation trap and wiping of the touch screen. The instrument is left on 24 h a day, so there is no extra start-up time involved. Weekly maintenance requires up to 30 min.

The touch-screen monitor allows easy access to all applications needed for performing the various steps in an assay. These steps include running of daily controls, calibration, and reporting of patient results. Although daily calibration is not required, we did so.

Each assay described below was performed on the STA-R instrument, and results were compared with those of the MDA (bioMerieux) and the BFA (Dade Behring) already set up in the laboratory. The final step in the