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Nanotechnology and its Potential in Forensic DNA Analysis

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INTRODUCTION

In recent years there has been an explosion of interest in developing applications involving nanotechnology. Nanotechnology can be defined as techniques that use materials and measurements at the submicron level. According to the United States government's National Nanotechnology Initiative (**www.nano.gov/**), materials at the nanometer scale produce unique phenomena that can enable novel applications. This is because at this scale it is possible to manipulate materials at the level of individual molecules and atoms. With these techniques, one can visualize alternative methods of manufacturing based on the use of atoms as basic building blocks to construct and produce useful machines. This technology is not just science fiction. A good argument can be made that the PCR[a] is just such a nanoscale processing system, using *Taq* DNA polymerase as a molecular machine to produce DNA. Thus forensic scientists are already using a nanoscale manufacturing process every day.

At the nanoscale interface, quantum mechanical phenomena become more prevalent, and interactions between light and matter become important. These properties can lead to useful materials such as quantum dot nanocrystals, which produce an intense fluorescence that has none of the spectral band broadening due to the variety of different electronic energy levels in the dye molecule. Thus, current problems with fluorescent dye matrices could be greatly reduced. Individual DNA molecules can be labeled with these materials, and simply changing the size of the nanocrystal produces different color characteristics (**http://probes.invitrogen.com/products/qdot/**).

Nanoscale methods of manufacturing often involve the same lithographic techniques used in silica computer chips. These techniques are also being used to synthesize short single-stranded DNA fragments to produce vast arrays of over 250,000 DNA single nucleotide polymorphism (SNP) probes on a single silicon chip (**www.affymetrix.com/products/arrays/specific/500k.affx**). In addition, lithographic procedures can be used to reduce the sizes of chemical instrumentation such as capillary electrophoresis (CE) and liquid chromatography. New analytical devices are now being developed that may replace our current chemical instrumentation. These systems, known as labs on a chip, can use miniature pumps or electrophoresis to isolate molecules, perform chemical reactions, separate the products and detect them. Pyrosequencing is another application, similar to PCR, in which molecular systems are manipulated to produce a desired output (1).

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The question then becomes "How will the new advances resulting from nanotechnology benefit forensic measurements?" The rapid development of technology in DNA analysis, from silver-stained slab gels to multiplexed capillary-array electrophoresis, can be said to have arisen from the human genome project, a similar government effort. Thus it is important for forensic analysts to understand the current status of research in the development of nanoscale processes.

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as alternatives to CE for analysis and detection of nanoliter volumes of DNA. While there are still many details to work out to understand and control the problems of clogging, electric field effects and wall interactions, microfluidic systems are beginning to appear in many laboratories. While there is a lot of hype concerning lab-on-a-chip systems, including advantages of cost, speed and sensitivity, remember that the basic physical and chemical properties governing CE also apply to microfluidic systems. Their increased speed is generally due to the shorter length of the channels used. CE systems are equally fast when used with short capillaries (2). Unfortunately, the gain in speed claimed for microfluidic systems is generally accompanied by a loss of resolution. However, these systems are more compact than standard capillary and gel electrophoresis systems and can be disposable. Because of their small size, the potential of such devices to be used at the crime scene is widely mentioned, but before this can occur, a lot of technical and legal issues need to be resolved. Instead, it is the convenience of a rapid, disposable device that requires minimal cleanup and maintenance that will drive the technology.

At present the most widespread forensic application of microfluidic systems is post-PCR quantitation. The commercially available Agilent 2100 bioanalyzer uses an array of multiple channels to inject and quantify nanoliter amounts of 12 double-stranded DNA samples in less than 30 minutes. These systems are currently being used in several forensic laboratories to perform post-PCR quantification of mitochondrial DNA and have run times of less than 2 minutes per sample (**www.chem.agilent.com/scripts/LiteraturePDF.asp?iWHID=40498**). The 2100 bioanalyzer also has the potential to screen genomic DNA but at present does not have sufficient resolution to analyze STRs. Figure 1 illustrates the separation and detection of 2 D1S80 alleles in less than 2 minutes using this system.

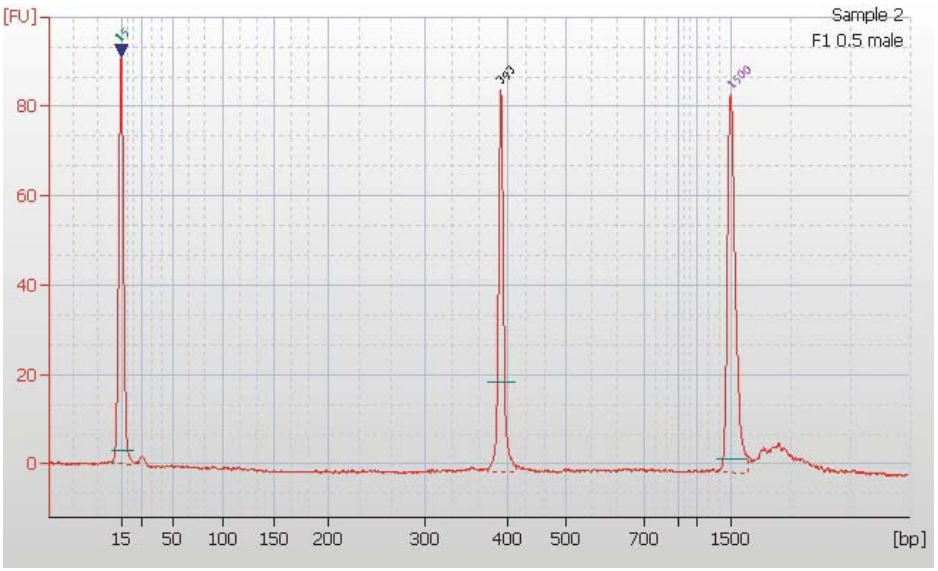

Figure 1. The analysis of a sample of human DNA amplified using the D1S80 VNTR locus and analyzed using the Agilent 2100 bioanalyzer microfluidic electrophoresis system. The first and third peak in this assay are internal sizing standards. The middle peak is a homozygous allele. Run time for this analysis is less than 2 minutes. Figure courtesy of Desiree Diaz, Florida International University.

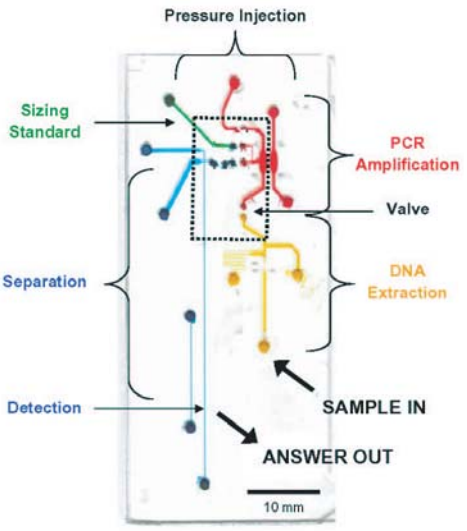

Figure 2. An integrated DNA chip. This system, currently under development by James Landers at the University of Virginia, has the potential to integrate all current processes in the forensic laboratory in one device.

Microchip-based electrophoretic systems to separate STRs are also being developed; however, due to inherent constraints involved in STR typing, these "chips" have channel lengths of approximately 20cm, making them appear more like microplates than microchips (3). Loading can also be a problem due to issues with pipetting, evaporation and clogging. Nevertheless, impressive sample capacities with up to 384 sample lanes are possible (4).

Using similar technology, chip-based, real-time PCR systems have been developed, and the reduced volumes possible with these devices may greatly decrease cost and reaction time (5). A number of research groups have produced systems to integrate PCR amplification, separation and detection (6–8). Systems for DNA extraction and purification have also been developed (9). Figure 2 illustrates an example of a potential integrated device that permits the entire processing of a DNA sample from extraction to genotyping (10).

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An alternative to DNA analysis by electrophoretic methods involves a variety of hybridization-based assays. However, these systems are usually limited to the detection of single nucleotide polymorphisms and other sequence variations, as it is difficult to detect STRs by hybridization due to sequence redundancy. SNP typing holds some promise in human identification, especially due to its potential to discriminate on the basis of genetic origin; however, the technique has rather poor capabilities to detect mixtures. Lithographic techniques can be used to produce these chips, and the synthesis of arrays of thousands of short single-stranded DNA capture probes on a single chip is possible. Techniques such as whole genome amplification can be used to target the entire genome (11), and more focused assays may be used to target mitochondrial SNPs (12).

Perhaps the ultimate application of nanotechnology is the development of tools that directly read DNA sequence one molecule at a time. Procedures currently exist to immobilize DNA molecules on gold pads or carbon nanotubes, where they can be probed by atomic force microscopy (AFM) or other techniques. In a study by Tanaka and coworkers, different species of tuna were identified by examining double-stranded DNA mismatches directly using AFM (13). Measurements of charge transport along DNA strands have been used to probe the conductivity of DNA and determine the effect of sequence-specific strand breaks (14,15). Figure 3 illustrates the AFM image of a disulfide-labeled strand of DNA stretched across a 1.2µm gap using an alternating electric field and visualized with atomic force microscopy. Such experiments can be used to examine the effect of DNA structure on its conductance.

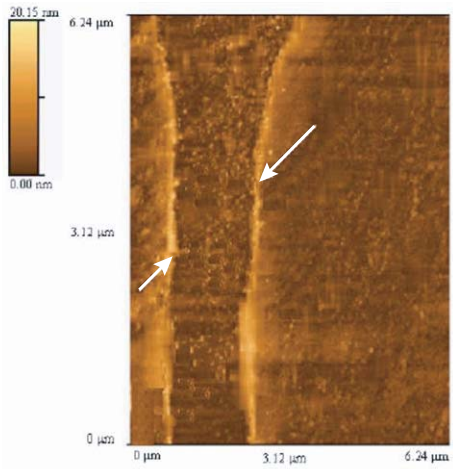

Figure 3. The isolation of a single disulfide-labeled DNA strand across an electrode gap of 1.2 microns. The DNA was stretched across the electrodes using an alternating electric field and visualized using AFM. This technique has been used to detect differences in DNA conductance resulting from sequence-specific strand breaks (15). Figure courtesy of Brittany Hartzell Baguley.

The ability to transfer charge along a DNA molecule may help explain the ability of biological repair enzymes to detect DNA strand breaks (16). These same properties may be used to sense the electrostatic charge distribution along a single strand of DNA as it passes through a nanopore and thus sequence the molecule (17).

CONCLUSIONS

This brief review can only hint at the potential of this new area of research. Of the major advances discussed, the potential integration of all laboratory operations onto a single platform is perhaps the most interesting development, as it may further minimize laboratory contamination issues. Newer capabilities such as enhanced SNP typing, quantum dot detection and single-molecule sequencing may also revolutionize this field by providing new directions for data collection and enhanced sensitivity for detection of trace DNA. Ultimately, detection and sequencing of single DNA molecules left at a crime scene may not be far away.

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