APPLICATION OF NANOTECHNOLOGY IN FORENSIC DNA AND HELP TO INVESTIGATIONS ON THE CRIME SCENE ANALYSIS

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ABSTRACT

Forensic applications of nanotechnology go beyond using nanoparticles. A classic tool of nanotechnology labs, the Atomic Force Microscope (AFM), could help with the paper message found in the crime scene described at the beginning of this article. This device allows details of a surface at the nano-scale level to be seen. It measures the forces exerted among a microscopic sharp tip and the atoms of the surface. This procedure can be used to examine ink crossings and determine which of two lines has been written over the other. This information could prove crucial in understanding certain documents, such as a contract with a contentious amendment. Because of applying knowledge and techniques from natural science, forensic science aims to identify, individualize and evaluate evidence. Evidence will then be used to reconstruct crime scenes, guide investigations and bring criminals to justice. Nanotechnology has been applied towards these purposes. Among the various nanotechnologies, nano-analysis is most commonly seen in forensic science with instrumentations including transmission electron microscope (TEM), scanning electron microscope (SEM), atomic force microscope
(AFM) and Raman micro spectroscopy (Micro-Raman). This paper will introduce the principles of nanotechnology, instrumentations, and known forensic applications. In addition, the toxicity of nanomaterials and future prospects will be discussed.

**KEYWORD:** Forensic, crime scene, criminalities, electron microscope, nanotechnology.

1. **INTRODUCTION**

Nanotechnology is making valuable contributions to various scientific fields in science and technology today. Generally, it is defined as the study, design, creation, synthesis, manipulation, application of functional materials, devices, and systems through control of matter at the nanometer scale. It has been applied to many areas of study\(^1\) including electronic engineering, physical sciences, materials sciences, biomedical sciences and many others. Recently, many new nanoscale sample analysis techniques in genetic, medicine, analytical chemistry have been applied to fields of forensic sciences. Nanotechnology contributes to forensic sciences in two ways. Since it can detect and analyze samples in the nanoscale, critical evidence that could not be collected and analyzed before due to the detection limits of the instruments can now be analyzed and used to support the investigations.\(^2\) In addition, nanomaterials possess novel properties that can assist the collection and detection of evidence which cannot be acquired previously. Some examples include trace amounts of gunshot residues, heavy metals, explosives, DNA on fingerprint or palm prints, and so on. Foreign law enforcement agencies have already begun to consider sponsoring more research projects on the forensic applications of nanotechnology. According to the National Nanotechnology Initiative (www.nano.gov) fiscal year 2006 budget request, the National Institute of Justice (NIJ) of United State Department of Justice (USDOJ) has two separate project areas that incorporate nanotechnology – DNA Research and Development ($1.0 million) and Chemical and Biological Defense ($1.0 million). The DNA Research and Development program consists of fundamental research and the demonstration of chip-based or micro-device technologies to analyze DNA in forensic applications. The Chemical and Biological Defense program is focuses on developing a wearable, low-cost device to provide warning of exposure to unanticipated chemical and biological hazards in sufficient time for its wearer to take effective protective measures.\(^2\) 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. 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. 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.\[^3\]

2. FORENSIC APPLICATIONS

Most of the known forensic applications of nanotechnology focus on the development and improvement of DNA microchips and array.\[^4\] Since little is known about utilizing nanotechnology on other types of evidences, various forensic applications will be discussed in the following paragraphs. Latent Fingerprint Enhancement of CdS The late Dr. Menzel was the pioneer of the usage of photo luminescent CdS semiconductor nanocrystal capped with dioctyl sulfosuccinate to enhance latent fingerprint detection.\[^5\] His concept was to apply nanocrystal fluorescent dye on articles that have been pre-fumed with cyanoacrylate ester and
also on the sticky side of electrical tape without pre-fuming. Nano-Fingerprint Residue Visualization Worley and coworkers at Los Alamos National Lab developed a novel method using micro-X-ray fluorescence (MXRF) to detect images of latent fingerprints\textsuperscript{[6]} Unlike mcommon chemical reagents methods where latent prints are developed via reactions between reagents and amino acid, or fatty acid from the fingerprint, MXRF generates latent fingerprints images by detecting inorganic elements in the prints. It is more advantageous due to the non-destructive nature of the analysis, as well as the stability of the inorganic residues. During analysis, fingerprints remain intact and can be used for additional tests, such as elemental analysis for gunshot residue, and prints can still be imaged up to an average of eight months under appropriate evidence storage. The most commonly observed inorganic residues in fingerprints are potassium and chloride ions. Other elements that can be found in latent prints by MXRF include silicon, calcium, aluminium, and so on. However, this method also has one drawback. A sebaceous fingerprint left by one subject was successfully imaged by MXRF, but sebaceous prints left by a different person were undetectable, indicating that print elemental composition may be the person and/or diet dependent, and this technique cannot be applied to all cases Because MXRF actually provides an elemental analysis of the inorganic elements found in fingerprints, substances foreign to the hands may also be visualized including sweat, lotion, saliva, and sunscreen. For example, lotion and sunscreen can be detected due to residual TiO\textsubscript{2} or ZnO nanoparticles, while sweat can be detected due to its inorganic components. Furthermore, MXRF can be used to investigate food consumption by linking elements detected in saliva and food residues found in fingerprints to investigate missing children cases. Gold Nanoparticles to Enhance PCR Efficiency Lin and colleagues found that Au nanoparticles can be used to dramatically enhance polymerase chain reaction (PCR) efficiency\textsuperscript{[7]} When 0.7 nM of 13 nm Au nanoparticles was added into the PCR reagent they found the reaction time is decreased while heating/cooling thermal cycle rates is increased. Their results also showed that the it has been suggested that sensitivity improved 5~10 times in conventional PCR, and more than 10,000 times in real-time PCR. The marked improvements in PCR efficiency is attributed to the superb heat transfer property of Au nanoparticles, another research groups have also begun to utilize nanoparticles to forensic biology related researches.

\section*{2.1 AFM and Questioned Documents}
Khanmy-Vital’s group in Switzerland first used AFM to examine ink crossing in documents to determine sequence of pen strokes\textsuperscript{[8]} AFM can study the 3-D surface morphology, which
provides essential information for determining the sequence of lines made by ball pen ink and ribbon dye. They suggest that AFM images present the same qualitative information as obtained by SEM images. Furthermore, since AFM can be operated under ambient conditions without vacuum and conductive coating of samples, potential damages to the sample during the experiment can be avoided. The depth of ink crossing, amplitude and phase images of ink can be clearly determined.

2.2 AFM and the Time of Death

Cai and Chen first reported the application of AFM to resolve one of the most crucial issue in forensic science – the estimation of the time of death[9] The morphological changes of blood cells can be useful for the quantitative assessment of the time of death. The deformation of cell and membrane surface of unfixed erythrocytes with time lapse is observed. Fissures and cell shrinkage took place in half a day. More protuberances on erythrocytes began to reveal in 2.5 days. The number of protuberance increases with time, so it can be used as an indication for the estimation of the time of death. Protuberance can come from several sources. One is when hemoglobin in cytoplasm flows outward when dehydration induces the formation of holes in cell mem-branes. The other possible source is the integral membrane proteins, such as band 3 protein, glycophorin A and others. In addition, the cytoskeleton proteins reveal that membrane became thinner due to dehydration. Their results suggest AFM is a new potential tool in forensic medicine (the estimation of the time of death), and can also analyze other tissues, membranes and biological samples. The authors also investigated the time-dependent surface adhesive force and morphology of red blood cells (RBC), and cellular viscoelavity vs. distance curve under: 1) controlled, roomtemperature (temp: 25°C, humidity: 76%); 2) uncontrolled, outdoor-environmental (temp: 21.2–33.7°C, humidity: 38.4–87.3%); and 3) controlled, low-temperature (temp: 4°C, humidity: 62%) condition by AFM.[10] 21RBC exhibits typical biconcave shape on a mica substrate, whereas either the biconcave shape or flattened shape was evident on a glass substrate. The mean volume of RBCs on mica was significantly larger than that of cells on glass, but surprisingly, the adhesive property of RBC membrane surfaces was substrate-independent. Over time, the changes in cell volume and adhesive force of the RBC under controlled room-temperature condition were similar to those under the uncontrolled outdoor-environmental condition. Under the controlled low-temperature condition, however, the changes in cell volume occurred mainly due to the collapse of RBCs, and the curves of adhesive force showed the dramatic alternations in
viscoelasticity of RBC. More researches on various environmental factors such as humidity, pH value, temperature, and light are needed to estimate blood age accurately.

2.3 AFM Force Spectroscopy and Bloodstain
Thalhammer’s group reported the age determination of dry bloodstain by AFM force spectroscopy.\textsuperscript{[11]} In this preliminary study the changes in erythrocytes elasticity on a nanometer scale was analyzed via a two-step procedure. In the first step, an overview image was generated showing the presence of several red blood cells, which could be easily detected by their typical “doughnut-like” appearance. Subsequently, AFM was used to test the elasticity by recording force-distance curves. The measurements were performed immediately after drying and after 1.5 h, 30 h and 31 days. The conditions were kept constant at room temperature (20°C) and 30% humidity. The elasticity pattern decreased over time, which is most likely influenced by the alteration of the bloodstain during the drying and coagulation processes. Once the calibration curve of the elasticity over time is developed, the age of bloodstains can be estimated and used to assist in criminal investigations.

2.4 AFM and Trace Evidence
Adya’s group applied AFM to the analyses of textile fibers\textsuperscript{[12]} and pressure sensitive adhesives\textsuperscript{[13]} In the fiber study, natural (cotton and wool), and regenerated cellulose (viscose) textile fibers exposed to various environmental stresses for different lengths of times were analyzed by AFM. AFM images were used to quantitatively measure the surface texture parameters of the environmentally stressed fabrics as a function of the exposure time. The finest nanoscale details of the surfaces of three weathered fabrics can be observed and clearly distinguish between the detrimental effects of the imposed environmental conditions. Three kinds of fibers were exposed to two different soils (town and riverside) and two different types of water (ponds and water) for zero, two, four and six weeks. The surface morphology of each sample was analyzed for average maximum peak heights (Hpm), average maximum heights (Hz), average maximum valley depths (Hvm), peak-to-valley distances (Rz), the root mean square roughness (Rrms) and other parameters to quantify the changes under the different circumstances. This study demonstrated that AFM is a very powerful tool in forensic examination of fiber evidences due to its capability to distinguish between different environmental exposures or forced damages to fibers. Pressure sensitive adhesive (PSA), such as those used in packaging and adhesive tapes, are very often used in criminal activities. Packaging tapes may be used to seal packages containing drugs, explosive devices, or
questioned documents, while adhesive and electrical tapes are used to tie up the victims in kidnapping cases. The AFM phase images show dark and bright areas corresponding to the soft polymer molecules and the rough surfactants, respectively, on three investigated PSA tapes. The mechanical properties of various tapes can be differentiated by the maximum adhesive force of the particles forming the film to the tip (Fmax), the maximum distance of deformation of these particles (dmax), and the adhesion energy (e) of the F-d curves. This is the first study to accurately analyze various tapes by AFM imaging and force mapping. Several studies have also reported other applications of AFM in criminal investigations. One example is a computational method that calculates cuticle step height from AFM images for the quantitative assessment of human hair. Another example is in the analysis of particle size distribution of powder spray-enhanced the latent fingerprint imaging. Besides the image and surface analysis capabilities of AFM, AFM microcantilever can also be used for selective detection. A review paper by Carrascosa et al. discussed many interesting applications of specific target detections performed in the nano and pico levels. Some applications include the analysis of DNA hybridization, detection of two isoforms of prostate specific antigens; C-reactive proteins; Salmonella enterica; Vaccinia virus; explosives as trinitrotoluene (TNT), Pentaerythritol Tetranitrate (PETN), and Cyclotrimethylenetrinitramine (RDX). Microcantilever based sensor have become an important device for detecting low-level molecular interactions with high accuracy. It detects molecules by utilizing the appropriate coatings on the cantilever surface. The microcantilever sensor detects the target molecule when the molecule interacts with the coating molecule. As described in the AFM introduction, any tiny position shift due to molecular interaction, recognition, adsorption, or desorption can be observed. When more target molecules accumulate on the cantilever surface, the additional weight caused more bending of the level that leads to more deflection.

2.5 NANOTECHNOLOGY IN DNA TYPING LABORATORIES

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. Perhaps the area of nanotechnology with the greatest potential to affect present operations in a DNA analysis laboratory is the development of microfluidic systems 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, micro fluidic 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 micro fluidic 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.[16] Unfortunately, the gain in speed claimed for micro fluidic 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 micro fluidic systems is post-PCR quantitation. The commercially available Agilent 2100 bio analyzer 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. The 2100 bio analyzer also has the potential to screen genomic DNA but at present does not have sufficient resolution to analyze STRs 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 micro plates than microchips.[17] 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.[18] 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.[19] A number of research groups have produced systems to integrate PCR amplification, separation and detection.[20–22] Systems for DNA extraction and purification have also been developed.[23-24]

2.6 NANOTOXICOLOGY

Nanotechnology has great potential to benefit the society; however those nanomaterials with unknown novel properties can also cause risks to the environment. The risks from nanomaterials are largely due to their unknown health impacts. After the use of “Magic
Nano” spray in Germany, more than 80 people complained of fever, headache and difficulty in breathing, and several went to the hospital due to pulmonary edema. The spray was designed to enhance water and dirt resistance for glass and ceramic tiles. German Federal Institute of Risk Assessment issued a warning against the usage of nanoparticles-containing household products and has resulted in first nanotech-product withdrawn from German market in 2006. But, direct evidence to conclude that all nanomaterials are harmful to the environment and health is limited. Generally, the smaller the nanoparticle the more toxic it is because smaller particle can penetrate more areas in the body. For the same amount of sample, the smaller particles can come in contact with a larger surface area, thus can potentially react with more active sites. Generally speaking, nanomaterials can enter our body through four entry routes, inhalation, digestion, skin absorption and ingestion. The seriousness of nanotoxicity has been acknowledged and emphasized in a review by the group of Oberdorster.[25] They mentioned that after inhalation, nanoparticles around the respiratory tract can enter into cells, blood stream, and lymph circulation. Subsequently, they can penetrate into the bone marrow, lymph nodes, spleen, and heart. Additionally, it has been observed that nanoparticles can cross the blood-brain barrier and penetrate into the central nervous system and ganglia, causing even more severe damage to the human body. Nanotoxicology is still a new field of research, but the reduction – and eventual removal - of toxicity associated with novel nanomaterials, nanostructures and nanodevices is of paramount importance.

3. 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. The constant development of nanotechnology, forensic scientists will be encountering various evidences in the nanoscale in the future. When professionals process these nano-evidences, they might raise questions below: How could I process this type of evidence correctly? Will these nano-evidences be toxic to me? How would I protect and my colleagues and myself? Forensic scientists will need to know more information in nanotechnology related fields.
Taiwan has great potential and capability to become one of the leading countries in applying nanotechnology to forensic sciences. From the author’s personal experience, the “average” qualification and general knowledge of forensic scientists in Taiwan are better than others in most countries. Therefore, to combine forensic science with nanotechnology and establish world-leading environment is not “mission impossible”. It can be achieved by putting an emphasis on developing educational researches to help provide the skilled workforce and supporting infrastructure/tools needed to advance nanotechnology. This would also require better utilization of forensic lab instrumentations, in conjunction with equipments that can perform nanoscale analysis. Finally, to further develop novel forensics and related studies, long-term exchange opportunities with international forensic scientists must be sought to ensure our awareness of the latest development in forensic science and nanotechnology. Develop education researches, skilled workforce and the supporting infrastructure and tools to advance nanotechnology.

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