A review on the role of emerging revolutionary nanotechnology in forensic investigations

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Abstract
Due to the unique properties of nanoparticles, it has gained prominence in lots of fields with extensive research being carried around it. With lots of novel applications arising from this field, Forensic science seems to be one of the fast-growing fields in nanotechnology. The growing and extensive use of nanotechnology being applied in forensic investigations is promising and could soon be the tipping point in the discipline. Applications mainly have been related to evidence identification and analysis in the broad major fields in Forensic Science such as single-crystalline semiconductor CdS nano slabs for explosives detection, functionalized TiO$_2$ nanorods for organophosphorus chemical warfare agents in Forensic Chemistry, the use of Nannopowders for latent print visualization in Forensic physics and Gold nanoparticle protein nanopore for detection of single-stranded DNA in Forensic biology. Nanotechnology has also been employed in illegal drug detection in recent times. These and other applications of Nanotechnology provides prompt and precise results with reduced methods due to the limited instruments used for analyzing evidence as well as providing sensitive and selective ways of detecting evidence. As evidence is notable in forensic investigations, nanotechnology’s use in identifying and detecting these has potential in enhancing and providing efficient and rapid means for investigations and unravelling leads into crimes. This review emphasizes some disciplines in forensic sciences in which nanotechnology is having an impact, novel methods and newly developed instruments and also takes into account its challenges as well as perspectives into the future.

Keywords: Forensic science, Forensic evidence, Investigations, Nanotechnology, Nanoparticles

INTRODUCTION
The ever increasing and sophisticated means of crime demands a more advanced and fast means of detection, investigation and reconstruction of events in order to apprehend perpetrators of such acts or establish a commission of a crime. Forensic science, since its inception, has been crucial in this role in most criminal investigations attesting crime commission, establishing identity and corroborating testimonies and providing facts in relation to the occurrence of a crime. (Fisher, 2004). Notwithstanding the massive improvement over the years, new technologies and fields in forensics are further advancing crime investigations. Forensic science’s extensive ambit has incorporated new techniques from the natural sciences to obtain criminal or other evidence of legal importance (Srividiya, 2016), one of which is Nanotechnology. Nanotechnology involves the managing of matter to produce materials of various types at nanoscale level. It deals with materials and structures that have at least one-dimension size ranging from 1 to 100 nm. The importance of these materials to researchers is that their smaller size can have an influence on substances physico-chemical properties (Khan et al., 2017). Nanoparticles, products from nanotechnology, has been extensively used in areas such as gas sensing (Mansha et al., 2016); herbal and nutraceuticals (Gopi et al., 2016); medicine; diagnostics and vaccinations (Shaalan et al., 2016); advanced drug delivery (Safari and Zarnegar, 2014) and later in various aspects of the field of forensic sciences.

Knowledge and applications from nanotechnology have resulted in the new discipline or subspecialty in forensics known as Nano-forensics, which has adapted techniques from nanotechnology in helping to improve upon investigations. This novel field is connected with the expansion of nanotechnical methods
and procedures as well as nanosensors for real-time crime scene investigations and enquiries into explosions or explosive gases as well as terrorist activities and biological agents, mediators and residues. The application of this technology and practices is providing a massive boost and augmenting forensic scientists in their capacity in testing illegal substances, unraveling hidden evidences and improving upon the time period for investigations to be conducted either in the analysis of evidences in the lab or spot-on on the crime scene. This piece profiles some of the applications of nanotechnology in forensic science and how it is changing the face and pace of the field in criminal investigations and justice delivery.

**FORENSIC EVIDENCE AND NANOTECHNOLOGY**

Forensic science deals with analyzing evidence from incident scenes in order to identify culprits or establish the perpetuation of a crime. These evidence lead to the arrest and prosecution of offenders or the eventual solution of a crime (McEwen, 2010). According to Henry Lee (Lee et al., 2004), evidence can be classified as biological, weapon, fingerprint, drug, impression, trace etc., it is also classified in terms of its state physically, the nature of the evidence and its composition, the type of crime as well as the questions to be resolved. Acknowledgement of these classifications is an important step in the investigation process. The failure of recognition, collection and proper preservation, as well as testing, can greatly impact the forensic value of the evidence leading to a reduction or loss of it which eventually affects justice delivery (Lee, 2013). Evidence from crime scenes, therefore is crucial in the forensic discipline to exonerate or incarcerate. Hence, a well-improved technology or process is needed to enhance analysis and investigations and this is what Nanotechnology has brought on board in improving recognition, detection and analysis of related evidence from crime scene.

The American Academy of Forensic Sciences (2020) has categorized forensic sciences into eleven distinct divisions/disciplines for forensic scientist, which is represented in Fig. 1. These are areas in forensic science with a vast number of cases and hence much focus and emphasis are placed on them to develop techniques and methods to improve on the quality, accuracy and speed in the analysis. As forensic science comprises of so many fields in the science discipline, nanotechnology is not alone discipline either but incorporates sciences such as biology, physics, chemistry, material science in developing new multifaceted technologies or processes enhancing and bringing refinement in techniques and processes available (Saini et al., 2010). In the emerging utilization of nanotechnology, certain techniques and instruments used in forensic laboratories have been exploited for nanomaterial’s characterization. They include the Atomic Force Microscopy, Scanning Electron Microscopy, Raman Micro Spectroscopy and the Transmission Electron Microscopy (Chen, 2011; Tiede et al., 2008). Most of the techniques from nanotechnology and Nano-forensics are to either analyze evidences of Nano-scale size or use the effects of the nanomaterial in recognizing, assembling, collecting or detecting evidence in criminal investigations. With regards to tools development as a new field of nano-forensics, Nano-sensors have become one important novel tool to the forensic scientist in criminal investigations (Prasad et al., 2020a). This with many other products and effects of nanotechnology has helped unravel lots of mysteries regarding certain crimes through identification and analysis of evidence which hitherto would not be identified or would have taken long periods in recognition and analysis.

**SUBDIVISIONS IN FORENSIC SCIENCE WITH NANOTECHNOLOGY APPLICATIONS**

Forensic science, as a discipline has many subdivisions with various scopes of functioning. These fields have various techniques and protocols in the performance of their functions in criminal investigations. Nanotechnology, since its inception, has found usefulness in enhancing the various existing techniques or creating advanced methods or mediums for investigations. Table 1 shows some interesting techniques or ways in which this new field has been utilized in enhancing forensic investigations. These applications represent a few of the techniques nanotechnology is being applied in the forensics field with lots of research also underway aimed at providing reliable and accurate results and speed of time as well as targeting particular challenges in terms of analysis of evidence in forensic investigations. Of the applications of nanotechnology, nano sensors seem to have been explored and utilized a lot for detection of explosives and heavy metals, enhancing fingerprints, detection of gun residues and in DNA fingerprinting. It is known as one of the novel approaches that provide conclusive evidence and eases the work of the forensic scientist.

**Nanotechnology in forensic chemistry and toxicology:** Forensic chemistry and toxicology encompass the area of forensics that deals with explosives, chemical warfare agents, fire and arson, petroleum products, drug detection, poisons and drugs and it is a critical discipline in the field of forensics with many cases. With the advancement in detection and analysis using nanotechnology, single-crystalline semiconductor CdS nano slabs were used on a silver surface in the detection of DNT (2,4-dinitrotoluene) (Ma et al., 2014), micro and nanofabricated structures, quantum dots, nanowires, nanotubes, nanobelts etc. are all nanosensors being used for explosives (TNT, RDX, HMX, DNT, PETN, TDX, Ammonium nitrate) detection and this is due to their heightened sensitivity and selectivity as well as low power consumption with im-
proved stability (Nanowerk, 2013). Chemical warfare agents have gained popularity in the execution of criminal activities in recent times and their detection is a major security concern to ensure the safety of both civilians and military personnel exposed to these chemicals (Zhao et al., 2016). These agents are extremely poisonous even at minute doses. Unfortunately, methods of detection of these agents lack sensitivity, selectivity, portability and rapid response requiring centralized laboratories, vast human and analytical resources and therefore cannot be used on the field. Hence the need for micromechanical sensors such as microcantilevers with functionalized TiO$_2$ nanorods for the detection of organophosphorus chemical agents (Biapo et al., 2019). Additionally, trace organophosphorus molecules were detected with the help of a metal-organic framework (MOF) Uio-66 film on Parylene-patterned resonant microcantilever which is highly sensitive to OP vapors (Shengran et al., 2019).

Forensic chemistry and toxicology also address issues related to drugs and their detection. The number of drug-facilitated crimes (DFC) keeps increasing over the years, which includes rape or other sexual assault, robbery, money extortion etc. The conventional techniques of TLC, HPLC, GC-MS, LC-MS, HPTLC, HPLC/MS/MS, FTIR, NMR, MS, UV-Vis and Raman spectroscopy are normally used, but these are methods that require the skills of a professional, are tedious, costly, time-consuming and can only be carried out in a laboratory. For this reason, portable nanosensors that can be used in the field where these drugs are seized have gained popularity in the field of analytical chemistry. Their excellent sensitivity and selectivity enable them to be the technique of choice for drug detection and analysis (Lad et al., 2016). For the detection of morphine, cobalt oxide nanoparticles, graphene and ionic liquid crystal modified on a carbon paste electrochemical sensor was fabricated in the study conducted by Atta et al. (2019). Dashtian et al. (2016) detected morphine in urine samples using new imprinted polymer-supported on multiwalled carbon nanotubes magnetized with Fe$_3$O$_4$ nanoparticles (MWNT-Fe$_3$O$_4$-NPs) which yielded satisfactory response feasible for analysis of morphine in urine and water. Subsequently, magnetic solid phase extraction based silane-modified magnetic nanoparticles by HPLC/diode array detection (DAD) was applied for the detection of ultra-trace amounts of morphine in human hair samples for the diagnosis of morphine addiction (Boojaria et al., 2015). Another narcotic substance of forensic interest is methamphetamine which is a recreational drug that stimulates the central nervous system and used for its euphoric properties. Its abuse continually grows and is the cause of major social problems. Its detection in urine is the easiest means of analysis but the conventional methods of liquid-liquid extraction and solid phase extraction (SPE)
### Table 1. Applications of nanotechnology in Forensic science.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Forensic Science</th>
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<td>(Ma et al., 2014)</td>
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<td>Drug detection</td>
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References:
- (Kasas, 2015)
- (Sivakumar et al., 2016)
- (Ranville, 2018)
- (Thayer et al., 2019)
- (Promsawan et al., 2019)
- (Sivakumar et al., 2016)
- (Prasad et al., 2020b)
- (Ranville, 2018)
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- (Ranville, 2018)
- (Promsawan et al., 2019)
- (Sivakumar et al., 2016)
which have a high recovery rate, greater selectivity and sensitivity, producing less toxic waste requires a more simple and easy method and hence the application of magnetic nanoparticles (MNPs) such as nano graphene oxide (Taghvimi et al., 2016). This method was confirmed by Fourier transform infrared spectroscopy (FTIR). Jing Wu et al. (2017), used magnetic lateral flow strip (MLFS) on magnetic beads (MB) and smartphone camera for the quantitative detection of cocaine in urine samples which could be read with an unmanned eye or smart phone camera.

With respect to poisons and toxins, nanotechnology has been used for forensic toxicological analysis where magnetic particles (MPs) in nanoscale is coupled with carbon nanotubes (CNTs) (Razmi and Jabbari, 2015), graphene oxides (GO) (Taghvimi et al., 2016), silicas, metal organic frameworks (MOFs) (Zhang et al., 2018) and molecularly imprinted polymers (MIPs) (Lingxin, 2016) has been used for extraction purposes (Liu et al., 2019; Kamaruzaman et al., 2017). Nanotechnology has also been used for the detection of illegally added dexamethasone to cosmetic products which has adverse effects such as skin atrophy, cutaneous reactivity, and some systematic side effects such as hypertension, diabetes mellitus etc. In its detection, a quick and efficient screening method has been developed by the preparation of magnetic molecularly imprinted polymers for selective dexamethasone recognition, extraction and determination (Du et al., 2018).

**Nanotechnology in Forensic biology and Biotechnology:** Forensic biology and biotechnology is an area of forensic science that deals with the analysis of biological evidence such as blood, semen, saliva, sweat, tears for the purpose of identification through DNA analysis and genetics. This area over the years has used short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) which fail to identify donors of biological trace evidences found at crime scenes through comparative DNA profiling. Therefore, the need for alternative methods capable of investigating, identifying and locating perpetrators. Forensic DNA phenotyping uses skeletal remains of missing persons and victims of disasters for identification purposes. However, eye, hair and skin color from DNA can be predicted using HirisPlex-S systems which in conjunction with SNP genotyping achieve essentially useful accuracies and have been validated for forensic analysis (Wirken et al., 2018). Identification is an important aspect of crime scene investigation with special importance to body fluids collected for analysis, nanotechnology has been used in this means using Raman microspectroscopy on the identification of individual red blood cells (Muro and Lednev, 2016).

Gold nanoparticle-protein nanopore, which is highly sensitive and specific has been used in detecting single-stranded DNA, illustrating a possibly rock-hard alternative for amplification-free, hybridization-based exogenous nucleic acids detection (Mereuta et al., 2020a). Post-PCR (polymerase chain reaction) lab is the utmost extensive forensic nanotechnology application in the field of forensic biology and biotechnology as it is fast and portable due to their small size and hence easy to use on the crime scene. For the extraction of DNA for quality PCR, magnetic nanoparticles, silica based magnetic nanoparticles and copper nanoparticles are used on biological evidences (significant body fluids and skeletal remains) (Lodha et al., 2016).

In relation to postmortem interval or time since death estimation by a pathologist (forensic pathologist) in forensic medicine, silver nanocluster (AgNCs) probe stabilized by DNA with simple and highly sensitive fluorescence aptasensors has been developed which selectively detect K⁺ ions in the vitreous humour of the eye (Ding et al., 2017) for PMI analysis. Subsequently, enzyme-PDA-CNPs-based electrochemical biosensor has been developed for the detection of hypoxanthine in vitreous humor to estimate post-mortem interval in forensic cases (Liao et al., 2020).

Saliva as a biological fluid commonly found in crime scenes contains various biochemical constituents such as water, hormones, enzymes, electrolytes, minerals, buffer, lipids, carbohydrates and cells which can be tested by DNA analysis for identification. The glycosylation of proteins in saliva which is affected by the biochemical environment (the body’s physiological and pathological states: disease, disorders, and host-microbiome interaction) can also be analyzed for identification purposes. This has necessitated the use of nanotechnology in this area by examining the post-mortem changes in saliva glycans by nano LC/MS and LC/MS/MS in determining the possibility of saliva glycosylation as a potential for PMI estimation (Kim et al., 2018).

**NANOTECHNOLOGY IN FORENSIC PHYSICS**

**Fingerprint:** The science behind fingerprint identification and development is the use of fingerprint powder which binds to the sweat and residue produced on the palm. This procedure of powder method has a downside due to its ability to adhere to both fingerprint and the background which makes obtaining clear images of the prints cumbersome thereby affecting the process of identification. Current advanced studies have applied nanotechnology to minimize and eliminate such challenges by using non-destructive micro X-ray fluorescence method, nanopowders for latent print visualization, photoluminescent CdS semiconductor nanocrystals capped with diocysulfo-succinate and ZnO-SiO₂ nanopowders for fingerprint development (Pandya and Shukla, 2018).

Nano-fingerprints is a new area that employs the use of nanotechnology in enhancing fingerprint development. Patent prints visible to the unaided eye are directly used in the process of investigation, but latent prints have to be developed prior to analysis, silver
nanoparticles stabilized by cationic surfactants was a new development in nanotechnology used but it also suffers lack of reproducibility on poor contrast and hence an advanced process of Multimetal Deposition (MMD) method. This (MMD) uses AuNPs through ionic interactions to bind to the fingerprint residue. In recent times, there have been novel nanotechnology applications in fingerprint development such as zinc sulfide/cadmium selenide nanoparticles used to enhance and visualize fingerprints under UV light (Kaushik et al., 2017); the silver physical developer (Ag-PD) method, silica nanoparticles (SiO$_2$-NPs) and aluminium oxide nanoparticles used in fingerprint development (Prasad et al., 2020b).

**Hairs and fibres:** Evidences as minute as fibre and hair can be found by carefully and strategically searching the crime scene. These evidences are easily escaped due to their size and hence the extensive studies using optical microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray microdiffraction. Currently, the non-destructive method of Nanoscope III multimode atomic force microscopy (AFM) is used to facilitate investigations of the structure of hair and fibre (Dupres et al., 2004). Also, using a nano-indentation technique where a sharp diamond indenter is pressed against the hair sample gives an accurate result. Hence, the correlation between the nanomechanical properties and the cellular structure of human hair to determine how the environment affects the identification of hair samples (Å and Chen, 2006). There is also the nano-chemical measurements using atomic force microscopy nanoindentation for micro-fibre analysis in determining the radius of the fibre, the effect of coating on the fibre's nanomechanical measurements (Clifford et al., 2012).

**Firearms and Ballistics:** A major area of forensic interest is that of ballistics (the science that deals with the study of firearms). Here, gunshot residues are collected and analyzed using SEM-EDX and AAS for the detection of the inorganic elements lead, antimony, and barium. These methods are precise and sensitive, time consuming and yet requires the services of an expert and ultramodern equipment (Aksoy et al., 2015; Goudsmits et al., 2015; Promsuwan et al., 2019) thus, the need for a highly sensitive and selective method which is easy and rapid as preliminary means of testing. For this purpose, the recent construction of electrochemical sensors based on the chemical alteration of electrodes using noble metal particles like platinum (Pt), gold (Au), silver (Ag) and palladium (Pd) on the nano and microscale level, as it heightens the active surface area as well as the electrocatalytic properties of an electrode (Promsuwan et al., 2019). To further increase the catalytic properties of these noble particles especially Pd, they can be synthesized on carbon nanotubes, graphene, and carbon microspheres (Li et al., 2017; Zhang et al., 2015; Yang et al., 2015).

**Bloodstain analysis:** Bloodstains found on the crime scene are evident that someone was harmed and upon analysis allow for identification of either the victim or the perpetrator through DNA profiling. Also, the bloodstain pattern allows crime scene investigators and forensic scientist to understand what happened on the scene and then be able to reconstruct it. This has necessitated studies using force spectroscopy (FS) to estimate the age bloodstain at a crime scene, from the time of deposition in order to estimate the time of occurrence of the incident (Smijn et al., 2016). Likewise, atomic force microscopy (AFM) has been used in estimating the time since death (TSD) of red blood cells in bloodstains (Cavalcanti and Silva, 2019).

**Questioned documents:** The sophistication of crime does not exclude the forgery of documents for criminal purposes. These include forging of signatures, hand-writings, stamps, passports, banknotes, licenses, certificates, identity cards, tickets and cheques. The examination is usually done on the chemical composition of the ink (pens, pencils, photocopiers, labels, stamps etc.) which uses optical microscopes with filters to augment the disparity between inks and optical excitation using different wavelengths. Document examination is done by analyzing, comparison, evaluation and verification/validation using excellent eyesight, hand lens, stereomicroscope, electrostatic detection device (EDD) and video spectral comparator (VSC). This requires analytical methods analyzing chemical compositions of inks by determining the relative age (timing) and identifying crossing lines (Rodrigues et al., 2019). Various methods has been used including spectroscopy in the IR region, Raman spectroscopy (Mar et al., 2015), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary electrophoresis, GC-MS, mass spectrometry coupled electrospay ionization (ESI-MS) etc. Advancements in technology allow interfacial engineering use via polymer-grafted nanoparticles to improve the performance of parts created by 3D printing (Street et al., 2020). Coupling Raman spectroscopy with atomic force microscopy (AFM) gives a high resolution providing both chemical and morphological properties for the analysis of Brazilian driver licenses, national and international banknotes, as well as distinguish between genuine and forged documents (Almeida et al., 2016).

**CHALLENGES AND FUTURE PERSPECTIVES**

Nanotechnology, with its improvement in various areas as well as Forensic science, has a lot of challenges. The novelty of the field and its application brings to bare concerns regarding its impact on the health and safety of users’ or workers (Iavicoli et al., 2014) in this case, a forensic scientist. There is also a growing concern on the toxicological profile as well as the fate of materials from nanotechnology in the environment which environmentalist are concerned about in terms
of bioaccumulation in microbes, plants and animals which also poses a risk.

Another overwhelming challenge is the cost involved; nanotechnology is an expensive area taking into account development and manufacture of equipment and research towards it hence only the developed countries or well-resourced forensic labs can purchase and utilize its related equipment.

These challenges will require some extra efforts from various quarters including governments and forensic research institutions to limit the associated risks as well as cost involved in the manufacture and purchase of nanotechnology materials for use by forensic labs. Future developments in this field need to be focused on on-field equipment as well as readily and potable materials. Also, lots of training needs to be done to enlighten various forensic scientists on this newly emerging field to be abreast with the latest developments and techniques in the field in relation to nanotechnology.

Conclusion

Overall, this review highlights the significance of this multidisciplinary scientific field in forensic investigations discussing applications such as the use of nanographene oxide in the detection of methamphetamine, the use of single-crystalline semiconductor for identification of explosives as well as gold nanoparticles in enhancing DNA identification. Some accounts have also been given on cobalt oxide nanoparticles modified into detecting morphine and Post mortem analysis using silver nanocluster probes for K+ estimation. Some significant nano-based substances used in latent fingerprint’s development and detection has also been discussed. These and many other advancements and developments in this review shows how promising nanotechnology is and the tremendous potential and advantages its application has to the various disciplines in forensics compared to the conventional methods. This field will bring a total transformation in crime investigations speeding up the time for analysis and providing better and accurate results as well as assembling evidences which earlier techniques failed to identify or locate. Despite all the numerous merits, further research has to be done to resolve the challenges in relation to safety and cost.

Conflict of interests

The authors declare that they have no conflict of interests.

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