

Research Paper: A Novel Nanoparticle-Based Method to Isolate DNA From Dried Saliva and Semen Samples



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Citation Bagban MA, Mansuri R, Jain N. A Novel Nanoparticle-Based Method to Isolate DNA From Dried Saliva and Semen Samples. JAMSAT. 2018; 4(1):13-20.



Article info:

Received: 01 Nov 2017

Accepted: 10 Feb 2018

Available Online: 01 Mar 2018

Keywords:

Nanoparticles, DNA, Saliva, Semen

ABSTRACT

Objectives: Saliva and semen stains may be found at crime scene (suicide, homicide, kidnapping, rape, sexual assault, poisoning, etc.). Through, which DNA can be isolated and individual can be identified. DNA can be of great importance in forensic science investigation to identify individuals. Currently, there are enormous methods to isolate DNA from biological fluids. The current study employed nanoparticle–biomolecule conjugate method to isolate DNA.

Materials & Methods: A nanoparticle-biomolecule conjugate is a magnetic nanoparticle with biomolecules attached to its surface. Saliva and semen were collected using cotton swabs from different surfaces (tile, steel, aluminum, glass, iron, wood, etc.) on which saliva and semen were poured and allowed to dry earlier as to create simulated crime scene. Magnetic nanoparticles (Fe₃O₄) act as solid phase support to isolate DNA from saliva and semen stains. In the current study, cells and spermatozoa were extracted from saliva and semen swabs, respectively. Cell lysis was performed to extract epithelial cells from saliva and spermatozoa from semen and magnetic nanoparticles and biomolecule conjugate were added to the lysed cells to bind to DNA.

Results: Application of external magnetic field helped to settle down nanoparticle-biomolecule conjugate and thus separate DNA from other cell components. Spectrophotometer Nanodrop technology was employed to perform the readings, which confirmed the presence of DNA at 260/280 ratio and quality of DNA was compared with the DNA isolated using the two different methods.

Conclusion: The current study aimed at developing all new methods to isolate DNA from dried saliva and semen samples using magnetic nanoparticles by reviewing the methods developed earlier to isolate DNA from blood and tissue using magnetic nanoparticles. With modifications in the method and chemical preparation, a new method was successfully developed. DNA was isolated successfully; and confirmation was made by analyzing DNA through Nanodrop spectrophotometer.

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Highlights

- A newly method is developed to isolate DNA based on nanoparticles from dried saliva and semen samples.
- This method is cost effective and provide a good quality and quantity of DNA.
- Novel nanoparticle-based method was compared with two other methods.

Plain Language Summary

Saliva and semen found at crime scenes can be considered significant and treated as such. Traditionally saliva at crime scenes has been documented and collected for identification of the suspect or victim through blood grouping and DNA analysis at a crime laboratory. Semen can also be useful for identification of an individual by DNA analysis. Saliva and semen samples, which may found in any crime scene, are in dried condition. Thus, for isolating the DNA from such dried samples, costly kits and skilled handling are required. In the present study, we have developed a nanoparticles-based method which is not only cost effective but also as effective as kit-based methods. The qualitative and quantitative analysis of isolated DNA from dried saliva and semen samples was done and comparison was also made with two different methods.

1. Introduction

DNA Isolated from saliva turned into an attractive alternative fluid to the application of blood-derived DNA in molecular field, and currently it is widely used in numerous applications; concern in quick and less destructive analytical tests exponentially grew up in the previous era, which led to widespread research on saliva as a biological material for clinical diagnostic analysis [1]. The salivary fluid has certain benefits compared to urine and blood, two of the utmost used diagnostic fluids in laboratories. Despite the ease in the collection of the saliva sample and noninvasive demanding and comparatively modest instructions for collection, it retains lower protein content, fewer complication and variable composition than serum [2].

The DNA analysis procedure conducted in criminological laboratory is occasionally time consuming, ensuing in the statement that the outcome is not significant and capable to efficiently contribute to the preliminary stage of the criminal case investigation by the police services [3]. Reference samples should be collected along with the crime scene samples in an appropriate manner, which is fast and trouble-free and does not affect the suspect or victim. Usually, swabs are prepared for this determination [4]. Biological evidence is considered as the utmost significant kind of evidence (particularly semen); subsequently, it is quite valuable to prove the occurrence of physical/sexual intercourse and recognize a culprit through DNA identification in rape cases [5-9].

Semen (spermatozoa in the seminal liquid): it is hardly present in oral cavity up to six hours, in anorectal cavity up to 24 hours, and in vaginal cavity up to 72 hours after sexual intercourse [10]. Due to the low possibility of collecting semen sample from victim's body just after a few hours, semen samples collected from the various surfaces and clothing and fabric materials of suspect or victim supported the identification.

The nonappearance of spermatozoa may happen if the suspect is vasectomized, azoospermic, or semen stains dry [9]. Since it is valuable to prove the incidence of sexual intercourse and identify the accused, currently, biological evidence for DNA analysis is considered the most significant evidence for legitimate proof in courts of law [11]. DNA extraction procedures are progressively more in effect since it is possible to get purified DNA from biological evidence. These kinds of DNA extraction procedures develop with respect to the time and some advanced studies in progress.

At present, most of the frequently used procedures of DNA isolation are based on organic extraction in which SDS (Sodium Dodecyl Sulfate) and proteinase-K are used to breakdown the cell membrane and protein digestion. During this procedure, accumulation of proteinase-K quickly deactivates nucleases such as DNases enzyme and RNases enzyme, which might damage DNA during the extraction procedure. Subsequently, DNA is purified by phenol-chloroform mixture; centrifugation is done, and then DNA is precipitated out using alcohol (especially ethanol) and formerly it was re-suspended in buffer containing low salt [12].

Magnetic nanoparticles can be influenced by magnetic fields. Such particles usually comprise two constituents, a magnetic material, which comprises iron, cobalt, and nickel and a chemical constituent that has functionality. The purification of the plasmid or genomic DNA using magnetic materials such as matrix or bead is already tried in various biological samples [13]. The naked or uncoated magnetic nanoparticles (Fe_3O_4) have a specific property to bind reversibly to the DNA under certain circumstances. There are numerous characteristic advantages in which elements are getting adsorbed to the magnetic substance.

The particle size is smaller (100 nm) due to lack of polymer coating, which offers a better surface area to bind to biomolecules. This furthermore permits the particles to have the greatest susceptibility to the magnetic field applied from outside. Additionally, magnetic nanoparticles act as steady colloidal suspension, which do not aggregate or permit even for distribution in a reaction blend. Thus, Saiyed Z M et al., recently stated that naked magnetic nanoparticles can be used as support material to extract or isolate genomic DNA from the cells of mammals [14]. The current study aimed at creating a novel approach to extract genomic DNA using magnetic nanoparticles from dried saliva and semen samples.

2. Materials and Methods

Sample collection and preparation

Saliva and semen samples were the major requirements for the study. Saliva samples were collected from volunteers of Department of Life Sciences, Gujarat University, Ahmedabad, India with care and under the guidance of expert with consent. Semen samples were collected from the Sunflower Laboratory with official consent. In almost all crime scenes when saliva and semen are found as evidence they are in dried form. Therefore, in the current study, the crime scene was simulated in the laboratory.

For the forensic analysis, the collected saliva and semen were left to dry on different surfaces. DNA isolation was performed by preparing the cotton swabs similar to the forensic laboratories. Saliva control and saliva swabs from all surfaces were made from the first day at the interval of five days up to 60 days. Each swab was used for DNA isolation using different methods mentioned below: 1. Conventional method, which is widely used [15], was slightly modified 2. By GeneiPure™ Mammalian Genomic DNA Purification Kit; and 3. Magnetic nanoparticles-based method.

Preparation of magnetic nanoparticles

For this purpose, 1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (2.78 g in 10 mL distilled water) (Merck) was mixed with 2 M FeCl_3 (3.24 g in 10 mL distilled water) (FINAR); 8 M NH_4OH was added with constant stirring at 24°C. Precipitations were observed. Impurities such as sulfate ions and chloride ions were eliminated by washing with warm distilled water. Precipitate particles were left to dry at 60°C in hot air oven for about 24 hours. Yield precipitates possess magnetic properties. These magnetic particles were stored in Tris EDTA (ethylenediaminetetraacetic acid) buffer (pH=8-8.5). Nanoparticle characterization was addressed using Malvern Analytical Nano sight NS300 instrument, which allows automated examination of the concentration and size dispersal of all types of nanoparticles ranging 10 to 2000 nm in diameter.

DNA isolation using magnetic nanoparticles

Saliva and semen swabs collected from different surfaces were left to dry. Each swab was kept in a microfuge tube. A 200- μL of Tris EDTA buffer was added to saliva swabs and 200 μL of acidulated water was also added to semen swabs for extraction of epithelial cells and spermatozoa, respectively. Mixed well for 30 minutes and swabs were removed and centrifuged at 3000 rpm for five minutes. Supernatant was removed carefully so that pellet of cells remained undisturbed. To this pellet, 200 μL of cell lysing buffer-I (hav-

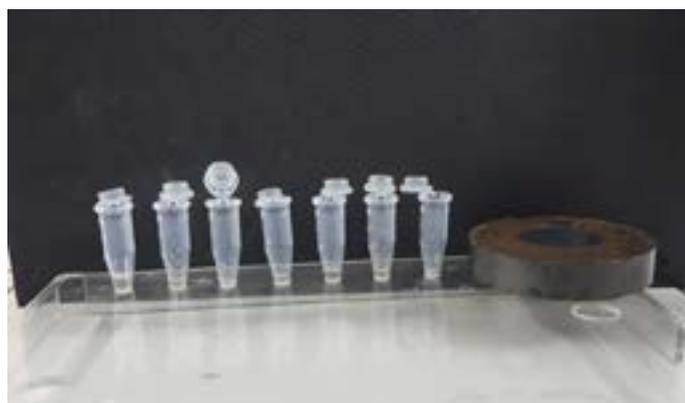
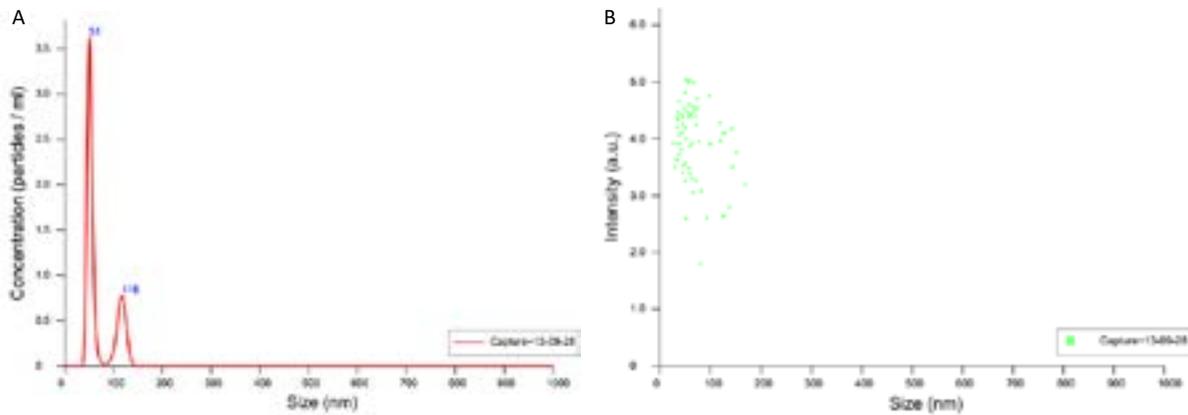


Figure 1. Application of external magnetic field to magnetic-biomolecule conjugate



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Figure 2. FTLA concentration/size and intensity/size graph for experiment

(A) Validation of a particle tracking analysis method to determine the size of nanoparticles; (B) Second graph is indicating that the high amount of intensity of magnetic nanoparticles ranged 0 to 100 nm size and can be observed as green scattered dots

ing 10 mM Tris buffer, 10 mM KCl, 10 mM MgCl₂, 0.5 M NaCl, 2 mM EDTA, 1% SDS dissolved in 50 mL distilled water) for saliva and cell lysing buffer-II for semen (having 10 mM Tris HCl, 75 mM NaCl, 25 mM EDTA, 1% SDS and 21 µl DTT dithiothreitol) was added.

Solutions were incubated for 20 minutes at room temperature and then, 75-80 µL of magnet nanoparticle solution was added. Then 150 µL of binding buffer (1 M NaCl+10% ethylene diol) was added. The mixture was incubated for 30 minutes at 4°C. DNA bounded to magnetic nanoparticles to form magnetic-biomolecule conjugate [16]. With the help of external magnetic field this magnetic-biomolecule conjugate was separated from all other cell components in the solution as shown in Figure 1. Supernatant was discarded, 70% ethanol was added to wash magnetic-biomolecule conjugate and the solution was left to dry; 200 µL of Tris EDTA buffer was added and incubated for 20 minutes at 65°C on shaking in between (DNA was separated from magnetic nanoparticles in the solution). With the help of

external magnetic field, these magnetic nanoparticles were allowed to settle down leaving DNA in the solution. Tris EDTA solution residing dissolved DNA was collected in another microfuge tube.

DNA analysis

DNA extracted from the saliva and semen control and saliva and semen swabs were analyzed using agarose gel electrophoresis and Nanodrop spectrophotometer (Epoch Biotech). Each DNA sample to be analyzed was dissolved in 500µL of Tris EDTA buffer and absorbance ratio of 260/280 and the quality was observed by Nanodrop.

3. Results

Nanoparticle validation on nano-sight

According to Figure 2 the size of magnetic nanoparticles ranged 10 to 118 nm in which the high peak was obtained

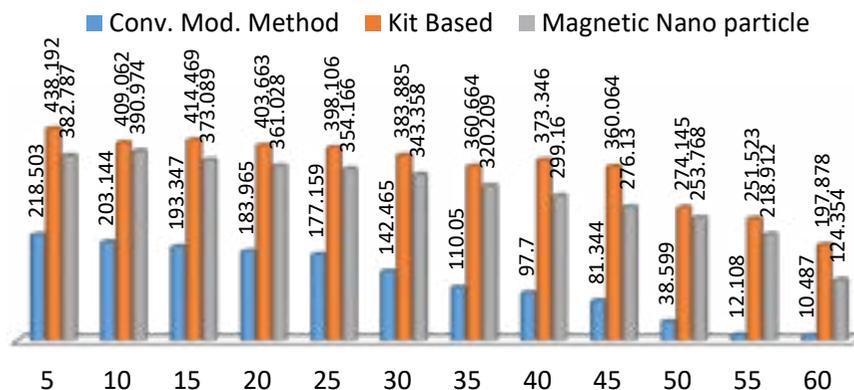


Figure 3. Comparison graph showing quantitative analysis of DNA extracted from saliva sample

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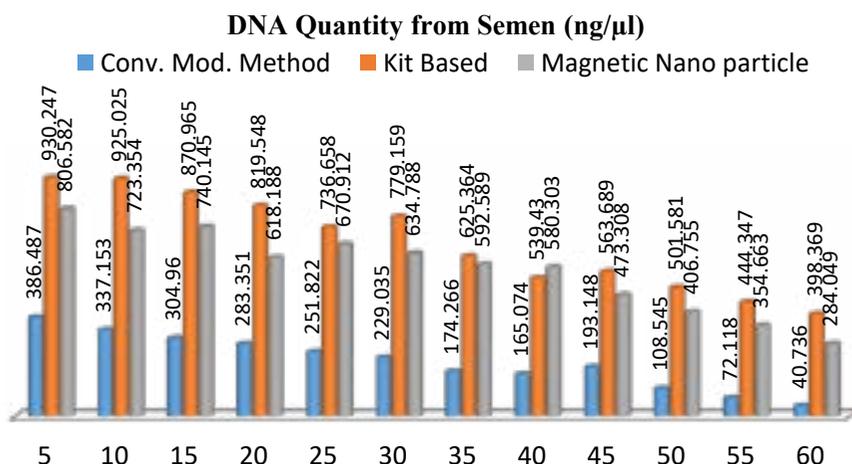


Figure 4. Comparison graph showing quantitative analysis of DNA extracted from semen sample

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Table 1. Qualitative data of DNAs extracted from Saliva and semen samples based on time period

Day	Ratio	Saliva Sample			Semen Sample		
		Conv. Mod. Method	Kit-Based	Magnetic Nanoparticle	Conv. Mod. Method	Kit-Based	Magnetic Nanoparticle
5	260/280	1.583	1.809	1.824	2.000	1.705	1.870
10	260/280	1.512	1.900	1.999	1.945	1.780	1.767
15	260/280	1.535	1.807	1.876	1.678	1.897	1.874
20	260/280	0.980	1.976	1.881	1.643	1.864	1.800
25	260/280	1.461	1.707	1.799	1.564	1.859	1.799
30	260/280	1.339	1.876	1.870	0.793	1.780	1.791
35	260/280	1.263	1.873	1.689	0.932	1.901	1.679
40	260/280	1.567	1.899	1.749	0.547	1.711	1.697
45	260/280	0.710	1.794	1.946	1.364	1.719	1.638
50	260/280	1.381	1.799	1.899	1.304	1.607	1.867
55	260/280	0.631	1.677	1.976	0.913	1.673	2.009
60	260/280	0.854	1.601	1.902	0.994	1.669	1.933

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at 51 nm size. For successful isolation of DNA from the sample, the size of magnetic nanoparticle should range 10 to 100 nm. As the maximum concentration of nanoparticles size was 51 nm, it can be useful to isolate DNA.

DNA analysis

For qualitative analysis of isolated DNA, the absorbance was measured at 260/280 nm ratio to evaluate the purity of DNA and RNA using Nanodrop Spectrophotometer. A ratio of ~1.7 is generally accepted as “pure” for DNA. If the

range is lower than 1.7, it shows the impurity of protein and if it is above 2.0 then it shows impurity of RNA.

Qualitative analysis of DNA

According to Table 1, for the saliva samples, 260/280 ratio ranged 0.631 to 1.583 by mod.¹ Conv.² method indicated the high amount of protein contamination up to 60 days while by kit-based method the ratio ranged 1.601 to 1.976 show-

- 1. Modified
- 2. Conventional

ing decent quality of DNA; and by magnetic nanoparticle-based method, the ratio ranged 1.749 to 1.999 showing good quality of DNA up to 60 days. For semen samples, 260/280 ratio ranged 0.793 to 2.000 for mod. Conv. method showed high contamination of protein as well as RNA, while by kit-based method ratio ranged 1.607 to 1.897 indicating good quality of DNA; and by magnetic-nanoparticle-based method, the ratio ranged 1.638 to 2.009 indicating slight contamination of RNA, which may be due to the interfering microorganisms in the beginning of DNA analysis.

Quantitative analysis of DNA

Quantity of the DNA is much more beneficial than that of the DNA in forensic examination. Figure 3 indicates the graphical representation of comparison of all the three methods for quantitative analysis of DNA extracted from saliva samples. Quantity of DNA obtained by mod. conv. method was 218.503 ng/ μ L on the day 5, which showed consistent descending manner and on the day 60 only 10.487 ng/ μ L. While in the kit-based and nanoparticle-based methods 438.192 and 382.787 ng/ μ L were amounts of DNA respectively, obtained on the day 5, and up to the day 60 quantity downgraded up to 197.878 ng/ μ L and 124.354 ng/ μ L by kit-based and nanoparticle-based methods, respectively. The amount of DNA obtained by magnetic-nanoparticle-based method on the day 60 was closely related to the amount obtained by kit-based method and the amount was also sufficient for further DNA analyses such as Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP).

Figure 4 indicates the graphical representation of comparison of all the three methods for quantitative analysis of DNA extracted from semen sample. Quantity of DNA obtained by mod. conv. method was 386.487 ng/ μ L on the day 5, which showed consistent descending manner and on the day 60 only 40.736 ng/ μ L obtained, while in the kit-based and nanoparticle-based methods the amounts of DNA were 930.247 and 806.582 ng/ μ L, respectively obtained on the day 5, and up to the day 60 quantity downgraded up to 398.369 and 284.049 ng/ μ L by the kit-based and nanoparticle-based methods, respectively. The amount of DNA obtained by magnetic-nanoparticle-based method on the day 60 was closely related to that of obtained by kit-based method and the amount was sufficient for further DNA analyses such as PCR and RFLP.

4. Discussion

Magnetic separation techniques based on magnetic particles covered with diverse polymers such as silica or agarose are used progressively for molecular-based bio-

logical applications [17]. Hawkins TL et al., explained the mechanism of carboxyl coated magnetic particles such as BioMag coated with carboxyl used to adsorb the nucleic acids (DNA) in pure form in high-salt environments. They also showed that magnetic (Fe_3O_4) particles produced by the co-precipitation technique precisely adsorbed the amphoteric hydroxyl ($-\text{OH}$) group that leftovers on the particles between pH range of 6.0 to 10.0 [16]. The naked or uncoated magnetic nanoparticles (Fe_3O_4) allow us to utilize their characteristics to bind to DNA reversibly under specific circumstances. There are numerous intrinsic benefits of the application of such naked nanoparticles, where magnetic support allows direct adsorption of biomolecules. The smaller particle sizes (≤ 100 nm) provide a higher surface area for the attachment of the biomolecules due to the absence of polymer coating; this furthermore permits the particles to have the greatest magnetic susceptibility to the applied magnetic field [16].

5. Conclusion

The current study aimed at developing all new methods to isolate DNA from dried saliva and semen samples using magnetic nanoparticles by reviewing the methods developed earlier to isolate DNA from blood and tissue using magnetic nanoparticles. With modifications in the method and chemical preparation, a new method was successfully developed. DNA was isolated successfully; and confirmation was made by analyzing DNA through Nanodrop spectrophotometer. A kit-based method is expensive whereas mod. conv. method shows poor results as compared with the other two methods. Thus, the developed magnetic-nanoparticle-based method is reliable and cost effective as well as less time consuming compared with the other methods.

Ethical Considerations

Compliance with ethical guidelines

This study have been approved by the Research Ethics Committee of Gujarat University. Informed consent was obtained from all individual participants included in the study.

Funding

The current study was funded by UGC MANF Scheme (MANF-2013-14-MUS-GUJ-23554).

Authors contributions

Conceptualization: All authors; Methodology: MohammedAzim Bagban & Mansuri Reshma; Investigation: MohammedAzim Bagban & Reshma MANSuri; Writ-

ing –original draft: MohammedAzim Bagban; Writing –review & editing: MohammedAzim Bagban & Nayan Jain; Funding acquisition: MohammedAzim Bagban; Resources: All authors; Supervision: Nayan Jain.

Conflict of interest

The authors declared no conflict of interest.

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