Application of Gelatin-Coated Magnetic Particles for Isolation of Genomic DNA from Bones

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Objective: *To develop a method for human genomic DNA extraction from bone using gelatin-coated magnetic particles.*
Material and Method: *Thirty human metacarpal with the bone age ranging from 36 to 93 years were included in the present study. Genomic DNA was extracted from bones using gelatin-coated magnetic particles. The concentration and purity of DNA were analyzed in comparison with a reference method. In addition, the quality of extracted DNA was examined for sex determination by conventional polymerase chain reaction (PCR).*
Results: *The average DNA concentration using gelatin coated magnetic particles exhibited approximately 15 times higher than a reference method with an insignificantly difference of the DNA purity in both methods. Twelve (40%) and fifteen (50%) samples out of thirty DNA isolated using established and reference method, respectively, could be amplified and sex correctly determined by PCR.*
Conclusion: *Gelatin coated magnetic particle is rapid, simple, and well-suited for isolation of DNA from bones.*

Keywords: *Gelatin-coated magnetic particles, Sex determination, polymerase chain reaction (PCR)*

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The identification of victims is necessary whether it is from disasters, traffic accidents, or crimes. In these events, sometimes bones are the only remaining source of DNA. During this decade, a number of sex determination assays using DNA analysis have been demonstrated. The most common gene for sex typing is amelogenin and it can be performed in conjunction with short tandem repeat (STR) analysis[1]. Several methods have been developed for isolation of genomic DNA from bones such as traditional phenol-chloroform extraction, combination of cetyltrimethylammonium bromide (CTAB) and isoamyl alcohol-chloroform extraction[2], and silica-based membrane purification[3]. Magnetic particles have been synthesized for isolation and purification of several biomolecules including DNA[4]. In addition, polymer-coated surface of magnetic particles such as silica[5], aminosilane[6], and poly-ethylenimine[7] enable improving the separation ability and recovery yield of DNA. Recently, gelatin-coated magnetic particles were formulated for isolation of DNA from bacterial cells[8] and human dried blood[9].

In the present study, gelatin-coated magnetic particles were developed for isolation of human genomic DNA from bones. Human metacarpal bones were utilized as a model for DNA isolation. The DNA quantity and purity were measured in comparison to a reference silica membrane purification kit. In addition, sex determination detected by the conventional polymerase chain reaction (PCR) targeting to amelogenin gene was conducted for DNA quality determination from both methods. Finally, the overall extraction time and cost effectiveness between the validated method and the commercial kit was compared.

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Material and Method

Bone samples

Thirty left metacarpal bones of Thai cadavers consisting of 15 male and 15 female were

collected from the Cadaveric and Surgical Training Center, Department of Anatomy, Faculty of Medicine, Chiang Mai University, Thailand. After dissection, the bone surface was removed, cleaned with 6% sodium hypochlorite, 70% alcohol, and exposed to UV light for 24 hours[10]. The treated bone was milled using pulverizer (Spex Certiprep 6750 Freezer/Mill Cryogenic Grinder, USA) and stored at -20°C until DNA extraction.

Preparation of gelatin-coated magnetic particles

Artificial gelatin-coated magnetic particles were prepared according to the procedure described elsewhere[8,9]. Briefly, microwave-melting gelatin (15%, w/v) was mixed with divalent and trivalent iron ions and coprecipitated in alkaline solution. The coated magnetite suspension was washed repeatedly with nitrogen-flushed deionized water and lyophilized for overnight. Dried magnetic particles were stable at 4°C for at least one year. The mean particle size was analyzed by a particle size analyzer (ZetasizerNano ZS, Malvern Instruments) and was revealed to be 600 nm.

DNA extraction using gelatin-coated magnetic particles

Gelatin-coated magnetic particles were applied for genomic DNA extraction in the present study. One hundred milligram of powdered bone was added with 600 µl alkaline lysis solution (0.2M NaOH and 1% [w/v] sodium dodecyl sulfate), vortexed vigorously and allowed to stand at room temperature for five minutes. The mixture was added with 50 µl proteinase K (10 mg/ml) and further incubated at 56°C for overnight. After centrifuged at 12,000 rpm for five minutes, the supernatant was carefully transferred to a new microcentrifuge tube. Fifty microliter of gelatin-coated magnetic particles (10 mg/ml) in binding buffer (4M NaI and 20% (w/v) polyethylene glycol) was added, followed by the addition of 400 µl binding buffer, gently inversed, and incubated at room temperature for five minutes. The magnetic particles were immobilized by the external magnet, and the supernatant was completely removed. The magnetic pellet was washed twice with cold 70% ethanol and dried at room temperature for 10 minutes. Finally, the magnetic particles were suspended in 30 µl of TE buffer (50 mM Tris-HCl and 1 mM EDTA, pH 8.0), and the bound DNA was eluted by incubation at 65°C for five minutes with gentle agitation. The unbound DNA solution was transferred to a new microcentrifuge tube and stored at -20°C until determination.

DNA extraction using the commercial kit

In the present study, silica based membrane purification kit (QIAamp DNA Micro Kit, Germany) was utilized as a reference method for genomic DNA extraction from bones. The procedure was performed according to the manufacturer's instruction. Briefly, 100 mg of fine powdered bones was added with 360 µl of ATL buffer, 20 µl proteinase K and 1 µl carrier RNA (1 µg/µl) and incubated at 56°C for overnight. Three hundred microliters of AL buffer were added and incubated at 70°C with shaking for 10 minutes. After centrifugation, the supernatant was carefully transferred to the column. Column was washed twice with AW buffer and DNA was eluted with 30 µl AE buffer in a clean microcentrifuge tube and kept at -20°C until determination. All buffers used herein were supplied from the kit.

Determination of quantity and purity of DNA

DNA quantity of the validated method was analyzed in comparison to a silica based membrane technology by UV spectrophotometer (BioTek Instruments, Inc., USA). In addition, an average OD260/OD280 ratio was measured for DNA purity analysis.

PCR reaction

Conventional PCR was performed for DNA quality determination through amelogenin gene amplification. PCR reaction in 25-µl containing 12.5 µl of 2x pre-mixed reaction, 10 pmol of X- and Y-specific primers (Table 1), and approximately 5 µl of DNA template. The PCR profiles were as followed: pre-denatured at 94°C for two minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 30 seconds, with a final extension at 68°C for five minutes. After amplification, the amplified products of X (108 bp) and Y (203 bp) was electrophoresed through a 3% agarose gel, stained with ethidium bromide and visualized by

Table 1. The nucleotide sequence of primers used in the present study

Primer	The nucleotide sequences (5'-3')
X-specific forward	TCTGACCAGCTTGGTTCTA
X-specific reverse	AGTGTGACTATCTTAGAATCAGGAG
Y-specific forward*	GGTCCCAATTTTACAGTTCC
Y-specific reverse*	CTGGTCAGTCAGAGTTGAC

* Followed by Nogami et al.[16]

Table 2. The summary of recovery yield, purity and cost per test of gelatin coated magnetic particles and commercial kit

Method	Average recovery yield (ng/ml)	Purity (OD ratio 260/280)	Cost per test (Baht)
QIAamp DNA micro kit	10.6 (0.0-101.5)	1.49 to 2.01	240
Gelatin coated magnetic particles	152.3 (11.7-791.1)	1.48 to >2.00	10

UV transilluminator (G:BOXChemi XRQ gel doc system, UK).

Result and Discussion

Gelatin coated magnetic particles were established for genomic DNA extraction from bone in the present study. The absorbance at 260 nm and the OD ratio of 260/280 nm were measured for DNA quantity and purity determination, and compared to the commercially available DNA extraction kit. The result indicated that genomic DNA recovered by the developed method was approximately 15 times higher than the kit with an average DNA of 152.3 µg/ml (11.7-791.1 µg/ml) and 10.6 µg/ml (0.0-101.5 µg/ml), respectively. The OD ratio of each bone sample extracted by in-house method and kit was compared and they seemed to be insignificantly different ranging from 1.48 to >2.00 and 1.49 to 2.01, respectively (Table 2). Generally, the OD ratio of approximately 1.8 of extracted DNA was suitable for further analysis with negligible protein contamination[11]. However, the low and high OD ratio observed after extraction in some of these samples (19 samples for the validated method and 21 for the kit) would be involved with the contamination of biomolecules such as proteins, nucleotides, RNA, and single-strand DNA[11]. Moreover, the acidity change in the samples would be the cause of the variation of OD ratio in which acidic and basic solution could under and over-represent OD ratio by 0.2 to 0.3, respectively[12]. Due to the lack of decontamination step, increasing absorbance to 260 nm may come from nucleotides, RNA, and single stranded DNA, which could be absorbed. Therefore, magnetic particles have an advantage in the greater ability to adsorb DNA than proteins and single-strand RNA in the presence of high salt concentration[5,13].

Sex determination using amelogenin gene amplification by PCR was subsequently performed for quality determination of extracted DNA. Initially, the previously reported single pair of primers specific for the first intron of amelogenin was investigated. A quantity of 106-bp and 112-bp PCR product was generated from the X and Y homologues[14]. The 3.5% agarose gel electrophoresis was performed and the result gave a good outcome whereas an ambiguous result in some samples were observed with the presence of a thick band of 106-bp, which was indiscriminated from the two bands of 106-bp and 112-bp and lead to a misidentification of sex (data not shown). Although the PCR reaction with primer specific for X and Y chromosome utilized in the present study was optimized for multiplexing, some of non-specific amplification was observed (data not shown). Thirty bone samples in which DNA were individually extracted from both methods were examined of which only 12 (40%) and 15 samples (50%) using established and reference method, respectively, could be amplified. The positive and negative amplification by PCR of some samples extracted from both methods were shown in Fig. 1. Several factors could affect the ability of gene amplification including the bone age and PCR inhibitors in individual samples[15]. In addition, the average time for the whole extraction process were compared and found insignificantly different between the two methods. The cost per test of the validated method was approximately 24 times cheaper than the commercial DNA extraction kit (Table 2).

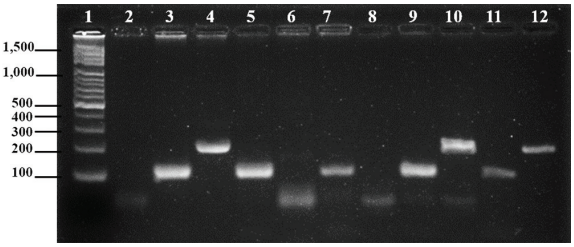

Fig. 1 The 3% agarose gel electrophoresis of approximately 108 and 203 bp amplified products specific for human amelogenin gene on X- and Y-chromosomes, respectively. DNA was extracted from metacarpal bones using a reference silica based membrane purification kit and the validated gelatin-coated magnetic particles. Lane 1, standard DNA marker; lane 2, negative amplification control (distilled water); lanes 3-4, positive amplification control of X and Y chromosome, respectively; lane 5-6 and 9-10, amplified product of female (M5) and male (M28) sample extracted by using the kit; lane 7-8 and 11-12, amplified product of female (M5) and male (M28) sample extracted by using magnetic particles.

Conclusion

In conclusion, gelatin-coated magnetic particles are implemented in the present study for human genomic DNA isolation from metacarpal bone. This technique is rapid, simple, and cost-effective. More studies with larger number of bone samples should be performed to ensure possible application in forensic medicine.

What is already known on this topic?

The identification of individuals is necessary and bones are sometime the only remaining source of DNA. The most common gene for sex typing is amelogenin. This can be analyzed in conjunction with STR analysis. Several methods have been developed for isolation of genomic DNA from bones. Magnetic particles have been synthesized for isolation and purification of several biomolecules including DNA. In addition, polymer-coated surface of magnetic particles enable improving the separation ability and recovery yield of DNA. Gelatin-coated magnetic particles were formulated for isolation of DNA from bacterial cells and human dried blood.

What this study adds?

Gelatin-coated magnetic particles are implemented for human genomic DNA isolation from metacarpal bone. This technique is rapid, simple, and cost-effective.

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Potential conflicts of interest

None.

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การประยุกต์ใช้อนุภาคแม่เหล็กเคลือบด้วยเจลาตินเพื่อการแยกจีโนมิกส์ดีเอ็นเอจากกระดูก

พงษ์ศักดิ์ ขันธ์เพ็ชร, สรศักดิ์ อินทรสูต, สุคนธ์ ประสิทธิ์วัฒนเสรี, กานดา เมฆใจดี, ผาสุก มหรรฆานุเคราะห์

วัตถุประสงค์: *เพื่อพัฒนาวิธีการแยกจีโนมิกส์ดีเอ็นเอมนุษย์จากกระดูกโดยใช้อนุภาคแม่เหล็กเคลือบด้วยเจลาติน*

วัสดุและวิธีการ: *การศึกษาในครั้งนี้ได้เก็บรวบรวมกระดูกฝ่ามือของผู้อุทิศร่างกายจำนวน 30 ชิ้น ที่มีอายุอยู่ระหว่าง 36 ถึง 93 ปี ทำการสกัดจีโนมิกส์ดีเอ็นเอจากกระดูกโดยใช้อนุภาคแม่เหล็กเคลือบด้วยเจลาติน เปรียบเทียบความเข้มข้นและความบริสุทธิ์ของดีเอ็นเอที่ได้กับชุดสกัดสำเร็จรูปแบบการทำให้บริสุทธิ์ด้วยเยื่อวุ้นสารประกอบซิลิกา ตรวจสอบคุณภาพดีเอ็นเอที่สกัดได้เพื่อใช้ในการตัดสินเพศโดยเทคนิคพีซีอาร์แบบดั้งเดิม*

ผลการศึกษา: *ค่าความเข้มข้นเฉลี่ยของดีเอ็นเอที่แยกโดยใช้อนุภาคแม่เหล็กเคลือบด้วยเจลาตินมีความเข้มข้นสูงกว่าวิธีการสกัดที่ใช้อ้างอิงโดยประมาณ 15 เท่า โดยความบริสุทธิ์ของดีเอ็นเอที่ได้ไม่มีความแตกต่างกันในทั้งสองวิธี จากจำนวนตัวอย่าง 30 ตัวอย่าง สามารถเพิ่มปริมาณดีเอ็นเอและตัดสินเพศจากกระดูกด้วยเทคนิคพีซีอาร์ได้อย่างถูกต้องโดยใช้อนุภาคแม่เหล็กเคลือบด้วยเจลาติน 12 ตัวอย่าง (40%) และจากวิธีการสกัดที่ใช้อ้างอิง 15 ตัวอย่าง (50%)*

สรุป: *เทคโนโลยีอนุภาคแม่เหล็กเคลือบด้วยเจลาตินเป็นวิธีที่รวดเร็ว ง่าย และเหมาะสมในการตรวจพิสูจน์เพศจากกระดูกฝ่ามือมนุษย์*