Isolation of DNA from Human Saliva


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CONTENTS

I. INTRODUCTION
II. MATERIALS AND METHODS
III. RESULTS
IV. DISCUSSION
V. CONCLUSIONS
REFERENCES
KOREAN ABSTRACT

I. INTRODUCTION

The application of concepts and techniques developed in molecular genetics, immunogenetics, and developmental biology to the study of craniofacial-oral-dental disorders becomes a central issue in dentistry. Many scientists appreciate that with the possible exception of diseases caused by trauma, essentially all human diseases have some genetic components. These diseases include not only inherited diseases, but also viral, bacterial, and fungal infectious diseases, neoplastic diseases, cardiovascular and cerebrovascular diseases, and other craniofacial diseases. With developed methodologies, the diagnostic PCR (polymerase chain reaction) has been used for the diagnosis of several infectious diseases and forensic purposes, and has been also used as screening tests for breast cancer and cystic fibrosis in general population.

There have been also several reports on the application of DNA-based diagnosis in the field of dentistry, including detecting severity indicator of periodontitis, diagnosis of head and neck cancer, and screening of diagnostic markers associated with susceptibility to infectious and autoimmune diseases.

van Schie and Wilson analyzed Fc receptor allelic polymorphism of IgG using genomic DNA extracted from saliva and reported that saliva appeared to be an excellent source of DNA as a substitute of blood. The extraction procedures of DNA from saliva and saliva-stained materials for forensic use were also reported. However, there is little information on the standardized procedures associated with DNA from saliva.

The implication of saliva as diagnostic fluid has been recently refocused because of availability of molecular biological methodologies. Amount of analyte or concentration of molecule are no longer limitation of diagnostics because the sensitivity and specificity in diagnostic procedure were increased due to the development of newer micromethodologies and increasing availability of immunological and molecular biological reagents. In addition, the collection of saliva is very simple, safe, and non-invasive.

The purpose of the present study was to isolate genomic DNA from saliva and to compare DNA

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from saliva with that from blood and to investigate the changes of genomic DNA from saliva according to the storage temperature and period of saliva samples. Based on this study, the diagnostic ability and forensic application of DNA from saliva will be considered.

II. MATERIALS AND METHODS

1. Collection and storage of saliva

The first experiment was about to compare extracted DNA from blood with one from saliva. Unstimulated and stimulated whole saliva samples were collected from 10 healthy adults (mean age 36.9 ± 11.5 years). Collection of saliva was performed between 9:00 am - 12:00 am to minimize effects of the diurnal variability in salivary compositions. About 2 ml of salivary samples from the subjects were collected into the chilled sterile 15 ml polypropylene tube before meals or at least two hours after meals. Unstimulated whole saliva was collected by the spitting method (i.e. after swallowing, saliva is collected with closed lips, and then all saliva is expectorated into a vessel one or two times per minute). In the case of stimulated whole saliva, the chewing of gum base (about 1 g) was used as mechanical stimulus. Obtained saliva sample was used immediately for extraction of DNA.

The second experiment was about to investigate the changes of DNA from saliva stored at various temperatures during different periods. About 20 µl of stimulated whole saliva samples were collected from 10 healthy adults. Salivary aliquots were made by distribution of each 600 µl saliva into sterile 1.5 ml microcentrifuge tube and were stored at room temperature, 4°C, -20°C and -70°C until examination for 5 months.

2. Extraction of genomic DNA from saliva

Genomic DNA was extracted from saliva using a commercial DNA purification kit (Wizard® Genomic DNA Purification kit, Promega Co., WI, USA). Each 600 µl of prepared saliva sample was added into the 1.5 ml microcentrifuge tube containing 900 µl of cell lysis solution and the tube was inverted 5-6 times to mix. Samples were incubated for 10 minutes and centrifuged at 13,000-15,000 xg for 20 seconds at room temperature. Supernatant was carefully removed as possible without disturbing the visible white pellet. Nuclei lysis solution (100 µl) was added into the tube, and the mixture was pipetted 5-6 times and incubated at 37°C until the clumps are disrupted. If the clumps were still visible after 1 hour, nuclei lysis solution was added. Into the tube, 1.5 µl of RNase solution was added, and the mixture was incubated at 37°C for 15 minutes, and then cooled to room temperature. Protein precipitation solution (100 µl) was added to the nuclear lysate. The sample was mixed thoroughly and centrifuged at 13,000-16,000 xg for 3 minutes at room temperature. The supernatant was transferred to a clean 1.5 ml microcentrifuge tube containing 300 µl of isopropanol and the sample was gently mixed by inversion, and centrifuged at 13,000-16,000 xg for 1 minute at room temperature. The supernatant was removed and one sample volume of 70% ethanol was added. The tube was gently inverted several times to wash the DNA pellet and centrifuged at 13,000-16,000 xg for 1 minute at room temperature. After careful removal of the ethanol, the tube was inverted on clean absorbent paper and dried by air for 10-15 minutes. Finally 65 µl of DNA rehydration solution was added, and the tube was incubated at 65°C for 1 hour. Extracted DNA yield was determined spectrophotometrically at 260 nm (Ultrspec 3000, Pharmacia Biotech Ltd., Cambridge, UK). The purity of DNA was determined as rate of optical density at 280 nm and 260 nm. The extracted DNA was stored at -20°C until examination.

3. Extraction of genomic DNA from blood

Peripheral venous blood was obtained with standard venipuncture technique into glass tube containing 250 µl of 0.5 M EDTA (pH 8.0). DNA
was extracted from the anticoagulated blood with the same method with exception of 300 μl of sample volume as previously described in saliva using a commercial DNA purification kit (Wizard® Genomic DNA Purification kit, Promega Co., WI, USA).

4. DNA gel electrophoresis

High molecular mass genomic DNA from blood and saliva was examined by electrophoresis. Electrophoresis were performed on 1% agarose submarine gel at constant 100 V in TBE buffer (pH 8.8) (134 mM Tris, 75 mM boric acid, 25 mM EDTA) for 1 hour.

III. RESULTS

Mean concentration of genomic DNA extracted from blood was 18.06±3.98 μg/ml and those from stimulated whole saliva and unstimulated whole saliva were 4.17±5.00 μg/ml and 2.61±2.45 μg/ml, respectively. There were significant differences of concentration of DNA between blood and saliva (p<0.001). Although the concentration of DNA from stimulated whole saliva was higher than that from unstimulated whole saliva, there was no significant difference. The purity of DNA from saliva was higher than that from blood (Table 1). High molecular mass genomic DNA from saliva was located at the same position of that from blood in electrophoretic analysis (Fig. 1).

Stimulated whole saliva was used in the second study on the effects of various storage conditions on

![Isolation of DNA from Human Saliva](image)

**Table 1. Concentration and purity of DNA from saliva and blood**

<table>
<thead>
<tr>
<th>Sample (n=10)</th>
<th>Blood</th>
<th>Saliva</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration(μg/ml)</td>
<td>18.06±3.98</td>
<td>4.17±5.00</td>
<td>2.61±2.45</td>
</tr>
<tr>
<td>Purity</td>
<td>1.51±0.20</td>
<td>1.89±0.24</td>
<td>1.83±0.27</td>
</tr>
</tbody>
</table>

***: p<0.001 ; *: p<0.05

SWS: stimulated whole saliva ; UWS: unstimulated whole saliva

![Fig. 1. Electrophoretic pattern of high molecular mass genomic DNA from lane a: blood, lane b: stimulated whole saliva, lane c: unstimulated whole saliva. DNA was isolated from 300 μl of blood, 600 μl of saliva. Seven μl of final 65 μl of DNA from blood and 15 μl from saliva were loaded on the gel. Electrophoresis were performed on 1% agarose submarine gel at constant 100 V in TBE buffer for 1 hour, with ethidium bromide.](image)

437
Table 2. Differences of concentration and purity of DNA from stimulated whole saliva according to period of storage at room temperature

<table>
<thead>
<tr>
<th>Sample (n=10)</th>
<th>Time of DNA extraction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>1 M</td>
</tr>
<tr>
<td>Concentration(μg/ml)</td>
<td>6.40±5.95</td>
<td>2.14±1.69</td>
</tr>
<tr>
<td>Purity</td>
<td>1.90±0.12</td>
<td>1.68±0.14</td>
</tr>
</tbody>
</table>

**p<0.01 ; *: p<0.05
B: Baseline ; M: Month after collection time

Table 3. Differences of concentration and purity of DNA from stimulated whole saliva according to period of storage at 4°C

<table>
<thead>
<tr>
<th>Sample (n=10)</th>
<th>Time of DNA extraction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>1 M</td>
</tr>
<tr>
<td>Concentration(μg/ml)</td>
<td>6.40±5.95</td>
<td>4.93±4.17</td>
</tr>
<tr>
<td>Purity</td>
<td>1.90±0.12</td>
<td>2.01±0.15</td>
</tr>
</tbody>
</table>

**p<0.01 ; *: p<0.05
B: Baseline ; M: Month after collection time

Table 4. Differences of concentration and purity of DNA from stimulated whole saliva according to period of storage at -20°C

<table>
<thead>
<tr>
<th>Sample (n=10)</th>
<th>Time of DNA extraction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>1 M</td>
</tr>
<tr>
<td>Concentration(μg/ml)</td>
<td>6.40±5.95</td>
<td>6.23±3.01</td>
</tr>
<tr>
<td>Purity</td>
<td>1.90±0.12</td>
<td>1.93±0.06</td>
</tr>
</tbody>
</table>

*: p<0.05
B: Baseline ; M: Month after collection time

Genomic DNA from saliva. Changes of DNA from saliva stored at room temperature were shown in Table 2. Gradual reduction of DNA concentration was occurred and the concentration of DNA after 1 month differed from initial concentration significantly (p<0.05). Purity of DNA was also decreased gradually and significantly. In the case of DNA stored at 4°C, the reduction of concentration was occurred which was similar to DNA stored at room temperature. The concentration was, however,
Table 5. Differences of concentration and purity of DNA from stimulated whole saliva according to period of storage at -70°C

<table>
<thead>
<tr>
<th>Sample (n=10)</th>
<th>Time of DNA extraction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>1 M</td>
</tr>
<tr>
<td>Concentration (µg/ml)</td>
<td>6.40±0.96</td>
<td>6.13±5.59</td>
</tr>
<tr>
<td>Purity</td>
<td>1.90±0.12</td>
<td>1.77±0.20</td>
</tr>
</tbody>
</table>

*: p<0.05 ; N.S.: not significant
B: Baseline ; M: Month after collection time

decreased significantly after 3 months (Table 3). Purity of DNA from saliva stored at 4°C was maintained above 1.5 for the whole storage period of 5 months. In the case of saliva stored at -20°C, there was no change of genomic DNA until 3 months. But after 5 months, concentration of DNA decreased significantly (p<0.05) (Table 4). Although concentration of genomic DNA from saliva stored at -70°C for 5 months decreased as compared with that from fresh saliva, there was no difference according to storage period (Table 5). Purity of DNA from stimulated whole saliva stored at -20°C and -70°C was maintained above 1.5 for the whole storage period of 5 months.

IV. DISCUSSION

Blood has been used as the most popular sample for the diagnosis of diseases in medicine. Urine, cerebrospinal fluid, synovial fluid, sweat, and semen also provide useful diagnostic information. In the field of dentistry, the possibility of saliva as a diagnostic fluid has been speculated. It has been paid attention to possibility of saliva as diagnostic analyte by simplicity and safety of collection that is the greatest advantage with advent of incurable diseases such as AIDS. Recently, remarkable advance in diagnostic procedures is increased sensitivity and specificity due to the development of newer micromethodologies and increasing availability of immunological and molecular biological reagents. The outcome of researches already provide DNA probes and antibodies that can be used as clinical diagnostic screening reagents for various diseases including hereditary disorders. The dental and craniofacial diagnostics will be also developed with same strategy.

In this present study, the concentration of DNA from saliva was lower significantly than that from blood. There was a tendency that concentration of DNA from stimulated whole saliva was higher than that from unstimulated whole saliva. It seems because the number of epithelial cells shed from oral mucosa increases by mechanical rubbing during gum chewing. Compared with data from the previous study, the concentration of DNA from stimulated whole saliva in this study was slightly low. Only stimulated whole saliva was used in the second experiment about the effects of various storage conditions of saliva samples on the extraction of genomic DNA, because stimulated whole saliva is easily collected than unstimulated whole saliva and concentration of DNA from stimulated whole saliva was higher than that of unstimulated one. As expected, in the present study the concentration of DNA was maintained well in low temperature. The concentration of DNA from saliva stored below -20°C did not change even when the storage period was extended to 3 months. However, the concentration of DNA from saliva stored at room temperature showed apparent decreasing pattern within 1 month of storage period. The concentration of DNA from saliva stored at 4°C showed decreasing pattern when the storage period was extended to 3 months. The concentrations of DNA from stimulated saliva in the first and second
experiments were 4.17 μg/ml and 6.40 μg/ml, respectively. This difference could be explained by the variation in the sample collection. When the commercial kit developed for blood sample was used for desquamated epithelial cells in saliva sample, there might be difficulty for getting consistent data. However, the results showed that it is possible to get enough amount of DNA for further analysis. Further researches are necessary to prove the practical usability of extracted DNA for the diagnostic purpose, especially in the case of samples stored for long periods because the degradation of DNA usually happens.

Laboratory tests based on DNA or RNA analysis become routine examinations in contemporary medicine. There has been a report that mutations in regulatory and structural genes as well as chromosomal anomalies are associated with approximately 14 percent of the major craniofacial—oral—dental malformations, and approximately three-quarters of all congenital malformations affect the craniofacial—oral—dental and neck regions. With advanced techniques and strategies of developmental biology and molecular genetics, biomedical scientists have successfully mapped to human chromosomes approximately 70 genes related to craniofacial anomalies, 20 genes related to clefting defects, and 3 genes related to craniosynostosis. This kinds of topics are still main theme of biomedical researches and the products from those researches will lead to new diagnostic reagents and methodologies. Saliva can be obtained atraumatically and repeatedly in sufficient quantities from nearly all patients as well as healthy population. The PCR-based assays were reported to detect mutations and markers associated with diseases using DNA from saliva instead of blood. These kinds of information will be used for screening purpose to detect high-risk groups for certain oral and maxillofacial diseases in the general population. Some preventive measures might be also possible to protect this high-risk groups from the threat of diseases. There is, of course, still difficulty of determining whether a mutant gene that causes a disease in high-risk family also causes that same disease in the general population. The DNA diagnostics could be applied for congenital craniofacial syndromes and malignancies.

Forensic medicine and dentistry are another field for the application of genetic information. The sex determination by amplification of amelogenin gene and individual identification by amplification of VNTR (variable number of tandem repeats) or more sensitive STR (short tandem repeats) become already routine procedures and matching probability of individual identification was reported.

The knowledge on saliva composition is rapidly growing because the technical limitations were overcome by the development of scientific methods. Saliva provides an easily available, non-invasive diagnostic medium for widening ranges of diseases and clinical situations. Saliva can be also obtained repeatedly from almost all subjects, even children and handicapped individuals. In addition, saliva can be used for large-scale epidemiological study because non-trained personnel could collect saliva samples in the field without biohazardous trash.

The fact that the decrease of DNA concentration at room temperature was great tells us the need of standardized protocols for the preservation and transfer of saliva samples. Therefore, the development of convenient and standardized collection kits for saliva is mandatory. The development and modification of diagnostic kits for saliva, which overcome the possible problems is also necessary.

The concepts and technologies developed in the fields of molecular genetics, clinical genetics and immunogenetics lead to the diagnostic reagents for the detection of bacteria and virus and commercial products for forensic medicine. This move will be applied to the field of oral and maxillofacial diseases. This means that the results of basic researches can directly change to new products in the biotechnology sector. This present study also provides basic knowledge which will be used for further cooperative researches.
V. CONCLUSIONS

The purpose of the present study was to isolate DNA from fresh saliva and saliva samples stored in various conditions and to compare DNA from saliva with that from blood. Whole saliva sample was collected from 10 healthy adults and DNA was isolated. The concentration of DNA from saliva was lower than that from blood. The concentration of DNA from stimulated whole saliva was higher than that from unstimulated one. The concentration of DNA from saliva stored at room temperature showed apparent decreasing pattern within 1 month of storage period. The concentration of DNA from saliva stored at 4°C showed decreasing pattern when the storage period was extended to 3 months. However, the concentration of DNA from saliva stored below -20°C did not change even when the storage period was extended to 3 months. Although saliva is useful diagnostic fluid containing enough amount of DNA for diagnostics, standardization of procedures and modification of commercial kit are needed to get more consistent results.

REFERENCES


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441
국문초록

인체 타액으로부터 DNA의 분리

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김영구 · 고용섭 · 정성창 · 이승우

최근 진단분야에 있어서의 획기적인 진로로는 다양한 면역화학물질과 분자생물학적 시약의 활용이 가능해짐에 따라 항상된 진단술학의 민감도와 특이도를 높일 수 있으며, 이와 더불어 진단용 기구의 수준 향상으로 가능해진 미세 손실을 줄 수 있다. 이 분야의 기술적 진보는 다양한 용기를 분석하고 DNA를 제공할 수 있는 결과로서 타액을 고려할 수 있게 하였다. 본 연구의 목적이 인체의 타액에서 genomic DNA를 분리하고 이를 혈액에서 분리한 genomic DNA와 비교 검토하여 볼으므로써 타액 검체의 진단학적, 범죄의학적 활용도를 살펴보는데 있다. 또한, 타액 검체의 다양한 보관 과정에 genomic DNA의 분리에 미치는 영향을 살펴보고자 하였다. 건강한 성인의 타액으로부터 혈액과 전진악을 체취하고 이들로부터 DNA를 분리하였다. 본 연구 결과, 타액 DNA 농도는 혈액에 비해 유의하게 낮았으며 자극적 분비된 전진악으로부터 추출된 DNA 농도가 비자극적 분비된 전진악의 경우와 비교하여 높은 경향을 보였다. 실험에서 보한 타액의 경우, DNA 농도의 감소가 1 달이내에 두드랐으며, 4℃에 보관한 경우 3개월 경과 후에 그 감소가 두드랐으며, -20℃에 보관한 경우 5개월이 경과된 후에 DNA 농도가 감소된 경향을 관찰할 수 있었다. 본 연구의 결과를 볼 때, 타액은 진단을 위한 충분한 양의 DNA를 제공하여 주고 단 입관된 결과를 얻기 위해서는 혈액에서 이용된 방법의 적절한 변형과 표준화 과정이 요구되었다.