SUMMARY

The oral mucosa is susceptible to tissue injury from many causes, including infection, autoimmune disorders, surgical and accidental trauma, and gingival and periodontal inflammation. However, little is known about the events that influence wound healing in the mouth. This study investigated the changes in salivary thromboplastic activity prior to and after primary tooth extraction. Cytological smears and biochemical tests were also used in this study.

Salivary pH and salivary flow rate did not significantly change after primary tooth extraction. Salivary thromboplastic activity was not significantly higher after extraction than before extraction (p=0.068). Epithelial cells significantly decreased and leukocyte cells significantly increased in saliva after primary tooth extraction (p<0.001). It is concluded that oral cavity may aid the process of haemostasis and, perhaps, wound healing in the extraction area by increasing salivary leukocyte cells and thromboplastic activity. Thromboplastic activity may be a novel indicator of wound healing completion.

Keywords: Salivary cells; Salivary flow rate; Salivary pH; Salivary thromboplastic activity; Tooth extraction

Introduction

Wound healing is a dynamic, multi-faceted physiological process that involves a wide range of biological mediators. Although primary tooth extraction is a simple intervention, some complications can occur. The oral mucosa is susceptible to tissue injury from many causes, including infection, autoimmune disorders, surgical and accidental trauma, and gingival and periodontal inflammation; however, little is known about the events that influence wound healing in the mouth. Normally tooth extraction induces some changes in the oral cavity. Saliva is important in maintaining oral health and much of this protective activity is mediated by the variety of different salivary proteins.

Damaged tissue release a lipoprotein known as thromboplastin (tissue factor FIII) which activates the extrinsic coagulation pathway. Activated monocytes and endothelial cells also express this thromboplastin in their surface and participate in the coagulation. Thromboplastin initiates the coagulation system and is a component of cell membrane but not found active in blood. Like body fluids, saliva has also been known to have thromboplastic activity. Thromboplastin in saliva is thought to supply the haemostasis when injury takes place in the mouth and it facilitates the barrier function of buccal mucosa. Thromboplastin is related to cells and cell fragments in saliva. In the adult vertebrate body, physical integrity is continuously maintained by a tissue repair mechanism consisting of the vascular endothelium, tissue factor and circulating Factors VII, IX and X that governs localized thrombin elevations. The cellular effects of these thrombin elevations explain all aspects of tissue repair and maintenance.

It has been shown that oral cavity is also affected from the disturbances of the haemostatic system in which spontaneous bleeding of the dental tissues, petechies of oral soft tissues and ecchymosis are common in...
routine oral examination\textsuperscript{15}. Post operative examinations show that minor oral surgeries can also cause bleeding. Salivary thromboplastin may establish the haemostasis after oral trauma\textsuperscript{7,11}. Thromboplastin also contributes to wound healing, inflammatory response, tumour growth, metastasis and angiogenesis\textsuperscript{16,17}. The disturbance of haemostatic balance in the oral cavity is an indicator of an acquired or congenital defect of the coagulation system\textsuperscript{18}.

There is no study in the literature involving the relationship between the wound healing after primary tooth extraction and salivary thromboplastic activity. In this study, for the first time, wound healing in oral cavity is followed by the salivary thromboplastic activity after primary tooth extraction. Salivary pH and salivary flow rate were also determined and saliva imprint samples were also evaluated cytologically.

**Material and Methods**

This study was performed on 25 healthy children, age ranging 7-12 years. Children were randomly selected from individuals who attended Marmara University, Faculty of Dentistry. Oral dental examinations and restorative treatments were conducted by a pedodontist. Among the group, 80% of the children had experienced dental extractions before. Children were sent to the Oral and Maxillofacial Surgery Department for tooth extraction due to caries and pulpal pathology. Children had standard breakfast 1 hour before tooth extraction. 3 primary canine, 1 primary incisor, and 21 primary molar teeth were extracted from 25 children. Bleeding stopped in 60 minute in all children. Informed consent was obtained from each subject’s family before saliva collection.

All saliva samples were collected in resting position in the surgery department. First, the subjects rinsed their mouths with distilled water 3 times. Non-stimulated mixed saliva samples were collected by spitting into a funnel prior to and 1 hour after primary tooth extraction. Collection time was recorded and saliva volume was measured by pipetting the saliva into a new tube. Then the tubes were stored at -20ºC. Salivary flow rate was calculated by dividing the saliva volume to collection time. Saliva samples were analyzed for pH by using pH paper (Neutralit-pH 5.5-9.0; Merck).

Thromboplastic activity of saliva samples was evaluated according to Quick’s 1 stage method using normal plasma\textsuperscript{19}. This was performed by mixing 0.1 ml of saliva with 0.1 ml of 0.02 M CaCl\textsubscript{2}, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagents were in the reaction temperature (37ºC) before admixture. Since the clotting time is inversely proportional to the salivary thromboplastic activity (STA), the lengthening of the clotting time is a manifestation of the decreased STA.

For cytological examinations, saliva samples were smeared over a glass microscope slide and fixed with air. They were stained with Giemsa stain\textsuperscript{20}. All slides were examined microscopically (x100) for the presence of epithelium, erythrocyte, leukocyte and yeast cell.

The results were evaluated using a Student t-test, Wilcoxon test, Pearson correlation analysis and Spearman correlation analysis using the Unistat 5.0 statistical package programme.

**Results**

Mean age and body mass indices of 25 children were 9.88±1.39, and 16.70±2.23, respectively. According to sex, no significant differences were found in all parameters.

Salivary pH values and salivary flow rates did not significantly change after primary tooth extraction (Table 1). Salivary thromboplastic activity was moderately higher after extraction than before extraction (p= 0.068).

No significant difference was found concerning yeast cells in saliva imprint samples of both groups (p>0.1). There were no erythrocyte cells in saliva imprint samples (Table 2). Epithelial cells significantly decreased and leukocyte cells significantly increased in saliva imprint samples after primary tooth extraction (p<0.001).

Significant negative correlation was found between salivary flow rate and STA before primary tooth extraction (r= -0.413; p=0.04).

| Table 1. Comparison of salivary flow rate, pH and salivary thromboplastic activity (STA) prior to and after primary tooth extraction |
|-----------------|-----------------|-----------------|
|                  | Pre-extraction period (n=25) | Post-extraction period (n=25) | p (t-test) |
| Salivary flow rate (ml/min) | 0.21 ± 0.11 | 0.21 ± 0.08 | 0.782 |
| Salivary pH | 7.22 ± 0.45 | 7.34 ± 0.40 | 0.136 |
| STA (sec) | 72.64 ± 27.59 | 60.08 ± 26.23 | 0.068 |

Values are given as mean ± standard deviation. Since the clotting time is inversely proportional to the thromboplastic activity, the lengthening of the clotting time is a manifestation of decreased thromboplastic activity.
Table 2. Cytological examination of saliva samples

<table>
<thead>
<tr>
<th></th>
<th>Pre-extraction period (n=25)</th>
<th>Post-extraction period (n=25)</th>
<th>p (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(1)</td>
<td>16%</td>
<td>64%</td>
<td>0.001</td>
</tr>
<tr>
<td>(2)</td>
<td>28%</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>56%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Leukocyte cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0)</td>
<td>56%</td>
<td>4%</td>
<td>0.001</td>
</tr>
<tr>
<td>(1)</td>
<td>32%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td>4%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>8%</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>Yeast cells</td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>(0)</td>
<td>68%</td>
<td>68%</td>
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<tr>
<td>(1)</td>
<td>32%</td>
<td>32%</td>
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</tbody>
</table>

**Epithelial cells:** (1) 0-15 cells (normal) (2) 16-30 cells (medium) (3) more than 30 cells (too much)

**Leukocyte cells:** (0) nonexistent (1) 3-5 cells (2) 6-15 cells (3) more than 16 cells

**Yeast cells:** (0) nonexistent (1) present

**Discussion**

Wound healing process seem to be strictly regulated by multiple growth factors and cytokines released at the wound site\textsuperscript{18}. In this study, possible new marker - thromboplastin is monitored in saliva after tooth extraction. Alterations that disrupt controlled timely healing process would extend tissue damage and prolong repair\textsuperscript{18}.

Whole saliva is the most frequently used type of saliva for the diagnosis of systemic diseases, since it is readily collected and contains serum constituents. These constituents are derived from the local vasculature of the salivary glands and reach the oral cavity via the flow of gingival fluid. Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use\textsuperscript{21,22}. Salivary thromboplastin may establish haemostasis following oral trauma and facilitate barrier functions of oral mucosa\textsuperscript{7,11}. As STA has been measured by PT test, shortened clot formation time shows increased thromboplastic activity\textsuperscript{19}. STA was moderately increased after a single tooth extraction. Our preliminary assays (data not shown) showed a significant increase in STA after 2 teeth extraction. According to this finding, the size of an extraction area and the quality of the extraction may affect the STA.

Thromboplastin is related to the cells and cell fragments of saliva, so that high cell content (epithelial and leukocyte cells) of saliva increases the STA\textsuperscript{7,10,11}. Normally, leukocyte cells do not have thromboplastic activity; they can only secrete thromboplastin when they are exposed to vein media or collagen\textsuperscript{23-25}. Clot formation and wound healing process following tooth extraction are initiated by means of saliva. In the present study, leukocyte cell count increased 1 hour after tooth extraction. There was no significant difference in STA between pre and post tooth extraction; this may be due to the decrease in epithelial cell count or inhibition of thromboplastic activity by specific inhibitor like tissue factor pathway inhibitor. Saliva, as well as blood coagulation system, can play preventive role in oral cavity damages. Thromboplastin also plays an important role in wound healing, inflammatory response, tumour growth, metastasis and angiogenesis\textsuperscript{16,17}.

The relationship between salivary pH and salivary flow rate is well known\textsuperscript{12,13}. Fluctuations in salivary pH may change the secretion of thromboplastin from the cells present in saliva. Salivary pH and STA can be affected by changes of salivary flow rate. In the present study, salivary pH and flow rate did not change after primary tooth extraction. No significant difference in STA between groups may indicate wound healing after tooth extraction. The negative correlation between STA and salivary flow rate has been shown in healthy subjects\textsuperscript{10}. In the present study, there was also negative correlation between STA and salivary flow rate ($r=-0.413; p=0.04$) before tooth extraction; interestingly this correlation was not seen after tooth extraction. This finding may support the secretion of thromboplastin from the leukocyte cells in 60 minutes following tooth extraction.

It might be concluded that oral cavity aids the process of haemostasis and, perhaps, wound healing in the extraction area by increasing salivary leukocyte cells and thromboplastic activity. STA may be a novel indicator of wound healing completion.
References


