Salivary Thromboplastic Activity May Indicate Wound Healing after Tooth Extraction

SUMMARY

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 prior to extraction (p=0.068). Epithelial cells significantly decreased and leukocyte cells significantly increased in saliva after primary tooth extraction (p<0.001). In conclusion: 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

ORIGINAL PAPER (OP)


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. 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 coagulation. Thromboplastin initiates the coagulation system and is a component of cell membrane, but not found active in the blood. Like body fluids, saliva has also been known to have thromboplastic activity. Thromboplastin in saliva is thought to supply 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 dental tissues, petechiae of oral soft tissues and ecchymoses are common in routine oral examination\textsuperscript{15}. Post operative examinations show that minor oral surgery can also cause bleeding. Salivary thromboplastin may establish the hemostasis after oral traumas\textsuperscript{7,11}. Thromboplastin also contributes to wound healing, inflammatory response, tumour growth, metastasis and angiogenesis\textsuperscript{16,17}. The disturbance of hemostatic 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; 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 with the age range of 7-12 years. Healthy children were randomly selected from individuals who attended Marmara University Faculty of Dentistry. Oral dental examinations and restorative treatments were conducted by a paedodontist. Among the group, 80% of the children had experienced dental extractions before. Thereafter, they were sent to the Oral and Maxillofacial Surgery Department for tooth extraction as they had caries and pulpal pathology. Children had standard breakfast 1 hour before tooth extraction. 3 primary canines, 1 primary incisor, 21 primary molars were extracted from 25 children. Informed consent was obtained from each subject’s family before saliva collection.

All saliva samples were collected in the resting position in the surgery department. Firstly, subjects rinsed the mouth with distilled water 3 times. Unstimulated 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 -20°C. Salivary flow rate was calculated by dividing the saliva volume to the collection time. Saliva samples were analyzed for pH by using pH-paper (Neutralit-pH 5.5-9.0; Merck-pH indicate paper).

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 at the reaction temperature (37°C) before admixture. Since the clotting time is inversely proportional to the thromboplastic activity, the lengthening of the clotting time was counted as a manifestation of the decreased thromboplastic activity.

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 Student t-test, Wilcoxon test, Pearson correlation analysis and Spearman correlation analysis; the Unistat 5.0 statistical package programme was used.

**Results**

Mean age and body mass index of 25 children were 9.88±1.39 and 16.70±2.23, respectively. Salivary pH values and salivary flow rates did not significantly change after primary tooth extraction. Salivary thromboplastic activity was moderately higher after the extraction than prior to extraction (Tab. 1).

No significant difference of yeast cells was found in the values prior to and after the extraction in saliva imprint samples (p>0.1). Erythrocytes were not found in saliva samples. Epithelial cells significantly decreased and leukocyte cells significantly increased in saliva imprint samples after primary tooth extraction (Tab. 2).

Significant negative correlation was found between salivary flow rate and thromboplastic activity before primary tooth extraction (r= -0.413; p=0.04). No significant differences were found in any parameter were found in accordance with gender.

| Table 1. Comparison of salivary flow rate, pH and thromboplastic activity prior to and after primary tooth extraction |
|---------------------------------------------------------------|---------------------|-----------------------|
| **Pre-extraction** (n=25) | **Post-extraction** (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 |
| Salivary Thromboplastic Activity (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 the decreased thromboplastic activity.
Discussion

Wound healing process seem to be strictly regulated by multiple growth factors and cytokines released at the wound site\(^1\). In this study, possible new marker - thromboplastin was monitored in saliva after tooth extraction, as alterations that disrupt controlled timely healing process would extend tissue damage and prolong repair\(^1\).

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 derive from the local vascular bed of 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\(^2\). Salivary thromboplastin may establish haemostasis following oral trauma and facilitates barrier functions of oral mucosa\(^2\). As thromboplastic activity has been measured by PT test, shortened clot formation time shows increased thromboplastic activity\(^1\). Salivary thromboplastic activity was moderately increased after a single tooth extraction. Our preliminary assays (data not shown) showed a significant increase in salivary thromboplastic activity after 2 teeth extraction. According to this finding, we consider that the size of an extraction area and the quality of the extraction may affect the thromboplastic activity.

Thromboplastin is related to the cells and cell fragments of saliva; consequently, high cell content of saliva (epithelial cells and leukocytes) increases thromboplastic activity\(^7\)\(^1\)\(^1\). Normally, leukocytes do not have thromboplastic activity, they can only secrete thromboplastin when they are exposed to vein media or collagene\(^2\)\(^3\)\(^-\)\(^5\). Clot formation and wound healing process following tooth extraction are initiated by 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 time, possibly 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\(^1\)\(^6\)\(^7\).

The relationship between salivary pH and salivary flow rate is well known\(^1\)\(^2\)\(^3\). Fluctuations in salivary pH may change the secretion of thromboplastin from the cells present in saliva. Salivary pH and thromboplastic activity can also be affected by the changes of salivary flow rate. In the present study, salivary pH and flow rate did not change after primary tooth extraction. Absence of significant difference in STA between groups may indicate undisturbed wound healing after tooth extraction.

Negative correlation between salivary thromboplastic activity and salivary flow rate has been shown in healthy subjects\(^1\). In the present study, there was also negative correlation between thromboplastic activity and salivary flow rate before tooth extraction (r= -0.413; p=0.04); interestingly this correlation was not seen after tooth extraction. This finding may support the secretion of thromboplastin from the leukocytes 60 minutes following tooth extraction. Thromboplastic activity may be a novel indicator of wound healing completion.

### Table 2: Cytological examination of saliva samples

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

**References**


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