Periostin as a Periodontal Healing Indicator after Scaling and Root Planing

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Abstract
Periostin, a marker of periodontal health, maintains the periodontal integrity. This study aimed to evaluate the expression of periostin mRNA as a potential indicator of periodontal healing after scaling and root planing in patients with periodontitis.

Gingival crevicular fluid (GCF) samples were collected from three periodontally healthy subjects and four periodontitis patients with periodontal pocket depths (PPDs) of 4–6 mm at baseline and 1, 2, 4 weeks (D0, D1, D2, and D3 respectively) after scaling and root planing. Periostin mRNA was measured using quantitative real time-PCR (qPCR). The relationships between periostin mRNA expression and the clinical periodontal health parameters (PPD, bleeding on probing [BOP]) were analyzed using Wilcoxon and paired t tests.

Compared to healthy subjects, patients with periodontitis expressed low D0 levels of periostin mRNA, which increased at D2 but decreased at D3; however, these differences were not statistically significant ($p \geq 0.05$). PPD did not differ significantly over time; even so, the BOP scores were lower at later time points relative to D0 and controls ($p < 0.05$).

In conclusion, the GCF levels of periostin mRNA were highest at 2 weeks postconventional periodontal therapy and correlated with BOP. Further studies are needed to confirm these findings.

Keywords: Periostin, Periodontal ligament, Periodontitis, Healing process, Periodontal therapy.

Introduction
According to a 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, periodontitis is defined as the loss of periodontal attachment due to microbially associated and host-mediated inflammation.1 Presently, periodontitis is among the most prevalent oral diseases worldwide.2 Tadjoedin et al. found that 75.2% of patients included in a study at the Dental Teaching Hospital, Faculty of Dentistry, Universitas Indonesia experienced chronic periodontitis; of these, 29% cases were localized and the rest (46.2%) were generalized.2 The consequences of periodontitis can be severe. According to a World Health Organization database regarding the Community Periodontal Index Treatment Needs of Southeast Asia countries, severe periodontitis led to tooth loss in 5–15% of the adult population.3

In periodontitis, the chronic inflammation of periodontal tissue is caused by microorganisms such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola.4 These periodontopathogenic bacteria and their products initiate a potentially tissue-destructive host immune response via inflammatory mediators and cytokines.5,6 Consequently, the affected patients experience a clinical attachment loss, periodontal pocket formation, consistent bleeding on probing (BOP), gingival recession, and even bone loss that may be detectable on clinical and radiograph examinations.7

Periodontitis can be classified into four stages based on its severity. Stages I and II involve interdental clinical attachment losses (CALs) of 1–2 or 3–4 mm, respectively. Stage III involves interdental CAL of ≥5 mm and a loss of
fewer than four teeth. Eventually, stage IV involves interdental CAL of ≥5 mm and a loss of more than five teeth.\textsuperscript{1,7} Periodontitis is also differed by its rate progression in three categories: slow (grade A), moderate (grade B), and rapid (grade C) progression. Grade A has evidence of no CAL or radiographic bone loss over 5 years or <0.25 percentage of bone loss divided by age (% BL/age); grade B has evidence of <2 mm loss over 5 years or 0.25 to 1.0 % BL/age; while grade C has ≥2 mm loss over 5 years or > 1.0 % BL/age.\textsuperscript{1}

The protein periostin has recently emerged as a research topic of interest. This protein is secreted into the extracellular matrices of the connective tissues during growth, development, and healing processes.\textsuperscript{8} In the periodontal tissues, periostin, which is produced by gingival fibroblasts, plays an important role in tissue homeostasis by mediating collagen formation and controlling the structural and functional capabilities of the connective tissue via both physiological and pathological means.\textsuperscript{9-10} Accordingly, periostin is an important component of the periodontal tissue, considering its roles as a regulator of periodontal tissue cells and stabilizer of extracellular matrix.\textsuperscript{11} Under physiological conditions, periostin is present in high concentrations in the epithelial–connective tissue junctions between the periodontal ligament fibers; in contrast, periostin expression decreases in the context of periodontitis or other inflammation conditions.\textsuperscript{5,11,12} Inflammation suppresses the expression of periostin in the periodontal ligament fibroblasts, which results in extracellular matrix defects and ultimately weakens the structural and functional integrity of periodontal tissue.\textsuperscript{11,12}

A recent study reported increased levels of periostin after surgical therapy, which suggests that this protein may accelerate wound healing and periodontal tissue regeneration.\textsuperscript{11} In that study, healthy subjects maintained fairly stable periostin levels from before to after therapy; in contrast, subjects with periodontitis exhibited a significant increase in the periostin levels immediately after therapy, which persisted for up to 48 h thereafter and decreased gradually to a stable level within 2–4 weeks.\textsuperscript{11} An animal study by Rios et al. similarly observed increased levels of postoperative periostin in mice, as well as a positive correlation between the periostin levels and the healing process.\textsuperscript{11,13}

Successful periodontal wound healing and homeostasis require the presence of specific cells within the affected area and a particular sequence within a specified time period.\textsuperscript{12} In this context, periostin may be a useful biological marker as it participates in the regulation of collagen fibrillogenesis, which eventually promotes tissue mechanical stability and strength.\textsuperscript{8,11} The present study aimed to evaluate the expression of periostin mRNA in patients with periodontitis and to determine whether this variable could be used as a biomarker for the healing process after initial periodontal therapy.

**Materials and methods**

This research protocol was approved by the appropriate institutional ethical committee. Patients were selected for enrollment if they met the following inclusion criteria: an age of 35–65 years, periodontal pocket depth (PPD) of 4–6 mm, and willingness to provide consent. Patients who had received any periodontal treatment or used antibiotics, phenytoin, Ca-blockers, and/or cyclosporine within the last 3 months were excluded, as were those with a smoking habit, alcoholism, or systemic compromising disease, and those who were pregnant or breastfeeding. All included patients underwent an evaluation of clinical factors, including the PPD, loss of attachment, and BOP. Samples of gingival crevicular fluid (GCF) were then collected from the selected teeth by two calibrated dentists according to the research protocol.

The study sample included four patients with stage II, grade A periodontitis. Additionally, three periodontally healthy subjects were selected as a control group. GCF samples were collected by gently inserting a sterile paper point (no. 30) into the deepest periodontal pocket of each tooth for 30 s. The obtained samples were immediately placed in sterile microcentrifuge tubes containing 300 μl of Tris–EDTA (TE) buffer. Samples were collected from the experimental group before (D0) and 1 (D1), 2 (D2), and 4 weeks (D3) after scaling and root planing (SRP) therapy. Samples were collected from the control group only at D0. All collected samples were placed on ice and immediately transferred to the laboratory for further *in vitro* processing.

RNA was extracted from the samples using GENEzol\textsuperscript{TM} reagent (GZR100, Geneaid Biotech, New Taipei, Taiwan) according to the
manufacturer's protocol. The extracted RNA was reverse-transcribed to obtain cDNA using the ReverTra® qPCR RT Kit (TOYOBO Co Ltd, Japan), and the purity and concentration of the obtained cDNA was measured using a MetertechSP-8001 UV/Visible Spectrophotometer (Metertech Inc., Taiwan).

The level of periostin (POSTN) mRNA in each sample was then determined by qPCR. All reactions were performed in duplicate in MicroAmp™ Fast Optical 48-well plates, using an Applied Biosystems Step One Plus thermocycler (Applied Biosystems, Foster City, CA, USA). For this purpose, we used the primer sequences reported by Watanabe: forward, 5′-TTGAGACGCTGGAAGGAAT-3′ and reverse, 5′-AGATCCCGTAAAGTTGTTT-3′. The final volume of each reaction in each microtube was 10 μl, which contained 3 μl of cDNA, 0.5 μl each of forward and reverse primers, 5 μl of SensiFAST SYBR® Hi-ROX Kit reagent, and 1 μl of nuclease-free water. The qPCR amplification protocol included 45 cycles comprising an initial denaturation step at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s. Beta-actin (primers: forward, 5′-TAATGTCAGCAGCATTCCC-3′ and reverse, 3′-TCACCGACGCCGGCT-5′) was used as the reference gene according to Carneiro, using the following qPCR conditions: 2 min at 50 °C, 10 min at 95 °C, and 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Relative POSTN expression was calculated using the 2^{-\Delta\Delta CT} formula as reported by Livak and Schmittgen.

Differences in the gene expression between the experimental groups (D0, 1, 2, and 3) were analyzed using the Friedman test (SPSS Statistics, version 23; IBM, Armonk, NY, USA). The efficacy of SRP was evaluated by comparing the differences in PPD and BOP from before (D0) to after therapy (D3) using a paired t-test or Wilcoxon statistical analysis. The Spearman test was used to evaluate the correlations between changes in the POSTN expression and clinical parameter values. A p-value of <0.05 was considered indicative of a significant difference.

Results

Table 1 presents a distribution of the patients with periodontitis, comprising three men and one woman aged between 35 and 65 years. Table 2 presents the results of a normality test (Shapiro–Wilk) of the samples. Specifically, the POSTN expression analysis revealed an abnormal data distribution at D0. Accordingly, the Friedman test was used for the comparative analysis.

Table 1. Distribution of Characteristics Among Patients with Periodontitis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSTN expression before SRP (D0)</td>
<td>0.012</td>
</tr>
<tr>
<td>POSTN expression 1 week after SRP (D1)</td>
<td>0.491*</td>
</tr>
<tr>
<td>POSTN expression 2 weeks after SRP (D2)</td>
<td>0.407*</td>
</tr>
<tr>
<td>POSTN expression 4 weeks after SRP (D3)</td>
<td>0.365*</td>
</tr>
<tr>
<td>PPD before SRP</td>
<td>0.024</td>
</tr>
<tr>
<td>PPD 4 weeks after SRP</td>
<td>0.001</td>
</tr>
<tr>
<td>Bleeding index before SRP</td>
<td>0.272*</td>
</tr>
<tr>
<td>Bleeding index 4 weeks after SRP</td>
<td>0.272*</td>
</tr>
<tr>
<td>Fold change gene expression</td>
<td>0.004</td>
</tr>
<tr>
<td>PPD change</td>
<td>0.272*</td>
</tr>
<tr>
<td>BOP change</td>
<td>0.024*</td>
</tr>
</tbody>
</table>

Shapiro–Wilks test; *p ≥ 0.05, normal data distribution; POSTN, periostin mRNA; SRP, scaling and root planing; PPD, periodontal pocket depth; BOP, bleeding on probing.

Table 2. Results of Data Normality Testing.

The PPD data had an abnormal distribution, whereas the BOP scores exhibited a normal distribution; the comparative analyses of these variables were performed using the Wilcoxon test and paired t-test, respectively. The fold changes in the gene expression also exhibited an abnormal distribution and were correlated with changes in PPD and BOP using the Spearman test. Figure 1 presents the relative POSTN expression levels at D0, D1, D2, and D3. In the patient group, POSTN expression was highest at D2 and lowest at baseline (D0), and the levels were higher than those in the control group; however, a comparison of POSTN expression over time in the patient group found no significant differences (p ≥ 0.05; Table 3).
The PPD was measured at D0 and D3. As presented in Table 4, although the values decreased over time from the baseline, this difference was not significant (p ≥ 0.05). The BOP scores were also measured at the same time points, as listed in Table 5. Notably, the BOP score at D3 was significantly lower than the score at baseline (p < 0.05). Eventually, the fold change in the POSTN expression was correlated negatively with the changes in PPD and BOP over time (D0 vs D3; Figure 2); however, neither of these correlations were found to be significant (both p ≥ 0.05; Table 6).

**Table 3.** Comparative Analysis of POSTN Expression Before and After SRP.

<table>
<thead>
<tr>
<th>Fold expression change of POSTN gene</th>
<th>Median (Min–Max)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SRP (D0)</td>
<td>1.12 (0.63–9.37)</td>
<td></td>
</tr>
<tr>
<td>After 1 week SRP (D1)</td>
<td>2.88 (0.61–8.82)</td>
<td></td>
</tr>
<tr>
<td>After 2 weeks SRP (D2)</td>
<td>7.36 (1.76–24.13)</td>
<td>0.272</td>
</tr>
<tr>
<td>After 4 weeks SRP (D3)</td>
<td>4.40 (0.48–16.96)</td>
<td></td>
</tr>
</tbody>
</table>

Friedman test; *p < 0.05, significant difference; POSTN, periostin mRNA; SRP, scaling and root planing.

**Table 4.** Comparative Analysis of The PPD Score Before (D0) and 4 Weeks After (D3) SRP.

<table>
<thead>
<tr>
<th>PPD</th>
<th>Median (Min–Max)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SRP (D0)</td>
<td>5.50 (5–6)</td>
<td></td>
</tr>
<tr>
<td>After 4 weeks SRP (D3)</td>
<td>3.0 (3–4)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Wilcoxon test; *p < 0.05, significant difference; PPD, periodontal pocket depth; SRP, scaling and root planing.

**Table 5.** Comparative Analysis of The BOP Score Before (D0) and 4 Weeks After (D3) SRP.

<table>
<thead>
<tr>
<th>BOP Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SRP (D0)</td>
<td>3.25 (0.96)</td>
</tr>
<tr>
<td>After 4 weeks SRP (D3)</td>
<td>0.75 (0.96)</td>
</tr>
</tbody>
</table>

Paired t-test; *p < 0.05, significant difference; BOP, bleeding on probing; SRP, scaling and root planing.

**Table 6.** Analysis of Correlations Between the Fold Change in POSTN Expression and PPD and BOP Scores Before and 4 Weeks After SRP.

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSTN expression – PPD change</td>
<td>–0.099</td>
<td>0.816</td>
</tr>
<tr>
<td>POSTN expression – BOP change</td>
<td>–0.291</td>
<td>0.484</td>
</tr>
</tbody>
</table>

Spearman test; *p < 0.05, significant correlation; POSTN, periostin mRNA; PPD, periodontal pocket depth; BOP, bleeding on probing; SRP, scaling and root planing.

**Discussion**

Periostin is a regulator of periodontal tissue homeostasis and has been identified as the most specific protein expressed in the periodontal ligament. This protein is secreted by gingival fibroblasts and is detected in the cytoplasmic processes of periodontal fibroblasts (i.e., cementoblasts) and the surrounding collagen fibrils, where it plays an important role in maintaining the structural and functional capabilities of connective tissue. Rios et al. previously demonstrated the importance of periostin in gene knockout mice, which developed periodontal-like disease. In a feedback loop, the chronic inflammation caused by periodontal disease reduces the localization of periostin in the periodontal ligament. Collectively, periostin levels decrease as the disease progresses, which eventually leads to deleterious effects on the tissue itself and further
acceleration of disease progression, as reported by Molina et al.\textsuperscript{11}

Notably, Molina et al. also found a reduction in these inflammatory stimuli after periodontal therapy led to a temporary increase in periostin, which promoted cell migration and proliferation and stabilized the extracellular matrix to facilitate the healing process.\textsuperscript{11} The findings of the present study are consistent with those of Molina and colleagues. Specifically, the lowest POSTN level was detected at baseline; this increased over time to a peak at 2 weeks after SRP, followed by a decrease at week 4 (D3). In these patients with moderate periodontitis, the observation of the lowest POSTN expression level at baseline (D0) suggests that a reduction in this factor due to chronic inflammation does indeed cause tissue damage. The high BOP score and PPD confirmed the inflammation and resulting tissue damage, respectively (Tables 4 and 5). Although the POSTN level decreased again at 4 weeks after SRP, it remained higher than at the baseline, suggesting the continued expression of this gene under normal conditions and especially during the tissue remodeling stage of the healing process. This was supported by the improvements in clinical attachment and BOP scores as mentioned in Tables 4 and 5.

Periodontal wound healing involves three overlapping stages: inflammation, new tissue formation, and tissue remodeling.\textsuperscript{19} As its expression peaks at 2 weeks, periostin is highly involved in both inflammation and new tissue formation. The latter requires a specific environmental condition to achieve periodontal regeneration rather than repair. Notably, Park et al. demonstrated that periostin could be used as a biomarker of periodontal regeneration in a tissue engineering study in rats.\textsuperscript{20} Similar effects have also been reported in other tissues, such as the skin, heart, and blood vasculature, which may suggest a role for periostin in the formation of new tissues.\textsuperscript{11} The present study therefore aimed to evaluate the potential usefulness of periostin as a biomarker of periodontal healing after SRP. Although the results were generally not statistically significant, they present a profile of periostin levels in humans that is consistent with the findings of the previous animal studies and, to the best of our knowledge, it has not previously been reported. Notably, a larger study population might provide more reliable results. Furthermore, the observed high expression of POSTN at 2 weeks after SRP (D2) suggests that this therapy successfully eliminated inflammation and might eventually lead to healing.

GCF sampling was selected for the present study due to its simplicity and relatively nontraumatic and noninvasive nature.\textsuperscript{21} Although this technique is limited by the potential for contamination and difficulties with volume determination and recovery from strips, GCF analysis is generally considered a promising option for the detection of early changes indicative of disease onset.\textsuperscript{22} For example, a previous study observed significant differences in the GCF periostin concentrations among the healthy individuals and patients with gingivitis and periodontitis, with the lowest levels observed in the latter group.\textsuperscript{21} The total periostin level was found to correlate positively with the severity of disease or the degree of inflammation, but negatively with clinical parameters (PPD, CAL), consistent with the present study.\textsuperscript{11,21}

Conclusions

In conclusion, this study demonstrated that periostin can be detected in the GCF samples from both patients with periodontitis and healthy individuals. In the former group, the POSTN level gradually increased after periodontal therapy but decreased after 2 weeks as the tissue healed. Further and broader studies of periostin expression patterns during the periodontal healing process are needed to obtain more objective results and confirm the potential usefulness of this protein or associated mRNA as a biomarker of periodontal tissue.

Declaration of Interest

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