Increasing oral PMN during experimental gingivitis and its reversal by prophylaxis

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Abstract

This investigation evaluated clinical parameters and the levels of polymorphonuclear leukocytes [PMN] collected in an oral rinse amongst subjects who refrained from dental hygiene for a period of 12 days. Methods: Study enrolled consenting adults and assigned to a non-prophy group (n = 16) and a separate prophy group (n = 27). Both groups underwent clinical evaluations and sampling for PMN at baseline and on days 3, 6, 9 and 12 of study initiation. The prophy group underwent supragingival prophylaxis at the conclusion of the no-hygiene phase and recalled for a final clinical evaluation and PMN assessment 1 week later.

Results: Progressive increases in oral PMN were noted due to abstinence from oral hygiene (p < 0.05). Subjects registered PMN increases ranging from 20% recorded three days following abstinence to the highest value of 298% at the 12-day evaluation (p < 0.05). One week after prophylaxis, average PMN scores were 22% lower than baseline (p < 0.05). Abstinence from dental hygiene led to progressive increases in clinical parameters for dental plaque, gingival inflammation and bleeding. Dental plaque, gingival index and gingival bleeding scores recorded increases of 59%, 64% and 126% respectively at the conclusion of the no-hygiene phase. Prophylaxis resulted in marked reductions in all clinical parameters.

Conclusions: Abstinence from dental hygiene corresponded with increasing scores for dental plaque, gingival inflammation and bleeding in conjunction with increasing oral PMN. These effects were irrespective of age or gender and were reversed by supragingival prophylaxis.

1. Introduction

The human mouth with its distinctive anatomical features that includes a significant mucosal interface and substantial vasculature represents a dynamic environment influenced by local and external local factors [1,2,3]. Primary features of this environment include the dentition supported by the underlying tissue and bone and the numerous environments that extend to the oropharynx [1]. A substantial effort has been instrumental in exploring oral health with a goal of maintaining health promoting factors and reducing the impact of deleterious influences in this dynamic environment [2].

Despite the extensive research and widely recognized factors that influence oral health, global surveys consistently report chronic conditions including caries, gingivitis and periodontal disease worldwide [1]. Features of this environment include the substantial microbial burden along with the influences of diet and external factors [1,2,4]. Contemporary concepts in clinical dentistry readily identify the inability to maintain optimal hygiene as an important confounder in the initiation and progression of chronic oral conditions [2,5]. Additionally, dietary influences promote microbial proliferation resulting in production of acids, metabolic products, toxins and cellular residues with immunological features [6].

A consistent and sustained host response is a feature central to inflammation with polymorphonuclear leukocytes [PMN] identified as the “first responders” [7–13]. The contributions of neutrophils at mucosal interfaces [14–17] are recognized. For instance, analysis of nasal wash samples indicates that microbial burden is correlated with neutrophil recruitment. PMN recruitment represents a critical step in clearing nasopharyngeal colonization by Streptococcus pneumoniae, responsible for otitis media and upper respiratory infection amongst children [18]. Neutrophils are identified in nasal cytology samples [19, 20], sputum [21], used to identify occupational exposures [22], examine

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inflammation in skin [23] and other regions including the eye [24]. The literature broadly describes oral neutrophils in the human mouth including teeth and implants [25–30] with studies reporting collection from distinct oral samples [31–35], along with functional aspects in disease [36–38]. Also available are PMN interactions with microorganisms [39,40,33], the influences of common oral hygiene ingredients [41,42], functional modulators [43–46] along with the effects of pharmaceuticals on neutrophil function [41,47]. Despite the available evidence, there are few reports evaluating the effects of abstinence from oral hygiene in an experimental gingivitis model on PMN [48]. Accordingly, the present effort was designed to examine the effects of abstinence from oral hygiene amongst adults with clinical features reflecting commonly observed levels of dental plaque and gingival inflammation. Subjects were evaluated over time for oral PMN in conjunction with widely established clinical parameters that included the dental plaque index, gingival index, bleeding index and periodontal pocket probing depths. In addition, the study determined the effects of supragingival prophylaxis on these outcomes.

2. Materials and methods

This was a single-site study that enrolled healthy volunteers. The study protocol was approved by the Institutional Review Board of the University at Buffalo School of Dental Medicine, Buffalo, New York. Subjects were enrolled after study approval and the entire study was conducted at the University of Buffalo.

2.1. Study subjects and clinical evaluations

Prospective subjects of either gender between the age of 18–70 years from the local area who expressed an interest in study participation were invited to attend an informational visit at the dental clinic. The dental examiner explained the study requirements and those who voluntarily completed the informed consent form were scheduled for screening to determine study eligibility. A dentist completed an oral examination that included the entire dentition, soft tissue regions, palate in the dental clinic under constant lighting. Subjects were interviewed for their medical history and underwent a whole mouth assessment. Those presenting at least 20 natural teeth, in good general health and registering whole mouth dental plaque and gingival index scores of 1.5 and 1.0 respectively by the Turesky-Modification of the Quigley Heins Index [49] and Löe-Silness [50] Index respectively were enrolled. Additionally, clinical evaluations recorded the bleeding index and periodontal examination to assess pocket depths [51]. Exclusion criteria included those who had recently participated in a clinical study, presenting oral respiratory, dental implants, dentures, orthodontic bands, caries, ulcers, periodontal disease or other soft tissue pathologies. Subjects reporting pregnancy, breast feeding or medical and dental procedures in the period preceding the screening visit were excluded. Also excluded were those with systemic conditions, under the care of medical professionals or prescribed medications. After study enrollment, subjects were instructed to refrain from oral hygiene procedures for the study period.

The study was conducted at the University at Buffalo School of Dental Medicine, Buffalo, New York. Subjects were enrolled after study approval and the entire study was conducted at the University of Buffalo.

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2.2. Study procedures

Following study enrollment, subjects underwent their baseline clinical examination for dental plaque, gingival index, bleeding index and periodontal pocket probing depths. A baseline oral sample for PMN was also collected during this visit utilizing procedures described in section below. Following study enrollment, subjects were instructed to refrain from oral hygiene and provided a study schedule. Subjects were recalled to the dental clinic on the mornings of day 3, 6, 9 and 12 after study initiation. During each recall visit, the clinical evaluations conducted at baseline were repeated and oral samples collected for PMN analysis.

After completing the evaluations on day 12, subjects in the non-prophylaxis group continued their study participation. The subjects in the prophylaxis group underwent supragingival prophylaxis and were issued a commercially available fluoride toothpaste and soft-bristled toothbrush for twice daily hygiene. This group of subjects were recalled seven days later for their final evaluation that included PMN sampling and clinical evaluations conducted at each recall. At each recall visit, subjects underwent an oral examination, were reminded of the study procedures and interviewed for any adverse events.

2.3. Sampling for oral PMN and laboratory analysis

Subjects rinsed their mouth with 10 ml of saline for 30 s that was collected for PMN analysis. Collected samples were transferred to the laboratory and stained with fluorescent dyes for fluorescence microscopy and enumerated utilizing procedures described previously [52].

3. Statistical analysis

Statistical analyses were conducted separately from the two subject groups representing those who were provided or not provided a prophylaxis at study conclusion. Subject demographics including age and gender were summarized for those completing the entire study and provided evaluable results. Microscopy results of polymorphonuclear leukocytes [PMN] recorded as counts per milliliter were log transformed (log10) for analysis. Within treatment comparisons from baseline to each post-treatment evaluation for PMN, gingival, plaque, bleeding index and pocket depths were conducted by paired t-tests. Percent differences from baseline to each post-treatment clinical evaluation were calculated with differences for PMN computed as described previously [52]. For each evaluated parameter, an increase from baseline is reported as a negative value with reductions from baseline registered as a positive number. Statistical tests were two-sided and statistically significant results reported at α = 0.05.

4. Results

The study enrolled forty-three subjects and a summary of subject demographics is presented in Table 1. Sixteen adults comprising 7 males and 9 females were assigned to the non-prophy group while 27 subjects comprising 10 males and 17 females were assigned to the prophy group. The age range in the non-prophy group and prophy group were 18–58 years and 20–60 years respectively. The average age [mean ± SD in years] for those enrolled in the non-prophy and prophy groups were 38 ± 14 and 37 ± 15 respectively with no significant differences between groups (p > 0.05). No adverse events were reported by either the subjects or the dental examiners over the study period.

Clinical and PMN parameters from the non-prophy group is summarized in Table 1 with all results presented as mean and SD at all evaluations. Notable findings in this group were the baseline PMN scores of 4.96 that increased to 5.19, 5.28, 5.38 and 5.56 at the 3-day, 6-day, 9-day and 12-day evaluations respectively. Corresponding observations were noted for all clinical parameters recorded. Subjects registered

| Treatment          | Number of Subjects | Age (years) |
|--------------------|--------------------|-------------|
|                    | Male   | Female | Total | Mean ± S.D. | Range |
| Non-Prophy Group   | 7      | 9      | 16    | 38.88 ± 14.20 | 18–58 |
| Prophy Group       | 10     | 17     | 27    | 37.41 ± 15.31 | 20–60 |

Summarized for all clinical parameters recorded. Subjects registered

| Treatment          | Number of Subjects | Age (years) |
|--------------------|--------------------|-------------|
|                    | Male   | Female | Total | Mean ± S.D. | Range |
| Non-Prophy Group   | 7      | 9      | 16    | 38.88 ± 14.20 | 18–58 |
| Prophy Group       | 10     | 17     | 27    | 37.41 ± 15.31 | 20–60 |

Subjects did not receive a prophylaxis at end of the study.

Subjects who received a prophylaxis at the end of the study.
Table 2
Summary of subject evaluations from the non-prophy group over the study period.

| Parameter            | Baseline Summary (Mean ± S.D.) | 3-Day Summary (Mean ± S.D.) | 6-Day Summary (Mean ± S.D.) | 9-Day Summary (Mean ± S.D.) | 12-Day Summary (Mean ± S.D.) |
|----------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| PMN log10 (Counts/ml)| 4.96 ± 0.25                     | 5.19 ± 0.20                 | 5.28 ± 0.25                 | 5.38 ± 0.24                 | 5.56 ± 0.24                 |
| Plaque Index         | 1.66 ± 0.42                     | 1.91 ± 0.55                 | 2.25 ± 0.31                 | 2.46 ± 0.48                 | 2.64 ± 0.48                 |
| Gingival Index       | 1.17 ± 0.44                     | 1.43 ± 0.40                 | 1.64 ± 0.25                 | 1.92 ± 0.07                 | 1.93 ± 0.19                 |
| Bleeding Index       | 0.41 ± 0.22                     | 0.53 ± 0.22                 | 0.63 ± 0.22                 | 0.82 ± 0.22                 | 0.93 ± 0.23                 |
| Pocket Depth (mm)    | 1.93 ± 0.19                     | 1.95 ± 0.20                 | 2.01 ± 0.12                 | 2.07 ± 0.13                 | 2.14 ± 0.19                 |

Results indicate Mean ± SD for Polymorphonuclear Leukocytes (PMN) and clinical parameters i.e. dental plaque, gingival index, bleeding index and pocket depth at all examinations.

Table 3
Summary of subject evaluations from the prophy group over the study period.

| Parameter            | Baseline Summary (Mean ± S.D.) | 3-Day Summary (Mean ± S.D.) | 6-Day Summary (Mean ± S.D.) | 9-Day Summary (Mean ± S.D.) | 12-Day Summary (Mean ± S.D.) |
|----------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| PMN log10 (Counts/ml)| 5.06 ± 0.31                     | 5.14 ± 0.33                 | 5.23 ± 0.30                 | 5.20 ± 0.31                 | 5.22 ± 0.27                 |
| Plaque Index         | 2.25 ± 0.35                     | 2.75 ± 0.45                 | 2.96 ± 0.49                 | 3.08 ± 0.48                 | 3.16 ± 0.42                 |
| Gingival Index       | 1.75 ± 0.17                     | 1.92 ± 0.16                 | 2.00 ± 0.15                 | 2.05 ± 0.11                 | 2.10 ± 0.11                 |
| Bleeding Index       | 0.94 ± 0.38                     | 1.15 ± 0.46                 | 1.33 ± 0.45                 | 1.43 ± 0.49                 | 1.53 ± 0.40                 |
| Pocket Depth (mm)    | 2.09 ± 0.17                     | 2.13 ± 0.18                 | 2.19 ± 0.18                 | 2.19 ± 0.20                 | 2.23 ± 0.18                 |

Results indicate Mean ± SD for Polymorphonuclear Leukocytes (PMN) and clinical parameters i.e. dental plaque, gingival index, bleeding index and pocket depth at all examinations.

baseline scores of 1.66, 1.17, 0.41 for their whole mouth dental plaque, gingival and bleeding index outcomes. An average pocket probing depth of 1.93 mm was recorded at baseline from the non-prophy group. Shown in Table 2 are results for clinical evaluations over the study period. Average clinical scores increased over the study period.

The summary of clinical and PMN parameters from the prophy group is summarized in Table 3. The results are presented as mean and SD at all evaluations. Average baseline PMN values were 5.06 with increases noted at all post-baseline evaluations prior to prophylaxis. Average PMN scores on the 3-day, 6-day, 9-day and 12-day were 5.14, 5.23, 5.20, 5.22 respectively. Prophylaxis reduced PMN scores with an average score of 4.94 which was lower than the baseline value. Whole mouth dental plaque, gingival, bleeding index scores at baseline were 2.25, 1.75, 0.94 respectively with average pocket probing depth of 2.09 mm. All clinical parameters increased over the study period (Table 3). The effects of prophylaxis were marked with the average scores for dental plaque, gingival index, bleeding index were 2.15, 1.67, 0.89 respectively and were numerically below baseline values. Correspondingly, the average

Table 4
Analysis of Polymorphonuclear Leukocyte log10 (Counts/ml) [Mean ± SD] at Each Time Point For Subjects Who Completed the Clinical Study.

| Treatment Group       | Time Point | Time Point Summary (Mean ± S.D.) | Within-Treatment Analysis |
|-----------------------|------------|----------------------------------|--------------------------|
|                       |            | Percent Change^c Sig.^d          |                         |
| Non-Prophylaxis Group | 3-Day      | 5.19 ± 0.20 ± -69.8% p = 0.001   |                          |
|                       | 6-Day      | 5.28 ± 0.25 ± -108.9% p = 0.001  |                          |
|                       | 9-Day      | 5.38 ± 0.24 ± -163.0% p = 0.001  |                          |
|                       | 12-Day     | 5.50 ± 0.24 ± -298.1% p = 0.001  |                          |
| Prophylaxis Group     | 3-Day      | 5.14 ± 0.33 ± -20.2% p = 0.193   |                          |
|                       | 6-Day      | 5.23 ± 0.30 ± -47.9% p = 0.016   |                          |
|                       | 9-Day      | 5.20 ± 0.31 ± -38.0% p = 0.097   |                          |
|                       | 12-Day     | 5.22 ± 0.27 ± -44.5% p = 0.015   |                          |
|                       | 19-Day     | 4.94 ± 0.32 ± 22.4% p = 0.113   |                          |

^a Subjects did not receive a prophylaxis at the end of study.
^b Subjects who received a prophylaxis at the end of the study.
^c Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
^d Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.

Table 5
Analysis of dental plaque index [mean ± SD] at each time point for subjects who completed the clinical study.

| Treatment Group | Time Point | Time Point Summary (Mean ± S.D.) | Within-Treatment Analysis |
|-----------------|------------|----------------------------------|--------------------------|
|                 |            | Percent Change^c Sig.^d          |                         |
| Non-Prophylaxis | 3-Day      | 1.91 ± 0.55 ± -15.1% p = 0.006   |                          |
| Group           | 6-Day      | 2.25 ± 0.31 ± -35.5% p < 0.001   |                          |
|                 | 9-Day      | 2.46 ± 0.48 ± -48.2% p < 0.001   |                          |
|                 | 12-Day     | 2.64 ± 0.48 ± -59.0% p < 0.001   |                          |
| Prophylaxis     | 3-Day      | 2.75 ± 0.45 ± -22.2% p < 0.001   |                          |
| Group           | 6-Day      | 2.96 ± 0.49 ± -31.6% p < 0.001   |                          |
|                 | 9-Day      | 3.08 ± 0.48 ± -36.9% p < 0.001   |                          |
|                 | 12-Day     | 3.16 ± 0.42 ± -40.4% p < 0.001   |                          |
|                 | 19-Day     | 2.15 ± 0.34 ± 4.9% p < 0.057    |                          |

^a Subjects did not receive a prophylaxis at the end of study.
^b Subjects who received a prophylaxis at the end of the study.
^c Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
^d Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.

Table 6
Analysis of gingival index [mean ± SD] at each time point for subjects who completed the clinical study.

| Treatment Group | Time Point | Time Point Summary (Mean ± S.D.) | Within-Treatment Analysis |
|-----------------|------------|----------------------------------|--------------------------|
|                 |            | Percent Change^c Sig.^d          |                         |
| Non-Prophylaxis | 3-Day      | 1.43 ± 0.40 ± -22.2% p = 0.001   |                          |
| Group           | 6-Day      | 1.64 ± 0.25 ± -40.2% p < 0.001   |                          |
|                 | 9-Day      | 1.92 ± 0.07 ± -64.1% p < 0.001   |                          |
|                 | 12-Day     | 1.93 ± 0.19 ± -64.9% p < 0.001   |                          |
| Prophylaxis     | 3-Day      | 1.92 ± 0.16 ± -9.7% p < 0.001    |                          |
| Group           | 6-Day      | 2.00 ± 0.15 ± -14.3% p < 0.001   |                          |
|                 | 9-Day      | 2.05 ± 0.11 ± -17.1% p < 0.001   |                          |
|                 | 12-Day     | 2.10 ± 0.11 ± -20.0% p < 0.001   |                          |
|                 | 19-Day     | 1.67 ± 0.12 ± -5.1% p = 0.001    |                          |

^a Subjects did not receive a prophylaxis at the end of study.
^b Subjects who received a prophylaxis at the end of the study.
^c Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
^d Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.
Comparisons (p < 0.001). The effects of prophylaxis on gingival and bleeding indices were noted in the prophy group at the 3-day, 6-day, 9-day and 12-day evaluations respectively with several achieving statistical significance (p < 0.001). Prophylaxis reduced PMN levels to 4.94 and were 22% lower than baseline but did not gain statistical significance (p = 0.026).

Time point Summary (Mean ± S.D.)

| Treatment Group | Time | Summary | Within-Treatment Analysis |
|-----------------|------|---------|---------------------------|
| Non-Prophylaxis | 3-Day| 1.95 ± 0.20 | –0.02 p = 0.027 |
| Group #1 | 6-Day | 2.01 ± 0.12 | –0.08 p = 0.106 |
| | 9-Day | 2.07 ± 0.13 | –0.14 p = 0.011 |
| | 12-Day | 2.14 ± 0.39 | –0.21 p = 0.079 |
| Prophylaxis Group | 3-Day | 2.13 ± 0.18 | –0.04 p = 0.026 |
| | 6-Day | 2.19 ± 0.18 | –0.10 p < 0.001 |
| | 9-Day | 2.19 ± 0.20 | –0.10 p < 0.001 |
| | 12-Day | 2.23 ± 0.18 | –0.14 p < 0.001 |
| | 19-Day | 2.05 ± 0.15 | 0.05 p = 0.083 |

Subjects did not receive a prophylaxis at the end of study.
Subjects who received a prophylaxis at the end of the study.
Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.

## 5. Discussion

Inflammatory outcomes from microbial proliferation and other signals include a consistent and sustained host response [1,8,13,53]. Polymorphonuclear leukocytes [PMN] are widely recognized as the “first responders” with central functions to contain the emerging stimuli [7,8,12,13]. PMN are reported from mucosal interfaces [14] including the eye [24], nasal [19,20] and respiratory regions [18]. Neutrophil assessments have been used to measure inflammatory outcomes due to exposure to work-place occupational hazards amongst smelter workers [54], wood workers [22], wildfire fighters [58] and occupational asthma [56].

Common oral diseases such as gingivitis and periodontal disease are reported widely due to their global incidence [1,2,4]. Recent efforts in clinical dental research have placed a greater focus on biomarkers and other emerging approaches to examine the transition from health to disease. The current effort was on examining the effects of abstinence from oral hygiene during over a duration of 12 days resulting in experimental gingivitis. Oral PMN [52] were evaluated over the course of the study in conjunction with clinical measures for dental plaque, gingival inflammation, bleeding index and periodontal pocket depths [49-51]. Associated with the effort was the assessment of supragingival prophylaxis on reversing the observed outcomes.

This study enrolled healthy adults who presented with widely described average clinical parameters for dental plaque and gingival inflammation in the population [57,58]. These subjects were over the age of 18 years and not requiring ongoing medical or dental care. Additionally, the study excluded those reporting pregnancy or systemic conditions. Over the period of oral hygiene abstinence, the sequential evaluation of subjects every three days allowed a progressive monitoring of clinical outcomes and oral PMN. All assessments were conducted in the morning to standardize sampling and evaluation procedures. While subjects were not instructed to change their diet, they were instructed refrained from oral hygiene.

Progressive changes in clinical measures and levels of oral PMN identify the adherence of subjects to the study procedures and are consistent with observations reported previously [48,59]. Additionally, the frequency of the recall visits offered further avenues to reinforce subject adherence to study procedures. Primary outcomes in this study were the concomitant increases in oral PMN and clinical outcomes over the no-hygiene period with the group provided prophylaxis at the conclusion of the study offering important insights. The prophylaxis group demonstrated improvements in their clinical features and

### Table 7

Analysis of gingival index [mean ± SD] at each time point for subjects who completed the clinical study.

| Group | Time | Summary (Mean ± S.D.) | Within-Treatment Analysis |
|-------|------|-----------------------|---------------------------|
| Non-Prophylaxis | 3-Day | 5.58 ± 0.31 | –0.113 p = 0.008 |
| Group#1 | 6-Day | 6.16 ± 0.41 | –0.15 p = 0.002 |
| | 9-Day | 6.34 ± 0.52 | –0.18 p = 0.001 |
| | 12-Day | 6.61 ± 0.63 | –0.21 p = 0.000 |
| Prophylaxis Group | 3-Day | 4.79 ± 0.39 | –0.10 p = 0.044 |
| | 6-Day | 5.07 ± 0.49 | –0.12 p = 0.000 |
| | 9-Day | 5.24 ± 0.61 | –0.15 p = 0.001 |
| | 12-Day | 5.49 ± 0.72 | –0.20 p = 0.000 |

Subjects did not receive a prophylaxis at the end of study.
Subjects who received a prophylaxis at the end of the study.
Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.

### Table 8

Analysis of pocket depth (mm) [mean ± SD] at each time point for subjects who completed the clinical study.

| Group | Time | Summary (Mean ± S.D.) | Within-Treatment Analysis |
|-------|------|-----------------------|---------------------------|
| Non-Prophylaxis | 3-Day | 1.95 ± 0.20 | –0.02 p = 0.027 |
| Group#1 | 6-Day | 2.01 ± 0.12 | –0.08 p = 0.106 |
| | 9-Day | 2.07 ± 0.13 | –0.14 p = 0.011 |
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| | 12-Day | 2.23 ± 0.18 | –0.14 p < 0.001 |
| | 19-Day | 2.05 ± 0.15 | 0.05 p = 0.083 |

Subjects did not receive a prophylaxis at the end of study.
Subjects who received a prophylaxis at the end of the study.
Percent change exhibited relative to the baseline mean. Negative values indicate increase in evaluated parameter at the time point examined.
Significance of paired t-test comparing the baseline to the time point examined. Results indicate p value.
associated reductions in oral PMN. Whereas oral PMN levels were markedly lower after prophylaxis than the baseline and corresponded with previous observations [48] these outcomes were not statistically significant. Furthermore, while dental plaque levels after prophylaxis were significantly lower than baseline, assessments for gingival index, bleeding index and pocket probing depths while lower remained above baseline. Several reasons likely influence these observations with differences in study designs likely representing an important factor. The present study enrolled subjects between 18 and 60 years to include a wider age range of subjects with commonly described levels of oral hygiene who were not provided any scaling and polishing at study inception in contrast to previous a previous report [48]. These considerations were intended to more closely replicate subject enrollment criteria used in studies to examine the effects of oral hygiene interventions [52]. In the present study design, the resolution phase was one week and shorter than those generally described. Nonetheless, the rapid reductions in PMN and clinical outcomes represents a noteworthy finding.

From the perspective of application, the significant oral density of PMN has led to additional investigations. For instance, in an interventional study with a chlorhexidine mouthwash, subjects demonstrated marked improvements in clinical parameters of oral hygiene in conjunction with reductions in oral PMN. Sequential reductions in oral PMN over the study period with this outcome substantially higher than clinical parameters provides an ongoing measure of the oral inflammatory burden [52]. The shorter study design highlighted in this investigation incorporating frequent assessments may offer guidance on stratifying subjects and efficient monitoring of oral health outcomes. Available in other areas of medicine are applications for PMN from the standpoint of cost-effective point-of-care diagnostics and assess recovery from disease. For example, biomarkers related to PMN activities such as lactoferrin and PMN-elastase have been cleared by the US FDA for clinical use in USA and Europe [60]. In interventional studies, dietary supplementation with vitamin E [61] or flaxseed and cassava powder [62] reportedly influence PMN function to aid in the recovery from pneumococcal disease for persons of all ages. It is noteworthy that cytological analyses for nasal inflammation have been explored for application from pneumococcal disease for persons of all ages. It is noteworthy that cytological analyses for nasal inflammation have been explored for application from pneumonia in the PERCH study, Clin. Infect. Dis. 48 (9) (2019 Sep) 1130–1136.

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