Effect of Macrophage Activity and Age on Periodontal Disease in a Mouse Model

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Effect of Macrophage Activity and Age on Periodontal Disease in a Mouse Model

by
Benjamin Halpern

THESIS
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My Family, Friends and Co-Residents

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Abstract

Effect of Macrophage Activity and Age on Periodontal Disease

Benjamin James Halpern

**Background:** Periodontal disease is an inflammatory disease that increases in prevalence with increasing age. The elderly demonstrate an elevated and dysregulated inflammatory response. Macrophages act as a key regulator of inflammation. The study aim was to evaluate the extent to which age-related changes and chemical inhibition in the macrophage affect periodontal disease in a mouse model.

**Methods:** Old (24 month) and young (3 month) mice were utilized for this study. Periodontal status was examined in mice at baseline. Periodontal disease was induced via *Porphyromonas gingivalis* (*P. gingivalis*) inoculated ligatures in old and young mice for 7 days. A second cohort had disease induced for 7 days, followed by ligature removal, and recovery for 7 additional days. Half the mice in each induction or recovery group were treated with Pexidartinib (PLX), which inhibits macrophage recruitment. Linear bone loss, alveolar bone volume, and macrophage quantification were examined by t test.

**Results:** PLX successfully inhibited macrophage numbers in all treatment groups. The young periodontal disease group had significantly more vertical bone loss (0.234±0.024mm) compared to the young periodontal disease PLX group (0.140±0.023mm)(p≤0.001) and less alveolar bone volume (0.460±0.016BV/TV) compared to the young periodontal disease PLX group (0.586±0.004BV/TV)(p≤0.001). The old periodontal disease group exhibited no difference in vertical bone loss (0.242±0.025mm) compared to the old periodontal disease PLX group (0.238±0.032mm)(p=0.825) and significantly less alveolar bone volume (0.536±0.030BV/TV) compared to the old periodontal disease PLX group (0.614±0.030BV/TV)(p≤0.01). The young recovery control group trended towards less vertical bone loss (0.170±0.020mm) compared to
the young recovery + PLX group (0.190±0.011mm)(p=0.055) and had no difference in alveolar bone volume (0.557±0.015 BV/TV) compared to the young recovery PLX group (0.545±0.011 BV/TV)(p=0.131). The old recovery control group exhibited no difference in vertical bone loss (0.241±0.019mm) compared to the old recovery + PLX group (0.218±0.035mm)(p=0.187) and had significantly less alveolar bone volume (0.567±0.021BV/TV) compared to the old recovery PLX group (0.617±0.023BV/TV)(p≤0.01).

Conclusion:

PLX prevents periodontal disease induction in old and young groups and improves recovery in older age groups. The difference in response during recovery could suggest that there are age related changes in the macrophage that affect disease progression.
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ABBREVIATIONS

PLX - Pexidartinib
CT – computed tomography
BV/TV – Bone Volume divided by Total Volume
IHC – Immunohistochemistry
Perio – Periodontal
Dz – Disease
CEJ – Cementoenamel Junction
ABC – Alveolar Bone Crest
FOV – Field of view
IL - Interleukin
I. Introduction
I. Introduction

Periodontal disease is a chronic inflammatory disease characterized by the destruction of the supporting structures of the teeth.\(^1\) Periodontal disease is characterized by gingival inflammation that leads to loss of alveolar bone and periodontal tissues.\(^2\) The etiology of the disease is primarily attributed to a dysbiosis between the host response and bacterial biofilm.\(^3,4\) Individuals with severe and rapidly progressing forms of the disease exhibit an excessive host response and currently no treatment exists to modulate this response.\(^5\)

Periodontal disease severity and prevalence increases with age. Approximately 46% of US adults, representing 64.7 million people, have periodontal disease, with 8.9% having a severe form of the disease.\(^6\) Of adults 35-49 years of age, 36.6% have periodontal disease, which increases to 68% in adults 65 and older.\(^6\) Further, with the aging demographic in the US it is expected that the incidence of periodontal disease will increase as the population ages.\(^7\)

The elderly demonstrate a chronically elevated and dysregulated inflammatory response. The term “inflamm-aging” has been used to describe the systemic pro-inflammatory status associated with aging that predisposes the elderly population to a wide range of age-associated disease and conditions.\(^3,8\) Osteoporosis, Alzheimer’s disease, Type II diabetes, atherosclerosis, and Parkinson’s disease all become more prevalent with increased age and all have an underlying inflammatory component associated with their onset and progression.\(^9-12\) It has been proposed that inflamm-aging results from a defect in the resolution of inflammation or from a chronic unknown mechanism that signals and prolongs the inflammatory response.\(^13\) Experimental gingivitis studies have shown that a similar process happens in the periodontium, as older populations develop more gingivitis than younger populations.\(^14\)

The immune response during periodontal disease utilizes components of both innate and adaptive immunity.\(^3\) The early innate immune response is characterized by the presence of
neutrophils and macrophages. Both of these cellular inflammatory mediators are implicated in the protective and destructive host response.\textsuperscript{15}

Macrophages act as a key regulator of inflammation, and thus play an important role in periodontal disease induction and recovery.\textsuperscript{16} The role of macrophages during the inflammatory response includes initiation of inflammation, activation of lymphocyte-mediated adaptive immunity, mediation of alveolar bone resorption, and resolution of inflammation and tissue repair.\textsuperscript{16} Macrophages have both pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes.\textsuperscript{17} M1 macrophages exhibit increased phagocytosis, increased antigen presentation, as well as increased production of the pro inflammatory cytokines TNF-\(\alpha\), IL-1\(\beta\), and IL-6.\textsuperscript{16,18} The M2 macrophage produces the anti-inflammatory cytokine IL-10 as well as growth factors required for wound healing: TGF\(\beta\), VEGF, PDGF.\textsuperscript{16,19} The M1 phenotype aids in promoting inflammation and killing bacteria, however, the M2 phenotype aids in tissue repair, tissue homeostasis and inhibition and resolution of inflammation. An imbalance in the M1/M2 ratio is common in the pathogenesis of many inflammatory diseases, including periodontal disease.

Age related molecular alterations in macrophages include compromised chemotaxis and phagocytosis, production of reactive oxygen species, and increased cytokine production.\textsuperscript{15} With increased age, macrophages exhibit reduced production of reactive oxygen species and cytokines, such as IL-6, TNF-\(\alpha\), MIP-1\(\alpha\), MIP-1\(\beta\), and MIP-2, and increased production of receptors involved in inflammation amplification (C5aR, TREM-1) and PGE\(2\) production.\textsuperscript{15,20} The age-related reduction in chemotaxis by macrophages was demonstrated in a murine model and may be due to a reduced production of chemokines, such as macrophage inflammatory protein-1\(\alpha\) (MIP-1\(\alpha\)), MIP-1\(\beta\), and MIP-2.\textsuperscript{15,21} Research on macrophage phagocytosis as a function of age, has been inconclusive and conflicting, with studies showing increased, decreased, and unaltered phagocytosis with increased age.\textsuperscript{15,20} In terms of periodontal disease, macrophages
from both young and old mice exhibit similar capacity to phagocytize a major periodontal pathogen, *P. gingivalis*.\textsuperscript{15,22} Reduced production for some inflammatory mediators, such as IL-6, TNF-\(\alpha\), IL-12, IFN-\(\gamma\), occurs with increasing age. However, some mediators may increase with age such as PGE\(_2\).\textsuperscript{15,20,23,24} IL-12 stimulates cell mediated immunity. The ability of PGE\(_2\) to inhibit production of IL-12 may decrease older individuals ability to clear infections.\textsuperscript{15,24} PGE2 is heavily involved with periodontal destruction.\textsuperscript{15,25} The elevated levels of PGE\(_2\) in old age could be one of the reasons for the increased severity of periodontal disease in the elderly population.\textsuperscript{15} In vitro findings have shown that age does not affect macrophage ability to kill *P. gingivalis*, however, in vivo studies have shown impaired killing.\textsuperscript{15} This suggests that age-dependent changes in the local environment may inhibit macrophage ability to kill periodontal pathogens.\textsuperscript{15}

The dichotomous nature of the macrophage make it a potentially useful target for creating novel treatments for periodontal disease. Inhibition of macrophage recruitment has been accomplished with the use of Pexidartinib (PLX) (Plexxikon, Berkeley, CA). PLX is a kinase inhibitor that antagonizes macrophage colony-stimulating factor-1 (CSF-1) receptor, which is a key regulator of monocyte differentiation and activation.\textsuperscript{26} PLX has been used previously in cancer treatment. It successfully inhibited tumor-associated macrophages and caused a delay in melanoma outgrowth.\textsuperscript{27} PLX also significantly decreased macrophage recruitment into the fracture callus, which resulted in improved healing of tibial shaft fractures in elderly mice.\textsuperscript{28} The ability of PLX to aid in the recovery of long bones suggests that it may be a useful pharmacologic agent that could assist in the recovery of alveolar bone lost due to periodontal disease.

Mouse models of periodontal disease have been used to study disease mechanisms and in the research of new therapeutic approaches.\textsuperscript{29–31} Mouse models are useful because the disease process and initiation of inflammation can be controlled through placement of a ligature. The
disease process and the resulting alveolar bone loss can be accomplished in a relatively short time; from three to ten days. Removal of the ligature allows for the study of the resolution of inflammation and the recovery process.

The purpose of this study was to examine the extent to which age-related changes to the macrophage affect the pathogenesis of periodontal disease. Currently, it is known that macrophages play a role in the immune response to periodontal pathogens and the concurrent tissue breakdown in the periodontium. However, the specific role of the macrophage in periodontal disease in the context of aging is not well known, and therefore merits further investigation. This study utilized an aged mouse model of periodontal disease and pharmacological inhibition of macrophage activity to better understand the biology of aging in this context and to provide insight into the treatment of periodontal disease.
II. Materials and Methods
II. Materials and Methods

Experimental model: All procedures were approved by the UCSF Institutional Animal Care and Use Committee. Old (24-month) and young (3-month) male C57BL/6 mice were utilized in 10 treatment groups with 4 to 7 mice in each group (Table 1). A power analysis was used to calculate the sample size to detect a change in alveolar bone height of 0.25mm (α=0.05, β=0.80). These values on mean amount of bone loss to be expected in a mouse model of experimental periodontitis were derived from the literature. An initial pilot experiment was carried out to ensure significance was attainable with the proposed sample size. Periodontal disease induction was accomplished by tying a 6-0 nylon suture inoculated with *Porphyromonas gingivalis* (ATCC 33277) around the second maxillary molars. The suture or ligature was placed bilaterally and in a subgingival position (Figure 1.1). Old and young controls did not receive a ligature, and therefore did not exhibit experimental periodontitis. These controls were utilized for analysis at baseline. Ligatures were placed for 7 days for induction of periodontal disease in old and young mice. A second cohort had disease induced for 7 days, then the sutures were removed and the mice were allowed to recover for 7 additional days. Half of the mice in each induction or recovery group were treated with PLX, which was placed in the chow and given ad libitum (Table 1). At the end of the induction of recovery period, mice were euthanized via CO2 overdose and cervical dislocation. The maxilla were retrieved immediately and prepared for evaluation of bone loss measurements or immunohistochemistry. To prepare for bone loss measurements, the maxilla were defleshed, soaked in 30% hydrogen peroxide overnight, then rinsed and stored in 70% ethanol at 4°C until subsequent imaging and analysis.

Macrophage Inhibition: Inhibition of macrophage recruitment was accomplished by administration of PLX. PLX is a small molecule inhibitor of the macrophage colony stimulating factor 1 (MCSF-1) receptor. Antagonizing the MCSF-1 receptor prevents activation and
subsequent differentiation of monocytes into macrophages. PLX was delivered via mouse chow ad libitum at an average dose of 40mg/kg/day. To evaluate the effect of macrophage activity during induction of periodontitis and during recovery (after ligature was removed), PLX was administered from days 0-7 or days 7-14 respectively. Non-treated groups received the control chow provided by the drug manufacturer.

Analysis:

**Vertical Bone loss:** After tissue preparation the maxillae were stained with 1% methylene blue for 5 minutes prior to obtaining measurements. Magnified images of the maxillae were taken using a dissecting microscope, and Image J (NIH at https://imagej.nih.gov/ij/) was used to measure the bone loss. A ruler was placed in the field of view for calibration. Bone loss was measured from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) at 6 sites per tooth. Sites included in the bone loss analysis in both the experimental and control groups were those limited to the areas directly in contact with the ligature. Sites included were the distal buccal of the first molar, distal palatal of the first molar, all 6 sites on the second molar, and the mesial buccal and mesial palatal of the third molar (Figure 1.2). The sites that were excluded were those not in contact with the ligature. Sites were further excluded if there was damage to the tooth or surrounding structures or avulsion of the tooth during preparation.

**Alveolar Bone Volume:** Micro CT was used to measure changes in bone volume in the area of induced periodontal disease. The 3-dimensional area of interest spanned from the CEJ to the apex of the teeth, from the buccal and lingual walls of the maxillary alveolar bone, and from the mid-point of the 1st molar to the mid-point of the 3rd molar. The total volume of the area was measured, and the bone volume in the area was measured. This is expressed as the bone volume divided by the total volume (BV/TV) (Figure 1.3).
**Macrophage Quantification:** Immunohistochemical analysis was used to quantify macrophages in the periodontal tissues. The maxillae were fixed in 4% paraformaldehyde for 24h, then decalcified with 19% ethylenediaminetetraacetic acid (pH 8) for 28 days. The decalcified specimens were dehydrated and embedded in paraffin. The tissue blocks were cut into sections (8 micrometers) in a mesiodistal direction. Sections were incubated with a purified rat anti-mouse F480 antibody (BD Biosciences) overnight at 1:200 dilution, followed by 1:500 dilutions in HRP conjugated goat anti-mouse IgG (H+L) secondary antibody against the primary antibody for 1 hour. The sections were then incubated with a 3,3'-diaminobenzidine (DAB) substrate and observed under microscopy. Photographs were taken at 200x, and 3 regions of interest per sample that were adjacent to the tooth, including the epithelium, connective tissue and bone in the coronal 1/3 of the root were analyzed at 400x (Fig 1.4). The region of interest had a field of view (FOV) of 0.23mm². F480+ macrophages were quantified using Image J imaging software. The 3 regions were averaged per sample for each mouse.

**Statistical Methods:** For vertical bone loss measurements, the mouse mean value was the unit used for statistical analysis. For the volumetric analysis, the BV/TV was calculated per sample. Bone loss and macrophage quantification measurements were first analyzed via ANOVA for significant differences, and between group differences were analyzed using a two tailed T-test. Significance was determined at p<0.05.
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<td>Young Control</td>
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<tr>
<td>2</td>
<td>Old Control</td>
<td>No Treatment</td>
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<td>7 Days Ligature</td>
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<td>4</td>
<td>Young Perio Dz + PLX</td>
<td>7 Days Ligature w/ PLX</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>Young Recovery Control</td>
<td>7 Days Ligature, 7 Days Recovery</td>
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<td>8</td>
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<td>7 Days Ligature, 7 Days Recovery w/ PLX</td>
</tr>
<tr>
<td>9</td>
<td>Old Recovery Control</td>
<td>7 Days Ligature, 7 Days Recovery</td>
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<tr>
<td>10</td>
<td>Old Recovery + PLX</td>
<td>7 Days Ligature, 7 Days Recovery w/ PLX</td>
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Figure 1: Description of Data Collection 1.1: Ligature in place 1.2: Vertical bone loss (CEJ-ABC) measurements 1.3: Alveolar Bone Volume area depicted 1.4: Macrophage Quantification, Gross image and regions of interest
III. Results
III. Results

Young Control vs Old Control

The young control group (n=5) had significantly less vertical bone loss (0.095 ± 0.015mm) compared to the old control group (n=4) (0.192 ± 0.007mm) (p ≤ 0.001) (Figure 2.1, 2.2). No differences in F 480+ macrophage quantity were found between the young control group (n=6) (11.7 ± 7.3 cells/FOV) compared to the old control group (n=7) (9.0 ± 6.6 cells/FOV) (p = 0.50) (Figure 2.3, 2.4).

Young Control vs Young Periodontal Disease

The young periodontal disease group (n=5) had significantly more vertical bone loss (0.234 ± 0.024mm) compared to the young control group (n=5) (0.095 ± 0.015mm) (p ≤ 0.001) (Figure 3.1, 3.2). The young periodontal disease group (n=5) had significantly more F480+ macrophages (50.33 ± 7.4 cells/FOV) compared to the young control group (n=6) (11.7 ± 7.3 cells/FOV) (p ≤ 0.001) (Figure 3.3, 3.4).

Old Control vs Old Periodontal Disease

The old periodontal disease group (n=6) had significantly more vertical bone loss (0.242 ± 0.025mm) compared to the old control group (n=4) (0.192 ± 0.007) (p ≤ 0.01) (Figure 4.1, 4.2). The old periodontal disease group (n=6) had significantly more F480+ macrophages (74.2 ± 27.7 cells/FOV) compared to the old control group (n=7) (9 ± 6.6 cells/FOV) (p ≤ 0.001) (Figure 4.3, 4.4).

Young Periodontal Disease vs Young Periodontal Disease + PLX

The young periodontal disease group (n=5) had significantly more vertical bone loss (0.234 ± 0.024mm) compared to the young periodontal disease + PLX group (n=5) (0.140 ± 0.023mm) (p ≤ 0.001) (Fig 5.1, 5.2). The young periodontal disease group (n=5) had significantly less alveolar
bone volume (0.460 ± 0.016 BV/TV) compared to the young periodontal disease + PLX group (n=5) (0.586 ± 0.004 BV/TV) (p ≤ 0.001) (Fig 5.3). The young periodontal disease group (n=5) had significantly more F480+ macrophages (50.3 ± 7.4 cells/FOV) compared to the young periodontal disease + PLX group (n=5) (11.7 ± 8.4) (p ≤ 0.001) (Figure 5.4, 5.5).

**Old Periodontal Disease vs Old Periodontal Disease + PLX**

The old periodontal disease group (n=6) had no difference of vertical bone loss (0.242 ± 0.025mm) compared to the old periodontal disease + PLX group (n=6) (0.238 ± 0.032mm) (p = 0.825) (Fig 6.1, 6.2). The old periodontal disease group (n=5) had significantly less alveolar bone volume (0.536 ± 0.030 BV/TV) compared to the old periodontal disease + PLX group (n=5) (0.614 ± 0.030) (p ≤ 0.01) (Fig 6.3). The old periodontal disease group (n=6) had significantly more F480+ macrophages (74.2 ± 27.7 cells/FOV) compared to the old periodontal disease + PLX group (n=6) (24.4 ± 13.0 cells/FOV) (p ≤ 0.01) (Fig 6.4, 6.5).

**Young Recovery Control vs Young Recovery + PLX**

The young recovery control group (n=6) trended towards less vertical bone loss (0.170 ± 0.020mm) compared to the young recovery + PLX group (n=6) (0.190 ± 0.011mm) (p=0.055) (Fig 7.1, 7.2) The young recovery control (n=6) had no difference in alveolar bone volume (0.557 ± 0.015 BV/TV) compared to the young recovery + PLX group (n=6) (0.545 ± 0.011 BV/TV) (p = 0.131) (Fig 7.3). The young recovery control group (n=6) had significantly more F480+ macrophages (54.6 ± 9.6 cells/FOV) compared to the young recovery + PLX group (n=6) (5.4 ±4.7 cells/FOV) (p ≤ 0.001) (Fig 7.4, 7.5).

**Old Recovery Control vs Old Recovery + PLX**

The old recovery control group (n=6) had no difference in vertical bone loss (0.241 ± 0.019mm) compared to the old recovery + PLX (n=7) (0.218 ± 0.035mm) (p = 0.187) (Fig 8.1, 8.2). The old
recovery control (n=6) had significantly less alveolar bone volume (0.567 ± 0.021 BV/TV) compared to the old recovery + PLX group (n=7) (0.617 ± 0.023 BV/TV) (p ≤ 0.01) (Fig 8.3). The old recovery control (n=6) had significantly more F480+ macrophages (47.8 ± 18.3 cells/FOV) compared to the old recovery + PLX group (n=7) (5.0 ± 2.3 cells/FOV) (p ≤ 0.001) (Fig 8.4, 8.5).

Figure 2: Young Control vs Old Control 2.1: Bar Graph of Vertical Bone Loss 2.2: Dissecting microscope images 2.3: Bar Graph of Macrophage Quantification 2.4: Immunohistochemistry images
**Figure 3:** Young Control vs Young Perio Dz

3.1: Bar Graph of Vertical Bone Loss

3.2: Dissecting microscope images

3.3: Bar Graph of Macrophage Quantification

3.4: Immunohistochemistry images
**Figure 4: Young Control vs Young Perio Dz**

4.1: Bar Graph of Vertical Bone Loss
4.2: Dissecting microscope images
4.3: Bar Graph of Macrophage Quantification
4.4: Immunohistochemistry images
Figure 5: Young Perio Dz vs Young Perio Dz + PLX

5.1: Bar Graph of Vertical Bone Loss
5.2: Dissecting microscope images
5.3: Bar Graph of Alveolar Bone Volume
5.4: Bar Graph of Macrophage Quantification
5.5: Immunohistochemistry images
Figure 6: Old Perio Dz vs Old Perio Dz + PLX
6.1: Bar Graph of Vertical Bone Loss
6.2: Dissecting microscope images
6.3: Bar Graph of Alveolar Bone Volume
6.4: Bar Graph of Macrophage Quantification
6.5: Immunohistochemistry images
**Figure 7: Young Recovery Control vs Young Recovery + PLX**

7.1: Bar Graph of Vertical Bone Loss  
7.2: Dissecting microscope images  
7.3: Bar Graph of Alveolar Bone Volume  
7.4: Bar Graph of Macrophage Quantification  
7.5: Immunohistochemistry images
Figure 8: Young Recovery Control vs Young Recovery + PLX
8.1: Bar Graph of Vertical Bone Loss 8.2: Dissecting microscope images 8.3: Bar Graph of Alveolar Bone Volume 8.4: Bar Graph of Macrophage Quantification 8.5: Immunohistochemistry images
### Table 2: Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Vertical Bone Loss (CEJ – ABC) (mm)</th>
<th>Alveolar Bone Volume (BV/BT)</th>
<th>Macrophage Quantification (Cells/FOV)</th>
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<tr>
<td>Young Control</td>
<td>0.095 ± 0.015</td>
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<td>Old Control</td>
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<td>Young Perio Dz</td>
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<td>Old Perio Dz + PLX</td>
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<td>0.218 ± 0.035</td>
<td>0.617 ± 0.023</td>
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### Table 3: Vertical Bone Loss Comparisons

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<th>Group</th>
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<th>Group</th>
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<td>Old Control</td>
<td>0.192 ± 0.007</td>
<td>P ≤ 0.001</td>
</tr>
<tr>
<td>Young Control</td>
<td>0.095 ± 0.015</td>
<td>Young Perio Dz</td>
<td>0.234 ± 0.024</td>
<td>P ≤ 0.001</td>
</tr>
<tr>
<td>Old Control</td>
<td>0.192 ± 0.007</td>
<td>Old Perio Dz</td>
<td>0.242 ± 0.025</td>
<td>P ≤ 0.01</td>
</tr>
<tr>
<td>Young Perio Dz</td>
<td>0.234 ± 0.024</td>
<td>Young Perio Dz + PLX</td>
<td>0.140 ± 0.023</td>
<td>P ≤ 0.001</td>
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<tr>
<td>Old Perio Dz</td>
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<td>Old Perio Dz + PLX</td>
<td>0.238 ± 0.032</td>
<td>P = 0.825</td>
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<tr>
<td>Young Recovery Control</td>
<td>0.170 ± 0.020</td>
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<td>0.190 ± 0.011</td>
<td>P=0.055</td>
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<tr>
<td>Old Recovery Control</td>
<td>0.241 ± 0.019</td>
<td>Old Recovery Test</td>
<td>0.218 ± 0.035</td>
<td>P = 0.187</td>
</tr>
<tr>
<td>Group</td>
<td>Alveolar Bone Volume (BV/TV)</td>
<td>Group</td>
<td>Alveolar Bone Volume (BV/TV)</td>
<td>P-value</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------------</td>
<td>------------------------</td>
<td>------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Young Perio Dz</td>
<td>0.460 ± 0.016</td>
<td>Young Perio Dz + PLX</td>
<td>0.586 ± 0.004</td>
<td>P ≤ 0.001</td>
</tr>
<tr>
<td>Old Perio Dz</td>
<td>0.536 ± 0.030</td>
<td>Old Perio Dz + PLX</td>
<td>0.614 ± 0.030</td>
<td>P ≤ 0.01</td>
</tr>
<tr>
<td>Young Recovery Control</td>
<td>0.557 ± 0.015</td>
<td>Young Recovery + PLX</td>
<td>0.545 ± 0.011</td>
<td>P = 0.131</td>
</tr>
<tr>
<td>Old Recovery Control</td>
<td>0.567 ± 0.021</td>
<td>Old Recovery Test</td>
<td>0.617 ± 0.023</td>
<td>P ≤ 0.01</td>
</tr>
<tr>
<td>Group</td>
<td>Macrophage Quantification (Cells/FOV)</td>
<td>Group</td>
<td>Macrophage Quantification (Cells/FOV)</td>
<td>P-value</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------</td>
<td>-------------</td>
<td>---------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Young Control</td>
<td>11.7 ± 7.3</td>
<td>Old Control</td>
<td>9.0 ± 6.6</td>
<td>$P = 0.50$</td>
</tr>
<tr>
<td>Young Control</td>
<td>11.7 ± 7.3</td>
<td>Young Perio Dz</td>
<td>50.3 ± 7.4</td>
<td>$P \leq 0.001$</td>
</tr>
<tr>
<td>Old Control</td>
<td>9.0 ± 6.6</td>
<td>Old Perio Dz</td>
<td>74.2 ± 27.7</td>
<td>$P \leq 0.001$</td>
</tr>
<tr>
<td>Young Perio Dz</td>
<td>50.3 ± 7.4</td>
<td>Young Perio Dz + PLX</td>
<td>11.7 ± 8.4</td>
<td>$P \leq 0.001$</td>
</tr>
<tr>
<td>Old Perio Dz</td>
<td>74.2 ± 27.7</td>
<td>Old Perio Dz + PLX</td>
<td>24.4 ± 13.0</td>
<td>$P \leq 0.01$</td>
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<tr>
<td>Young Recovery Control</td>
<td>54.6 ± 9.6</td>
<td>Young Recovery + PLX</td>
<td>5.4 ± 4.7</td>
<td>$P \leq 0.001$</td>
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<tr>
<td>Old Recovery Control</td>
<td>47.8 ± 18.3</td>
<td>Old Recovery Test</td>
<td>5.0 ± 2.3</td>
<td>$P \leq 0.001$</td>
</tr>
</tbody>
</table>
IV. Discussion
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The old control group had significantly more vertical bone loss than the young control group (Figure 2.1). This agrees with previous studies that older mice demonstrate an aged periodontal disease phenotype that represents a genuinely chronic model to study the mechanisms of periodontal destruction.\(^{34}\) In the study by Liang, old mice displayed significantly (p<0.05) increased bone loss, elevated proinflammatory cytokines (IL-1β, tumor necrosis factor α, IL-17A) as well as increased innate immune receptors involved in inflammation (Toll-like receptor 2, CD14, CD11b, CD18, complement C5a receptor). \(^{34}\) The fact that mice display a chronic disease process similar to humans provides validity to the use of a mouse model to develop novel therapeutic approaches for the treatment of periodontitis.

There was no significant difference in the number of macrophages between the old and young control groups (Figure 2.4). In the control groups there was a large standard deviation of the macrophage quantification. This may be explained by the “Random Burst” or “Asynchronous Multiple Burst” model of periodontal disease progression.\(^{35,36}\) Some areas might have been undergoing a burst of disease activity and thus had more macrophages present in their tissues. This shows that in the absence of disease, the baseline quantity of macrophages in the periodontal tissues is independent of age.

The ligature induced model was useful for inducing periodontal disease in both young and old mice. Both young and old periodontal disease groups had significantly more vertical bone loss and less alveolar bone volume compared to their respective controls (Figure 3.1, 4.1). This data agrees with previous studies that have induced periodontal disease in mice.\(^{32}\) The linear bone measurements attained in this study are similar to other ligature induced models. In the study by Li et al, young mice had approximately 0.1mm CEJ-ABC measurement at baseline which increased to approximately 0.2mm after 7 days of induction with the ligature.\(^{32}\) In addition to more bone loss, both disease groups also had significantly increased numbers of macrophages
in their periodontal tissues compared to their controls (Figure 3.3, 4.3). This shows that an increased number of macrophages in the periodontal diseased tissues is independent of age.

Macrophage infiltration of the periodontal tissues was inhibited with the use of PLX. The ligature induced periodontal disease model resulted in successful recruitment of macrophages into the periodontium. During the induction of disease both the young periodontal disease and old periodontal disease groups had significantly more macrophages present in their tissues than their respective controls tissues (Figure 3.3, 4.3). PLX treatment was successful in decreasing macrophage recruitment in all treated groups. During the induction of disease in both young and old mice, there were significantly less macrophages present in both the young and old periodontal disease + PLX groups compared to the non-PLX groups (Figure 5.3, 6.3). Similarly, during the recovery period, there were significantly less macrophages present in the PLX treated Old and Young recovery groups compared to the non-treated recovery groups (Figure 7.3, 8.3). The inhibition of macrophage recruitment is similar to what has been demonstrated in other studies that have shown PLX inhibiting macrophage recruitment in tumors as well during fracture healing. By effectively inhibiting macrophage recruitment in young and old mice, the extent to which age-related changes in the macrophage affect the pathogenesis of periodontal disease can be studied.

During the induction of disease, the young group treated with PLX had significantly less vertical bone loss and more bone volume compared to its respective control (Figure 5.1, 5.3). The old group treated with PLX did not have a significant difference in vertical bone loss but did have significantly more alveolar bone volume compared to its respective control (Figure 6.1, 6.3). This shows that the macrophage is involved with periodontal destruction and inhibiting its contribution to the disease process can slow the progression of disease.
During recovery of disease, the young and old PLX groups did not have significantly different vertical bone levels compared to their respective controls (Figure 5.1, 6.1). The old recovery PLX had significantly more alveolar bone volume compared it the respective control (Figure 8.3). The young recovery PLX group did not have any difference with its control (Figure 7.3). This suggests that inhibiting macrophages in old may have some benefit to preserving alveolar bone during the recovery of disease. This is promising, as periodontal disease predominately effects older age groups, and this may be a novel therapy that could aid in treating periodontal disease.

In terms of periodontal disease, the strongest effects were seen in the young perio disease groups where PLX decreased linear bone loss and increased bone volume. This could lead the way to novel treatment for stage III, grade C, molar incisor pattern periodontitis which is most commonly seen in younger age groups. There is a genetic component to this type of periodontal disease, so at risk individuals could be identified by family history and given the medication prophylactically in their youth when they are most susceptible to prevent the rapid bone loss seen in these individuals.

The increased bone volume seen in the Old Recovery + PLX group compared to the old recovery group is most applicable to treatment of periodontal disease. Typically, the disease is identified in the elderly population and then treatment is performed. The fact that PLX aided in the recovery indicates that it may have use as an adjunct to traditional periodontal treatments. PLX administration could be combined with traditional periodontal therapies such as scaling and root planning or periodontal flap surgery with or without regeneration to aid in recovery.

More research on the long term effects of systemic PLX administration are needed before its widespread use is adopted. In terms of periodontal disease, a locally delivered method would be preferable to prevent unwanted systemic effects. However, PLX works by blocking CSF-1 on
monocytes in the blood and preventing monocyte differentiation into macrophages. In its current form, a locally delivered method would likely not achieve the desired results. As further research is conducted into macrophage signaling and differentiation, it may be possible to create subclasses of the medication that could be made to only affect recruitment into the periodontal tissues.

Age related changes to the macrophage response can be further studied by looking at local pro-inflammatory macrophage markers to explain the difference in response between age groups. Analyzing cytokines such as IL-6, IL-12 TNF-α, MIP-1α, MIP-1β, MIP-2, C5aR, TREM-1, PGE₂ may help further elucidate differences between old and young macrophages and the mechanisms in which inhibiting macrophages has shown to have a beneficial effect on periodontal disease.

**Conclusion**

These data show that modulating the macrophage response has beneficial effects during induction and recovery of periodontal disease. This is a promising area of novel treatment for periodontal disease. Currently, there are a limited number of treatments for periodontal disease that modulate the host response. Novel treatments for periodontal disease, such as host response modulation are needed as the disease model of periodontitis has moved away from a plaque specific hypothesis to a broader dysbiosis between the host response and bacteria. New treatments are needed to address the changing disease progression models. A limitation of the current treatment modality is that it affects macrophages systemically, which may have detrimental effects in other areas of the body. In order for this treatment to become more acceptable and to justify its long term use in humans, a greater understanding of its systemic effects and the drug half-life are needed.
Works Cited
Works Cited


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