INNATE IMMUNE RECEPTOR EXPRESSION IN PERI-IMPLANT TISSUES OF PATIENTS WITH DIFFERENT SUSCEPTIBILITY TO PERIODONTAL DISEASE

By

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I dedicate this thesis to my wife, Katherine, and my family for all of their support during this journey.
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## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>6</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>7</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>8</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>9</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>11</td>
</tr>
<tr>
<td>2  BACKGROUND</td>
<td>14</td>
</tr>
<tr>
<td>Anatomy of the Periodontium</td>
<td>14</td>
</tr>
<tr>
<td>Periodontal Diseases</td>
<td>15</td>
</tr>
<tr>
<td>Dental Implants</td>
<td>17</td>
</tr>
<tr>
<td>Dental Implants in Periodontal Patients</td>
<td>20</td>
</tr>
<tr>
<td>Immune Response</td>
<td>23</td>
</tr>
<tr>
<td>3  MATERIALS AND METHODS</td>
<td>32</td>
</tr>
<tr>
<td>Experimental Groups</td>
<td>32</td>
</tr>
<tr>
<td>Dental Implant Surgeries and Soft Tissue Biopsies</td>
<td>33</td>
</tr>
<tr>
<td>Real-Time PCR</td>
<td>35</td>
</tr>
<tr>
<td>Western Blot</td>
<td>35</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>36</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>37</td>
</tr>
<tr>
<td>4  RESULTS</td>
<td>39</td>
</tr>
<tr>
<td>5  DISCUSSION</td>
<td>47</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>54</td>
</tr>
<tr>
<td>BIOGRAPHICAL SKETCH</td>
<td>61</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table       page

2-1  TLR-2 and TLR-4 ligands ................................................................. 31
4-1  Clinical parameters of periodontally healthy ........................................... 41
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Clinical description of the gingiva.</td>
<td>27</td>
</tr>
<tr>
<td>2-2</td>
<td>Anatomy of the alveolar mucosa</td>
<td>28</td>
</tr>
<tr>
<td>2-3</td>
<td>Diagram of the dentogingival fibers extending from the cementum</td>
<td>29</td>
</tr>
<tr>
<td>2-4</td>
<td>X-ray showing the three parts of an implant system.</td>
<td>30</td>
</tr>
<tr>
<td>3-1</td>
<td>Experimental design</td>
<td>38</td>
</tr>
<tr>
<td>4-1</td>
<td>Elevated levels of RAGE mRNA in biopsies from periodontally susceptible</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>individuals prior to implant therapy</td>
<td></td>
</tr>
<tr>
<td>4-2</td>
<td>Elevated levels of RAGE protein in biopsies from periodontally susceptible</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>individuals prior to implant therapy</td>
<td></td>
</tr>
<tr>
<td>4-3</td>
<td>Elevated levels of TLR2 mRNA in biopsies from periodontally healthy</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>individuals following implant therapy</td>
<td></td>
</tr>
<tr>
<td>4-4</td>
<td>Up-regulation of RAGE and TLR4 with concomitant down-regulation of TLR2</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>in biopsies from periodontally susceptible individuals following implant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>therapy.</td>
<td></td>
</tr>
<tr>
<td>4-5</td>
<td>Elevated cellular infiltrate in biopsies from periodontally susceptible</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>individuals prior to and following implant therapy</td>
<td></td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>Clinical attachment loss</td>
</tr>
<tr>
<td>CEJ</td>
<td>Cementoenamel junction</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>RAGE</td>
<td>Receptor for advanced glycation end-products</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TLR-2</td>
<td>Toll-like receptor-2</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Toll-like receptor-4</td>
</tr>
</tbody>
</table>
The effect of the innate immune system on implant therapies is not clear. Multiple innate immune receptors including the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs) are up-regulated on cells within inflamed gingiva and are responsible for initiation of detrimental host responses.

The aim of this study was to compare the expression levels of TLR-2, TLR-4 and RAGE in gingival tissues from periodontitis and non-periodontitis susceptible participants prior to and following implant therapy. We hypothesize that healthy tissues from periodontitis susceptible patients have higher expression of TLR-2, TLR-4 and RAGE receptors compared to non-periodontitis susceptible patients; and that peri-implant mucosa around dental implants placed due to periodontitis-associated tooth loss has higher expression of TLR-2, TLR-4 and RAGE receptors compared to peri-implant mucosa around dental implants placed due to non-periodontitis-associated tooth loss.

This was designed as a controlled study where two groups had, as part of their treatment, implant therapy in order to restore edentulism. Group A was diagnosed with
generalized chronic periodontitis (n=10), while Group B was diagnosed as periodontally healthy (n=10). Biopsies were collected from edentulous ridges prior to implant placement and again from the peri-implant mucosa two months following implant placement. TLR-2, TLR-4 and RAGE expression was evaluated by real-time PCR (qPCR), western blot and immunohistochemical analysis. A comparison between periodontitis and non-periodontitis susceptible patients for TLR-2, TLR-4 and RAGE expression was performed with Mann-Whitney U test.

Prior to implant therapy, elevated levels of RAGE were detected in gingival tissues from periodontitis susceptible participants when compared to those from periodontally healthy patients, while no differences in the expression of TLR-2 nor TLR-4 were detected. Following implant therapy, there was an up-regulation of RAGE and TLR-4 levels which coincided with a significant down regulation of TLR-2 levels in biopsies from periodontitis susceptible participants. Levels of RAGE and TLR-4 remained unchanged in biopsies from periodontally healthy participants, while TLR-2 levels were significantly up-regulated. Histologically, post-implant biopsies from periodontitis susceptible participants displayed higher levels of inflammatory infiltrate.

The findings of the study revealed elevated levels of inflammatory potential were found following implant therapy in periodontitis susceptible patients.
CHAPTER 1
INTRODUCTION

Periodontal disease is an inflammatory disease that affects the soft and hard tissue structures that support teeth\(^1\). The early stage of periodontal disease affects the soft tissues, or gingiva, around teeth and is called gingivitis. The gums become swollen and red due to inflammation, which is the body’s natural response to the presence of harmful bacteria.\(^2\) The more advanced and serious form of periodontal disease is called periodontitis, in which there is loss of supporting bone around teeth.\(^1\) As this bone loss progresses, teeth can become mobile, loose and potentially fall out.

Periodontitis does not affect everyone, it affects only those that are susceptible and harbor the proper bacteria. However, approximately 75% of adults in the United States are affected by some form of periodontal disease, ranging from mild cases of gingivitis to severe forms of periodontitis.\(^3\) The total true prevalence of periodontitis in the USA from the “gold standard” full mouth periodontal examination (FMPE) protocol was 22.4%, in which the most severe form of the disease is present in approximately 4.8% of the population, whereas the 17.5% exhibit moderate to mild signs of disease.\(^1\) Overall, periodontitis is thought to account for 30 to 35% of all tooth extractions.\(^1\)

In modern dentistry, rehabilitation of lost teeth using dental implant-supported prostheses is an established and widely used treatment modality. Over the past 30 years, research has validated the success of osseointegrated implants as a viable alternative to traditional fixed or removable dental prosthetic restorations. The term osseointegrated refers to the direct structural connection at the light microscopic level between bone and the surface of a load-carrying implant.\(^4\)
An important question regarding implant therapy in periodontitis susceptible patients is whether they also demonstrate an elevated risk for infection of the tissues surrounding the implant, resulting in loss of hard and soft tissues around the implant. There is limited information available regarding the outcome of implant therapy in periodontitis-susceptible patients. However, it seems that most studies show that periodontitis-susceptible patients can be successfully treated with dental implant therapy and have good long-term results.5-9

Periodontitis-associated microorganisms have shown to colonize around dental implants,10,11 and this can occur within minutes after implant placement.12 It has suggested that the inflammatory and immune response defense mechanisms to bacteria around implants is similar to that for bacteria around natural teeth.13-16 The peri-implant tissue response to bacteria may follow similar patterns to that of periodontitis in a susceptible host.

A fundamental aspect of the inflammatory response of the gingiva are toll-like receptors (TLRs), which are intracellular and extracellular proteins that form the first response to bacterial and viral infiltration. They elicit an inflammatory response to lipopolysaccharides, peptidoglycan, flagellin, foreign DNA and RNA, as well as bacterial byproducts.17 It has been shown that TLR-2, TLR-4 and RAGE are up-regulated in the presence of bacterial insult in the periodontium.18-21 Both periodontally diseased and healthy tissues have demonstrated the expression of innate immune receptors TLR-2, TLR-4 and RAGE, though there are differences in the expression levels. There is limited information regarding the expression of TLR-2, TLR-4 and RAGE receptors in peri-
implant mucosa around implants placed due to periodontitis and non-periodontitis-associated tooth loss.

The aim of this study was to compare the expression levels of TLR-2, TLR-4 and RAGE in gingival tissues from periodontitis and non-periodontitis susceptible participants prior to and following implant therapy.
CHAPTER 2
BACKGROUND

Anatomy of the Periodontium

The periodontium is the tooth supporting structures that consist of alveolar bone, cementum, periodontal ligament and the gingiva. The alveolar bone is the bone that supports and surrounds teeth. Cementum is the mineralized surface that covers the roots of teeth. The periodontal ligament is the ligament that attaches the tooth to the socket of the alveolar bone. The gingiva is the part of the oral mucosa that covers the alveolar bone and the cervical areas of teeth. The gingiva is divided into marginal gingiva (which is unattached and surrounds the teeth in a collar-like fashion) and attached gingiva, which is continuous with the marginal gingiva, but is firmly adhered to the alveolar bone. The attached gingiva is further continuous with the movable alveolar mucosa and they join at the mucogingival junction (MGJ) (Figures 2-1 and 2-2).

Gingiva is attached to the tooth surface firmly by the junctional epithelium (JE) and loosely by connective tissue. The connective tissue of the marginal gingiva is densely collagenous and contains a system of collagen fiber bundles called the gingival fibers. Gingival fibers function to brace the marginal gingival firmly around the tooth, to provide the rigidity necessary to withstand the forces of mastication without being deflected away from the tooth surface, and to unite the marginal gingiva with the cementum of the root and the adjacent attached gingiva.\(^22\) They are arranged in three groups: gingivodental, circular and transseptal.\(^23\) The gingivodental fibers are those on the facial (cheek side), lingual (tongue side) and interproximal (between the teeth) surfaces that attach the gingiva to the tooth. The circular fibers course through the connective tissue of the marginal and interdental gingiva and encircle the tooth in a ring-like fashion. The
transseptal fibers are located interproximally and form horizontal bundles that extend between the cementum of adjacent teeth into which they are embedded (Figure 2-3).

When a periodontal pocket is measured with a periodontal probe, it measures the depth of the gingival sulcus. The gingival sulcus is a pocket next to a tooth that is bounded by the tooth on one side and the sulcular epithelium of the gingiva on the other. In a normal, healthy patient, the gingival sulcus measures ≤3 mm and the pocket ends at the cementoenamel junction (CEJ). The CEJ is the area where the cementum of the root meets the enamel of the crown. If the pocket ends apical to the CEJ, or closer to the root apex, then clinical attachment loss (CAL) is considered to have taken place (Figure 2-2).

**Periodontal Diseases**

The periodontal pocket, which is defined as a pathologically deepened sulcus beyond 3 mm around a tooth, is one of the most important clinical features of periodontal disease. There are numerous types of periodontal diseases ranging from gingivitis, which is inflammation of the gingiva in response to a bacterial challenge, to periodontitis, which is inflammation of the supporting structures of the tooth that leads to bone loss. Periodontitis is always preceded by gingivitis, but not all gingivitis progress to periodontitis. Gingivitis will progress to periodontitis only in those people that are susceptible to bone loss around teeth. The prevalence of periodontitis in the United States is about 22.4%.¹

The transition from gingivitis to periodontitis is associated with changes in the composition of bacterial plaque. In advanced stages of the disease, the numbers of motile bacteria and spirochetes increase, whereas the numbers of coccoid rods and straight rods bacteria decrease.²⁴ The cellular composition of the infiltrated connective
tissue also changes with increasing severity of the lesion. Fibroblasts and lymphocytes predominate in gingivitis, while the number of plasma cells, blast cells, polymorphonuclear leukocytes (PMNs) and osteoclasts increase as the lesion progresses to periodontitis. Fibroblasts are cells that make collagen that helps form the gingiva. Lymphocytes and plasma cells are white blood cells that play a key role in adaptive (cell-mediated) immunity against microorganisms. PMNs are immune cells that play a key role in both innate and adaptive immunity. Blast cells are immature immune cells in bone marrow. Osteoclasts are cells that resorb, or eat up, bone. It is thought that they do this through the release of hydrogen ions. These ions act as an acid that removes the mineral content of bone. The resorption of bone by osteoclasts is the hallmark of periodontitis.

The two main types of periodontitis are aggressive periodontitis and chronic periodontitis. Aggressive periodontitis is characterized by rapidly progressing bone destruction, which can happen over a span of months to a few years. Chronic periodontitis is a more gradually progressing bone destruction that happens over years to decades. As mentioned earlier, the factors involved with bone and soft tissue destruction in periodontitis are bacterial and host mediated. Bacterial plaque products induce the differentiation of bone progenitor cells into osteoclasts and stimulate gingival cells to release mediators that have the same effect as osteoclasts. The end result is that the alveolar bone will be resorbed away and the collagen within the gingiva will become inflamed and destroyed.

Chronic periodontitis can be classified based on the extent and severity involved. The extent can be characterized as localized (<30% of sites around teeth are involved)
and generalized (>30% of sites around teeth are involved). The severity can be characterized based on the amount of clinical attachment loss (CAL) as follows: slight is 1-2 mm of CAL, moderate is 3-4 mm of CAL and severe is ≥5 mm of CAL. Stable or non-progressing chronic periodontitis means that there was past CAL; however, it is no longer progressing and is currently under control. For this diagnosis to be made, longitudinal records of CALs are needed. The periodontitis patients that were a part of this study were considered to be stable chronic periodontitis patients with non-active disease.

Traditional periodontal therapy involves removing local environmental factors that can cause the retention and colonization of bacteria. Dental plaque and calculus (also called tartar) are the main reservoirs for bacteria. Removal of these reservoirs will help stabilize periodontal destruction. This can be accomplished non-surgically through scaling and root planing, which constitutes the use of ultrasonic and hand instruments to remove plaque and calculus around teeth in the pockets. This can also be accomplished surgically through the various types of periodontal surgery. If the severity of the bone loss is severe enough that the tooth cannot be predictably saved, then extraction is the treatment of choice. Once the tooth is extracted, it can be replaced with a tooth-borne prosthesis, a removable prosthesis, or an implant-retained prosthesis.

**Dental Implants**

Modern dental implants have been used as a therapeutic treatment modality since the Swedish orthopedic surgeon Per-Ingvar Branemark placed the first titanium dental implant in the mid-1960s. Over the years, there have been many different design forms of dental implants. The three basic types of implants have been transosteal (which are
metal pins or U-shaped implants that are placed completely through the lower jaw bone from one side through the other), subperiosteal (which are metal framework implants that are placed on top of the bone beneath the periosteum) and endosseous implants (which are implants that are placed within the bone). The implant choice of today is the endosseous implant. An endosseous dental implant is basically a titanium screw in the shape of a single tooth root that is placed into an edentulous area of the jaw. When the implant is placed, it is given a certain amount of time (usually 2-6 months) to osseointegrate. The term osseointegration refers to the direct contact between vital bone and the implant surface without intervening soft tissue.\textsuperscript{31, 32} Basically, bone grows onto the implant surface and rigidly fixates it. Once osseointegration is achieved, implants can resist and function under forces of normal occlusion for many years. In order to attach a crown (which is a prosthetic tooth) onto the implant, an abutment is needed to connect the two. Therefore, when a natural tooth is replaced with an implant, it is really replaced by a three part system: the implant, abutment and crown (Figure 2-4).

Implants can be placed via a one-stage or two-stage technique. A one-stage technique refers to the implant being placed with a transmucosal healing abutment. This type of healing abutment is connected to the implant via a screw and it has a portion that sticks out of the gum tissue into the oral cavity. The gums will heal around this abutment and the implant can be accessed by simply removing this abutment. A two-stage implant technique refers to the implant being placed with a cover screw. A cover screw is designed to be screwed into the implant and not stick out into the oral cavity. It lies flush with the implant surface. When a cover screw is used, the gum tissue is
sutured over the implant and the implant is allowed to heal beneath the gums undisturbed. The benefit of a two-stage technique is that the gum tissue protects the implant as osseointegration occurs. However, a second surgery is needed in the future to access the implant once enough time has elapsed for osseointegration to have taken place. The benefit of using a one-stage technique is that it does not require a second surgery to access the implant. The gum tissue heals and conforms to the shape of the healing abutment. A one-stage technique is desirable in most situations. However, if at that time of implant placement, the implant was not stable in the bone or extensive bone grafting was needed to properly support the implant, then a two-stage technique is required.

Longitudinal biomechanical assessments indicate that during the first few weeks after placement of one-stage implants, decreased rigidity is observed. This is due to normal bone remodeling as it heals around the implant surface. Therefore, it is considered normal care to not remove a healing abutment from a one-stage implant during the first 6 weeks of healing because it is thought that the implant is potentially not stable enough to withstand the reverse torque of removing the healing abutment. There is a risk that the implant can be removed while trying to remove the healing abutment if enough time has not elapsed for osseointegration to have taken place. It is acceptable that at about 2 months after implant placement, the stability of the implant can be verified and the healing abutment can be removed so that the implant can be restored with a crown.

The survival of osseointegrated implants varies depending on where they are placed in the jaw and the local environment in which they are placed. Implants can be
placed in fresh extraction sockets, in healed bone after a tooth has been extracted or in
crafted bone. In general, dental implants have been shown to be up 98% successful.
Most studies show about a 1% failure rate per year for dental implants.\textsuperscript{36-39} The failure
of an implant can be due a number of variables. Overheating the jaw bone when drilling
an osteotomy (or hole) for the implant, heavy occlusion (or bite force) on the implant
crown and the body rejecting to integrate with the implant are the most common causes
of implant failures.

\textbf{Dental Implants in Periodontal Patients}

It has become a common form of oral rehabilitation to place implants in
periodontitis-susceptible patients who have lost some or all of their dentition.\textsuperscript{40} It is not
yet completely understood if periodontitis-susceptible patients may show an elevated
risk for peri-implant tissue destruction. Peri-implantitis is a term introduced in the 1980s
to describe a destructive inflammatory process affecting the soft and hard tissues
around osseointegrated implants, leading to the formation of a peri-implant pocket and
loss of supporting bone.\textsuperscript{41} This is different from peri-implant mucositis, which is the
presence of inflammation in the soft tissue surrounding a dental implant without signs of
bone loss.

There are studies that specifically examined whether periodontal patients have an
increased risk for peri-implantitis.\textsuperscript{42-46} The results of these studies show different
success rates for implants placed in periodontitis-susceptible patients.

Nevins and Langer reported on the successful outcome of implants for the
treatment of the recalcitrant, or difficult to manage, periodontal patient.\textsuperscript{6} The study
concluded that individuals with a strong susceptibility to periodontal disease can be
successfully treated with implant therapy and have a success rate of over 97%.\textsuperscript{6}
Ellegaard et al. showed similar results, with more than a 95% success rate for survival of implants placed in periodontally compromised patients.\textsuperscript{5} Sbordone et al. found that even though implants placed in patients with periodontally compromised teeth had more of the motile periodontal bacteria compared to implants placed in completely edentulous patients, there was no significant difference in terms of loss of attachment between the two groups over a 3 year period.\textsuperscript{47} In a systematic review, Karoussis et al. showed that there were no differences in both short-term and long-term implant survival between patients with a history of chronic periodontitis and periodontally healthy patients. However, patients with a history of chronic periodontitis may exhibit significantly greater long-term probing pocket depth, peri-implant marginal bone loss, and incidence of peri-implantitis compared with periodontally healthy subjects.\textsuperscript{48}

There are quite a few systematic reviews that show that there is indeed a difference in the success and survival rates of implants placed in periodontally compromised patients compared to those placed in periodontally healthy patients.\textsuperscript{42-46}

In a large multicenter report, Brocard et al. showed that implants placed in periodontally maintained patients had a success rate of 89%; this was lower than the cumulative success rate of 95% for implants placed in all patients in the study.\textsuperscript{42} In a systematic review, Van der Weijden et al. reviewed studies that specifically examined bone level outcomes around implants placed in partially edentulous patients with a history of periodontitis. The authors concluded that the outcome of implant therapy in periodontitis patients may be different compared to individuals without such a history as evidenced by loss of supporting bone and implant loss.\textsuperscript{43} Roccuzzo et al. showed that over a 10-year period, there were 4.7% of sites in periodontally healthy patients with ≥3
mm of marginal bone loss around their implants, while there were 11.2% of sites in moderate chronic periodontitis patients and 15.1% in severe chronic periodontitis patients. Patients with a history of periodontitis presented with a lower survival rate and a statistically significant higher number of sites with peri-implant bone loss. In a follow-up study by the same group, it was found that patients with a history of periodontitis had a statistically significant higher number of sites which required additional treatment compared to periodontally healthy patients. In a similar study, Matarasso et al. also showed that periodontitis patients had significantly more marginal bone loss around implants compared to periodontally healthy patients over a 10-year period.

It has been shown that implants placed in periodontally compromised patients with residual periodontal pockets have significantly more sites with deeper probing pockets, bleeding pockets and marginal bone loss. This can be realized by understanding that the residual periodontal pockets tend to harbor periodontal pathogens that exacerbate bone loss. However, it has also been shown that treated periodontal patients that are considered stable with no residual active disease still have statistically significantly higher marginal bone loss rates compared to periodontally healthy patients.

It is clear to see that the available data show conflicting results regarding the success rates and clinical outcomes of implants placed in periodontally compromised patients compared to those placed in periodontally healthy patients. Despite the variations in study design and outcome variables, most of the studies indicate that subjects with a history of periodontitis are at greater risk for peri-implant disease. This gives rationale for investigating whether a difference in the immune response of periodontally compromised patients plays a key role in the lower success rates of dental
implants and the higher levels of marginal bone loss seen around implants placed in these types of patients.

**Immune Response**

Dental plaque is essentially a build-up of bacterial colonies working together to survive. Though periodontal and peri-implant diseases are multi-factorial, dental plaque is the predominate cause of periodontal inflammation. Chronic periodontitis is characterized by deliberate long-term destruction of the periodontium from the constant irritation of bacterial plaque. The key periodontal pathogens involved in chronic periodontitis are *P. gingivalis*, *A. actinomycetemcomitans*, and *Tannerella forsythia*.24

Periodontal bacteria that harbor in the diseased periodontal pockets stimulate the host’s inflammatory response. Both Gram-positive and Gram-negative bacteria possess many structural components as well as products which stimulate host cells to activate a wide range of inflammatory responses that results destruction of the periodontal tissues.2 The principal immune cells involved in this process are PMNs, which phagocytize and eliminate bacteria. However, they release a wide variety of factors that can damage host tissues including reactive oxygen species, collagenases and other proteases.51

The immune response to local infection is influenced by both environmental and genetic factors.52 These responses are regulated by a variety of host factors including cell-signaling from Toll-like receptors and receptors for advanced glycation end-products (RAGE). Toll-like receptors are a family of pattern-recognition receptors that trigger an inflammatory response to bacterial invasion.17 There are more than ten different TLRs in humans. They are predominantly expressed on cells of the innate
immune system, including neutrophils, monocytes/macrophages and dendritic cells. These immune cells play a significant role in capturing and eliminating bacteria.

It has been shown that gingival epithelial cells and connective tissue cells of periodontitis patients have an increased TLR expression compared to healthy patients. In periodontitis, bacteria and pathogen-associated molecular patterns (PAMPs) are sensed by TLRs, which initiate intracellular signaling cascades that can lead to host inflammation. When a ligand binds to a TLR, a signal gets created and leads to the formation of pro-inflammatory cytokines that initiate innate immune responses critical for the induction of adaptive immunity. Because there is constant bacterial invasion of the gingival tissues surrounding teeth, theoretically there should be constant cell-signaling of Toll-like receptors on the localized immune cells.

In the presence of periodontal pathogens, TLR-2 and TLR-4 are up-regulated. TLR-2 signaling may contribute to the production of interleukins by gingival epithelial cells in response to P. gingivalis fimbriae and Staphylococcus aureus peptidoglycan, which is a cell wall polymer. Upon in vitro stimulation with P. gingivalis lipopolysaccharide (LPS), human gingival fibroblasts showed increased expression of TLR-2 and TLR-4, which suggests that this LPS may be responsible for the observed up-regulation of TLR-2 and TLR-4 in periodontitis.

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria and is from a wide variety of periodontal pathogens including Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides oralis, and Fusobacterium nucleatum. It may interact with human gingival fibroblasts and induce the expression of specific interleukins (IL) such as IL-1β,
IL-6, IL-8, and intercellular adhesion molecule-1 (ICAM-1).\textsuperscript{2} See Table 2-1. Interleukins are cell-signaling molecules that exacerbate a pro-inflammatory immune response.

In addition to playing a key role in organizing the initial steps of an inflammatory response, LPS also plays a role in the transition to a more adaptive immune response. This is carried out by LPS through TLR-4 signaling, which enhances the expression of co-stimulatory molecules.\textsuperscript{2} Many Gram-negative bacteria have appendages called fimbriae, which are the principle molecules by which bacterial cells adhere to gingival fibroblasts, epithelial cells and other bacteria. Fimbriae have been shown to be able to modulate inflammatory responses through the activation of TLR-2 and TLR-4.\textsuperscript{54, 56}

Gingival fibroblasts are the major constituents of periodontal connective tissue. They help maintain the structures of the periodontium by regulating collagen and proteoglycan synthesis.\textsuperscript{17} However, human gingival fibroblasts can express TLR-2 and TLR-4 mRNA, which can be up-regulated in the presence of bacterial LPS.\textsuperscript{55}

The human periodontium is highly vascularized and its microvasculature plays a key role in regulating immune and inflammatory responses. Activation of vascular endothelium by TLR-4 signaling results in the production of various pro-inflammatory cytokines and chemokines.\textsuperscript{57} The expression of TLR-2 on human endothelial cells (cells that line blood vessels) can be up-regulated by TLR-4, LPS and other pro-inflammatory cytokines.\textsuperscript{54}

RAGE is a member of the immunoglobulin superfamily that is capable of inducing a pro-inflammatory immune response.\textsuperscript{58} It is a cell receptor that is expressed by a variety of cell types including epithelial, endothelial, smooth muscle cells, neurons, and lymphocytes.\textsuperscript{59} When RAGE gets activated, it can trigger cytokines and chemokines to
induce a pro-inflammatory response.\textsuperscript{59} It has been demonstrated that RAGE expression on is seen on gingival epithelial cells, fibroblasts and osteoclasts.\textsuperscript{20, 60} The expression on these cell types gets up-regulated in periodontally diseased gingival tissues compared to periodontally healthy tissues (unpublished data from Katz group). It is assumed that endogenous ligands for RAGE that are released as part of the inflammatory response of periodontal disease result in a sustained ligand-receptor interaction, which results in further up-regulation of RAGE.\textsuperscript{21}

Both periodontally diseased and healthy tissues have demonstrated the expression of innate immune receptors TLR-2, TLR-4 and RAGE, though there are differences in the expression levels. There is limited information regarding the expression of TLR-2, TLR-4 and RAGE receptors in peri-implant mucosa around implants placed due to periodontitis and non-periodontitis-associated tooth loss.
Figure 2-1: Clinical description of the gingiva. A = marginal gingiva, B = attached gingiva, C = movable alveolar mucosa, and D = mucogingival junction.
Figure 2-2: Anatomy of the alveolar mucosa.
Figure 2-3: Diagram of the dentogingival fibers extending from the cementum (1) to the crest of the gingiva, (2) to the outer surface, and (3) external to the periosteum of the labial plate. Circular fibers (4) are shown in cross-section.
Figure 2-4: X-ray showing the three parts of an implant system.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
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<tr>
<td></td>
<td>Lipoprotein/lipopeptides</td>
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<tr>
<td></td>
<td>Peptidoglycan</td>
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<tr>
<td>TLR-2</td>
<td><em>Porphyromonas gingivalis</em> lipopolysaccharide</td>
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<td></td>
<td><em>Porphyromonas gingivalis</em> fimbriae</td>
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<td></td>
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<td></td>
<td><em>Escherichia coli</em> lipopolysaccharide</td>
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<tr>
<td>TLR-4</td>
<td><em>Porphyromonas gingivalis</em> lipopolysaccharide</td>
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<tr>
<td></td>
<td><em>Actinobacillus actinomycetemcomitans</em> lipopolysaccharide</td>
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<tr>
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<td><em>Fusobacterium nucleatum</em> lipopolysaccharide</td>
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CHAPTER 3
MATERIALS AND METHODS

Experimental Groups

The experiment was designed as a controlled study in which the participating cohort of patients will have dental implant therapy to restore an edentulous area of their mouths. There were two different groups of subjects recruited for the study: Group A will consist of periodontally healthy patients (n = 10), while Group B will consist of patients diagnosed as having generalized chronic periodontitis patients (n = 10).

**Inclusion criteria:** For the Group A subjects, there were to be no sites around teeth that exhibited a probing pocket depth (PPD) of $\geq 5$ mm, no sites that had a clinical attachment loss (CAL) of $\geq 1$ mm, no more than 10% of all the sites probed around teeth that had bleeding upon probing (BOP), and the patients had to have at least one tooth missing or the need of one tooth to be extracted because of dental caries (tooth decay), endodontic failure (failed root canal therapy), or a tooth fracture. For the Group B subjects, there were to be at least 30% of all the sites around teeth that exhibited CAL of $\geq 3$ mm, at least 5 sites that exhibited PPD of $\geq 5$ mm, at least 5 sites that had BOP, and at least one tooth missing or the need of one tooth to be extracted because of advanced loss of periodontal support (advanced bone loss around the tooth).

**Exclusion criteria:** The excluded patients were those that had Type I or Type II diabetes, clinically significant neurological, hepatic, renal, gastrointestinal, hematologic, dermatologic, metabolic, autoimmune or immune deficiency diseases that would affect the compliance or periodontal status of the patient. Also, patients receiving immunosuppressive, antibiotic, glucocorticoid or radiation therapy were also excluded. Finally, smokers or oral tobacco users and alcohol or drug abusers were also excluded.
Pre-treatment of Periodontal Disease: In order to control the active periodontal infection of the Group B patients, they underwent an initial phase of treatment consisting of scaling and root planing (deep cleaning under local anesthesia), motivation and oral hygiene instructions, extractions of severely advanced periodontally compromised teeth. If active infection persisted after the initial phase of treatment, then those patients with remaining active disease underwent periodontal surgery to eliminate periodontal pockets and to restore health around the periodontally compromised teeth. Once periodontal therapy was completed and a healthy periodontium was achieved, then the implant therapy portion of the study was started.

Dental Implant Surgeries and Soft Tissue Biopsies

In this study, dental implant surgery was done only in sites that had been edentulous for a minimum of 3 months. For those patients that required a tooth to be extracted prior to implant placement, a minimum of 3 months time is required to allow for proper bone remodeling to take place prior to implant placement. The implant surgery was done under local anesthesia by making an incision across the edentulous ridge where the implant was to be placed, and an incision in the sulci of the adjacent teeth. The gingiva was lifted off of the bone in both the buccal and lingual directions until enough of the alveolar bone was visible to allow for proper implant placement. Only one stage implant placement was accepted for this study, meaning that there is a transmucosal healing abutment placed at the time of implant placement. The first soft tissue biopsy was taken at this time from the gum tissue immediately adjacent to the transmucosal healing abutment. This tissue is normally discarded to allow for proper approximation of the gingiva around the healing abutment. The gingiva was sutured and allowed to heal for 10 to 14 days prior to suture removal. Implant patients were given
the following antibiotic regimen: 2 grams of amoxicillin 1 hour prior to implant placement, then 500 milligrams three times daily for 7 days, or if the patient was allergic to amoxicillin, then 600 milligrams of clindamycin was given 1 hour prior to implant placement and 150 milligrams taken four times daily for 10 days. The patients were also instructed to rinse with Chlorhexidine 0.12% every morning and night for 14 days.

The second soft tissue biopsy was taken 2 months after the implant placement surgery. Under local anesthesia, a small incision (about 1 mm deep by 3 mm wide) was made on the lingual soft tissue immediately adjacent to the healing abutment. No sutures were needed after this biopsy was taken. No antibiotic tablets or oral rinse was given.

**Tissue Preservation:** TLR-2, TLR-4 and RAGE expression was evaluated by real time PCR (qPCR), western blot and immunohistochemical analysis. The biopsied tissue samples from both groups of patients were immediately placed in three different solutions for preservation until the laboratory portion of the experiment could be started. Those samples that would be examined through real-time PCR were stored in RNAlater® (Invitrogen) solution. This solution is a nontoxic storage medium that rapidly permeates tissues to stabilize and protect cellular RNA. It minimizes the need to immediately process tissue samples or to freeze samples immediately. Those tissue samples that would be examined using a western blot were stored in a transfer media. Finally, those tissue samples that would be examined using immunohistochemical analysis were stored in formalin, which is a colorless solution of formaldehyde and water that can store samples without the need of refrigeration.
Real-Time PCR

Total RNA (tRNA) from periodontal tissues was extracted using an RNeasy®
extraction kit (Qiagen). RNA was reverse transcribed to generate cDNA using a reverse
transcription master mix (5X buffer, 1mM DTTs, 2.5mM dNTPs, SupperScript® III
(Invitrogen), and oligonucleotides). hGAPDH (5’ TCC ACC ACC CTG TTG CTG TA 3’
(reverse), 5’ ACC ACA GTC CAT GCC ATC AC 3’ (forward)), hRAGE (: 5’ GGA CTT
CAC AGG TCA GGG TTA C 3’ (reverse), 5’ GAC TCT TAG CTG GCA CTT GGA T 3’
(forward)), (Integrated DNA Technologies, Coralville, IA), hTLR2 RT² qPCR primers or
hTLR-4 RT² qPCR primers (SABiosciences, Frederick, MD) and 2xqPCR Syber® Green
Master Mix (BioRad, Hercules, CA) were used for qPCR reactions. Each gene was
detected in independent reactions. Data are expressed as relative expression
normalized to GAPDH. Normalized mRNA expression = [raw Ct value] x [GAPDH
corrective ratio]. GAPDH corrective ratio = [lowest GAPDH Ct value within sample set] / [GAPDH Ct value for cell of interest]. Relative expression = 40 – normalized mRNA
expression.

Western Blot

The protein was harvested from the tissue samples stored in the transfer media
using a cell extraction buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM
EGTA, 1 mM NaF, 20 mM Na₄P₂O₇, 2 mM Na₃VO₄, 1% Triton™ X-100, 10% glycerol,
0.1% SDS, 0.5% deoxycholate) (Biosource™), supplemented with a
phenylmehtanesulphylfluoride or phenylmethylsulphonylfuoride (PMSF) and a protease
inhibitor cocktail (Sigma-Aldrich) according to the manufacturer’s protocol.

The extracted proteins were denatured by using a loading buffer that contained the
anionic denaturing detergent SDS (sodium dodecyl sulfate) and then boiled at 70°C for
10 minutes. The denatured proteins were then loaded on a gradient gel and run through electrophoresis (BioRad) in an SDS 1X running buffer. The separated proteins were transferred to a nitrocellulose membrane and probed with goat antibodies specific for either hRAGE, hTLR-2 or hTLR-4. Reactivity was detected with anti-goat HRP (horseradish peroxidase) and developed using ECL (enhanced chemiluminescence) reagents (Pierce) and x-ray films (Kodak). Reactivity was semiquantified using densitometric analysis and CapturePro® software (BioRad).

**Immunohistochemistry**

Immunohistochemical analysis (IHC) is done to detect specific antigens (proteins) in cells in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. The tissue specimens stored in the formalin were sent for paraffin embedment and sectioning into 5 µm slices and mounted onto slides. Sections were rehydrated using three washes of xylene for 5 minutes each, then in two washes of 100% ethanol for 10 minutes each, then in two washes of 95% ethanol for 10 minutes, then in two washes of 70% ethanol for 10 minutes each, and finally in two washes of deionized water PBS (phosphate buffered saline). After washing in ddH₂O, sections were subjected to haematoxylin and eosin staining and cover slipped with mounting media. Levels of infiltration were scored in a blinded fashion on an arbitrary scale from 1 (no infiltration) to 5 (highly infiltrated). Three sections 100 microns apart were scored at 20x magnification for each participant at each time point evaluated. Data are presented as an infiltration score, which is an average of the score received on each tissue section. Representative sections were acquired on a Zeiss microscope at 20x magnification.
**Statistical Analysis**

The clinical data was compared using calculated means and standard deviation values. Comparison between periodontitis and non-periodontitis susceptible patients for TLR-2, TLR-4 and RAGE protein and gene expression was performed using GraphPad (GraphPad Software, La Jolla, CA,) where one-way ANOVA with Bonferroni’s multiple comparisons test was used to determine significance. *p value* <0.05 was considered significant.
Figure 3-1. Experimental design.
CHAPTER 4  
RESULTS

In order to determine if different micro-environments can affect the therapeutic outcome of implant therapy, participants needing implant therapy were stratified based on etiology of tooth loss. Specifically, participants were categorized as periodontally susceptible (PS), which were those participants in which tooth loss was due to loss of periodontal stability, or periodontally healthy (PH) which were those participants in which tooth loss was due to reasons other than loss of periodontal support.

Table 4-1 shows the clinical parameters of the periodontitis susceptible and periodontally healthy patients. Participants within the PH group consisted of 4 males and 6 females aged 23 to 69 years and presented with a mean PD of 2.31+/− 0.39mm, mean BOP of 6.28 ± 3.32% and a mean CAL 0.49+/− 0.21mm. Participants within the PS group consisted of 5 males and 5 females aged 29 to 79 years and exhibited a mean PD 3.08+/−0.45 mm, mean BOP of 40.4 ± 23.27% and a mean CAL 4.21+/−0.85mm.

In order to evaluate the inflammatory potential at the site of implant therapy, TLR2, TLR4 and RAGE mRNA and protein levels were evaluated in biopsies taken during implant placement. RAGE mRNA levels in gingival biopsies from periodontally susceptible participants were significantly higher than those observed in biopsies from periodontally healthy participants (Figure 4-1). There was no difference in the mRNA levels of TLR4 and TLR2 between the participant groups prior to implant placement (Figure 4-1). Similarly, RAGE protein levels were also elevated in gingival biopsies from periodontally susceptible participants prior to implant placement when compared to
those observed in biopsies from periodontally healthy participants, while TLR4 and TLR2 protein levels were similar (Figure 4-2).

TLR2, TLR4 and RAGE mRNA and protein levels were also evaluated in biopsies take two months following implant placement in order to evaluate the inflammatory potential two months post-implant. While there was no difference in the expression levels of TLR4 and RAGE mRNA, TLR2 mRNA levels were significantly lower in tissues from periodontally susceptible participants following implant therapy compared to those detected in gingival tissues from periodontally healthy participants (Figure 4-3). Interestingly, there was a significant up-regulation of both RAGE and TLR4 protein with a concomitant down-regulation of TLR2 protein in tissues from periodontally susceptible participants post-implant (Figure 4-4). On the other hand, there was a significant down-regulation of both RAGE and TLR4 protein with a concomitant up-regulation of TLR2 protein in tissues from periodontally healthy participants post-implant (Figure 4-4).

The level of cellular infiltrate was semi-quantified in biopsies pre- and post-implant using histological analysis (Figure 4-5A). Biopsies taken during implant placement from periodontally susceptible participants presented with increased levels of immune infiltrate when compare to those from periodontally healthy participants (Figure 4-5B). Similarly, while a slight, but not statistically significant increase in cellular infiltrate was observed in periodontally healthy participants following implant therapy, biopsies from periodontally susceptible participants revealed significant cellular infiltrate two months post-implant therapy (Figure 4-5B).
Table 4-1. Clinical parameters of periodontally healthy (PH) and periodontally susceptible (PS) participants. Clinical measurements were taken at the initial screening and diagnostic visit. PPD = probing pocket depth, CAL = clinical attachment loss, BOP = bleeding on probing.

<table>
<thead>
<tr>
<th></th>
<th>Mean PPD (mm ± SD)</th>
<th>Mean CAL (mm ± SD)</th>
<th>Mean BOP (%) ± SD</th>
<th>Age (years ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>2.31 ± 0.39</td>
<td>0.41 ± 0.21</td>
<td>6.28 ± 3.32</td>
<td>52.18 ± 13.96</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PS</td>
<td>3.08 ± 0.45</td>
<td>4.21 ± 0.85</td>
<td>40.4 ± 23.27</td>
<td>62.30 ± 14.84</td>
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<td>(n = 10)</td>
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</table>
Figure 4-1. Elevated levels of RAGE mRNA in biopsies from periodontally susceptible individuals prior to implant therapy. During implant installation excess soft tissue as a result of flap adaptation was collected from 10 periodontally healthy (PH) and 10 periodontally susceptible (PS) participants. RNA was isolated and subjected to reverse transcription. The resulting cDNA was probed for the expression of rage, tlr4 and tlr2 using gene specific primers. Data are expressed as relative units of expression. *p value <0.05 one-way ANOVA with Bonferroni’s multiple comparisons test.
Figure 4-2. Elevated levels of RAGE protein in biopsies from periodontally susceptible individuals prior to implant therapy. Immediately following implant installation excess soft tissue as a result of flap adaptation was collected from 10 periodontally healthy (PH) and 10 periodontally susceptible (PS) participants. Protein was isolated, subjected to SDS-PAGE and probed for the expression of RAGE, TLR4 and TLR2 using antigen specific antibodies, after which reactivity was quantified using densitometry. (A) Data are expressed as optical density. *p value <0.05 one-way ANOVA with Bonferroni’s multiple comparisons test. (B) Representative western blot. L= ladder in kDa.
Figure 4-3. Elevated levels of TLR2 mRNA in biopsies from periodontally healthy individuals following implant therapy. Two months following implant installation a tissue biopsy from the peri-implant mucosa of the initially sampled site was collected from 10 periodontally healthy (PH) and 10 periodontally susceptible (PS) participants. Protein was isolated, subjected to SDS-PAGE and probed for the expression of RAGE, TLR4 and TLR2 using antigen specific antibodies, after which reactivity was quantified using densitometry. Data are expressed as fold change over pre-implant protein expression. *$p$ value < 0.05 one-way ANOVA with Bonferroni’s multiple comparisons test.
Two months following implant installation a tissue biopsy from the peri-implant mucosa of the initially sampled site was collected from 10 periodontally healthy (PH) and 10 periodontally susceptible (PS) participants. Protein was isolated, subjected to SDS-PAGE and probed for the expression of RAGE, TLR4 and TLR2 using antigen specific antibodies, after which reactivity was quantified using densitometry. Data are expressed as fold change over pre-implant protein expression. *p value <0.05 one-way ANOVA with Bonferroni’s multiple comparisons test.
Figure 4-5. Elevated cellular infiltrate in biopsies from periodontally susceptible individuals prior to and following implant therapy. Prior to and two months following implant installation tissue biopsies were collected from 10 periodontally healthy (PH) and 10 periodontally susceptible (PS) participants. After fixation and paraffin embedding, sections were stained with hematoxalin and eosin. Levels of infiltration were scored in a blinded fashion on an arbitrary scale from 1 (no infiltration) to 5 (highly infiltrated). Three sections 100 microns apart were scored for each participant at each time point evaluated. (A) Data are presented as an average of the score received on each tissue section.\(^*\)\textit{p value} < 0.05 one-way ANOVA with Bonferroni’s multiple comparisons test. (B) Representative histological sections at 20x magnification. White arrows indicate areas of infiltration. Biopsies taken from (i-ii) edentulous ridges prior to implant installation and (iii-iv) peri-implant mucosa two months following implant placement in (i,iii) periodontally healthy and periodontally susceptible participants.
CHAPTER 5
DISCUSSION

Inflammation plays a key role in the initiation and progression of periodontal diseases.\textsuperscript{10,11} The hyper-reactive immune response of the host to bacterial byproducts plays a key role in the destruction of the supporting bone around teeth. It has been shown that TLR-2, TLR-4 and RAGE are up-regulated in the presence of bacterial insult in the periodontium.\textsuperscript{18-21} They elicit an inflammatory response to lipopolysaccharides, peptidoglycan, flagellin, foreign DNA and RNA, as well as bacterial byproducts.\textsuperscript{17} Both periodontally diseased and periodontally healthy tissues have demonstrated the expression of innate immune receptors TLR-2, TLR-4 and RAGE, though there are differences in the expression levels.\textsuperscript{18-21}

An important question is whether periodontitis susceptible patients demonstrate an elevated risk for infection of the tissues surrounding dental implants, resulting in loss of hard and soft tissues around the implant. There is limited information available regarding the outcome of implant therapy in periodontitis-susceptible patients. However, it seems that most studies show that periodontitis-susceptible patients can be successfully treated with dental implant therapy and have good long-term results.\textsuperscript{5-9}

Periodontitis-associated microorganisms have shown to colonize around dental implants,\textsuperscript{10,11} and this can occur within minutes after implant placement.\textsuperscript{12} It has been suggested that the inflammatory and immune response defense mechanisms to bacteria around implants is similar to that for bacteria around natural teeth.\textsuperscript{13-16} The peri-implant tissue response to bacteria may follow similar patterns to that of periodontitis in a susceptible host.
Thus far, there has been limited information regarding the expression of TLR-2, TLR-4 and RAGE receptors in peri-implant mucosa around implants placed due to periodontitis and non-periodontitis-associated tooth loss. Therefore, it was our aim to compare the expression levels of TLR-2, TLR-4 and RAGE in gingival tissues from periodontitis and non-periodontitis susceptible participants prior to and following implant therapy. Our hypothesis was that healthy tissues from periodontitis susceptible patients have higher expression of TLR-2, TLR-4 and RAGE receptors compared to non-periodontitis susceptible patients. We also hypothesized that peri-implant mucosa around dental implants placed due to periodontitis-associated tooth loss has higher expression of TLR-2, TLR-4 and RAGE receptors compared to peri-implant mucosa around dental implants placed due to non-periodontitis-associated tooth loss.

The results of our study showed that prior to implant therapy, elevated levels of RAGE were detected in gingival tissues from periodontitis susceptible participants when compared to those from periodontally healthy patients, while no differences were found in the expression of TLR-2 and TLR-4. Following implant therapy, there was an up-regulation of RAGE and TLR-4 levels which coincided with a significant down regulation of TLR-2 levels in biopsies from periodontitis susceptible participants. Levels of RAGE and TLR-4 remained unchanged in biopsies from periodontally healthy participants, while TLR-2 levels were significantly up-regulated. Histologically, post-implant biopsies from periodontitis susceptible participants displayed higher levels of inflammatory infiltrate.

Thus far, the roles of RAGE, TLR-2 and TLR-4 have not been investigated in relation to their effect on peri-implant tissues. It has been demonstrated that innate
immune system recognizes a limited number of germ-line encoded receptors. These receptors evolved to recognize products of microbial metabolism. Toll-like receptors play a major role in pathogen recognition and initiation of inflammatory and immune responses. Stimulation of Toll-like receptors by microbial products leads to the activation of signaling pathways that result in the induction of antimicrobial genes and inflammatory cytokines.

RAGE, TLR2 and TLR4 have multiple ligands, allowing them to function as sensors of environmental cues and therefore play a crucial role in the regulation of both homeostasis and pathogenesis. Cells of the periodontium including gingival epithelial cells, fibroblasts, macrophages and osteoclasts express RAGE, TLR-2 and TLR-4, and this expression can be elevated in periodontally diseased tissues. Therefore, it can be speculated that greater expression of these receptors may indicate a greater potential for the development of an inflammatory reaction around implants placed in patients that are susceptible to periodontal diseases.

The development of an inflammatory reaction in response to plaque accumulation around teeth and implants has been investigated in both animal and human studies. Through these studies it was shown that the bacterial biofilm formation around implants is comparable to that formed around teeth. A study that compared the clinical and microbiological parameters of experimental gingivitis and experimental peri-implantitis in human participants showed that the biofilm formation around teeth and implants was similar in development and substance after 3 weeks of accumulation. Also, the cause-effect relationship between the accumulation of bacterial plaque and the
development of peri-implant mucositis was similar to that seen in the experimental gingivitis models around teeth.\textsuperscript{68, 71}

A study that examined soft tissue biopsies around teeth and implants in a clinically healthy situation and after 3 weeks of experimental biofilm formation revealed that the connective tissue surrounding both teeth and implants displayed an increased volume of inflammatory cells (i.e. T- and B-lymphocytes) as a consequence of abolished oral hygiene practices.\textsuperscript{69} The biopsies showed that there were no statistically significant differences in the host immune response to bacterial plaque induced gingival inflammation around implants compared to that around natural teeth. It was also found that biopsies from healthy peri-implant mucosa were found to contain a small number of inflammatory cells.\textsuperscript{69}

The results of this current study show similar findings with regard to the small cellular infiltrate detected around implants placed in periodontally healthy participants. These results are in agreement with several other studies.\textsuperscript{13, 15, 16} However, this current study revealed that different inflammatory responses are seen in biopsied tissue of periodontally susceptible versus periodontally healthy subjects. The biopsies taken during implant placement from periodontally susceptible participants presented with increased levels of immune infiltrate when compared to those from periodontally healthy participants. Similarly, while a slight, but not statistically significant increase in cellular infiltrate was observed in periodontally healthy participants following implant therapy, biopsies from periodontally susceptible participants revealed significant cellular infiltrate two months post-implant therapy. Given that the treatment for both the periodontally healthy and periodontally susceptible participants was identical, this increase in cellular
Infiltrate may be attributed to an inherent difference in the participants’ immune response. However, a major limitation to this study is that no clinical data were recorded when peri-implant biopsies were taken, even though all implants and peri-implant mucosa appeared healthy.

Previous studies have shown that peri-implant mucosa has different structural alterations and was found to contain significantly higher numbers of inflammatory cells compared to masticatory mucosa. Berglundh et al. 1992 showed that the placement of a dental implant leads to the formation of a barrier epithelium and connective tissue integration at the implant and mucosa interface. Liljenberg et al. 1996 suggested that the enhanced numbers of inflammatory cells in the peri-implant mucosa were related to the existence of a permeable barrier epithelium and a microbial challenge at implant sites. In this current study, differences were observed in inflammatory infiltration prior to and two months following implant therapy for both the periodontally healthy and periodontally susceptible groups. It is not certain if bacterial plaque accumulation played a major role in this effect; however, Furst et al. 2007 showed that early bacterial colonization of implant surfaces occurs within 30 minutes after implant placement.

The results of this current study show that two months following implant installation, there was a significant up-regulation of both RAGE and TLR-4 protein expression with a concomitant down-regulation of TLR-2 protein expression in peri-implant tissues from periodontally susceptible participants. On the contrary, there was a significant down-regulation of both RAGE and TLR-4 protein expression with a concomitant up-regulation of TLR-2 protein expression in tissues from periodontally healthy participants. The reasons for the down-regulation of TLR-2 protein expression
following implant therapy in periodontally susceptible participants and the up-regulation of the same protein expression on periodontally healthy participants is not fully understood. Although the main function of TLRs is to recognize various pathogens through their signature patterns, interaction of TLRs with endogenous ligands have been reported in non-infectious conditions.\textsuperscript{73} Thus, both bacterial and host-derived affects on TLR responsiveness are plausible and not mutually exclusive.

RAGE, TLR2 and TLR4 expressed on the cells of the periodontium respond to both exogenous and endogenous ligands. TLR-2 has traditionally been described as a receptor for gram-positive ligands such as peptidoglycan and bacterial lipoproteins, while TLR-4 is the principal signaling receptor for gram-negative bacterial lipopolysaccharides (LPSs).\textsuperscript{74-78} This ligand interaction can lead to increased receptor expression and inflammation.\textsuperscript{17,21} Thus, increased TLR2, TLR4 and RAGE expression in healthy and recently formed peri-implant mucosa of periodontitis susceptible patients may in part explain the observation of clinical studies that subjects with a history of periodontitis are at greater risk for development peri-implant disease.\textsuperscript{40}

It is possible that generation and accumulation of endogenous TLR and RAGE ligands may affect the physiological homeostasis in vivo and alter their expression levels and responsiveness. Therefore, it is plausible that elevated expression of TLR-4 and RAGE from the peri-implant mucosa of clinically healthy implants from periodontally susceptible patients may in part explain a greater risk for development peri-implant disease through a non-bacterial mediated pathway.

The results of this current study do not support our hypotheses that healthy tissues from periodontitis susceptible patients have higher expression of TLR-2, TLR-4 and
RAGE receptors compared to non-periodontitis susceptible patients, and that peri-implant mucosa around dental implants placed due to periodontitis-associated tooth loss has higher expression of TLR-2, TLR-4 and RAGE receptors compared to peri-implant mucosa around dental implants placed due to non-periodontitis-associated tooth loss. For periodontally susceptible participants, it was found that there was an increase in RAGE and TLR-4 expression, but a decrease in TLR-2 expression after implant placement. For periodontally healthy participants, it was found that RAGE and TLR-4 remained unchanged, but there was an increase in TLR-2 expression after implant placement. It would be of great interest to longitudinally follow the participants in this study in order to evaluate whether the expression of TLR2, TLR4 and RAGE can act as predictor for peri-implant disease.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Dominick Catania was born in Brooklyn, New York on October 21, 1979. He grew up in Ft. Lauderdale, Florida and attended Bryant University in Smithfield, Rhode Island where he studied Business Finance. He had worked as a mutual fund accountant for one year when he decided to pursue a career in dentistry. He graduated from the University of Florida College of Dentistry in 2009 with a Doctor of Dental Medicine degree and attended the same institution for his specialty training in Periodontology. Upon graduating in the spring of 2012, he plans to join a private practice in the southeastern area of Florida and aspires to provide quality healthcare to all those who are in need.