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# Experimental Peri-Implant Mucositis in Humans. A Pilot Study

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**Experimental Peri-Implant Mucositis in Humans. A Pilot  
Study.**

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**M.S., The Ohio State University, 2005**

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# APPROVAL PAGE

Master of Dental Science Thesis

## **Experimental Peri-Implant Mucositis in Humans. A Pilot Study.**

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## **1. Introduction**

Over the past 20 years, implant therapy has been used as an alternative to tooth replacement, to retain removable appliances, and to support fixed prostheses. Implant therapy has become the gold standard in tooth replacement with over four and a half million implants placed each year across the globe. While osseointegration of dental implants occurs in more than 90% of the cases[1], once exposed into the oral cavity, implants will be immediately colonized by bacterial plaque[2]. The consequent interaction of the bacterial biofilm with the peri-implant soft tissue interface results in an inflammatory reaction. The inflammatory state around the implant mucosa has been termed mucositis. The periodontal literature has extensively characterized the tissue response to the biofilm formed around the natural dentition. However, it is still unclear whether the tissue response to bacterial biofilm around the natural dentition differs to that around implants.

Peri-implant mucositis is defined as a reversible inflammatory process involving the soft tissue surrounding an implant without accompanying bone loss[3]. The prevalence of peri-implant mucositis is estimated at 79.2% of patients and 42.2% of implants[4]. Evidence from animal studies supports that a persistent inflammatory condition may progress to peri-implantitis[3, 5, 6] and eventually to implant loss.

The biological processes regulating the inflammatory response in the peri-implant mucosa are not clearly defined. Several studies have shown a similar response in the tissue around both teeth and implants to bacterial plaque[7-9]. At the same time, other studies have demonstrated differences between teeth and implant soft tissue in terms of

the immune response[10], the quality and quantity of the inflammatory infiltrate[8], and the quality of the microbial flora[11]. This study utilizes a human model of experimental peri-implant mucositis to evaluate the progression of the inflammatory process of the soft tissue around implants and to compare that to the inflammatory process of gingiva around teeth. This thesis seeks to clarify how the peri-implant mucosa and gingiva compare in response to undisturbed plaque accumulation, using clinical indices of health and disease.

## **2. Background and Significance**

### **2.1 Gingivitis**

#### **2.1.1 Definition and Prevalence**

Gingivitis is defined as the presence of gingival inflammation without the loss of connective tissue attachment[12]. The prevalence of gingivitis is difficult to quantify. This is partly due to a deficient methodology to objectively measure gingival inflammation. However, several studies attempted to quantify the prevalence of gingivitis in America[13, 14].

The 3<sup>rd</sup> National Health and Nutrition Examination Survey (NHANES III) conducted in 1988-1991 gives us some measure to determine the prevalence of gingivitis. However, NHANES III did not directly attempt to quantify gingivitis. The data collected included bleeding on probing, which we can then correlate to a moderate inflammation using the index for assessment of gingivitis introduced by Löe and Silness[15]. The periodontal evaluation in NHANES III included a total of 7,447 dentate individuals 13 years and older. The examinations were partial-mouth periodontal exams, performed on two

randomly selected quadrants, on 2 sites per tooth, with a maximum of 14 permanent teeth eligible for assessment (third molars were excluded). Brown et al. [13] reported that bleeding on probing was found in 62.9% for all persons aged 13 and older. However, these data have to be considered with caution, since a partial-mouth periodontal assessment has been shown to underestimate the prevalence of the disease[16]. Furthermore, the criteria to detect gingivitis include the visible signs of color change and texture. Thus, a large percentage of individuals with gingivitis without bleeding on probing were not included in the above statistic. A recent study by Li et al. [14] sought to determine the prevalence of gingivitis in American adults using the Löe and Silness Gingival Index (GI) and found that 93.9% of individuals had a mean GI > 0.50. This percentage decreased to 55.7% for individuals with a mean GI  $\geq$  1.0. These studies suggest that the prevalence of gingivitis in the American population ranges between 55.7% and 93.9% depending on the definition used and method of calculation.

### **2.1.2 Etiology**

The etiology of gingivitis was elegantly demonstrated in 1965 by Löe [17] (see section 2.1.4). In this experiment, a direct cause and effect relationship was found between plaque accumulation and gingival inflammation. Hence, this study offered proof that bacterial biofilm causes gingivitis[17].

Under experimental gingivitis conditions, a significant shift was observed in the bacterial composition from health to gingivitis[17]. The bacteria of healthy gingiva were composed predominately of Gram-positive cocci and rods. When localized gingivitis was first clinically detectable (GI = 1 on one tooth surface), Gram-negative cocci and



rods were found in increasing percentages in the microflora. There was a shift again as generalized gingivitis was clinically identified (GI = 1 for the individual). Here, Gram-positive and negative filaments, fusobacteria, spirilla, and spirochetes were more readily measurable. In the study by Theilade et al. [18] a correlation between gingival status and plaque composition was established. Theilade's work details the growing complexity of the microflora composition as plaque accumulates[18, 19]. The authors' results indicate that gingivitis is not due to an infection by a single pathogen, but rather a plaque ecology that is unfavorable to the host[20].

### **2.1.3 Histopathology**

The histological features of healthy gingiva include a keratinized oral epithelium continuous with a junctional epithelium (JE) that is attached to the tooth surface by hemidesmosomes. Polymorphonuclear leukocytes (PMN), while small in overall number, are present in the healthy sulcus as a normal reaction to the biofilm present around teeth even in a meticulously cleaned mouth[21]. If sufficient plaque is allowed to accumulate, gingivitis will ensue. Page and Schroeder described the pathogenesis of gingivitis and periodontitis as a continuum of increasing inflammatory response by the host to microbial plaque[21].

The first stage of gingivitis is the *initial lesion*. It is formed within 2-4 days of the initiation of plaque accumulation. The characteristics of the initial lesion are vasculitis adjacent to the JE, increased flow of gingival crevicular fluid (GCF), and increased migration of PMNs into the JE and sulcus. Additionally, an altered morphology of epithelial cells in the coronal part of the JE and loss of perivascular collagen can be seen.

Neutrophils adhere to vessel walls and migrate to the connective tissue, JE, and sulcus. At this stage bacterial invasion is not evident and the inflammatory response is thought to be related to plaque-derived chemotactic substances[21].

The second stage of gingivitis is called the *early lesion*. It is mostly an accentuation of the initial lesion and occurs at days 4 through 7. There is an accumulation of lymphoid cells (approximately 75% of the total infiltrate) adjacent to the JE, further loss of collagen fiber network supporting the marginal gingiva and the beginning of proliferation of the basal cells of the JE. The primary distinguishing feature here is the migration of lymphoid cells (predominately T cells with very few B cells) into the lesion[21].

The third and final stage of gingivitis is the *established lesion*. It is formed at least 7 days after the initiation of plaque accumulation. The histo-pathological hallmark of this condition is the predominance of plasma cells. Immunoglobulins are seen extravascularly in the connective tissues and JE. There is a continuing loss of connective tissue matrix elements and proliferation, apical migration, and lateral extension of the JE leading to the formation of a pocket epithelium[21]. This established lesion can remain stable for months or years without progressing. The *established lesion* is still reversible. Further persistence of the established lesion may eventually progress to the *advanced lesion*, which is defined as irreversible and accompanied by attachment loss. Once attachment loss occurs, the condition is defined as periodontitis.

#### **2.1.4 Experimental Gingivitis**

The model of experimental gingivitis, which requires patients to refrain from oral hygiene measures for a period of time, was first proposed by Löe et al.[17]. In that study, experimental gingivitis was achieved by disengaging all oral hygiene practices for 21 days in healthy subjects. The development of gingivitis varied by individual and occurred as early as 10 days in some subjects. By 21 days all participants showed clinical signs of gingivitis. Following the experimental phase, oral hygiene practices were reinstituted resulting in the return to health in about 1 week[17].

The experimental gingivitis model has been used ubiquitously over the past 50 years to measure a variety of outcome variables. One of these outcome variables is whether age plays a role in susceptibility to gingivitis. In an experiment by Holm-Pedersen et al. [22], gingivitis developed more rapidly and more severely in elderly than in young persons. The authors suggest that with age comes an altered host response to the bacterial plaque. The effect of age on the host response to plaque accumulation was confirmed by Matsson [23], who compared pre-school aged children with young adults. The author found that the younger group had a statistically significantly lower amount of gingival exudate and gingival bleeding than the older group. Winkel et al. [24] tested the effect of age on the rate of development of gingivitis in persons not susceptible to periodontal destruction. The findings of this study were that in individuals not susceptible to periodontal destruction, age was not a factor in the development of experimentally induced gingivitis. Winkel et al. and Matsson use different classifications of age, making direct comparisons of their studies difficult. Thus, there may be a critical age difference required to detect a true difference in gingivitis susceptibility to plaque accumulation.

The experimental gingivitis model has also been used to elucidate the histopathologic and immunologic features of gingival inflammatory changes. Payne et al. [21, 25] used the experimental gingivitis model to describe the pathologic alterations in gingival tissues in early plaque accumulation. They found that within the first 8 days of plaque accumulation, a lesion resembling a delayed hypersensitivity developed. Seymour et al. [26] took biopsies at four time points through a 21-day oral hygiene abstention study and found that the nature of the composition of the infiltrate did not change significantly, as the degree of inflammation increased.

Another use of the experimental gingivitis model was to evaluate techniques and procedures to reverse gingivitis or to mitigate its onset. Bosman and Powell [27] demonstrated that a once daily rinse with 0.2% chlorhexidine (CHX) mouthrinse regained gingival health in 4 days versus 10 days when only brushing was performed. Gusberti et al. [28] investigated the ability of two therapeutic mouth rinses, 0.12% CHX and 1% hydrogen peroxide ( $H_2O_2$ ), to mitigate the development of gingivitis when all other oral hygiene measures were removed. They found 0.12% CHX to significantly reduce gingivitis incidence, plaque, bleeding on probing, and specific facultative and obligate anaerobes compared to a placebo. Conversely, 1%  $H_2O_2$  did not have a significant difference over the placebo[28]. Ultimately, these types of studies aid in distinguishing the therapeutic value of different chemotherapeutic agents in treating gingivitis.

The early experimental gingivitis studies hinted at the possibility of differences in subject susceptibility to plaque accumulation. This response variability was interpreted as related to differences in plaque accumulation rates or differences in microbial composition[17,

18]. More recently, Trombelli et al. [29] in an experimental gingivitis trial were able to identify two subgroups of subjects that differed in their response to similar plaque exposure. These were labeled the “High Responder” (HR) and “Low Responder” (LR). The identification of these two subpopulations was based on their amount of GCF volume. This finding supports the hypothesis of the existence of host-related factors implicated in the individual variability of the inflammatory response to plaque. The implication of susceptibility to gingivitis due to a subject’s genetic make-up or host response versus a purely microbial reaction has changed how the disease process is studied. More efforts are looking into the effect of genetic variation and other factors that could further elucidate the pathogenesis of gingivitis.

## **2.2 Peri-implant Mucositis**

### **2.2.1 Definition and Prevalence**

The definition of peri-implant mucositis is inconsistent in dental literature[30]. The different definitions are described in the Table 1. Roos-Jansåker and colleagues [4] evaluated 216 patients and 987 implants after 9-14 years after baseline (one-year post delivery of prosthetic suprastructure). They found 79.2% of patients and 42.2% of implants had peri-implant mucositis<sup>1</sup>. Another study by Fransson et al. [31] selected a group of 82 subjects previously identified as having progressive bone loss in 1 or more implants beyond normal physiologic remodeling 1 year after loading. The prevalence of

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<sup>1</sup> Prevalence values were taken from Table 8, where BOP was detected at implant level or patient level at implants where no bone loss was identified between 1-year radiographic control and final examination. If prevalence was determined by presence of Bleeding on Probing and Pocket Probing Depth  $\geq 4$ mm irrespective of bone levels then prevalence would be 76.6% at the patient level and 48.1% at the implant level (Table 6).

peri-implant mucositis on implants with no additional bone loss after baseline (n=285 implants) was 90.9% at implant level analysis.

*Table 1.* Definitions of peri-implant mucositis in the current literature.

<b>Author</b>	<b>Conditions defined as peri-implant mucositis</b>
Albrektsson and Isidor [3]	A reversible inflammatory reaction in the soft tissues surrounding a functioning implant with no loss of supporting bone
Roos-Jansåker et al. [4, 32]	When one or more of the following parameters are satisfied and no radiographic bone loss beyond 3 threads after 1 year of loading: <ul style="list-style-type: none"> <li>• Bleeding on Probing</li> <li>• Suppuration on probing</li> <li>• Pocket Probing Depth <math>\geq 4\text{mm}</math></li> <li>• No radiographic bone loss beyond 3 threads after 1 year of loading</li> </ul>
Ferreira et al. [33]	When one or more of the following parameters are satisfied and no radiographic bone loss beyond 3 threads after 1 year of loading: <ul style="list-style-type: none"> <li>• Bleeding on Probing</li> <li>• Suppuration on probing</li> <li>• Pocket Probing Depth <math>\leq 4\text{mm}</math></li> <li>• No radiographic bone loss beyond 3 threads after 1 year of loading</li> </ul>
Karbach et al. [34]	When both of the following parameters are satisfied: <ul style="list-style-type: none"> <li>• Bleeding on Probing</li> <li>• Pocket Probing Depth <math>\geq 5\text{mm}</math></li> </ul>
Koldslund et al. [35, 36]	When both of the following parameters are satisfied: <ul style="list-style-type: none"> <li>• Bleeding on Probing</li> <li>• No bone loss</li> </ul>
Maximo et al. [37]	When one or more of the following parameters are satisfied and no radiographic bone loss beyond 3 threads after 1 year of loading: <ul style="list-style-type: none"> <li>• Bleeding on Probing and/or gingival marginal bleeding</li> <li>• No radiographic bone loss beyond 3 threads after 1 year of loading</li> </ul>
6 <sup>th</sup> , 7 <sup>th</sup> , and 8 <sup>th</sup> European Workshops on Periodontology [38-40]	Inflammatory lesion limited to the mucosa seen clinically as: <ul style="list-style-type: none"> <li>• Soft tissue redness</li> <li>• Edema</li> <li>• Bleeding on Probing</li> <li>• No bone loss subsequent to time of placement of prosthetic suprastructure “baseline”</li> </ul>

A recent systematic review and meta-analysis by Atieh et al. [41] found 63.4% of subjects and 30.7% of implant sites had peri-implant mucositis. As demonstrated, the prevalence of peri-implant mucositis cited in the literature varies dramatically. This variability could be explained by the difference in the methods to diagnose this condition.

### **2.2.2 Etiology**

Peri-implant mucositis is caused by bacterial plaque. To establish the relationship between plaque formation and inflammation around implants, Pontoriero et al. [7] conducted a three-week experimental gingivitis peri-implant mucositis study in partially edentulous subjects restored with dental implants. Phase contrast microscopy was used to evaluate submucosal and subgingival plaque samples. Coccoid cells, motile rods, and spirochetes were found in similar proportions at baseline and at the end of the three-week experimental period on both the implants and teeth. The period of oral hygiene abstention demonstrated the same cause and effect relationship between plaque accumulation and inflammation for implants and mucositis as for teeth and gingivitis. The results were similar to those found decades earlier by Löe et al. [17] and Theilade et al.[18]. A more recent study by Salvi et al. [10] also confirmed the cause and effect relationship between plaque formation and inflammation around the soft tissues of implants. In this study the peri-implant mucosa appeared to have a stronger response to plaque compared to the gingiva. Nonetheless, a well-established causal relationship between plaque accumulation and peri-implant mucosal inflammation has been consistently validated.

Oral bacteria colonize on the implant as early as 30 minutes to two weeks following implant placement. Furthermore, the microbial flora at implants presents a composition almost identical to the adjacent teeth[42, 43]. This may suggest that in partially dentate patients, adjacent pockets harboring periodontal pathogens can act as a reservoir for colonization and establishment of a microflora that may not be supportive of mucosal health[44].

Colonization of the implant occurs similarly to that of a tooth. When the implant is exposed into the oral environment, a salivary pellicle forms on the surface. The pellicle forming on a titanium surface is similar to that forming on enamel, including salivary alpha-amylase and proline-rich proteins[45]. The “pristine” pockets of a newly inserted titanium implant abutment are not only colonized rapidly, but with a complex subgingival microflora that mirrors the subgingival plaque of shallow gingival crevices[2].

There are two differences between bacterial colonization on teeth and implants that have been identified thus far. First, the numbers of the red and orange complex bacteria, while present, appear to be lower on implant sites than for tooth sites[2]. Quirynen et al. [2] explained this by suggesting that some taxa are able to colonize a clean site on their own, while other taxa require the establishment of appropriate conditions by “pioneer” species. In addition, Buchmann et al. [46] found that implants can be colonized by a complex microflora that included *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. However, this microbial colonization was compatible with peri-implant mucosal health. The second difference in bacterial colonization of teeth and implants is the specific bacterial profile in the presence of periodontitis and peri-



implantitis. Bacterial species not traditionally found in periodontitis such as staphylococci, enteric species, and yeasts have been isolated at implant sites with peri-implantitis [47, 48]. Therefore, while several studies show similarities in the bacterial colonization of teeth and implants, there is evidence that the quantity and quality of this colonization may differ.

In a study designed to explore potential differences of the bacteria at sites of peri-implant health, peri-implant mucositis, and peri-implantitis, Renvert et al. [49] looked at 40 species from 213 subjects and 976 implants using the checkerboard DNA-DNA hybridization method. The only statistical difference in bacteria was found with *Eikenella corrodens*, which was higher in peri-implant mucositis when compared to healthy peri-implant mucosal samples. The prevalence of *P. gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *A. actinomycetemcomitans* were low and did not differ by implant status. This study demonstrated that there were essentially no differences in the microbial profile between implant status, nor between teeth and implant when teeth were present. This may suggest that different pathogens than those studied were responsible for the differences in implant status or that implant status may be more influenced by specific host immune factors[49].

### **2.2.3 Normal Histology of the Peri-implant Mucosa**

The histological features of peri-implant mucosa in health include a keratinized oral epithelium continuous with a thin barrier epithelium (similar to the junctional epithelium at teeth) that attaches to the implant surface by hemidesmosomes. The barrier epithelium is approximately 2mm long, which is then continuing with a connective tissue attachment

(also attaching via hemidesmosomes to a titanium oxide implant surface [50]) that is between 1 to 1.5 mm long. While the principal fibers of teeth invest in the root cementum and fan out in lateral, coronal and apical directions from the root, the implant equivalent fibers invest in the bone crest and run parallel to the implant[51]. Differences in the vascular supply are also evident between the gingiva and the peri-implant mucosa. The vascular supply for the gingiva originates from the periosteum and the periodontal ligament. The vascular supply for the peri-implant mucosa comes solely from the periosteum[52]. Leukocytes are present in small numbers in a normal reaction to the biofilm present around implants, which is similar to that found in healthy gingiva[9]. The connective tissue around implants was found by Berglundh et al. [9] to have greater collagen and lower density of fibroblasts than the corresponding gingival connective tissue. Despite these noted differences, the peri-implant mucosa and gingiva are remarkably similar.

As outlined previously for teeth, if sufficient plaque is allowed to accumulate around the implants, mucositis will ensue. Animal[5, 9, 53] and human[7, 8] studies have shown a comparable reaction to an experimental biofilm accumulation in gingiva and soft tissues adjacent to dental implants, when examined both clinically and histologically. This is described in more detail in section 2.2.4.

Peri-implant mucositis is a reversible inflammatory process [54]. Evidence supports that a persistent inflammatory condition may progress to peri-implantitis[5, 6, 55] – an irreversible condition, with accompanying bone loss[3]. Similarly to what has been reported for gingivitis and periodontitis, not all implants with mucositis progress to

implantitis. Nonetheless, mucositis appears to be a required state prior to the development of peri-implantitis[54].

#### **2.2.4 Experimental Peri-implant Mucositis in Animal Studies**

The experimental peri-implant mucositis model was largely used to determine the histopathological features of the inflammatory response in the peri-implant mucosa versus gingiva. Ericsson et al. [53] in a dog model, induced plaque accumulation for 3 months at teeth and implant sites. The long-standing plaque accumulation resulted in the establishment of an inflammatory cell infiltrate in both the gingiva and peri-implant mucosa. The two lesions had several commonalities including: the length of the junctional epithelium, the percent volume in the infiltrate connective tissue (ICT), the vascular structures, and the prevalence of plasma cells. However, the peri-implant mucosa had a larger average distance from free margin to bone crest (4.2mm versus 3.2mm), a larger apical extension of the ICT (1.2mm versus 0.9mm), and a larger residual tissue of the inflammatory cell infiltrate (nerves, matrix, lymph, etc.) when compared to the gingival unit. The gingiva had larger percent volumes of fibroblasts, macrophages, lymphocytes and PMNs in the ICT. These results imply that the mucosal tissue may be less effective in preventing the apical propagation of the inflammatory infiltrate than gingiva[53].

Using a similar experimental model, Berglundh et al. [9] evaluated the changes of gingiva and peri-implant mucosa after 21 days of plaque accumulation. The peri-implant mucosa and gingiva had similar reactions to early plaque formation. Both had an increase in leukocyte transmigration, similar apical extensions of the ICT, and similar

volumes of inflammatory cells in the connective tissue lesions. The results of the study point out that teeth and titanium abutments acquire similar plaque quantity after 21 days of accumulation, and that both gingiva and peri-implant mucosa respond similarly to the accumulated plaque[9].

The beagle dog model was used to explore the nature of lesions resulting from subgingival and submucosal ligature placement for six weeks[5]. Clinical evaluation demonstrated that signs of tissue destruction were more pronounced at implants than teeth. Biopsies revealed that the implants had a larger soft tissue lesion than teeth. The inflammatory infiltrate on teeth was contained in the connective tissue whereas lesions on the implant site extended into the bone marrow [5]. This study suggests that long lasting inflammation in the peri-implant mucosa can progress faster into the supporting bone when compared to teeth.

In summary, the series of experiments utilizing the dog model indicate that peri-implant mucosa and gingiva respond similarly under conditions of plaque accumulation from a clinical perspective. However, structural differences between the peri-implant mucosa and gingiva may render the peri-implant mucosa less effective in preventing the progression of an inflammatory infiltrate.

### **2.2.5 Experimental Peri-implant Mucositis in Human Studies**

A human experimental peri-implant mucositis model by Pontoriero et al. [7] sought to compare clinically and microbiologically gingiva and peri-implant mucosa after three weeks of oral hygiene abstention. The results of this study showed no differences in any

of the clinical parameters or in the composition of the microbiota between peri-implant mucosa and gingiva.

Similarly, Zitzmann et al. [8] investigated the soft tissue reactions to “*de novo*” plaque accumulation over a three-week period in humans. In this study, 12 subjects, each with two implant sites and two control teeth sites, had two biopsies taken at baseline and after three weeks of plaque formation. From these biopsies, histological, histometric, and immunohistochemical evaluations were performed. The clinical parameters evaluated (PII and mGI) were not different between tooth and implant sites. On histological observations both gingiva and peri-implant mucosa had large cell infiltrates present in the connective tissue lateral to the junctional epithelium. Comparisons were made with histomorphometry and immunohistochemistry. No differences in the infiltrate between the gingiva and the peri-implant mucosa were observed at any time point. However, the authors point out that mean cell densities in the infiltrate of the gingiva were consistently higher than in the peri-implant mucosa[8]. Zitzmann and Pontoriero both support the concept that both gingiva and peri-implant mucosa have a similar inflammatory response to plaque accumulation.

Salvi et al. [10] used an experimental gingivitis model to clarify the pathogenesis of gingival/mucosal inflammation with regard to specific clinical, microbiological, and host-derived factors. As stated earlier, they found a comparable cause and effect relationship regarding bacterial challenge and host response around both natural teeth and implants. A stronger inflammatory response to plaque accumulation was seen in the mucosa surrounding implants versus natural teeth (i.e. statistically significant increase in GI,

Pocket Probing Depth [PPD], and MMP-8). No difference between the subgingival and the submucosal microbiota was observed. Additionally, the authors examined the response to reinstitution of oral hygiene measures on the resolution of inflammation. Reversibility was seen at the biomarker level for both mucositis and gingivitis. The authors found that 3 weeks of resumed plaque control appears insufficient to bring gingival and peri-implant mucosa back to baseline levels[10].

## **2.3. Clinical Assessment of Gingivitis and Peri-implant Mucositis**

### **2.3.1 Gingival Crevicular Fluid**

The estimation of the gingival inflammatory response to plaque accumulation has traditionally relied on very subjective assessments (i.e. Gingival Index). Quantitative analysis of the GCF has been used as an objective measure of the inflammatory status of gingiva [56-58]. GCF, in addition to epithelial cells and leucocytes, contains transudates and exudates of interstitial fluid, which includes plasma proteins, antibodies, complement, and protease inhibitors. GCF flow has been found to increase as gingival inflammation increases [59, 60]. Also, qualitative analysis of GCF has been used to determine pro-inflammatory cytokine levels [61].

Two methods have been utilized for quantitating GCF volume: the ninhydrin area method (NAM) and the Periotron. The NAM relies on staining serum alpha-amino proteins absorbed on a filter paper with ninhydrin and quantifying the resulting stained area. The Periotron measures electrical capacitance of the fluid absorbed on the filter paper strip, which changes in dielectric insulating properties as the quantity of fluid absorbed changes[57]. There have been several versions of the Periotron: the Periotron

600, Periotron 6000, and the most recent Periotron 8000. The Periotron is most appropriate to evaluate longitudinal changes of GCF in a particular individual, but not between individuals[62].

### **2.3.1 Bleeding on Probing**

Bleeding on probing (BOP) around teeth has long been one of the primary clinical indicators of inflammation. Lang et al. [63], evaluated BOP as a potential predictor for future attachment loss. Lang found an increasing incidence of BOP corresponded with clinical attachment level loss of  $\geq 2$  mm over two years. However, the presence of BOP was not a highly sensitive indicator of disease. Rather, the absence of BOP showed a nearly 100% predictability for health.

BOP has been also evaluated around dental implants. In an experimental peri-implant mucositis and experimental peri-implantitis study, Lang et al. [64] demonstrated that healthy peri-implant sites had zero BOP, sites with experimental peri-implant mucositis had a mean BOP of 66.7%, and sites with experimental peri-implantitis had a mean BOP of 90.9%. Thus, destruction of peri-implant tissues was found to be associated with BOP.

To answer the question of how reliable BOP is as a predictor of future attachment loss around implants, Jepsen et al. [65] evaluated 25 patients with 54 implants (mean loading time of 41 months). A minimum threshold of 1mm of attachment loss was selected to be positive for site breakdown. BOP was found to have a high negative predictive value for peri-implant attachment loss (82% at the implant level and 97% at the site level).

Similarly to teeth, the absence of BOP around implants can serve as an indicator for

stable peri-implant conditions[65]. When the threshold for disease progression is defined as an annual increase in PPD  $\geq 0.5\text{mm}$  or  $2.5\text{mm}$  in 5 years, BOP at  $\geq 50\%$  of sites had a specificity of 100% (compared to 73% for teeth) and a positive predictive value of 100% (compared to 40% for teeth)[66].

Costa et al. [55] performed a retrospective study on a group of patients identified as having peri-implant mucositis five years earlier. In these patients the presence of BOP was significantly associated with further breakdown to peri-implantitis at five years. Specifically, subjects who had  $\geq 50\%$  of sites with BOP had an odds ratio (OR) of 17.69 for developing peri-implantitis.

Thus, these results seem to indicate that BOP may be a predictor for peri-implant bone loss. However, gingival and peri-implant mucosa are structurally different and lighter pressure during probing around implants has been recommended. Gerber et al. [67] showed the mean BOP percentage at implants (13.7%) and teeth (6.6%) increase as the probing pressure increased from 0.15N to 0.25N. The authors conclude that a probing force of 0.15N should be applied around implants to avoid false positive BOP[67]. While BOP as a predictor of disease activity is limited, it may be the most useful clinical predictor we have during implant maintenance programs.

There are multiple methods to measure gingival and peri-implant mucosal bleeding due to inflammation. The Angulated Bleeding Index (AngBI) [68], the method by which the periodontal probe is placed to the level of the marginal gingiva at a 60-degree angle, is one example. In an experimental gingivitis model, this method has been shown to be a sensitive indicator of early changes in gingival inflammation [68]. A modification of this



technique was recently described by Trombelli et al. [29] and is known as the Angulated Bleeding Score (AngBS). While this method has been used in several experimental gingivitis studies[29, 68], it has yet to be employed in an experimental peri-implant mucositis model.

### **2.3.3 Gingival Index**

The evaluation of inflammation and diagnosis of gingivitis and mucositis is not only based on BOP. Clinical evaluation of the color and texture of the gingiva and mucosa provide valuable information as to the inflammatory status of the soft tissue. The accuracy of such scales is imperative for prevalence statistics in epidemiological studies. The Periodontal Index (PI) System developed by Russell [69] provided a scale that ranged from health to terminal periodontal disease. The parameters of this scale were as follows:

- 0 = Negative. There is neither overt inflammation in the investing tissues nor loss of function due to destruction of supporting tissue.
- 1 = Mild Gingivitis. There is an overt area of inflammation in the free gingiva, but this area does not circumscribe the tooth.
- 2 = Gingivitis. Inflammation completely circumscribes the tooth, but there is no apparent break in the epithelial attachment.
- 6 = Gingivitis with Pocket Formation. The epithelial attachment has been broken and there is a pocket (not simply due to swelling of the free gingiva). There is no interference with normal masticatory function, the tooth is firm in its socket, and has not drifted.
- 8 = Advanced Destruction with Loss of Masticatory Function. The tooth may be loose; may have drifted; may sound dull on percussion with a metallic instrument; and may be depressible in its socket.

Essentially a mean score below 2 would be considered in a range of health to gingivitis, while a score above 2 would be considered periodontitis.

The most widely used and accepted classification system was developed by Loe and Silness[15]. The criteria for the GI System proposed was as follows:

- 0 = Absence of inflammation.
- 1 = Mild Inflammation. Slight change in color and little change in texture.
- 2 = Moderate Inflammation. Moderate glazing, redness, edema, and hypertrophy. Bleeding on pressure.
- 3 = Severe Inflammation. Marked redness and hypertrophy. Tendency to spontaneous bleeding. Ulceration.

In an effort to define parameters more appropriate for peri-implant mucosa, Mombelli et al. [70] applied a modified Sulcus Bleeding Index (mBI), which is often referred to as modified Gingival Index (mGI), to describe the level of inflammation. In other words, the mBI replaces GI for implants. The criteria for the mBI system proposed was as follows:

- 0 = No bleeding when a periodontal probe is passed along the mucosal margin adjacent to the implant.
- 1 = Isolated bleeding spots visible.
- 2 = Blood forms a confluent red line on the margin.
- 3 = Heavy or profuse bleeding

Unfortunately, utilization of either the GI or mBI to describe the inflammation around the peri-implant mucosa requires the investigator to sweep the mucosal margin with a periodontal probe. This becomes a problem in an experimental gingivitis and mucositis study where the objective is undisturbed plaque formation. Trombelli et al. [29] conducted an experimental gingivitis study in which the GI system is modified to remove the bleeding on probing component. In this study, subjects were subjectively assessed as to whether there was an absence of inflammation (GI = 0); the presence of mild

inflammation, or moderate inflammation solely due to observation of the gingival color and level of edema (GI = 1 or 2); and potentially on spontaneous bleeding if it occurred (GI = 3). The limitation of this method is the ability to distinguish GI = 1 or 2 due to the elimination of the bleeding component.

#### **2.3.4 Plaque Index**

The accumulation of bacterial plaque on teeth and implants as the causal agent for gingivitis and mucositis is well established. The most widely used and accepted index system for the quantification of bacterial plaque, and thus the measure of a subject's oral hygiene status, was developed by Silness and Löe[71]. The Plaque Index (PII) System was proposed as follows:

0 = No plaque.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.

2 = Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye.

3 = Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin plaque.

Mombelli et al. [70] also supplied a modification to the Plaque Index (mPII) in order to make it more fitting for peri-implant mucosa. The criteria for the mPII system proposed was as follows:

0 = No detection of plaque.

1 = Plaque only recognized by running a probe across the smooth marginal surface of the implant. Implants covered by titanium spray in this area always score 1.

2 = Plaque can be seen by the naked eye.

3 = Abundance of soft matter.

The mPII suffers from the same limitations described above with the GI and mBI, where in the context of certain experimental gingivitis and mucositis models, sweeping the plaque retained on an implant or tooth would interfere with the experimental design.

Ultimately, the use of the standard PII described by Silness and Loe[71], which permits the use of disclosing solution in order to distinguish between a score of 0 or 1, allows for plaque to be quantified and remain undisturbed.

## **2.4 Risk Factors for Peri-implant Diseases**

Many studies have attempted to identify risk factors for peri-implant disease. Often the outcome variable is implant loss. This underestimates the true impact of the risk factor on peri-implant disease, as implant loss is the last and most severe stage in the disease process. When the literature reports peri-implant disease as an outcome variable, it is often referring to peri-implantitis. Less frequently will a study separate out peri-implant mucositis as an outcome of interest. It has already been described that peri-implant mucositis is a prerequisite stage prior to disease progression to peri-implantitis.

Therefore, it must be inferred that a risk factor for implant loss and/or peri-implantitis must also be a risk factor for peri-implant mucositis. Herein, unless explicitly stated as a risk for peri-implant mucositis, the outcome variable will be noted as peri-implant disease, intended to include both peri-implant mucositis and peri-implantitis. The literature has identified four main risk factors for peri-implant disease: poor oral hygiene; history of periodontitis; smoking; and uncontrolled diabetes.

### **2.4.1 Poor Oral Hygiene**

The measure of oral hygiene is determined by plaque control, which is measured by the PII described previously. Several studies detailed in section 2.2.4 established the relationship between bacterial challenge (plaque accumulation) and host response (gingival inflammation), and demonstrated a similar response around both natural teeth and implants[7, 8, 10]. Few studies take this relationship further and explicitly explore poor oral hygiene as a risk factor to peri-implant mucositis. However, in their investigation to elucidate risks for peri-implant disease in 212 Brazilian subjects, Ferreira et al. [33] found oral hygiene to be an important factor in both peri-implant mucositis and peri-implantitis. The odds ratio for peri-implant mucositis and peri-implantitis in patients with poor oral hygiene were 1.9 (95% CI 1.2-2.3) and 3.8 (95% CI 2.1-6.8), respectively. This increases to 2.9 (95% CI 2.0-4.1) and 14.3 (95% CI 9.1-28.7) for peri-implant mucositis and peri-implantitis, respectively, for very poor oral hygiene. This study suggests that as oral hygiene worsens, patients are more likely to develop peri-implant mucositis and peri-implantitis.

### **2.4.2 History of Periodontitis**

There is evidence indicating that patients with a history of periodontal disease are at a significantly greater risk for peri-implant diseases[32, 72, 73]. In a 10-year prospective study of patients who were under regular supportive periodontal therapy (SPT), Karoussis et al. [74] found a significant difference in the incidence of peri-implantitis in patients who lost their teeth due to periodontal disease (28.6%) when compared to individuals who lost their teeth due to other reasons (5.8%). In a systematic review and meta-

analysis by Schou et al. [75], the authors found a significant increase in the number of patients with peri-implantitis after a 10-year follow-up, when their tooth loss was due to periodontal disease, risk ratio (RR) 9 (95% CI 3.94-20.57). Marginal bone loss after 5 years around implants in patients whose tooth loss was due to periodontitis was significantly increased compared to patients whose tooth loss was due to other reasons.

Ferreira et al. [33] reported an OR of 3.1 (95% CI 1.1-3.5) for patients with a history of periodontitis to develop peri-implantitis. This relationship was not found to be statistically significant for peri-implant mucositis. In their five-year retrospective study of patients diagnosed with peri-implant mucositis at baseline, Costa et al. [55] found the OR for these patients to develop peri-implantitis was 9.2 (95% CI 1.53-55.32) if they currently had periodontitis. The OR increased to 11.43 (95% CI 1.11-117.33) if these patients with periodontitis were not in a routine maintenance program.

Simonis et al. [76] evaluated the long-term results of implants in 55 patients with 131 Straumann tissue level implants after 10-16 years. The long-term survival rate was 82.94%, with frequent biological (mucositis and implantitis) and technical complications. Patients with a history of periodontitis showed a prevalence of peri-implantitis of 37.93%, while in subjects without a history of periodontitis, peri-implantitis was present in 10.53% of the cases. This translated to an OR of 5.1 (95% CI 1.92-14.06). The literature consistently found that subjects with history of periodontitis were much more likely to develop peri-implant disease than persons without periodontitis.

### 2.4.3 Smoking

Smoking is a risk factor extensively evaluated in the implant literature. Several studies have demonstrated that smokers have significant increased risk for peri-implant mucositis[34, 77, 78]. Karback et al. [34] reported smoking to be associated with peri-implant mucositis with an OR of 3 (95% CI 1.141-7.916). In a prospective study of 24 implants in 10 smokers and 18 implants in 4 nonsmokers of at least one-year follow-up, Ataoglu et al. [79] found that smokers had a significant increase in the inflammation-related clinical parameters PPD, mPI, mGI, and GCF flow rate.

A retrospective study by Hass et al. [80] evaluated 1366 implants in 421 patients (366 implants in 107 smokers and 1,000 implants in 314 nonsmokers) with an average recall time of 22 months. Smokers were found to have increased bleeding index, PPD, degree of peri-implant mucosal inflammation, and radiographic bone loss. These differences were significant in the maxilla compared to mandible in smokers and in the maxilla between smokers and nonsmokers. These findings may indicate that implants in the maxilla are more susceptible to the effects of smoking than in the mandible.

Fransson et al. [31] evaluated 82 subjects previously identified as having peri-implant bone loss and described the clinical characteristics of the peri-implant mucosa. Subjects identified as smokers had a statistically significant difference in mean number of affected implants when compared to nonsmokers, 3.2 versus 1.7 respectively. Additionally, smokers had a significantly greater prevalence of implants with suppuration (25% versus 6%), greater number of PPD  $\geq$  6mm (40% versus 20%), and an OR of 2.2 (95% CI 1.5-3.3) of having a history of progressive bone loss around implants[31]. A systematic

review by Strietzel et al. [81] found implant failure in smokers had an implant related OR of 2.25 (95% CI 1.96-2.59), and a patient related OR of 2.64 (95% CI 2.26-5.77). Thus indicating that smokers had a significantly increased risk of biological complications around implants.

There is mounting evidence that smoking in combination with a history of periodontal disease may add additional risks than either factor alone[82, 83]. Heitz-Mayfield and Huynh-Ba [83] evaluated how a history of periodontitis and smoking both separately and in combination affected dental implants. They found that there was an increased risk of peri-implantitis in smokers versus nonsmokers (OR ranged from 3.6 to 4.6) and an increased risk of peri-implantitis in patients with a previous history of periodontitis. In a 10-year retrospective analysis, Aglietta et al. [82] evaluated implants placed in smokers with a history of treated periodontitis, who were also enrolled in supportive periodontal therapy. Subjects with a history of treated periodontitis and smoking had lower implant survival rates and higher marginal bone loss than implants placed in smokers without a history of periodontitis[82]. This may suggest an increased risk for peri-implant disease and implant failure when a patient has multiple risk factors.

#### **2.4.4 Diabetes**

The risks of implant failure and biologic complications associated with diabetes are more controversial in the literature. This may be due to a significant heterogeneity between studies that investigated the effects of diabetes on implant health. While some studies investigate the implant outcomes for patients with poor diabetic control[33], others evaluated whether there are differences in implant success in patients with good diabetic



control[84]. Lastly, some studies do not specifically note the level of diabetic control of the patients[85-87]. With regard to patients with good diabetic control, Abdulwass and Dhanrajani [84] reported no increased risk of implant failure in patients that had HgA1c levels  $\leq 7\%$  and are in a supportive periodontal therapy program, when compared to non-diabetics. Conversely, Ferreira et al. [33] showed that patients with poor diabetic control have an increased risk of peri-implant mucositis and peri-implantitis.

In studies where the level of diabetic control is not reported, the conclusions are controversial[85-87]. Using the results of the two studies described above as examples, well-controlled diabetes does not appear to cause increased risk of biologic complications around implants, while uncontrolled diabetes is a risk factor for peri-implant disease. By not stratifying this variable, the true effect of diabetes on implants cannot be fully elucidated.

### **3. Aim, Hypothesis and Objectives**

#### **3.1 Aim**

The aim of the present pilot study is to evaluate and compare peri-implant soft tissue response to that of gingival tissue in the same subjects in a naïve condition, during *de novo* plaque accumulation, and after resolution.

#### **3.2. Hypothesis**

There is a different clinical response to plaque accumulation in peri-implant mucosa when compared to gingiva.

### **3.3 Objectives**

#### **3.3.1 Primary Objective**

To assess changes in Plaque Index, Gingival Index, Angulated Bleeding Score, and Gingival Crevicular Fluid volume between peri-implant mucosa (test) and gingiva (control) over the two experimental phases (stimulation and resolution of inflammation).

#### **3.3.2 Secondary objectives**

- To describe differences between test and control at the naïve condition.
- To describe the effectiveness of the methodology to induce plaque accumulation.
- To describe the prevalence of complications during the experimental phase.

## **4. Study Design and Methods**

### **4.1 Study design**

The study was a controlled clinical trial with a cross arch design to evaluate and compare the peri-implant soft tissue response to *de novo* plaque accumulation to that of the gingiva around natural teeth. Clinical parameters were evaluated in the patients' natural state, after professional debridement, over 21 days of oral hygiene abstention (experimental peri-implant mucositis and gingivitis) and following 21 days of re-instatement of oral hygiene practices.

## **4.2 Patient Selection**

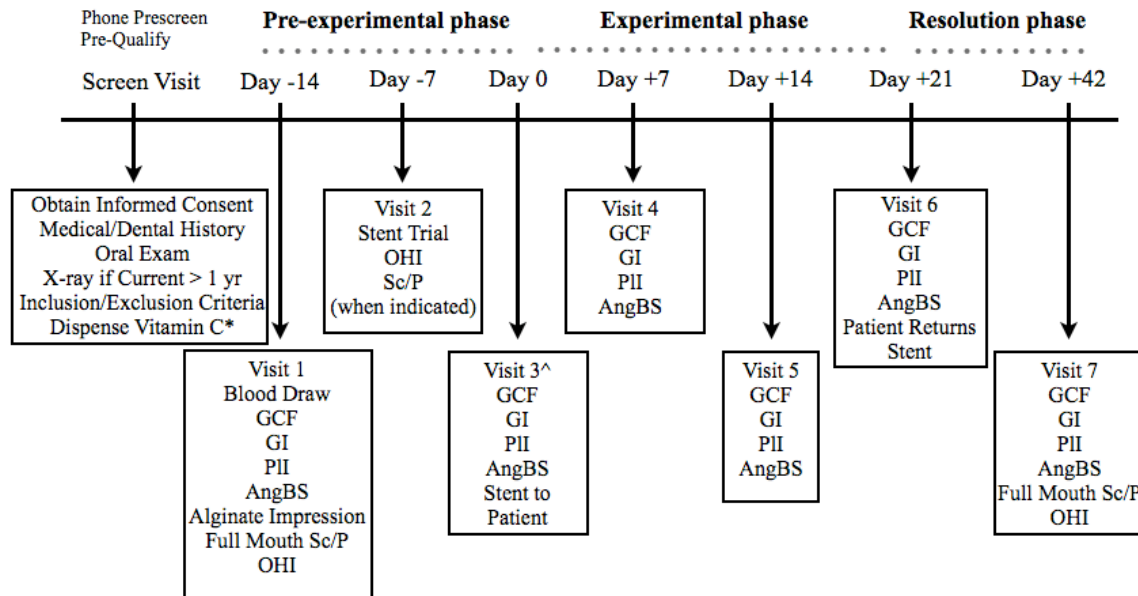
The research protocol was approved by the Institutional Review Board at the University of Connecticut. Participants were recruited among the patient population formerly treated for implant therapy at the University of Connecticut School of Dental Medicine. Once a patient was determined to be eligible for participation, he/she signed informed consent. Participants were required to have two implants (test) in the premolar or molar positions and two natural teeth (control) in premolar or molar position in the same dental arch. The inclusion/exclusion criteria for selecting the study participants are presented in Table 2.

Table 2. Inclusion and exclusion criteria.

Inclusion Criteria	
Age	≥ 21 years
Implants (test)	<ul style="list-style-type: none"> <li>• 2 Straumann Tissue Level or Bone Level implants</li> <li>• Implants must be in the molar or premolar position in the same dental arch</li> <li>• Fixed restoration ≥ 1 year in function</li> </ul>
Teeth (control)	<ul style="list-style-type: none"> <li>• 2 natural teeth in corresponding position as the implants</li> <li>• Teeth must be in the same dental arch as the implants</li> </ul>
Gingival/Mucosal condition	Absence of active infection at test and control sites
Bone Height	Absence of radiographic bone loss at test and control sites
Pocket Depths	≤ 4mm at both test and control sites
Exclusion Criteria	
Antibiotic use	Use within 1 month before screening
Systemic disease requiring the use of:	<ul style="list-style-type: none"> <li>• Chronic anti-inflammatory medications</li> <li>• Antibiotics</li> <li>• Anticoagulants</li> <li>• Medications initiated &lt; 3 months prior to the start of the study</li> </ul>
Organ diseases	<ul style="list-style-type: none"> <li>• Impaired kidney function</li> <li>• Heart murmur</li> <li>• Rheumatic fever</li> <li>• Bleeding disorder</li> </ul>
Active infections	<ul style="list-style-type: none"> <li>• Hepatitis</li> <li>• Tuberculosis</li> <li>• HIV</li> </ul>
Oral disease	<ul style="list-style-type: none"> <li>• Caries</li> <li>• Periodontal disease</li> </ul>
Smoking	Tobacco use of any kind
Medications	Any medication known to affect periodontal disease
Pregnancy	Currently pregnant or planning to become pregnant in the following 3 months
Allergy	History of allergy to common toothpaste or mouth rinse ingredients
Events during the study that would cause excluding the participant	<ul style="list-style-type: none"> <li>• Changes in medical status or medications that meet exclusion criteria</li> <li>• Use of antimicrobial rinses</li> <li>• Use of irrigating devices</li> <li>• Noncompliance with taking Vitamin C</li> <li>• Noncompliance with wearing the stent during the experimental phase</li> <li>• Use of anti-inflammatory drugs (acute use of acetaminophen was permitted)</li> <li>• An acute dental/oral condition requiring treatment</li> </ul>

### 4.3 Experimental Design and Procedures

The experimental schema is reported in Fig. 1.



*Figure 1.* Experimental design. GCF = gingival crevicular fluid volume; GI = gingival index; PII = plaque index; AngBS = angulated bleeding score; Sc/P = scaling and polishing; OHI = oral hygiene instructions; \*only after the patient qualified for the study and agreed to participate; ^ PII of 0 and GI of 0 needed to be achieved prior to proceeding with the experimental phase.

Additional explanations for specific visits are described below:

**Screening visit:** The experimental procedure was explained and a written informed consent was presented for patient acceptance. Inclusion and exclusion criteria were verified with a medical questionnaire, clinical oral exam, and dental radiographs. The radiographs, taken only if current (within 1 year) films were not readily available for review, were used to determine the presence or absence of bone loss around implants and teeth. The qualified patients were then provided with 56 tablets of 250 mg vitamin C supplement to be taken daily during the pre-trial and trial period. The purpose of the

vitamin C was to prevent any possibility of a deficiency that could affect the patient's susceptibility to inflammation.

Visit 1 (day -14): General OHI was provided to each patient. A toothbrush, floss, and fluoridated toothpaste were provided to each patient (Colgate-Palmolive Company, New York, NY, USA). Peripheral blood (10 mL) was collected in EDTA tubes and immediately stored at -80°C until further analysis (results to be reported elsewhere).

Visit 2 (day -7): All patients received a review of OHI, stent trial, and a second round of scaling and polishing if needed. Experimental procedures were reviewed.

Visit 3 (day 0): Healthy gingiva ( $GI = 0$ ) and excellent plaque management ( $PII = 0$ ) was verified. If subjects did not satisfy this prerequisite, they were provided another round of scaling and polishing and oral hygiene instructions and asked to return in one week.

Visit 6 (day +21): Stents were collected and patients were instructed to reinstitute oral hygiene. A new toothbrush and fluoridated toothpaste were provided to each patient (Colgate-Palmolive Company, New York, NY, USA).

Visit 7 (day +42): Scaling and polishing and a review of oral hygiene instructions followed collection of clinical parameters.

## Clinical Parameters

Clinical parameters were collected as listed below from the test and control sites selected for each patient.

- GCF was collected as described by Trombelli et al. [29] and measured using the Periotron 8,000 (OraFlow Inc., Plainview, NY, USA). The specific site was isolated using a cotton roll to avoid salivary contamination and gently air dried in an apico-coronal direction. Supragingival plaque was not disturbed. A sterile paper strip (Periopaper, OraFlow Inc,) was placed into the sulcus until mild resistance was sensed. The paper strip was held in place for five seconds and immediately measured chair-side by the calibrated Periotron 8,000.
- GI according to Löe and Silness [15] and the modification by Trombelli et al. [29], which removed the bleeding on probing component.
- PII according to Silness and Löe [71] and applied by the method described by Furuichi et al. [88]. Plaque was first identified as either visible or not visible. If visible, the plaque was categorized as either a score of 2 or 3. If not visible, a PII score of 0 or 1 was determined by staining the tooth or implant with erythrosine solution (Red Cote, Butler, Chicago, IL, USA) using a syringe applicator.
- AngBS was performed as described by Trombelli et al. [29]. The gingiva or peri-implant mucosa was lightly air-dried. A periodontal probe was inserted into the sulcus at an angle of approximately 60 degrees to the long axis of the tooth or implant. The scoring method was: 0 = no bleeding; 1 = bleeding after probe stimulation; 2 = spontaneous bleeding.

## **Stent Fabrication**

Cast models were prepared from the alginate impressions taken on visit 1. Wax was placed (2 mm thick) from coronal to the height of contour to 4-5 mm apical to the gingival/mucosal margin on both the buccal and lingual/palatal surfaces of the test and control sites. This was done to allow the plaque to accumulate undisturbed. A duplicate of this altered model was made. Stents were fabricated using 1.5 mm thermo-forming material (Henry Schein Inc., Melville, NY, USA) and a vacuum forming machine (Henry Schein Inc., Melville, NY, USA). The stents were trimmed and adjusted as needed in the patient on visit 2. Patients were instructed to insert the stent prior to brushing during the experimental phase. This allowed the remainder of the dentition to be brushed without removal of plaque from the experimental sites.

## **4.4 Data Analysis**

For the clinical parameters of GI, PII, and AngBS, 2 recordings from each tooth and implant were taken. The means were calculated for both test and control sites for each patient. The averages of two readings from the Periotron 8,000 of each paper strip for the GCF measurement were recorded for one tooth and one implant. The patient was considered as the statistical unit. Thus, the patient was represented by a single control and a single test value for each parameter at each time interval.

A minimum of 15 participants was considered necessary for statistical analysis. This number of subjects for our pilot study was based on previous experimental peri-implant



mucositis studies in humans [7, 8, 10]. Considering 20% attrition, a total of 18 subjects were enrolled.

The null hypothesis was that no significant clinical differences assessed during the experimental three-week period of absent oral hygiene would exist between the peri-implant mucosa and the gingiva. The Kolmogorov-Smirnov goodness fit test was computed to test the normal distribution for each parameter. All the variables were found not normally distributed. Therefore, inter-individual comparisons were performed using the Mann-Whitney Test. The Wilcoxon Signed Rank Test was used to analyze intra-individual comparisons. The Spearman Rank Correlation Test determined correlations between the four clinical parameters. Significance for all variables was set at 95% ( $\alpha=0.05$ ).

## **5. Results**

### **Demographics and protocol deviations**

Eighteen patients were enrolled. For the purpose of this report we are presenting the results relative to 14 completed subjects. One participant has not yet completed the trial. During the study, 3 patients exited the experiment. Patient flow is reported in Fig. 2. One patient was excluded from the study due to the development of an acute episode of swelling of the mucosal margin of a test site with drainage observed at the d.14 visit. The site was immediately treated with debridement of the area, thorough irrigation and one week of 0.12% chlorhexidine mouth rinse. The condition completely resolved at one week. The other two participants were excluded from the study due to a change in medical condition unrelated to the study proceedings.

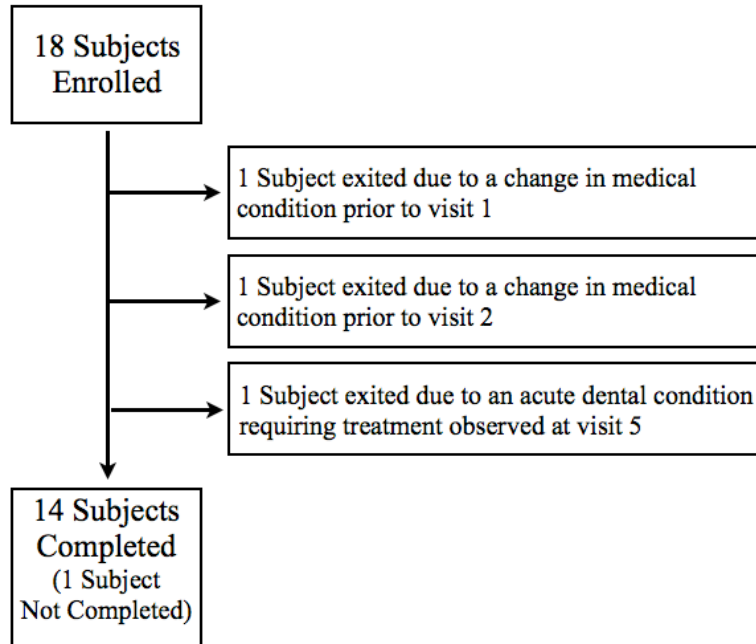


Figure 2. Enrollment and subject completion flow chart.

Demographic description of the patient population is reported in Table 3.

Table 3. Demographic descriptions.

Gender		Age (years)		Dental Arch		Implant Design	
Male	4	Male	71.3 ± 7.1	Maxilla	6	Tissue Level	28/28
Female	10	Female	60.8 ± 11.7	Mandible	8	Bone Level	0/28
Tooth Position		Implant Position		Ceramo-metal Restorations			
Premolar	23/28	Premolar	12/28	Control	14/28		
Molar	5/28	Molar	16/28	Test	28/28		

### Plaque Accumulation

The clinical data of the median and range of the PII are presented in Table 4. Both control and test groups showed a statistically significant decrease in PII from the naïve state (d.-14) to day 0 (d.0) ( $p=0.002$  and  $p=0.008$ , *Wilcoxon Signed Rank Test*).

Compared to d.0, there was a statistically significant increase in PII at each time point of

plaque accumulation for the control and test groups over the experimental phase (Table 5, Fig. 3a and b). There was statistically significantly greater plaque on d.42 than on d.0 in both groups ( $p=0.004$  control and  $p=0.008$  test, *Wilcoxon Signed Rank Test*). In neither group was there a statistically significant difference between the d.-14 and d.42. The control group had a significantly greater PII than the test groups on d.14 and d.21 ( $p=0.036$  and  $p=0.048$ , *Mann-Whitney Test*) (Table 5 and Fig. 4).

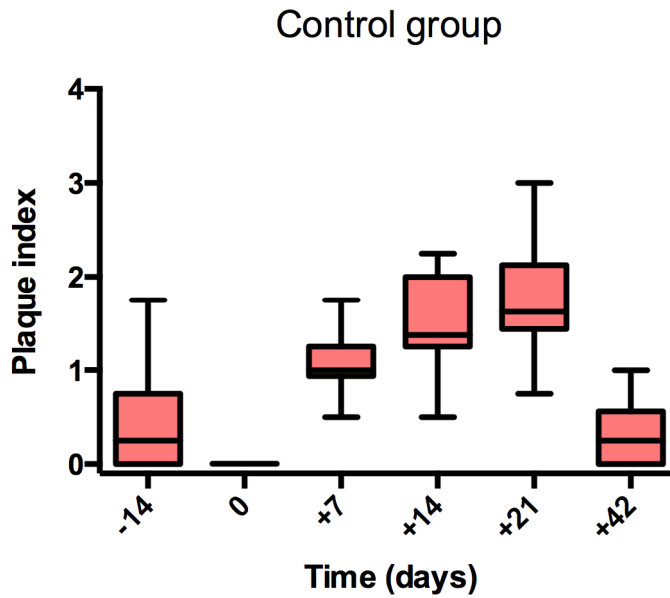
Table 4. Clinical data for PII, GI, AngBS and GCF for control and test sites.

	Day -14	Day 0	Day +7	Day +14	Day +21	Day +42
PII	median (range)					
Control	0.25 (1.75; 0)	0 (0; 0)	1.0 (1.75; 0.5)	1.38 (2.25; 0.5)	1.63 (3.0; 0.75)	0.25 (1.0; 0)
Test	0.25 (1.0; 0)	0 (0; 0)	1.0 (2.0; 0.25)	1.0 (1.75; 0.5)	1.25 (2.75; 0.5)	0.25 (0.75; 0)
GI	median (range)					
Control	0.13 (1.0; 0)	0 (0; 0)	0.38 (0.75; 0)	1.0 (1.0; 0)	1.0 (1.0; 0.25)	0.13 (0.75; 0)
Test	0.13 (1.0; 0)	0 (0; 0)	0.13 (1.0; 0)	0.5 (1.0; 0)	0.88 (1.0; 0)	0 (0.5; 0)
AngBS	median (range)					
Control	0 (0.5; 0)	0 (0.25; 0)	0.13 (0.75; 0)	0 (0.5; 0)	0.25 (0.75; 0)	0 (0.25; 0)
Test	0.13 (1.0; 0)	0 (0.5; 0)	0 (0.75; 0)	0.25 (0.75; 0)	0.25 (0.75; 0)	0.25 (0.75; 0)
GCF	median (range) ( $\mu$ l)					
Control	0.17 (0.88; 0.06)	0.15 (0.43; 0.04)	0.13 (0.40; 0.06)	0.33 (0.68; 0.05)	0.33 (1.25; 0.07)	0.20 (0.76; 0.06)
Test	0.14 (1.25; 0.04)	0.22 (0.58; 0.02)	0.29 (0.93; 0.07)	0.36 (1.17; 0.14)	0.34 (0.93; 0.09)	0.27 (0.58; 0.13)

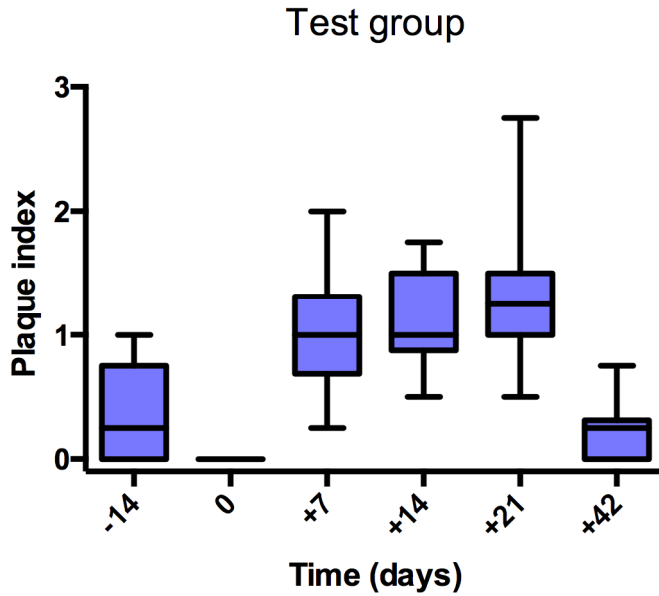
Table 5. Within group and between group (shaded) comparisons among median PII measured at different time points in control and test groups.

	Day -14		Day 0		Day +7		Day +14		Day +21		Day +42	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Day -14												
Control	-											
Test	*	-										
Day 0												
Control	‡		-									
Test		‡	*	-								
Day +7												
Control	‡		‡		-							
Test		‡		‡	*	-						
Day +14												
Control	‡		‡		‡		-					
Test		‡		‡		*	‡	-				
Day +21												
Control	‡		‡		‡		‡		-			
Test		‡		‡		‡		*	‡	-		
Day +42												
Control	*		‡		‡		‡		‡		-	
Test		*		‡		‡		‡		‡	*	-

\*Not significant. ‡Significance level: < 0.05. Mann-Whitney Test was used to determine significance between groups (shaded) and Wilcoxon Signed Rank Test was used for within group analysis.



a



b

Fig. 3. Box-Whisker plot for plaque index over pre-experimental, experimental, and resolution phases in control (a) and test (b) groups ( $n = 14$ ).

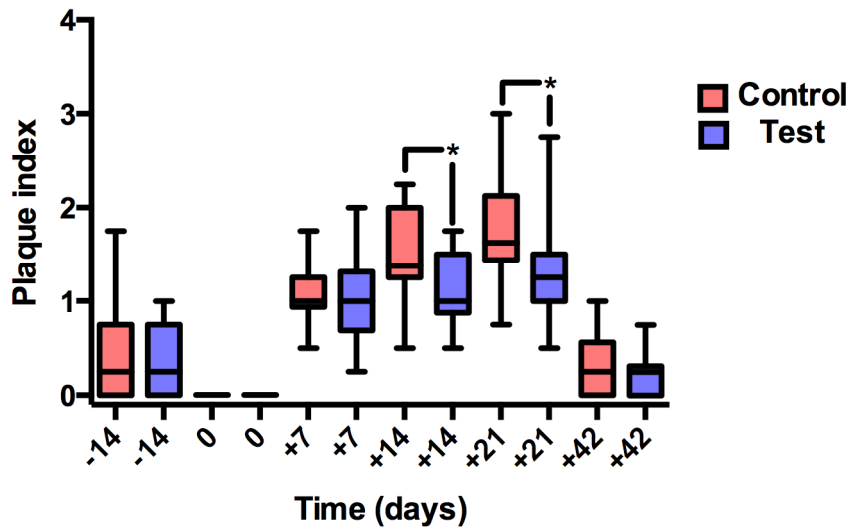


Fig. 4. Box-Whisker plot for plaque index over pre-experimental, experimental, and resolution phases comparing control and test groups ( $n = 14$ ). \*Significance level:  $< 0.05$ , Mann-Whitney Test used for between group analysis.

### Gingival Inflammation

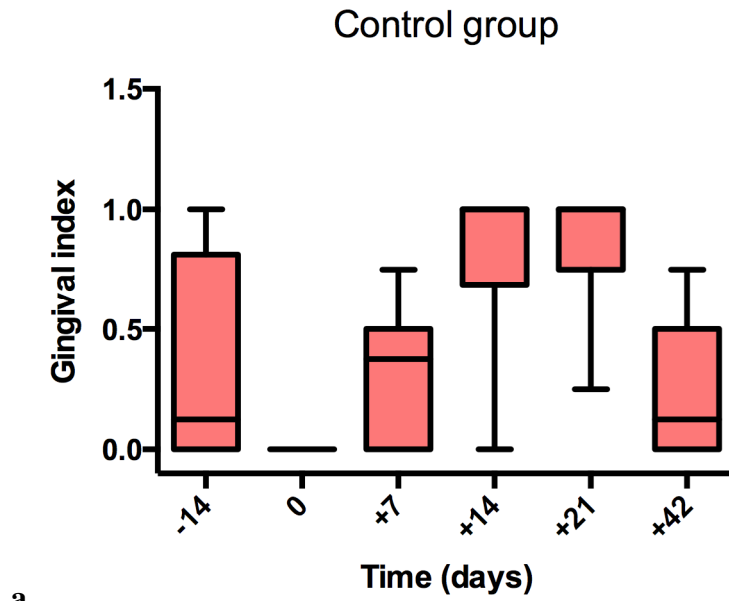
The clinical data of the median and range of the GI are presented in Table 4. Both groups showed a statistically significant decrease in GI from the d.-14 to d.0 ( $p=0.016$ , Wilcoxon

*Signed Rank Test*). Compared to d.0, there was a statistically significant increase in GI at each time point for the control and test groups over the experimental phase (Table 6 Fig. 5a and b). The GI was statistically significantly greater on d.42 than on d.0 in both groups ( $p=0.016$  control and  $p=0.031$  test, *Wilcoxon Signed Rank Test*). In neither group was there a statistically significant difference between the d.-14 and d. 42. Significant differences were not observed between the control and test groups at any time point (Table 6 and Fig. 6).

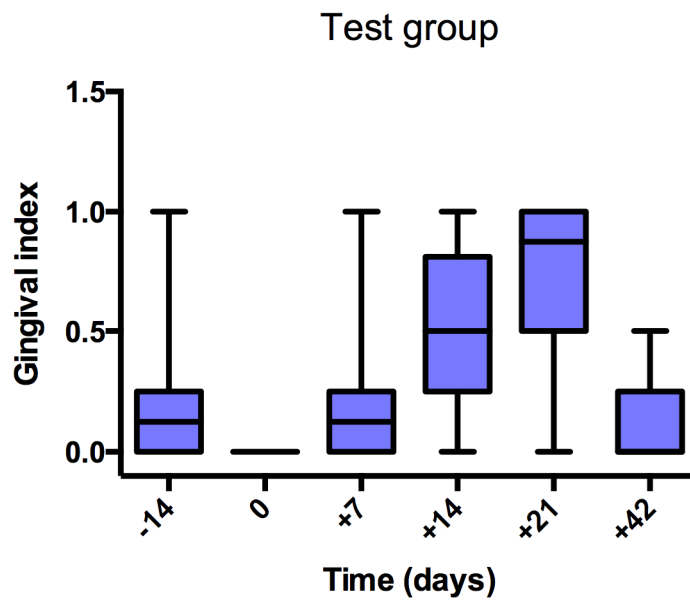
**Table 6.** Within group and between group (shaded) comparisons among mean GI measured at different time points in control and test groups.

	Day -14		Day 0		Day +7		Day +14		Day +21		Day +42	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Day -14												
Control	-											
Test	*	-										
Day 0												
Control	‡		-									
Test		‡	*	-								
Day +7												
Control	*		‡		-							
Test		*		‡	*	-						
Day +14												
Control	‡		‡		‡		-					
Test		‡		‡		‡	*	-				
Day +21												
Control	‡		‡		‡		*		-			
Test		‡		‡		‡		‡	*	-		
Day +42												
Control	*		‡		*		‡		‡		-	
Test		*		‡		*		‡		‡	*	-

\*Not significant. ‡Significance level: < 0.05. Mann-Whitney Test was used to determine significance between groups (shaded) and Wilcoxon Signed Rank Test was used for within group analysis.



a



b

Fig. 5. Box-Whisker plot for gingival index over pre-experimental, experimental, and resolution phases in control (a) and test (b) groups ( $n = 14$ ).

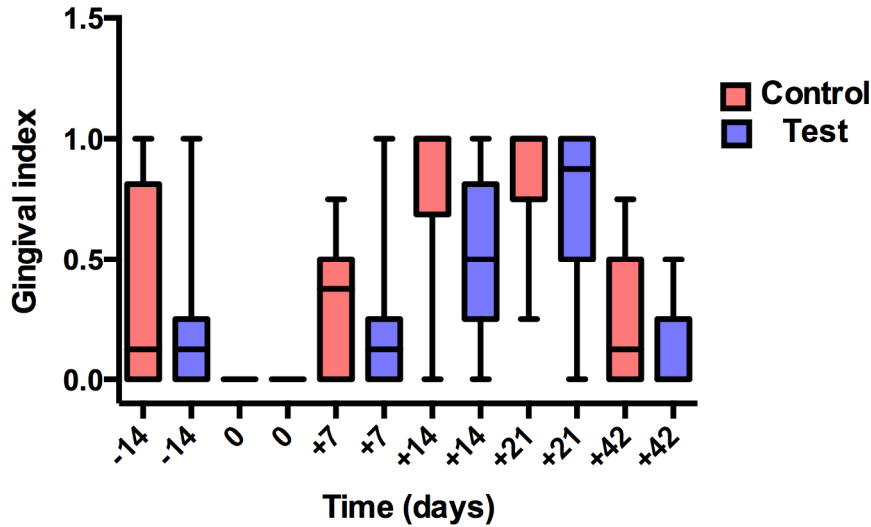
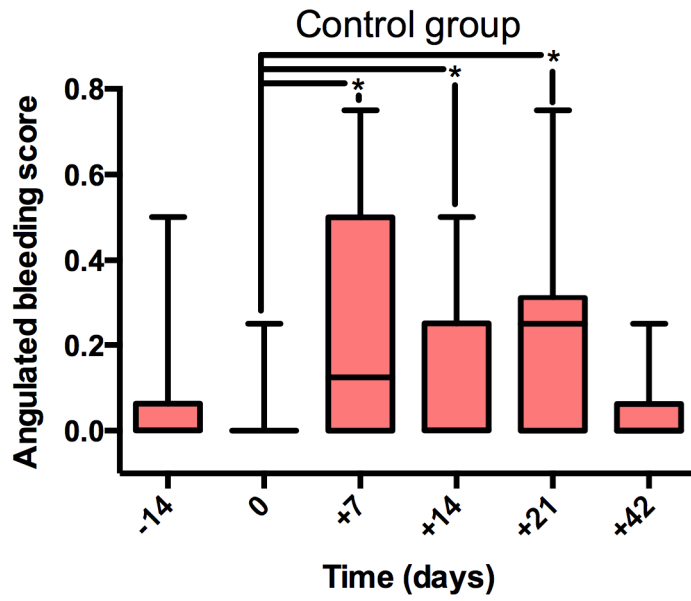


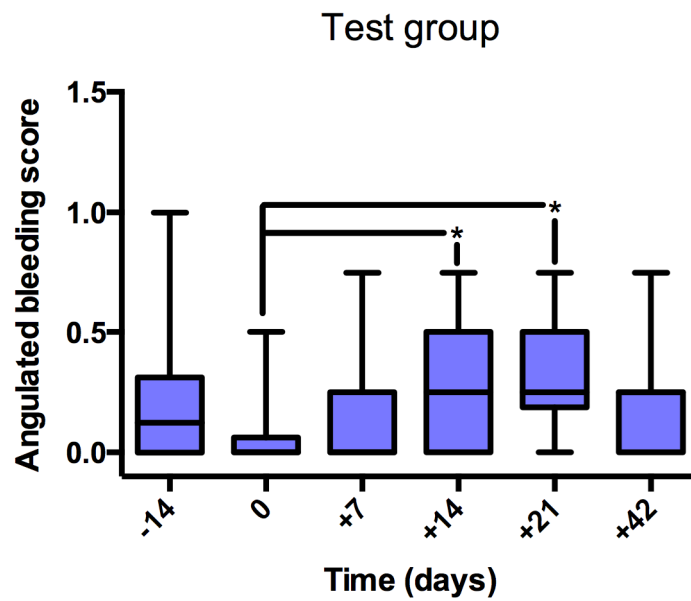
Fig. 6. Box-Whisker plot for gingival index over pre-experimental, experimental, and resolution phases comparing control and test groups ( $n = 14$ ).

The clinical data of the median and range of the AngBS are presented in Table 4. The control group demonstrated a statistically significant increase in AngBS from d.0 to d.7, d.14, and d.21 ( $p=0.016$ ,  $0.031$ , and  $0.008$ , *Wilcoxon Signed Rank Test*). The test group demonstrated a statistically significant increase from d.0 to d.14 and d.21 ( $p=0.016$  and  $0.018$ , *Wilcoxon Signed Rank Test*). There were no other statistically significant differences in AngBS within groups over time (Fig. 7a and b). The test group had a significantly greater AngBS than the control on d.42 ( $p=0.043$ , *Mann-Whitney Test*) (Fig. 8).





a



b

*Fig. 7.* Box-Whisker plot for angulated bleeding score over pre-experimental, experimental, and resolution phases in control (a) and test (b) groups ( $n = 14$ ).  
\*Significance level:  $< 0.05$ , Wilcoxon Signed Rank Test used for within group analysis.

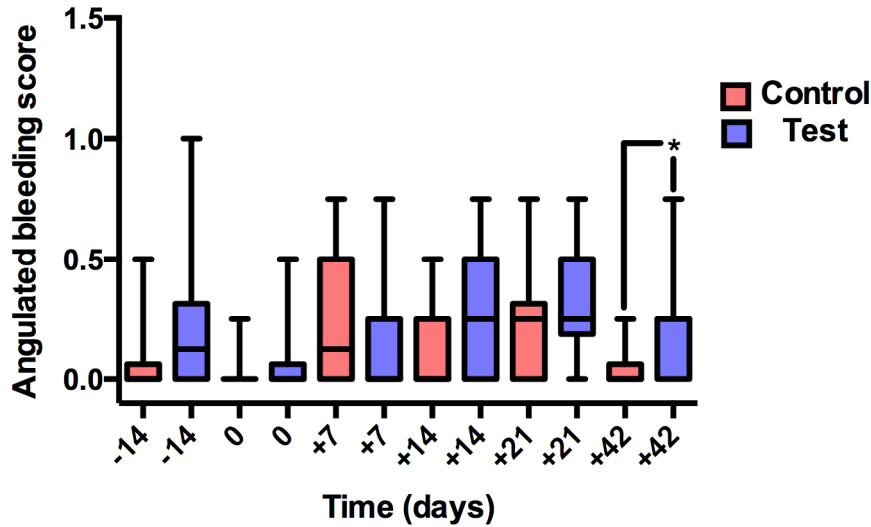
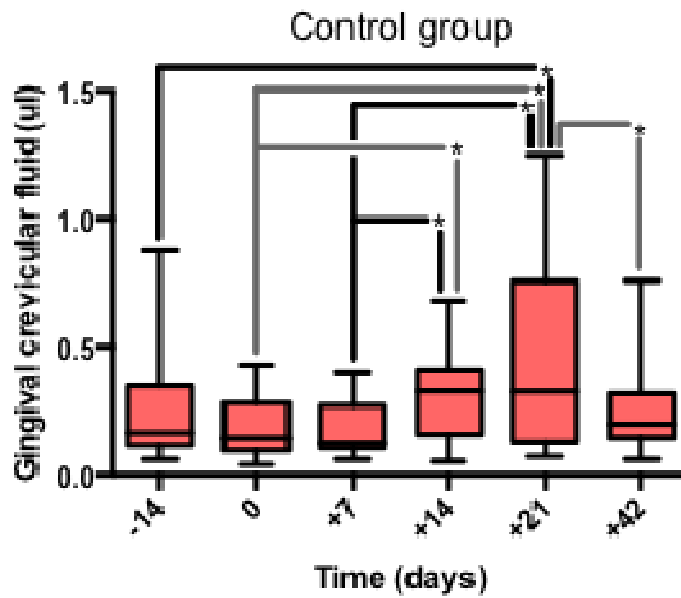
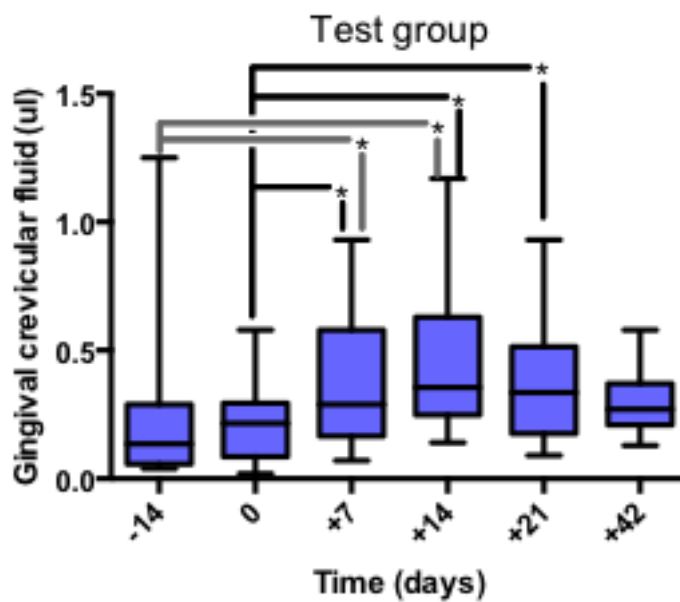


Fig. 8. Box-Whisker plot for angulated bleeding score over pre-experimental, experimental, and resolution phases comparing control and test groups ( $n = 14$ ). \*Significance level:  $< 0.05$ , Mann-Whitney Test used for between group analysis.

The clinical data of the median and range of the GCF are presented in Table 4. The control group demonstrated a statistically significant increase in GCF between the d.-14 and d.21 ( $p=0.023$ , *Wilcoxon Signed Rank Test*). Significant differences were also seen between multiple time intervals (Fig. 9a). The test group demonstrated a statistically significant increase from d-14 to d.7 and d.14 ( $p=0.016$  and  $0.044$ , *Wilcoxon Signed Rank Test*). Additionally, statistical differences were observed from d.0 to each of the other time intervals in the experimental phase (Fig. 9b). The test group had a significantly greater GCF than the control on d.7 ( $p=0.038$ , *Mann-Whitney Test*) (Fig. 10).



a



b

Fig. 9. Box-Whisker plot for gingival crevicular fluid volume over pre-experimental, experimental, and resolution phases in control (a) and test (b) groups ( $n = 14$ ).  
\*Significance level:  $< 0.05$ , Wilcoxon Signed Rank Test used for within group analysis.

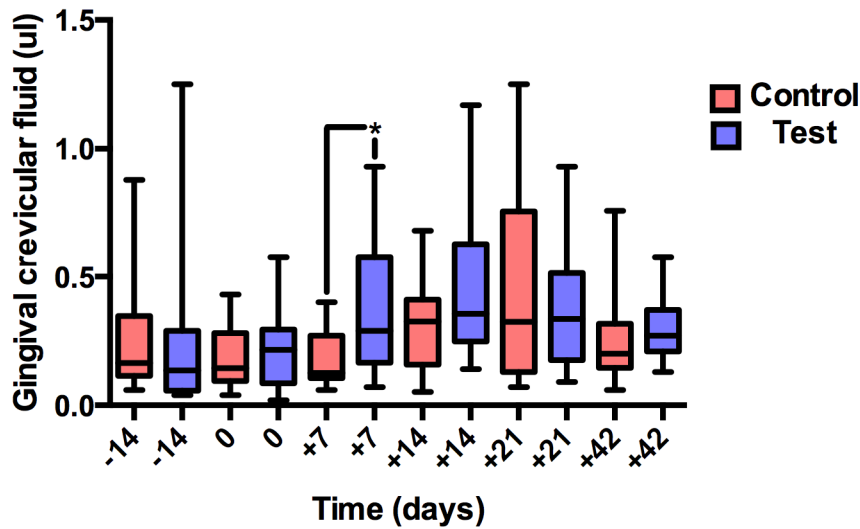


Fig. 10. Box-Whisker plot for gingival crevicular fluid over pre-experimental, experimental, and resolution phases comparing control and test groups ( $n = 14$ ).  
\*Significance level:  $< 0.05$ , Mann-Whitney Test used for between group analysis.

Correlations between PII, GI, AngBS, and GCF volume were analyzed. A correlation between GI and AngBS was found at d.21 for the test group ( $p=0.026$ ,  $r_s=0.609$ , *Spearman Rank Correlation Test*). Also in the test group, a correlation between GI and PII was seen at d.7 ( $p=0.02$ ,  $r_s=0.625$ , *Spearman Rank Correlation Test*). No other correlations were observed between any of the clinical parameters at any time point in either the test or the control groups.

## 6. Discussion

The primary objective of this study was to assess changes in the PII, GI, AngBS, and GCF volume between gingiva and peri-implant mucosa over a 21 day period of plaque accumulation (experimental phase), and following a 21 day period of resumption of oral hygiene measures (resolution phase). The control and test groups demonstrated a significant effect of time on PII score. Statistically significant increases in PII over the

experimental phase, and statistically significant decreases in PII over the resolution phase were observed. This finding demonstrates that the study methodology to induce plaque accumulation was effective. When comparing the two groups, the PII was significantly higher in the control group at d.14 and d.21 when compared to the test. This result was expected. Ceramo-metal restoration have showed lower plaque accumulation when compared with unrestored teeth[89]. Natural teeth accumulate more plaque than crowned teeth under the same conditions due to their differences in surface roughness. The lower PII in the test sites was observed despite the presence of full coverage crowns in 50% of the control sites. Additionally, the median PII scores on teeth and implants in our study were consistent with PII scores reported in the other experimental peri-implant mucositis studies in humans[7, 8, 10].

This study sought to investigate differences between gingiva and peri-implant mucosa in their responses to plaque accumulation. The GI, AngBS, and GCF volume are indirect measure of the peri-implant mucosal/gingival inflammatory response[90].

The control and test groups demonstrated a significant effect of time on GI score. Statistically significant increases in GI over the experimental phase, and statistically significant decreases in GI over the resolution phase were observed in both control and test groups. However, there were no statistically significant differences seen in GI scores between gingiva and peri-implant mucosa at any time point during the pre-experimental phase, the experimental phase, or the resolution phase, respectively. One of the limitations of this study is that for enrollment convenience we did not exclude subjects with full crown coverage in the control sites. This may raise concern in relation to a

possible effect of the crown margin on the response of the gingival tissue. A retrospective study by Carnevale et al. [91] assessed the PII and GI of 510 crowned teeth and 510 natural teeth in patients under a strict maintenance program. The results demonstrated that there were no differences in PII and GI between teeth with and without crowns. This held true regardless of where the crown margin was placed (supragingival, at the gingival margin, or subgingival).

The lack of significant difference in GI between test and control observed in our study differed from the results reported by Salvi et al. [10]. In that study, statistically significant differences were observed between the gingiva and peri-implant mucosa during the experimental and resolution phases. The peri-implant mucosa had greater GI than the gingiva. One reason for this difference could be related to the methodology used to measure GI. The Salvi et al. [10] study utilized the mGI described by Mombelli et al. [70], while in our investigation we utilized the GI method according to L  e and Silness [15] modified by Trombelli et al.[29]. We used this method because one of the priorities in this study was to leave the plaque undisturbed throughout the whole experimental phase. As detailed in section 2.3.3, by removing the bleeding component, the ability to distinguish between a GI score of 1 or 2 is limited. Hence, the lack of significant difference in GI should take into consideration that this methodology may have resulted in a less sensitive assessment of GI.

The GI in both groups on d.42 was significantly greater than the GI on d.0 (baseline). This result, also observed by others[10], suggests that the resolution of an established inflammatory state may require a longer period of plaque control to return to baseline.

However, the GI in both groups on d.42 was not statistically different than the GI on d-14 (naïve condition).

The AngBS is another clinical measure of the inflammatory response to plaque accumulation. Angling a periodontal probe into the marginal gingiva has been shown to be a sensitive indicator of early changes in the gingival condition in an experimental gingivitis model[68]. Other methods of measuring gingival bleeding due to inflammation involve probing while sweeping the marginal gingiva (thus disrupting the plaque biofilm), probing to the bottom of the sulcus, and probing parallel to the tooth. Each of these methods have been found to elicit significantly greater number of bleeding points than the angled probing method. Extrapolating these findings to peri-implant mucosa where the resistance to probing forces are less than gingiva[67], we would expect a significant overestimation of the level of peri-implant mucosal inflammation. The AngBS was chosen in our study in order to provide a sensitive test of peri-implant mucosal and gingival inflammatory change, without disruption of plaque accumulation. A statistically significant increase in AngBS was observed in both the control and treatment groups during the experimental phase. However, a statistically significant decrease in AngBS was not seen in either group following the resolution phase. In comparing the two groups, the test group had a significantly greater AngBS than the control group on d.42. This finding may suggest that the peri-implant mucosa does not recover as quickly as gingiva. This finding was consistent with that reported by Salvi et al. [10]. The Salvi et al. study found that peri-implant mucosa had a statistically greater level of inflammation than the gingiva at d.42. Although AngBS was not measured in their paper, the authors evaluated the mGI, which included a bleeding component. Their results led to the same conclusion

that inflammation at peri-implant mucosal sites did not resolve as quickly as inflammation at gingival sites.

The GCF volume as a clinical parameter of gingival inflammation has been demonstrated to have the highest correlation with PII[29]. The GCF volume increased throughout the experimental phase and decreased after the resolution phase. On d.7, the test group had a significantly greater GCF volume compared to the control group. This could suggest that the peri-implant mucosa responds more immediately to experimental plaque accumulation than gingiva. The traditional method for collecting GCF from the gingival or peri-implant mucosal sulcus involves the removal of supragingival plaque; after which a sterile paper strip is placed into the sulcus for 5 to 30 seconds[60, 92]. Supragingival plaque has been shown to significantly increase the GCF measurements in subjects with healthy gingiva[60]. However, removing the supragingival plaque would not be compatible with our study design of undisturbed plaque accumulation. Thus, the potential influence of plaque on our GCF measurements cannot be ruled out. This is the first study to our knowledge that directly compares the GCF in the peri-implant mucosal sulcus to that of the gingival sulcus using this methodology. Further investigations are needed to confirm these data.

One of the objectives in our study was to describe the clinical parameters between the control and test groups in the naïve condition (d.-14). For each clinical parameter evaluated at this time point, no differences were observed between the two groups. This observation was never reported in previous investigations. Previous experimental peri-implant mucositis/gingivitis studies in humans all consider baseline to be after



professional debridement with oral hygiene instruction and motivation[7, 8, 10]. Their aim was to have baseline PII and/or GI scores close or equal to zero prior to commencing the experimental phase. In our study, by evaluating participants at their first visit comparisons could be made to each subsequent step in the experimental and resolution phases. We found that while PII and GI scores remained significantly elevated in both control and test groups when comparing d.0 to d.42, there were no differences when comparing d.-14 to d.42.

The lack of significant difference for the clinical parameters analyzed in our study must be taken with caution due to the small sample size used. However, these data will be utilized to determine the sample size in future studies.

## **7. Conclusion**

In conclusion, the results from this study support a cause and effect relationship between plaque accumulation and clinical parameters of gingival and peri-implant mucosal inflammation. These results are in agreement with those found in several experimental peri-implant mucositis studies in humans[7, 8, 10]. We showed that 21 days of undisturbed plaque accumulation results in similar clinical inflammatory responses from both gingiva and peri-implant mucosa. Future studies of the GCF and plaque composition will further clarify whether there are any molecular and bacterial differences at the two experimental mucosal sites. The reversibility of gingivitis and peri-implant mucositis was demonstrated by the reimplementation of oral hygiene measures over a second 21-day period. Reinstitution of oral hygiene was not sufficient to restore the gingival/mucosal conditions observed at baseline (d.0). However, a reduction in the

parameters of PII, GI, AngBS, and GCF volume back to naïve condition levels was achieved at the completion of the resolution phase.

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