COMPREHENSIVE OPTICAL ASSESSMENT OF PERI-IMPLANT MUCOSA

A Thesis presented by
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This thesis is dedicated to:

My loving and most wonderful husband, Dr. Kevin Oh
For his endless love and support.
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ABSTRACT

Esthetic outcomes with implants begin with proper implant placement, but the predictability of the peri-implant esthetic outcome is also affected by patient’s pre-existing or reconstructed local tissue. An unpleasant optical phenomenon where the peri-implant mucosa appears gray has been documented in the literature. However, it’s etiology and solutions have not yet been fully investigated. The overall goal of this project is to perform comprehensive optical examination and to establish the clinical guideline to achieve optimal peri-implant mucosa.

A. Specific Aim 1: Assess the optical properties of the peri-implant mucosa.

A total of 40 patients who has a healthy, single bone level implant in the maxillary anterior zone is recruited at HSDM. For each patient, the test site (peri-implant mucosa) and the control site (adjacent natural gingiva) are identified. Using a dental spectrophotometer, CIELAB color coordinates, translucency parameter (TP), and thickness of test and control site are measured. We found that the color of peri-implant mucosa of bone level implants is significantly different from adjacent gingiva (p=0.0003). We further found that while color of the peri-implant mucosa are significantly different from those of the adjacent gingiva, the thickness and TP do not contribute to this color difference.

B. Specific Aim 2: Evaluate the vascular morphology change of the peri-implant mucosa.

Studies have shown that a significant vascular reconstruction takes place around a dental implant. Therefore, using a narrow band imaging endoscope, interpapillary capillary loops (IPCL) around a dental implant are compared to those around a natural tooth. We found that there are more interpapillary capillary loops in peri-implant mucosa compared to gingiva (p=0.02).
C. **Specific Aim 3: Determine the threshold for soft tissue color discernment**

While many studies have demonstrated the color threshold for shades of teeth and restorations, there is very little information with respect to soft tissue colors. Therefore, in controlled *in-vivo* and *ex-vivo* settings, color threshold of soft tissue was investigated, and a correlation between the objective color threshold (ΔE) and subjective color threshold for soft tissue color were also determined. For soft tissue, objective threshold is found to be ΔE=6.50-6.99, and the correlation between subjective and objective evaluations is significant (r=0.67) in ex-vivo setting.

D. **Specific Aim 4: Evaluate the efficacy of the newly developed colored abutment on improving the optical property.**

In order to improve this gray optical phenomenon, a pink colored abutment system has been developed. In a randomized manner, we investigated the color of the peri-implant mucosa with pink and gray abutment. We found that this pink colored abutment can significantly improve the aforementioned optical phenomenon, especially in those with thin tissue (<2mm) (p=0.04) and those with pink neck implant (p=0.04). The clinical significance, however, still needs to be determined.
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INTRODUCTION

Dental Implant Success

As dental implant is a standard of care for many patients, significant efforts have been invested to make this a predictable one. Some of the milestones include roughened-surface implants that improve survival rate of the implant\(^1\), and platform-switched design to reduce bone remodeling\(^2\). Implant level parameter such as initial bone loss, annual bone loss, radiolucency, mobility, infection, and pain are some of the most commonly cited parameter for a success and endpoint of a dental implant treatment\(^3,4\). The literature documents survival rates for implant-supported single-tooth crowns with a follow up period of 5-10 years as 96%\(^5,6\). A more recent retrospective article with a mean follow up of 4.2 years documented a survival rate of 81.7\(^7\).

Conversely, peri-implant soft tissue level, prosthetic level, and patient satisfaction level criteria are cited far less as identified categories of success parameter\(^8\). Papaspyridakos’s literature search demonstrated in 2012 that while 100% of the articles on single implant studies reported on implant level success rate, only 71% of the papers addressed peri-implant soft-tissue level, 21% on prosthetic level, and 36% of patient satisfaction level. But as Vilhjálmssson demonstrated, soft tissue outcome can significantly affect how our patients perceive the outcome: Vilhjálmssson showed that out of 50 patients, 72% of the patients are very satisfied with the form of the crown, only 48% of the patients were very satisfied with the form and color of the adjacent mucosa. In fact, while 0% of the patients reported “very dissatisfied” with the form and color of the crown, 4% of the patients were “very dissatisfied” with the form and color of adjacent mucosa\(^9\). Given the higher
expectations of patients today, an outcome that encompasses all aspects of a dental implant treatment for an implant success, rather than implant survival, is equally important. Therefore, this study will focus on studying the color, thickness, and translucency of peri-implant mucosa.

**Physical Properties and the Physiology of Color Perception**

Color is the perception by a subject of a particular combination of wavelengths of light emitted by a light source, transmitted through space, or reflected off of an object\(^\text{10}\). All the wavelengths that are not absorbed by the illuminated object define reflection of light (Figure 1A). In humans, the perception of color is a phenomenon caused by the ability of different wavelengths of light to excite red, green, or blue color-perceiving photoreceptor cells called S cone, M cone, L cone (Figure 1B)\(^\text{11}\). Specifically, light visible to human eyes is approximately from 380nm to 730nm. Lighting condition, background lights, eye fatigue, age, sex, and other physiologic factors can influence this interpretation of color in humans\(^\text{12}\). Furthermore, a genetic mutation that results in either a deficiency or addition of cones adds to even more varying perception of color. Even in the absence of these biologic considerations, each individual will have a different interpretation, qualification, and verbal description, making accurate color communication challenging.

**Color Systems**

In order to communicate color, in 1905, an American Artist Albert Henry Munsell devised a method for expressing colors by these three categories: hue, value, and saturation. Hue refers to the color that we commonly refer to (i.e. red, yellow, blue).
Value refers to lightness of a color (i.e. white vs. black). Finally, saturation describes how vivid the color is (Figure 2A).

In 1976, Commission Internationale de L’eclairage (CIE) developed the L*a*b* color space that would provide uniform color differences in relation to visual differences. Commonly known as CIELAB, the coordinates L* (lightness), a* (red-greenness), and b* (yellow-blueness) are implemented as a means of comparing the perceptual color difference between two points in the color space (Figure 2B).

**Color in Dentistry**

In dentistry, creating a dental prosthesis that best resembles the lost structure has been an ongoing effort. Specifically, matching the color of the dental prosthesis to that of the adjacent tissue has been one of the most challenging factors.

Extrinsic factors such as quality of light can have a significant effect\(^\text{13}\). There are several types of light sources: incandescent light with high concentrations of yellow wavelengths, fluorescent light with high concentration of blue wavelengths, and natural or day light which is closest to the full spectrum of white light. It is important to note that when an object is viewed under lights dominating in particular wavelengths, this can change the reflectance pattern and perception of color. Therefore, color is best observed with a light-correcting source than under natural or any other light\(^\text{14}\). Another factor that influences our perception of color perception is metamerism. Two objects that appear to be of identical color under a one kind of light can appear quite different under another kind of
light. This problem of metamerism can be avoided by selecting a shade and confirming it under different lighting conditions (i.e. natural daylight and fluorescent light). Finally, material property such as the surface texture, translucency, volume, wetness can all affect the color perception because these will affect light scattering, reflectance, or absorbance.

The optical presentation of natural teeth is influenced by multiple factors including color, surface texture, fluorescence, opalescence, translucency, the layering effect of enamel and dentinal tissues, and the underlying structures below soft tissues\textsuperscript{15,16,17}.

Even less is known about the color spectrum of human soft tissue\textsuperscript{18,19}. Attempts to qualify gingival color have also faced a number of challenges such as subjectivity of classification, health of the tissue, variability of gingival soft tissue within a single patient according to age, the fluctuations of gingival optical characteristics in health and disease, and the lack of comprehensive shade matching tools\textsuperscript{20,21,22}.

\textit{Color Measurement Techniques}

Traditionally and most popularly, visual determination\textsuperscript{23} of color is the application of Munsell color system, represented in three dimensions\textsuperscript{24}: Value; lightness ranging from white to black, Chroma; saturation ranging from achromatic gray to a highly saturated color, and hue; what we commonly know as color. While visual color determination is most frequently applied, inconsistencies may result from observer’s physiologic and psychological responses, fatigue, aging, emotions, lighting conditions, previous eye exposure, object and illuminant position and metamerism.
Color measuring devices offer significant advantages over visual color determination because instrument readings are objective and quantitative. Many variables are removed by employing a color-measuring device. Specifically, spectrophotometers employ CIELAB coordinates: L*, a*, and b*. L* refers to lightness coordinate (ranging from 0 for perfect black to 100 for perfect white), and a* and b* refer to chromaticity coordinates in the red-green axis and yellow-blue axis respectively. Positive a* values reflect the red color range and negative values indicate the green color range. Similarly, positive b* values indicate the yellow color range while negative values indicate the blue color range (Figure 3). In addition, spectrophotometers use uniform 7-Band LED light source, standardize the object distance, 45° incident light angle, and block out ambient light.

**Perception of Color Difference: Tooth structure**

The Euclidean distance ($\Delta E$)\(^{25}\) between the two color points corresponds to the perceptual difference between the two recorded colors (Figure 4). $\Delta E$ is defined as the color difference between two specimen and given by the equation: $\Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{\frac{1}{2}}$. $\Delta L*$ is defined as $L*_{\text{target}} - L*_{\text{standard}}$. Similarly, $\Delta a* = a*_{\text{target}} - a*_{\text{standard}}$ and $\Delta b* = b*_{\text{target}} - b*_{\text{standard}}$. Essentially, $\Delta E$ gives a standardized magnitude of color difference between two specimen, taking into account all three axes of the color spectrum. A classic study defined $\Delta E$ in oral environment as $3.7\Delta E$ units in the CIELAB color space\(^{26}\). In another words, within an oral environment, the human eye can perceive two colors as two distinct colors when $\Delta E$ is greater than 3.7. This clinical threshold value has been
commonly referenced and utilized in several dental studies. Furthermore, a spatial color difference of 1ΔE unit can be perceived by approximately 50% of experienced observers. Finally, different studies have established different levels of perceptibility threshold for differences in varying prosthetic applications. Levels of clinical acceptance with respect to color difference have ranged from ΔE = 4.2 for denture teeth to ΔE = 2.69 for all-ceramic crowns. With respect to soft tissues, one study found that perfect matching subjective evaluation presented values of ΔE = 6.63, subjective values of good matching presented average values of ΔE = 8.54. Finally, the same study showed “clearly distinguishable” subjective evaluations corresponded to objective evaluation of ΔE15.54.

**Current assessment of Peri-implant Mucosa**

The Pink Esthetic Score (PES) is commonly used for the evaluation of peri-implant mucosa. The PES is based on seven variables: mesial papilla, distal papilla, soft tissue level, soft tissue contour, alveolar deficiency, soft tissue color, and texture. Each variable is assessed with a 0-2 scale, with 2 being the best and 0 being the poorest score. Unfortunately, these criteria have been poorly correlated to a patient’s subjective perception of the overall outcome. PES has been shown to have significant variability depending on the observer dentist’s specialty, this system is still widely utilized. Vilhjalmsson further demonstrated that the patient’s subjective perception is poorly correlated with the PES (Spearman’s rank correlation coefficient of 0.25). Thus far, there are no objective criteria for peri-implant mucosa that can be correlated to the patient’s perception in a reliable way.
**Gray Shine Through Effect of Peri-implant Mucosa**

In a study assessing a 5mm-wide area of peri-implant soft tissue, Park et al demonstrated that titanium abutments influence the esthetic appearance of the soft tissue\(^{32}\). Specifically, lower L* (i.e. darker) and b* (i.e. bluer) values were found in peri-implant mucosa when compared to the same area of adjacent or contralateral gingival site; Tissues resulted in dark, gray soft tissue. In 2007, Jung demonstrated *in vitro* that four different types of restorative material (titanium, titanium veneered with feldspathic ceramic, zirconia, and zirconia veneered with feldspathic ceramic) can induce overall color changes. He further showed that this color difference was diminished with increased in soft tissue thickness\(^{33}\). Consequently, a case report showed that thickening of the human tissue through a connective tissue graft can improve the color difference that was unsatisfactory to the patient\(^{34}\). However, the soft tissue dimensions around dental implants are not always predictable\(^{35,36}\), especially after a second stage\(^{37}\), and placement of the prosthetic restoration.

**Peri-implant Mucosa: Structure**

At the structural level around a tooth, the dentogingival unit is composed of connective tissue attachment, epithelial attachment, and sulcus coronal to alveolar bone structure\(^{38}\). Some intrinsic variations of optical properties of natural gingiva do exist due to racial variations and age\(^{39,40,41,42,43}\). Furthermore, a number of extrinsic and iatrogenic factors may contribute to optical properties of natural gingiva: pharmacologic agents such as tetracycline\(^{44}\), mineral tri-aggregate (MTA)\(^{45}\), amalgam
tattoo\textsuperscript{46,47}, underlying metal of the prosthetic material\textsuperscript{48,49}, previous root canal treatment\textsuperscript{50}, and inflammation\textsuperscript{51}(Figure 5).

At the structural level around an implant, the supporting structures of sulcus depth, junctional epithelium, and connective tissue contact are said to be similar to that around a tooth\textsuperscript{52,53,54}. In 1999, Moon demonstrated that the epithelial portion was about 1.5-2mm long, and zone of connective tissue attachment were about 1-1.5mm high, significantly more than the dentogingival unit observed around a tooth\textsuperscript{55}. It can be said, therefore, that the intrinsic morphogenesis of supporting structure leads to a longer “biologic width” around an implant. Potentially, this would mean that only the soft tissue may be covering the underlying abutment and the neck of the implant. The effects of these foreign underlying structures on the optical phenomenon have been explored by a number of researchers\textsuperscript{56}. Other intrinsic factors that may contribute to the optical effect of the peri-implant mucosa are thickness and translucency of peri-implant mucosa.

\textit{Peri-implant Mucosa: Potential Contributing Factors of the gray-shine through effect}

Most notably, the thickness of soft tissue around the implant has been investigated in both \textit{in vitro} and \textit{in vivo}\textsuperscript{56,33}. Using $\Delta E = 3.6$ as a clinical threshold\textsuperscript{26}, Jung investigated the effect of soft tissue thickness on masking the color of underlying prosthesis \textit{in vitro}. He demonstrated that once the soft tissue is thicker than 3mm, the soft tissue can mask the color of underlying prosthesis to below the clinical threshold\textsuperscript{33}. However, Bressan\textsuperscript{56} showed \textit{in vivo} that even thick tissue (i.e. $>2$mm )
does not mask the color of the underlying prosthesis. Therefore, there are conflicting findings regarding the effect of thickness on this optical phenomenon.

Another potential contributing factor for this optical phenomenon is the translucency parameter (TP). Translucency is defined as the ability to allow appearance of an underlying background through its substance. Because translucency is also a function of wavelength and opposite of opaque, the TP can be measured. Johnston was first to describe TP as “how much can one observe white and black through an object”. If the object were completely opaque (as is the bottom box of Figure 6), the color difference between the area over the black strip and that over the white strip would be 0. If the object were completely translucent (as is the top box of Figure 6), the color difference would be 100. The TP values are calculated by using the following equation\textsuperscript{57}: \[ TP = \left[ (L_b^* - L_w^*)^2 + (a_b^* - a_w^*)^2 + (b_b^* - b_w^*)^2 \right]^{1/2}, \]
where “b” refers to color coordinates on the black background and “w” refers to color coordinates on the white background.

In 2013, Jun et al demonstrated a general direct correlation between the thickness of buccal area (including both soft tissue and hard tissue) and translucency parameter around an implant\textsuperscript{58}. A general correlation found, although no statement regarding TP’s effect on optical outcome was made.

Because the independent effect of thickness and translucency on this optical phenomenon around a dental implant is not identified, we will comprehensively study the physical property of the peri-implant mucosa in comparison to each patient’s own gingiva in Specific Aim 1.

\textit{Wound Healing}
Vascularization of any tissue is crucial for wound healing, bone remodeling, and adequate immune system to address iatrogenic or physiologic insults. Brief summary of classical stages of wound repair include the hemostatic phase, inflammatory phase, granulation tissue formation phase, and long-term remodeling phase. As shown by animal models, oral wound healing can be faster than skin wounds with less scarring\textsuperscript{59,60,61}. Other studies, however, demonstrated that some oral wounds were delayed compared to dermal wounds possibly due to inflammatory cytokine IL-1\textsuperscript{62}, saliva\textsuperscript{63,64}, or oral commensal bacteria\textsuperscript{62}.

Aside from osseointegration, the soft tissue collar called “peri-implant mucosa”\textsuperscript{65} serves as a biological seal, serving to ensure healthy conditions around an implant. Around teeth, a sophisticated soft tissue collar seals the tissue of tooth support against oral cavity\textsuperscript{66}. While this dentogingival unit develops with tooth eruption, the peri-implant mucosa forms after the placement as a part of the wound healing process.

**Vascular Topography via Biopsy**

In 1994, Berglundh studied the topography of vascular systems in periodontal and peri-implant tissues in two beagle dogs\textsuperscript{67}. Because the periodontal ligament (PDL) space is missing in implant sites, Berglundh found that peri-implant mucosa is lacking vasculature from the PDL space. Following this study, a number of other biopsy studies found that peri-implant mucosa, especially in the connective tissue attachment zone, is lacking the vascular network \textsuperscript{54,68,69}. Specifically, Moon found that compared to teeth, there were less vascular structures in the supra-crestal soft connective tissue.
near the implant than at a corresponding location around teeth.\textsuperscript{55}

**Vascular Topography in vivo**

As vascularization of peri-implant tissue represents the key factor in obtaining a successful result in implantology, an analysis of \textit{in vivo} vascular patterns may provide a better understanding of healthy peri-implant mucosa.

\textit{In vivo} videocapillaroscopy has been in use in medicine for many years\textsuperscript{70-73}. A specialized form of intra-vital microscopy that provides noninvasive access to skin microvascular hemodynamics has been in use to study Raynaud’s syndrome, lupus, and many other autoimmune disorders\textsuperscript{71,72}. Oral application of such videocapillaroscopy included comparing microvasculature of gingiva in healthy patients against chemotherapy patients\textsuperscript{70}, Systemic lupus erythematosus (SLE) patients\textsuperscript{71}, and diabetic patients\textsuperscript{72}. Most recently, an \textit{in vivo} evaluation of the vascular pattern in oral peri-implant tissue has been studied as well\textsuperscript{73}. This group found that the density and arrangements of capillary loops were significantly different in peri-implant mucosa compared to those in gingiva.

An endoscope is another instrument that has long been utilized in medicine to provide imaging\textsuperscript{68,69}. With development of fiber optic technology, the use of endoscopy has extended to many areas including the nasopharynx, esophagus, stomach, trachea, lungs, and colon. Conventionally, a \textit{white light emitting diode} is utilized to provide lighting for visualization of internal cavities during endoscopic procedures. \textit{Narrow-band imaging} (NBI) is a recently developed form of endoscopy, which utilizes narrow-
band wavelength filters to the endoscopic light source. The output wavelengths are restricted to narrow-band blue (415±15 nm) and green (540±15 nm) wavelengths. These wavelengths are selected because they are specific to hemoglobin absorption. Hemoglobin, and therefore blood vessels appear darker, and this adds to the increased contrast for better visualization against the background (Figure 7). Specifically, this allows clarity when visualizing mucosal vascularity. As many mucosal diseases are identified by vascularity patterns, this imaging modality is particularly useful in observing these changes more definitively and conveniently\textsuperscript{74,75}.

NBI was originally developed to diagnose gastrointestinal cancers. Since then, this technology has been extended to studying any condition that may have altered mucosal vascularity such as inflammatory bowel disease\textsuperscript{74}, Barrett’s Esophagus and esophageal cancer\textsuperscript{75}, chronic gastritis, gastric adenoma and gastric cancer, and ulcerative colitis\textsuperscript{76}.

More recently, NBI technology has been used as a diagnostic tool in the detection of a variety of oral mucosal diseases. Yang et al. have exhibited the ability of NBI to be used to evaluate oral mucosal diseases including oral leukoplakia\textsuperscript{77,78,79}, and squamous cell carcinoma\textsuperscript{80,81}. Specifically, Shibahara\textsuperscript{122} has been instrumental in categorizing stages of vascular pattern with respect to stages of oral cancer. These studies have demonstrated the ability of endoscopy equipped with NBI to observe and evaluate vasculature and microvasculature in the oral mucosa. When using NBI to evaluate oral lesions, the main criterion for evaluation is the density and level of complexity of the
intraepithelial papillary capillary loops (IPCLs). As inflammation increases, IPCL’s density increases along with the amount of disorder, appearing less organized and more complex.

**Perception of Color Difference : Soft Tissue**

The final outcome of dental treatment now includes implant-level, prosthetic-level, soft tissue-level, and patient satisfaction. Patient satisfaction, particularly for the anterior maxilla, has become a significant factor in treatment success. While a number of studies have been conducted on the esthetic outcome of the white component of the tooth, fewer have been dedicated to factors affecting the natural appearance of the surrounding soft tissue. Despite efforts to improve the color of the peri-implant tissues, studies have reported that the peri-implant mucosa color is still significantly different from the adjacent soft tissue surrounding natural teeth.

One of the challenges, however, of determining the esthetic success around the peri-implant mucosa is with the subjective interpretation of the patient. With the respect to tooth shades, Johnston and Kao were the first to compare subjective clinical observations to an objective ΔE. These researchers set ΔE = 3.7 as the average color difference among teeth rated as a match in the oral environment. Furthermore, different studies have established various levels of perceptibility for differences in varying prosthetic applications. Levels of difference required for discernment have ranged from ΔE = 2.6 for denture teeth to ΔE = 2.69 for all-ceramic crowns.
A few studies have investigated the shade of the gingiva using a spectrophotometer\textsuperscript{86,87,88}. In particular, Paniz studied the shade of the peri-implant mucosa compared with the shade of the gingiva at the adjacent tooth in a subjective and in an objective way. The threshold for the distinction of differences of soft tissue color by the human eyes was calculated to be $dE=8.74$\textsuperscript{89}.

\textbf{Ways to improve the Gray Shine Through Effect of Peri-implant Mucosa}

The knowledge that the underlying abutment can induce color changes, design alternatives for the underlying prosthesis have been investigated. A recent prospective multi-center study evaluated color differences between implants restored with titanium, gold alloy, and zirconia abutments to contralateral teeth using a spectrophotometer\textsuperscript{56}. The results showed that the color of the peri-implant soft tissue was significantly different from the gingiva around a contralateral tooth regardless of the material used.

In 2007, Ishikawa-Nagai et al demonstrated using a spectrophotometer that when a light-pink color strip was inserted under the peri-implant mucosa, the gray color of the underlying implant could be diminished\textsuperscript{90}. Based on this study, a pink abutment was manufactured. The pink color was achieved through a proprietary anodization process (Figure 8).

\textbf{Immediate Implant}

The extraction of a hopeless tooth and the immediate placement of an implant into the
socket offers advantages such as reduced overall treatment time and fewer surgical interventions. However, due to difficulty of placing an implant in an ideal position, compromised initial implant stability, and risk of mid-facial recession, case selection is crucial for an ideal outcome. Many studies have investigated the topic, especially of the midfacial mucosa level in randomized studies. Palatella compared immediate and early implant placement, and found that the midfacial mucosa recession occurred in both groups without statistically significant differences\textsuperscript{91}. Lindeboom compared immediate and delayed implant placement, and again no difference in the level of the midfacial mucosa was observed\textsuperscript{92}. Cooper further demonstrated that flapless surgery resulted in increased peri-implant mucosal tissue dimension\textsuperscript{93}. According to the latest systemic review, the evidence suggests that acceptable esthetic outcomes can be achieved with immediately placed implants\textsuperscript{94,95}. Some guidelines for successful outcomes of immediate implants include 1) placing the implant platform in the correct buccopalatal dimension\textsuperscript{96,97,98,99,100}; 2) maintenance of the buccal bone\textsuperscript{101,102,103}; 3) preexisting gingival biotype\textsuperscript{37,104,105,106}; 4) use of flapless or minimally invasive surgical implant placement\textsuperscript{97,107,108}; and 5) use of implant abutment or immediate abutment or provisional restoration\textsuperscript{109,110,111,112}. 
Significance and Innovation

Significance

Since the inception of modern dental implant treatment in the 1980’s, a tremendous amount of research has been dedicated in improving the success of dental implant treatment. Notably, novel surface treatment of implants\textsuperscript{113,114,115}, multiple site preparation techniques such as horizontal and vertical bone augmentation, site preparation materials such as autograft, allograft, xenograft, alloplast, and various implant placement techniques including irrigation to reduce bone necrosis\textsuperscript{116} have added to improved success. Successful dental implant treatment should also take into consideration the patient’s satisfaction with the procedure as well as the esthetic and functional outcomes of the treatment.

Specifically, for dental implants placed in the anterior maxilla, the esthetic outcome is a significant factor in the success of treatment. An optical phenomenon where the gray hue of a dental implant shines through the peri-implant mucosa has been documented in the literature for Straumann tissue level implants\textsuperscript{Error! Bookmark not defined.}. While a patient may perceive an implant exhibiting this phenomenon as an esthetic failure, we have very little information about factors that may be contributing to this problem.

The objective of this study is to assess the optical phenomenon of the peri-implant mucosa in a comprehensive way. By identifying the fundamental differences between the gingival tissue around a natural tooth and the peri-implant mucosa around a dental implant, this study aims to identify potential contributing factors that may lead to the discovery of ways to improve the overall outcome.
**Innovation**

Experiments in this study will employ a novel approach to study the peri-implant tissue. First, non-contact type dental spectrophotometer offers a mechanism for analyzing soft tissue color quantitatively and objectively. Non-contact operation eliminates risk of blanching for accurate color measurements, especially of the soft tissue color. Second, a narrow band imaging (NBI) is a recently developed form of endoscopy that allows clarity when visualizing mucosal vascularity. Using output wavelengths that are specific to hemoglobin absorption, NBI allows clear visualization of superficial tissue vasculature morphology without having to obtain a biopsy. Finally, no studies to date have explored the impact of using a pink colored abutment on the appearance of peri-implant mucosa.
Hypothesis / Specific Aims

The overall goal of this project is to perform comprehensive optical examination and to establish the clinical guidelines to achieve optimal peri-implant mucosa with respect to its health and appearance.

A. **Specific Aim 1: Assess the optical properties of the peri-implant mucosa.**

   A total of 40 patients who has a healthy, single implant in the maxillary anterior zone are recruited from the patient population at Harvard School of Dental Medicine. For each patient, the test site (midbuccal area measuring 2x2mm from the free gingival margin of peri-implant mucosa) and the control site (midbuccal area measuring 2x2 from the free gingival margin of gingiva of an adjacent tooth) are identified. CIELAB color coordinates, translucency parameter (TP) and thickness of test and control site are measured. We hypothesize that peri-implant mucosa exhibits optical properties that are significantly different from the patient’s natural gingiva. Further, we hypothesize that TP and thickness of the test site are associated with the optical properties.

B. **Specific Aim 2: Compare the vascular morphology of the peri-implant mucosa to that of gingival tissue.**

   Studies have shown that a significant vascular reconstruction takes place around a dental implant. Therefore, using a narrow band imaging endoscope, inter papillary capillary loops (IPCL) around a dental implant are compared to those around a natural tooth. Specifically, we investigate the orientation and density of IPCL in
peri-implant mucosa and in gingival tissue in vivo. In this pilot study, we hypothesize that the microvasculature of peri-implant mucosa can be compared to that of natural gingiva using a narrow-band imaging endoscope system. Furthermore, we hypothesize that the density of the IPCL will correlate with the a* value of the peri-implant mucosa.

C. Specific Aim 3: Determine the soft tissue color threshold

While many studies have demonstrated the color threshold for shades of teeth and restorations, there is very little information with respect to soft tissue colors. Therefore, in controlled in-vivo and ex-vivo settings, color threshold of soft tissue will be investigated. Furthermore, correlation between the objective color threshold (ΔE) and subjective color threshold for soft tissue color will be determined. We hypothesize that color threshold (ΔE) of soft tissue is greater than those for teeth and restorations. We also hypothesize that there is a correlation between subjective and objective evaluation of soft tissue color.

D. Specific Aim 4: Evaluate the efficacy of the newly developed colored abutment on improving the CIELAB coordinates.

In order to improve gray optical phenomenon, a pink colored abutment system has been developed. In this prospective randomized control study, we hypothesize that this colored abutment will significantly improve the gray shine through effect.
Chapter 1: Color of Peri-implant mucosa

Specific Aim 1: Analyze the optical property of the peri-implant mucosa

In this prospective study, we hypothesize that peri-implant mucosa exhibits optical properties that are significantly different from the patient’s natural gingiva. We further hypothesize that the thickness and translucency parameters of peri-implant mucosa affects this gray shine through phenomenon.

1A. Materials and Methods

Recruitment.

This study was approved by the Institutional Review Board of Harvard University. A total of three hundred patients were screened to participate in the study. Of those screened, fifty were eligible. A total of forty patients were enrolled in the study. All patients met the following inclusion criteria. Patients were in good health (ASA I, II)\textsuperscript{117}; patients had a single implant placed and restored in an esthetic zone between two teeth at least 6 months ago; patients had a clinically healthy implant (asymptomatic, probing depth < 4mm, bone loss limited to the success criteria defined by Albrektsson\textsuperscript{118}). Exclusion criteria were: uncontrolled systemic diseases or smoking (>10 cigarettes a day).

Color Measurements and Data Analysis.

CIELAB color measurement

A dental spectrophotometer (Crystaleye; Olympus, Tokyo, Japan; Figure 9) is utilized for
all color data acquisition. Prior to data collection, the Crystaleye spectrophotometer was calibrated using a reference plate installed at the edge of the cradle. This calibration allowed necessary standard color information for measuring to be obtained. Specifically, using this spectrophotometer, spectral CIELAB values were collected for the following: an area measuring 2x2mm immediately apical to the free gingival margin of the peri-implant mucosa, of the gingiva of an adjacent tooth, and of two teeth at the contralateral site. For example, if the patient had an implant at #7 site, the 2x2mm area apical to the free gingival margin was captured for #7i, #8 (adjacent tooth), #9, and #10 (contralateral pair to #7i and #8). The captured images and reflectance values were transmitted via a USB cable to a computer with the Crystaleye Application Master software for analysis (Figure 10).

Color difference ΔE

First, ΔE(Test) between the aforementioned area of interest of the implant site and that of its adjacent tooth was calculated. Then, in order to take into account the normal variance of natural gingiva, the ΔE(Control) between the same areas of two natural teeth at the contralateral sites was also calculated in the same patient. For example, if the patient had a single implant at 7i, the ΔE(Test) between 2x2mm area apical to free gingival margin of #7i and that of #8 was compared to the ΔE(Control) between the areas of interest in #9 and #10 in the same patient. These most apical areas are specifically chosen because they are often displayed upon smile and contribute to the overall esthetic outcome of the implant treatment.
Translucency Parameter (TP)

In order to measure the translucency of peri-implant mucosa, a small black strip and a small white strip is gently inserted underneath the peri-implant mucosa (Figure 11). Color spectra of the soft tissue immediately apical to the free gingival margin that is overlying the black strip was measured, and the following coordinates were acquired: L*$_b$, a*$_b$, b*$_b$. Then, the black strip was removed, and a white strip was inserted under the same area to acquire the following coordinates: L*$_w$, a*$_w$, b*$_w$. Using these coordinates, TP values for the peri-implant mucosa and the gingiva of the adjacent tooth were calculated according to Johnston$^{57}$: $TP= [(L^*_{b}-L^*_{w})^2+(a^*_{b}-a^*_{w})^2+(b^*_{b}-b^*_{w})^2]^{1/2}$, where “b” refers to color coordinates on the black background and “w” refers to color coordinates on the white background.

Tissue Thickness

In order to measure the tissue thickness, a strip of paper was gently inserted into the sulcus and an alginate quadrant impression (Algin x Ultra; Fast set) was obtained. This impression material was chosen for its ideal elastomeric and time stable traits along with optimal viscosity, wettability, stiffness and minimal distortion. Casts were then trimmed perpendicular to the buccal surface of implant crown containing the embedded strip. Using a caliper, the thickness of the buccal marginal peri-implant mucosa was measured 2mm apical to the gingival margin. Using a caliper directly on the soft tissue puts pressure on the soft tissue, pinching the area to be thinner than its natural form. Therefore, this technique of measuring the tissue thickness through the cast allows quantitative measurements of the soft tissue in its most natural form.
Statistical Analyses.

The difference between the L*, a*, b* values of peri-implant mucosa and its adjacent natural gingiva was evaluated using the Wilcoxon signed rank sum test for non-parametric data. The difference between $\Delta E$(Test) and $\Delta E$(Control) was also evaluated using the Wilcoxon signed rank sum test. Finally, the effects of translucency parameter and thickness on the optical phenomenon were also investigated through a linear regression analyses.

1B. Results and Discussions

Forty patients were included in the study. The group of patients was made up of 21 male and 19 female. Comparison of the CIELAB coordinates of the peri-implant mucosa to natural gingiva reveals that on average, the peri-implant mucosa has significantly lower L* value ($p=0.0003$) and lower b* (0.0001) value. [Table 1]

Table 1. Mean CIELAB values for the comparison between peri-implant mucosa and adjacent tooth gingival tissue

<table>
<thead>
<tr>
<th></th>
<th>Peri-implant mucosa</th>
<th>Gingiva</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>51.29 (0.80)</td>
<td>54.07 (0.82)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>a*</td>
<td>13.06 (0.50)</td>
<td>14.29 (0.53)</td>
<td>0.058</td>
</tr>
<tr>
<td>b*</td>
<td>11.97 (0.45)</td>
<td>14.95 (0.51)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Mean (SE)
The mean ΔE value between peri-implant soft tissue and gingiva around the adjacent tooth gingiva was ΔE = 7.65 (SE = 0.62). There are general variances of color across natural gingiva ΔE =5.82. Compared to the control site (difference between two sites of natural gingiva at the contralateral site), the test site (difference between peri-implant mucosa and gingiva of the adjacent tooth) was significantly different (p=0.0003). [Table 2]

<table>
<thead>
<tr>
<th>ΔE (Test): between peri-implant mucosa and adjacent gingiva</th>
<th>ΔE (Control): between two gingiva at the contralateral site</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.65 (0.62)</td>
<td>4.98 (0.35)</td>
<td>0.0003*</td>
</tr>
</tbody>
</table>

Mean(SE)

Furthermore to the color difference, the peri-implant mucosa 2mm apical to the free gingival margin is significantly thicker (p =0.0001) and more opaque (p=0.004) compared to the same area of the gingiva of the adjacent tooth (Figure 12, 13).

However, the variance of the color difference could not be explained by the thickness and translucency of the peri-implant mucosa (r² = 0.03) nor thickness and translucency of the adjacent gingiva (r² = 0.08).

1C. Discussions and Conclusions
Normal color variance exists at the population level based on age and ethnicity\textsuperscript{39-43}. Furthermore, even within a person, normal color variance exists\textsuperscript{120}. However, the gray shine-through effect of peri-implant mucosa is significantly outside of the normal color variance observed within a person’s gingival spectrum. The peri-implant mucosa appears darker and bluer in comparison to the gingiva of its adjacent tooth. As demonstrated by Vilhjalsson, this can contribute significantly to patient’s perception of an outcome\textsuperscript{9}.

This blue and dark hue is most likely the contribution of the following: the implant body, abutment, metal in the restoration, and or combination of all these factors. In addition, the individual variances in tissue thickness or translucency could mask or accentuate the underlying implant parts. It would be paramount to identify these additional risk factors that could contribute to the overall esthetic outcome before the commencement of the implant treatment.

Although we had originally hypothesized that the thickness would affect this gray shine-through effect, we did not see a clear correlation between them. This is in agreement with Bressan’s \textit{in vivo} study that demonstrated no difference in the display of the abutment color through the soft tissue in patients with thick (> 2mm) when compared to those with thin (<2mm) tissue.

Similar to the thickness, there was no correlation found between TP and the optical phenomenon of the peri-implant mucosa. In general, the peri-implant mucosa was
more opaque in comparison to the gingiva of the adjacent tooth.

We could not account for a number of variables such as history of bone or soft tissue graft, initial status of the recipient site such as previously infected or healthy, horizontal or vertical implant position, implant type, surgical expertise of the surgeon, and the type of restoration. Most significantly, the position of the implant would contribute significantly to this optical outcome. Therefore, a study that investigates the effect of the vertical and/or horizontal implant position on the gray shine though effect would be an appropriate next step of the study.

In conclusion, the peri-implant mucosa of bone level implants is significantly darker and bluer compared to the gingiva of the adjacent tooth. However, thickness and TP of the peri-implant mucosa do not contribute significantly to this optical phenomenon.
Chapter 2: Interpapillary capillary loops (IPCL) and soft tissue color

Specific Aim 2: Evaluate the vascular morphology change of the peri-implant mucosa.

In this pilot study, we hypothesize that the microvasculature of peri-implant mucosa can be compared to that of natural gingiva using a narrow-band imaging endoscope system. Furthermore, we hypothesize that the density of the IPCL will correlate with the a* value of the peri-implant mucosa.

2A. Materials and Methods

Recruitment.

This study was approved by the Institutional Review Board of the Harvard University. Twenty patients were examined. All patients met the following inclusion criteria. Patients were in good health (ASA I, II)\textsuperscript{117}; patients had a single implant placed and restored in the esthetic zone between two teeth at least 6 months ago; patients had a clinically healthy implant defined as asymptomatic, probing depth < 4mm, bone loss limited to the success criteria defined by Albrektsson\textsuperscript{121}. Patients with poorly managed diseases, smoking history (> 10 cigarettes/day), or peri-implant mucosa inflammation at the site or adjacent to it were excluded from the study.

Data Collection.

A narrow band imaging equipped endoscope (Olympus CV-190; Figure 14) is used to capture interpapillary capillary loops (IPCL) of peri-implant mucosa (i.e. test) and
gingiva (i.e. control) in the same patient. With NBI setting, 1.5x zoom, the microcirculatory characteristics were obtained using the optical probe to study the same area of both test and control site. A dental spectrophotometer (Crystaleye®; Olympus, Tokyo, Japan) is calibrated prior to data acquisition of spectral CIELAB values of the peri-implant mucosa and the gingiva of the adjacent tooth.

Data Analyses.

Endoscopic images are prepared by cropping to approximately 2mm x 2mm as close in proximity as possible to the free gingival margin. Two blinded, trained examiners viewed these images. Using a modification of Shibahara’s classification\textsuperscript{122}, the number of IPCL are identified, categorized, and counted. Category 1 is defined as a loop that is non-dilated, coursing perpendicular to the surface, appearing as a pinpoint. Category 2 is defined as a loop that courses parallel to the surface, appearing as a thin, linear loop. Category 3 is defined as dilated, tortuous in its arrangements. Examples of Shibahara’s classification along with examples that matches each category from our sample sets are shown in Figure 15. The difference between the numbers of IPCL found in test and control sites were evaluated using the Wilcoxon signed-rank test for non-parametric data; significance level: p< 0.05. The correlation between the numbers of IPCL and CIELAB values were evaluated using the Spearman’s rank correlation coefficient.

2B. Results

The IPCL analyses using the NBI endoscope showed many variations in capillary position, form, and organization. The architecture of the microcirculation in the test
sites was referable to Category 1 85% of the case, where only the apexes of the capillaries were visible. They often appeared as dots or commas, with perpendicular position of the loops with respect to the surface. The intercapillary capillary loops within the control site also displayed Category 1 approximately 85% of the case.

The vascular density, however, was significantly higher in the peri-implant mucosa group in comparison to the gingiva group (p = 0.02; Table 3).

Table 3 Mean number of IPCL in gingiva compared to peri-implant mucosa

<table>
<thead>
<tr>
<th>Category</th>
<th>Gingiva</th>
<th>Peri-implant mucosa</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>45.87 (5.63)</td>
<td>55.03 (5.70)</td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td>6.08 (1.59 )</td>
<td>7.03 (2.15)</td>
<td></td>
</tr>
<tr>
<td>Category 3</td>
<td>1.53 (0.58 )</td>
<td>2.32 (0.97)</td>
<td></td>
</tr>
<tr>
<td>Sum of all IPCL</td>
<td>53.47 (5.21)</td>
<td>64.37 (5.70)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Mean (SE)

No significant correlation was found between CIELAB values and IPCL density (Table 4).

Table 4 Correlation of vascular density to CIELAB values in peri-implant mucosa

<table>
<thead>
<tr>
<th></th>
<th>IPCL and L*</th>
<th>IPCL and a*</th>
<th>IPCL and b*</th>
</tr>
</thead>
</table>

2C. Discussions and Conclusion

In this present investigation, characteristics of the gingiva and peri-implant mucosa are evaluated \textit{in vivo} in order to assess vascular pattern, density, and orientation.

In terms of vascular orientation, Berglundh\textsuperscript{123} demonstrated that at implant sites, the supra-alveolar tissue is organized differently compared to those around a tooth. The lack of cementum on the implant surface directs the collagen fibers to stay more parallel to the implant surface, rather than perpendicular to it\textsuperscript{124}. More current studies have demonstrated through histologic studies that connective tissues can attach to some implant surfaces\textsuperscript{125}. Therefore, the orientation of the collagen fibers and microvasculature of the periodontal and peri-implant tissues may be becoming similar, although the timing of this phenomenon is unclear. In our investigation, vascular orientation of the IPCL in test sites did not vary significantly from those in the control sites.

In terms of vascular density, Burglundh’s qualitative study indicated that there may be less vasculature around the implant due to the lack of the PDL space\textsuperscript{53}. Likewise, in a zone close to the implant surface (i.e. 50-100$\mu$m away), no blood vessels were found, however, further away from the implant surface and close to the junctional and sulcular epithelium, blood vessels were observed\textsuperscript{54,126}. Therefore, the number of blood
vessels increased with increasing distance from the implant surface. Still, when compared to teeth, Moon found that there were less vascular structures in the supra-crestal soft connective tissue near the implant than at a corresponding location around teeth. In contrast, Scardina’s group in 2011 demonstrated using a videocapillaroscopy that there is statistically higher vascular density in a peri-implant mucosa of a test group compared to the natural gingiva of a control group (different population). In the present study, we were able to confirm this finding, in the same group of patients, using an individual’s natural gingiva as the control group.

Any wound healing sites such as implant surgical sites would have significant increase in the levels of VEGF, to induce neoangiogenesis around the peri-implant tissue. As demonstrated by Matsuo, new vessels could be confirmed only after 14 days following implant surgery, while osseointegration take far longer. It is most likely that the reconstitution of a vascular pattern formed to allow an adequate blood supply to the peri-implant tissues earlier in its wound healing stage.

In terms of the vascular quality, there are many categories and criteria to classify the loops. Our classification system is a modification from Shibahara who defined a classification for early oral cancer using the narrow-band imaging system. Similar to other classification systems for the large intestine, esophagus, pharynx, this classification for oral cancer also advances in its classification from Type I to Type IV with more dilated, inflamed, and tortuous vascular structures. All of the participants in this study had healthy peri-implant mucosa. Therefore, it is plausible that 85% of the
loops would be categorized as category 1, in both our test and control sites.

While there are some variance in the density of IPCL in peri-implant mucosa and natural gingiva, this does not correlate with the CIELAB values according to this particular data set. By having higher density IPCL in peri-implant mucosa, we hypothesized that this would contribute to more redness or higher a* value. However, as shown in Table 4, there is little correlation between the IPCL density and a* value. There may be two explanations for this phenomenon. One explanation is the limitation of our subject inclusion criteria. Since we have recruited only those who have clinically healthy peri-implant mucosa, our a* values in our sample population do not vary greatly. The second may be due to the limitations of the endoscope which can only capture relatively superficial IPCL.

While NBI technique is a novel and non-invasive tool, previous applications of NBI techniques were limited to mucosal tissues. Due to increased opacities in keratinized tissues that were investigated in our study, the degree of visibility of the microcirculation is reduced. This study allows us to conclude that the vascular pattern of healthy peri-implant sites is similar to that of healthy gingival mucosa in the same patient. In terms of the density, there was a higher number of IPCL detected within the marginal gingiva of the peri-implant mucosa. Finally, the density of IPCL did not contribute to the CIELAB values of the peri-implant mucosa. Future studies may include utilizing this NBI system to diagnose and detect early periodontitis and/or peri-implantitis.
Chapter 3: Threshold of soft tissue color

Specific Aim 3: Determine the color threshold for soft tissue

The purpose of this study is to study 1. Threshold for the distinction of differences of soft tissue color by the human eyes in ex vivo setting, and 2. The correlation between the objective and subjective evaluation in analyzing soft tissue color both in ex vivo and in vitro setting.

3A. Materials and Methods

Lab study in ex-vivo setting

Fifty pairs of gingival tissue images were prepared using a spectrophotometer. The spectrophotometer was managed by two operators who captured an area of about 2 x 5mm of soft tissue of the esthetic zone. All measured areas were analyzed using the Crystaleye software which allows the selection of a specific area. The results were recorded as CIELAB color value. All sites were free of inflammation. Images were set by pairs with ∆E between 0.36 to 23.13.

Thirty-five pre-doctoral students at the Harvard School of Dental Medicine were recruited. Color blind or deficient subjects were not included in the study. Therefore, all subjects were tested for color blindness. Using a MacAir laptop computer and Canon Realis SX80 Mark II projector, fifty pairs of gingival tissue images were projected onto a screen in a blinded fashion. Students were given 10 seconds per image to make an
assessment. Every pair is scored with one of the following: no difference, moderate difference, obvious in difference. An example of the pair is shown in Figure 16.

**In-Vivo**

Forty healthy adult patients who received an implant in the esthetic zone are identified and recruited. All patients were in good health (ASA I, II) with a single implant placed in an esthetic zone between natural teeth. Only clinically healthy implants, as defined by Albrektsson, and those that have been in function for at least 6 months were included.

Objective values of the peri-implant mucosa color and those of natural gingiva of adjacent teeth were obtained using a spectrophotometer. All measured areas were analyzed using the Crystaleye software which allows the selection of specific areas. The results were recorded through CIELAB color space. ΔE of the peri-implant mucosa and its adjacent gingiva rendered ΔE ranging from 1.7 to 19.0.

Patients’ subjective assessment were made by asking them to evaluate the color difference between the test (peri-implant mucosa) and the control site (adjacent gingiva) of their soft tissue. Inside a color-viewing booth (Macbeth Judge 2), patients’ lips were retracted using Sklar cheek retractor. Approximately 1 foot away from a large mirror, under a specific ambient light (daylight D65), patients were asked to observe the soft tissue color (Figure 17). Patient was asked evaluate and categorize the color difference between the peri-implant mucosa and its adjacent gingiva as one of the following: no difference, moderate difference, obvious in difference.
**Statistical Analysis**

For each subjective evaluation, the objective values corresponding to each category were analyzed with the calculation of the mean and standard error. To identify a correlation between the objective and subjective evaluation, the Pearson’s correlation coefficient was calculated.

**3B. Results**

In the lab (*ex vivo*) setting, 35 students evaluated 50 pairs of images. Thirty-five subjective scores for each pair were averaged. The analyses of subjective evaluations showed that 21 pairs displayed “no color difference”, 24 pairs displayed “moderate color difference”, and 5 pairs displayed “obvious color difference.” For the category scored as “no difference,” the mean objective value was $\Delta E = 3.54$. For the category scored as “moderate difference”, the mean objective value was $\Delta E = 6.99$. For the category scored as “obvious difference”, the mean objective value was $\Delta E = 16.03$. A full descriptive table is shown in Table 5.

**Table 5 Mean objective values of $\Delta E$, $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ for each subjective category in *ex vivo* setting**

<table>
<thead>
<tr>
<th>Category</th>
<th>No difference n=21</th>
<th>Moderate difference n=24</th>
<th>Obvious difference n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta E$</td>
<td>3.54 (0.90)</td>
<td>6.99 (0.76)</td>
<td>16.03 (3.55)</td>
</tr>
<tr>
<td>$\Delta L^*$</td>
<td>2.72 (0.42)</td>
<td>3.99 (0.58)</td>
<td>11.89 (1.34)</td>
</tr>
</tbody>
</table>
In clinical \textit{(in vivo)} setting, forty patients were included in the study. The group of
patients was made up of 21 men and 19 women. The analyses of their subjective
evaluations showed that 7 patients pointed to “no color difference, 22 patients pointed to
“moderate difference, and 10 patients pointed to “obvious difference”. For the category
scored as “no difference,” the mean objective value was $\Delta E = 5.90$. For the category
scored as “moderate difference,” the mean objective value was $\Delta E = 6.40$. For the
category scored as “obvious difference,” the mean objective value was $\Delta E = 10.76$. A full
descriptive table is shown in Table 6. A summary graph of table 5 and 6 are depicted in
Figure 18.

Table 6 Mean objective values of $\Delta E$, $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ for each subjective category in
\textit{in vivo} setting

|        | No difference $n=7$ | Moderate difference $n=22$ | Obvious difference $n=10$
|--------|---------------------|-----------------------------|-----------------------------
| $\Delta E$ | 5.90 (1.01)         | 6.40 (0.70)                 | 10.76 (1.48)                |
| $\Delta L^*$ | 3.36 (1.17)     | 3.90 (0.70)                 | 5.73 (1.60)                 |
| $\Delta a^*$ | 3.04 (0.75)     | 3.25 (0.47)                 | 4.43 (1.04)                 |
| $\Delta b^*$ | 2.50 (1.01)     | 2.64 (0.41)                 | 6.18 (0.78)                 |

Mean (SE)
Finally, the correlation between the subjective perception of color difference and the objective color difference was investigated. As described in Table 7, the correlation coefficient for overall color difference between the subjective scores and the objective values was 0.67 for *ex vivo* and 0.46 for *in vivo*. The correlation was much stronger in *ex vivo* setting compared to *in vivo* setting, and this was consistent in overall color difference $\Delta E$, $\Delta L^*$, $\Delta a^*$, and $\Delta b^*$. Of all the coefficient values, the strongest correlation was noted on $\Delta b^*$ axis for both *ex vivo* and *in vivo*.

**Table 7 Correlation between subjective and objective values in *ex vivo* and *in vivo***

<table>
<thead>
<tr>
<th></th>
<th>Pearson’s correlation coefficient</th>
<th>Pearson’s correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ex vivo</td>
<td><em>In vivo</em></td>
</tr>
<tr>
<td>$\Delta E$</td>
<td>0.67</td>
<td>0.46</td>
</tr>
<tr>
<td>$\Delta L^*$</td>
<td>0.43</td>
<td>0.22</td>
</tr>
<tr>
<td>$\Delta a^*$</td>
<td>0.52</td>
<td>0.20</td>
</tr>
<tr>
<td>$\Delta b^*$</td>
<td>0.76</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**3C. Discussions and Conclusion**

The literature has demonstrated significant spectrophotometric differences between the peri-implant tissue and periodontal tissue with $\Delta E$ values that ranges from 6.5 to 11$^{33}$. All studies to date have utilized the threshold references from those found with hard tissue (i.e. tooth structures of natural dentition or prosthetic materials). Paniz’ group in 2013 demonstrated correlation between the subjective and the objective evaluations of soft tissue by dental professionals, and found that the mean objective value of $\Delta E = 8.5$
corresponded to “good matching, but clinically distinguishable.” This value is much higher in comparison to the threshold found for teeth and prosthetic structures. A clinical threshold value for good match for tooth structures is $\Delta E = 2.69$ for all-ceramic crowns. It is plausible that the human eye could be more sensitive to the differences in the color spectrum that is adjacent to the white tissue of the teeth than to differences in the pink tissue of soft tissue.

The present study investigated the clinical threshold for soft tissue first as a laboratory environment by dental students in a blinded fashion. Images of soft tissue were obtained in the most controlled manner possible by using a spectrophotometer. Here we found that the average objective value of soft tissue pairs that were deemed to have “moderate color difference” was 6.99. We also investigated the clinical threshold in vivo environment with controlled ambient light. In this experiment, not only was the ambient light controlled, but also a truly patient-centered outcome was evaluated as the patients were asked to evaluate their soft tissue. In this in vivo experiment, the mean value for “moderate difference” was $\Delta E = 6.40$ between peri-implant mucosa and gingiva of the adjacent tooth, a lower value than the one presented for the ex vivo study. Furthermore, the average value of “obvious different” between peri-implant mucosa and gingiva of the adjacent teeth group was also much lower than that of the ex vivo experiment ($\Delta E = 10.76$ vs. 16.03). It must be noted, however, that the examined specimens were not the same in the two studies. In the in vivo experiment, the sample set had $\Delta E$ values that ranged from 1.7 to 19.0. The ex vivo experiment pairs had $\Delta E$ values that ranged from 0.63 to 23.
Overall, it can be concluded that the layperson can tell that the soft tissue color is “moderately different” compared to adjacent soft tissue with a mean objective values of \( \Delta E = 6.40-6.99 \). These clinical thresholds by the general population were lower than the threshold by dental professionals as demonstrated by Paniz(8.74)\textsuperscript{89}. Furthermore, it can be said that subjectivity plays a role not only in color perception, but also with a layer of bias when observing one’s own outcome.

As a second objective, the correlation between subjective and objective evaluation of soft tissue color discernment was investigated. From this experiment, we found that the objective and subjective evaluations correlated strongly (\( r = 0.67 \)) in \textit{ex vivo}. The correlation found \textit{in vivo} was moderate at \( r = 0.46 \). The same trend of stronger correlation in \textit{ex vivo} setting was observed in \( \Delta L^* \), \( \Delta a^* \), and \( \Delta b^* \). It is notable that the correlation between \( \Delta b^* \) and subjective perception was the strongest in both \textit{ex vivo} and \textit{in vivo}. One can postulate that our eyes may be most sensitive to changes in the yellow-blue axis. A more controlled follow up study with specimen pairs that have the same \( \Delta E \) with varying degrees of \( \Delta L^* \), \( \Delta a^* \), and \( \Delta b^* \) is needed to confirm these findings.

The limitations of this study are that the \textit{in vivo} and \textit{ex vivo} have different sets of samples. It may be interesting to repeat the \textit{ex vivo} experiment with the same sample obtained from the \textit{in vivo} experiment since those would be a better comparison.
Chapter 4: Effect of pink implant on soft tissue color

Specific Aim 4: Evaluate the efficacy of a new pink colored abutment on the appearance of the soft tissue.

The purpose of this prospective randomized clinical trial is to analyze the effect of a pink abutment system, on the overall esthetic appearance of peri-implant mucosa. In another words, compared to the gingiva around a natural tooth (Figure 19A), peri-implant mucosa appears gray due to the underlying structures such as the titanium abutment in the neck area, and titanium implant body in the body area (Figure 19B). By replacing these conventional parts with pink colored prosthetics, we hypothesize that this gray optical phenomenon could be ameliorated (Figure 19C).

4A. Materials and Methods

Recruitment.

Subjects, at least 18 years of age, with a restoratively hopeless tooth or teeth in the maxillary esthetic zone (2nd premolar ~ 2nd premolar) and one healthy adjacent tooth and/or healthy contralateral tooth for comparison were recruited (n=20). Anyone with the following condition was excluded: no posterior occlusion, uncontrolled or poorly controlled diabetes, use of I.V. bisphophonates, history of depression requiring hospitalization, immunosuppression medication use, active periodontal or endodontic diseases, smoker, alcohol or drug abuse. Presence of the buccal plate was confirmed by a CT scan (Resolution 0.3mm x 0.3mm x 0.3mm; Voxel size 0.3mm cubic; field of view: 8mm vertical by 16mm horizontal) prior to accepting the patient into the study.
Procedure.

On the day of the surgery, patient was randomized to either pink implant group or a gray implant group. Using a simple randomization method, patient’s group was determined in a blinded fashion where the surgeon picked a card out of an envelop that contained an even number of cards labeled “pink” or “gray.” Extraction of the compromised/hopeless tooth was performed using a conservative flapless approach to preserve the bone in the socket as much as possible. Once the socket has been examined, and the four walls confirmed intact, the chosen implant was placed. Sites were then copiously washed with sterile saline and customized healing abutment or a customized provisional crown was placed (Figure 20). Once the implant healed and the final restoration was being fabricated, each patient had two identical customized abutment fabricated along with one all ceramic crown; one was a conventional titanium abutment (control), and the other was a pink abutment (test) (Figure 21).

Data Collection and Analyses

The color of the peri-implant mucosa and that of the gingiva of the adjacent tooth was measured with a dental spectrophotometer (Crystaley, Olympus). First, the color was measured with a gray abutment and a ceramic crown in place. Secondly, the color was measured with a pink abutment and the same ceramic crown in place. Specifically, an area measuring approximately 2x2mm immediately apical to the free gingival margin was captured as this is the area that most significantly displays the color difference between the peri-implant mucosa and the gingiva of the adjacent tooth (Figure 10). All
measurements were completed with the ceramic crown in place. ΔE between peri-implant mucosa with gray abutment and adjacent gingiva was compared to that between peri-implant mucosa with pink abutment and adjacent gingiva using Wilcoxon signed rank sum test for non-parametric data. In another words, the optical effect of the pink abutments on the peri-implant soft tissue was analyzed quantitatively using CIELAB color system.

In order to measure the thickness, a strip of paper was gently inserted into the sulcus and a polyvinylsiloxane (PVS) quadrant impression was obtained. Casts were then trimmed perpendicular to the buccal surface of implant crown containing the embedded strip. Using a caliper, the thickness of the buccal marginal peri-implant mucosa was measured 1mm apical to the gingival margin.

**Power Calculation**

The sample size calculation is based on Fisher's exact test. It is estimated that 18 subjects for each group are needed for this study to have 80% power. Two-sided test with a 0.05 type I error rate and 5% adjustment for technical errors such as implant failure were taken into account.

**4B. Results**

In individual subjects, the objective a* and b* values with a gray abutment were significantly different compared to those with a pink abutment (Table 8). The benefit of having the new pink anodization of the abutment is added red and yellow hue of the peri-
implant mucosa.

Table 8 Mean CIELAB values of the peri-implant mucosa with gray abutment and pink abutment

<table>
<thead>
<tr>
<th></th>
<th>Gray abutment</th>
<th>Pink abutment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>n= 15</td>
<td>n=15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>50.42 (0.76)</td>
<td>50.13 (0.68)</td>
<td>p=0.53</td>
</tr>
<tr>
<td>a*</td>
<td>13.44 (0.55)</td>
<td>14.95 (0.41)</td>
<td>p=0.02*</td>
</tr>
<tr>
<td>b*</td>
<td>11.12 (0.42)</td>
<td>11.73 (0.45)</td>
<td>p=0.01*</td>
</tr>
</tbody>
</table>

Overall color change of the marginal soft tissue induced by changing the gray abutment with a pink abutment was ΔE = 4.4. This color change was more pronounced in patients with thin peri-implant mucosa (<2mm) compared to those with thick peri-implant mucosa (≥2mm). Those with thin peri-implant mucosa displayed ΔE = 4.96 simply by changing the abutment while those with thick peri-implant mucosa displayed ΔE of 3.32 from changing the abutment (Table 9).

Table 9 ΔE between peri-implant mucosa with gray abutment when compared to the same peri-implant mucosa with pink abutment

<table>
<thead>
<tr>
<th></th>
<th>Thin (&lt;2mm)</th>
<th>Thick (≥2mm)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=8</td>
<td>n=7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔE induced by pink abutment</td>
<td>4.96 (0.70)</td>
<td>3.32 (0.89)</td>
<td>p=0.043*</td>
</tr>
</tbody>
</table>

Mean(SE)
Finally, patients with a pink implant had a more pronounced color change effect of abutment change ($\Delta E=5.84$) when compared to those who had the gray implant ($\Delta E=2.33$) (Table 10). Specifically, this effect was most notable in the yellow-blue axis (Table 11, Table 12).

Table 10 $\Delta E$ induced by pink abutment in patients with pink implant compared to those with gray implant

<table>
<thead>
<tr>
<th></th>
<th>Pink Implant, n=7</th>
<th>Gray Implant, n=8</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta E$ induced by pink abutment</td>
<td>5.84 (0.68)</td>
<td>2.33 (0.46)</td>
<td>p=0.043*</td>
</tr>
</tbody>
</table>

Mean(SE)

Table 11 CIELAB values of the peri-implant mucosa with gray and pink abutment in patients with pink abutment

<table>
<thead>
<tr>
<th></th>
<th>Pink Implant, Gray Abutment, n=7</th>
<th>Pink Implant, Pink Abutment, n=7</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>51.86 (1.16)</td>
<td>50.54 (0.56)</td>
<td>p=0.04*</td>
</tr>
<tr>
<td>a*</td>
<td>12.16 (0.94)</td>
<td>15.06 (0.56)</td>
<td>p=0.09</td>
</tr>
<tr>
<td>b*</td>
<td>11.29 (0.80)</td>
<td>12.77 (0.80)</td>
<td>p=0.0002*</td>
</tr>
</tbody>
</table>

Mean(SE)
Table 12 CIELAB values of the peri-implant mucosa with gray and pink abutment in patients with gray implant

<table>
<thead>
<tr>
<th></th>
<th>Gray Implant, Gray Abutment n=8</th>
<th>Gray Implant, Pink Abutment n=8</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>49.20 (0.95)</td>
<td>49.76 (0.97)</td>
<td>p=0.15</td>
</tr>
<tr>
<td>a*</td>
<td>14.52 (0.53)</td>
<td>14.85 (0.59)</td>
<td>p=0.66</td>
</tr>
<tr>
<td>b*</td>
<td>10.99 (0.38)</td>
<td>10.83 (0.40)</td>
<td>p=0.50</td>
</tr>
</tbody>
</table>

ΔE between Gray Implant, Gray Abutment (GiGa) and adjacent gingiva, however, was close to ΔE between Pink Implant, Pink Abutment (PiPa) and its adjacent gingiva (Table 13). While close in quantitative ΔE values, closer analyses revealed that the directions of this color difference were significantly different (Figure 22A vs. Figure 22B). Furthermore, the degree of ΔL* masked the differences observed in Δa* and Δb* axes (Figure 23A vs. Figure 23B).

Table 13 Mean ΔE values between GiGa and Control vs PiPa and Control

<table>
<thead>
<tr>
<th></th>
<th>GiGa and Control n=8</th>
<th>PiPa and Control n=7</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔE</td>
<td>7.29 (1.88)</td>
<td>7.42 (1.13)</td>
<td>p=0.46</td>
</tr>
<tr>
<td>ΔL*</td>
<td>-3.61</td>
<td>-3.59</td>
<td></td>
</tr>
<tr>
<td>Δa*</td>
<td>-3.23</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Δb*</td>
<td>-3.56</td>
<td>-0.79</td>
<td></td>
</tr>
</tbody>
</table>

Mean(SE)
4C. Discussions and Conclusions

The present study evaluated the color change effect on marginal peri-implant mucosa of all ceramic prosthetic restoration. The variable of interest here was the color of the underlying custom abutment: conventional gray custom titanium abutment compared to the new pink custom titanium abutment. All measurements of two abutment materials were done in each patient with the same all ceramic crown in place.

As described on Table 8, significantly different a* and b* values were observed in peri-implant mucosa with gray abutment when compared to that with pink abutment. The benefit of having the new pink anodization of the abutment is the added red and yellow hue of the peri-implant mucosa, or reduction of blue and green hue of the peri-implant mucosa. Furthermore, this color improvement induced by the abutment was more pronounced two particular subpopulation: 1) Patients with thin tissue (<2mm), and 2) Patients with pink implants.

In terms of thickness, we found that those with thin tissue defined as <2mm saw greater benefit of the pink abutment compared to those with thick tissue. In other words, the added red and yellow hue of the peri-implant mucosa was more pronounced in patients with thin peri-implant mucosa. In the literature, there are some conflicting findings. While Bressan’s study\(^5\) demonstrated that the thickness of the tissue does not contribute to the peri-implant mucosa color, Jung’s\(^3\) paper found otherwise. This may be due to the fact that thick soft tissue was defined as greater than 3mm in Jung’s study compared to 2mm limit in Bressan’s study. No patient with soft tissue thickness 3mm was identified in
In terms of the effect of the color of the implant on the color change by the abutment, we found that the color improvement toward the appearance of the natural tooth gingiva of the pink abutment were especially more pronounced in those with pink implants (ΔE = 5.84) when compared to that in those with gray implant (ΔE = 2.33). In another words, those with pink implant had significantly more benefits when the pink abutment was used. While the implant, in theory, is completely submerged under the bone, depending on the thickness or presence of the buccal plate, the implant neck color may contribute to the peri-implant mucosa optical phenomenon.

More recently, increased attention has been dedicated to selecting various prosthetic materials for the abutment to significantly affect the peri-implant gingival shade. For instance, gold, zirconia, and titanium abutments have been compared\textsuperscript{56}. In Bressan’s study, the authors found that the peri-implant soft tissue color was different from the soft tissue color around natural teeth, no matter which type of restorative material was selected. This group did note that the gold and zirconia abutments reduce this color difference compared to titanium abutment. However, no statistically significant differences were present in the differences between the color of peri-implant mucosa and that of its adjacent gingiva regardless of the prosthetics used. Similar to this finding, while our results demonstrated statistical significance of pink abutment in those with thin tissue and pink implant, ΔE between peri-implant mucosa and natural gingiva was similar in GiGa group and PiPa group. We delved into this matter deeper, and found that while ΔE may be similar in number, the directions of color difference were significantly
different. Specifically, similar ΔL*’s of both GiGa and PiPa groups indicated that the peri-implant mucosa is darker compared to the gingiva of its adjacent tooth in both groups (-3.61 vs. -3.51). However, Δa* = -3.23 and Δb* = -3.56 of the peri-implant mucosa of GiGa to its control indicated that the peri-implant mucosa of GiGa is 3.23 units greener, and 3.56 units bluer compared to its control. In contrast, Δa* = 0.67 and Δb* = -0.79 between the peri-implant mucosa of PiPa and its adjacent gingiva indicate that the peri-implant mucosa of PiPa is 0.67 redder, and 0.79 units bluer in comparison to its control. Since this study presented only the preliminary data, the effects of the outliers could be significant. Future study with a larger sample size will be carried out to confirm the results of this study. Overall, we can say that the color improvement towards the appearance of the natural gingiva was observed in the PiPa group in this study.

We controlled for potential confounders with immediate placement, immediate provisionalization, and same implant type. However, there are still other variables such as the expertise of the surgeon, location of the implant that could confound the outcome.

Within the limitations of this study, we can conclude that the color of the peri-implant mucosa could be improved towards appearing more like the natural gingiva with a pink abutment. This improvement is especially pronounced in patients with thin mucosa and those with pink implant.
Chapter 5: Conclusions and Future Work

It can be concluded that the color between the peri-implant mucosa of bone level implants and the gingiva of adjacent tooth is significantly different. Specifically, the peri-implant mucosa displays greener and bluer hue compared to natural gingiva. This color difference is not only significant statistically, but also clinically, as the average color difference between the peri-implant mucosa and gingiva of adjacent tooth is greater than the average soft tissue color threshold for a layperson.

With respect to the contributing factors for this color difference, the soft tissue thickness, translucency, and superficial vasculature do not statistically impact this color difference in this particular study.

In trying to improve this gray shine-through effect, pink colored abutment and implant demonstrate efficacy, especially in patients with thin peri-implant mucosa. Overall, as we attempt to deliver dental treatment in the most biologically, functionally, and esthetically sound manner, we should include the soft tissue appearance around an implant as a significant implant success variable.
Figure 1A. Color perception by human eyes and interpretation by human brains. Picture from Precise Color Communication, Konica Minolta;

Figure 1B. Spectral function of the relative sensitivity of average human cones L, M, S. Picture from Color Ontology and Color Science; MIT Press. Chapter 1, 2010.
Figure 2A. Munsell Color System.

Figure 2B. Commission Internationale de L’Eclairage (CIE) developed L*, a*, b* space.

Picture from Precise Color Communication, Konica Minolta
Commission Internationale de L'eclairage (CIE) developed $L^*$, $a^*$, $b^*$ space.

Figure 3 Commission Internationale de L'eclairage (CIE) developed $L^*$, $a^*$, $b^*$ space.
Figure 4 Euclidean Distance. Picture from precise color communication, Konica Minolta.

\[ \Delta E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \]
Figure 5 Extrinsic factors that could affect optical properties around a tooth and around an implant.
Figure 6 Translucency Parameter measurement according to Johnston’s methods with a black strip and a white strip.
Figure 7 Narrow Band Imaging technology.

By Peter Lukes, Michal Zabrodsky, Jan Plzak, Martin Chovanec, Jaroslav Betka, Eva Foltynova and Jan Betka
Figure 8 Proprietary anodization method allows uniform TiO₂ layer that is 200nm thick to achieve pink hue around a dental implant abutment.
Figure 9 A dental spectrophotometer (Crystaley).
Figure 10 Screen capture of Crystaleyte software to demonstrate marginal gingiva and peri-implant mucosa that are measured and quantified.
Figure 11 Translucency parameter measurement intraorally using Johnson’s method.
Figure 12 Average soft tissue thickness 1mm apical to free gingiva margin of gingiva and peri-implant mucosa.
Figure 13 Average translucency parameter of gingiva and peri-implant mucosa.
Figure 14 A narrow band imaging equipped endoscope. (Olympus)
Figure 15 Shibahara’s type I, II, IV classification (Left) that are modified and used to categorize our samples into type I, II, III (right).
Figure 16 Example of a gingival tissue pair with specified area. Students evaluated the color difference between the two highlighted boxes.
Figure 17 Color viewing box Macbeth Judge II that allows ambient light control.
Figure 18 Mean objective values of $\Delta E$ and standard error for each subjective evaluation in ex vivo vs. in vivo. Clinical threshold for soft tissue lies between the range of $\Delta E = 6.40-6.99$. 
Figure 19 A. Normal periodontium around a tooth; B. Peri-implant mucosa with gray optical phenomenon contributed by titanium abutment and implant; C. Peri-implant mucosa with improved optical phenomenon with pink abutment and pink neck implant.
Figure 20 A. Atraumatic extraction; B. Flapless surgery; C. Genesis Implant (Left), Prima Connex (Right); D. Immediate implant placement; E. Immediate provisionalization; F. Three months following healing, each patient received both conventional gray and pink abutment.
Figure 21 A. Measurements of peri-implant mucosa with a gray abutment and its adjacent gingiva are measured; B. Measurements of peri-implant mucosa in the same patient with a pink abutment and its adjacent gingiva are measured.
Figure 22. Scatterplot of all the measurements. A: CIELAB coordinates of GiGa peri implant mucosa with respect to the reference. B: CIELAB coordinates of PiPa implant mucosa with respect to the reference. The lines demonstrate how delta E values are calculated, irrespective of the direct ion of the color difference.
Reference = Control (1mm apical to FGM of the adjacent tooth)

○ = CIELAB coordinates of PiPa peri implant mucosa with respect to the Reference

● = CIELAB coordinates of GiGa peri implant mucosa with respect to the Reference

Figure 23A: Average \( \Delta L^* \), \( \Delta a^* \), and \( \Delta b^* \) values of GiGa peri-implant mucosa with respect to the reference. Figure 23B: Average \( \Delta L^* \), \( \Delta a^* \), and \( \Delta b^* \) values of PiPa peri-implant mucosa with respect to the reference.
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