COMPREHENSIVE OPTICAL ASSESSMENT OF PERI-IMPLANT MUCOSA

A Thesis presented by

Mindy Sungmin Gil, DMD

To

The Faculty of Medicine

In partial fulfillment of the requirements for the degree of

Doctor of Medical Sciences in Oral Biology

Shigemi Nagai, DDS, Ph.D, MSD

Associate Professor

Harvard School of Dental Medicine

Harvard School of Dental Medicine

Boston, Massachusetts

March, 2015

This thesis is dedicated to:

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

COMPREHENSIVE OPTICAL ASSESSMENT OF PERI-IMPLANT MUCOSA

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.

Table of Contents

Section 1:

Section 2:

List of Figures

By Peter Lukes, Michal Zabrodsky, Jan Plzak, Martin Chovanec, Jaroslav Betka, Eva Foltynova and Jan Betka

List of Graphs and Tables

Acknowledgements

First, I would like to thank my advisor Dr. Shigemi Nagai. At both professional and personal level, she has been a guiding light for practically all of my time at Harvard School of Dental Medicine since 2006. As a teacher, starting with clinical restorative dentistry, various research projects such as caries project, peptide project, and finally color studies, she taught me everything I know today. As a researcher, her inquisitive mind never stops a simple solution. She goes over and beyond to apply science to understand and solve a problem, reaches out to various disciplines such as immunologists, chemists, business schools, technology development office to truly foster a sense of mutually beneficial, cooperative environment for success. As a mentor, she has always been a balance between a mom, professor, aunt, and friend all in one. Her never ending enthusiasm and energy demonstrated by endless phone calls, texts, emails, driving to school on snow days, super strong encouragement to participate in oral and poster presentations, patient care till well past 8pm are truly inspirational and admirable. In my life, I have never met someone who works harder than she, and this is one of many lessons I will take with me for the rest of my life. Finally, her personal dimensions that include empathy, generosity, quirky sense of humor, and appreciation for honesty on top of her glowing work ethics truly make her one of a kind mentor.

Second, I would like to thank my oral qualifying committee members (Drs. Toshi Kawai, Lanske, Yi), proposal committee (Drs. John Da Silva, Lanske, Kristiansen), and finally, defense committee (Drs. John Da Silva, Karimbux, Sakai). I would also like to thank PI's

of the labs I rotated through: Dr. Julie Glowacki of BWH and Dr. Toshi Kawai of Forsyth. Each and every member of the committee has taught me valuable ways to learn, think, and make sense of the information, so that I could become more than a simple gatherer of information.

Thirdly, I could not be where I am today without my friends, co-residents and mentors of OMII. Drs. WaiYin Chan, Sharon Jin, Marisa Zarchy Tae Kwon, Chie Hayashi, Jeff Wang, Rita Han, Jamie Chung, Marcelo Freire, Wichaya Wisitrasameewong, Alex Movila amongst many truly enriched my time here at Harvard School of Dental Medicine. My experience would not have been the same. I would like to also thank Dr. Howard Howell for his everlasting words of poignant wisdom and guidance, Dr. Nadeem Karimbux for demonstrating professional conduct through daily practice layered with a sense of humor, Dr. John Da Silva for his calm and practical point of views, Dr. David Kim for his organization, humanitarian efforts, and professional mentorship, Dr. Giseppe Initi for being a crucial bridge between residents and faculty by lending an honest and valuable ear with an open door, Dr. SooWoo Kim for personable and practical wisdom, and finally Dr. Samuel Koo for always relaying detailed knowledge on any question I had. Finally, for everything our part time faculty and previous residents have imparted to me, I cannot thank them enough.

Finally, I would like to thank my family for their endless love and support. I thank my mother and father Myung Jong and Eun Dok Gil, and my brother Ethan Gil who have unquestionably supported me in all the endeavors I ever pursued. I truly want to spotlight

my wonderful husband, Dr. Kevin Oh; I could not have done any of this without his generous and never ending support and love.

Looking back, I am truly grateful and blessed for all the mentors and friends in my life; my 9 years at Harvard School of Dental Medicine would not have been the same without them. I cannot imagine my life before having met each and everyone here. Thank you again.

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 Vilhjálmsson demonstrated, soft tissue outcome can significantly affect how our patients perceive the outcome: Vilhjálmsson 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[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)[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[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[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[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[15,16,17].

Even less is known about the color spectrum of human soft tissue[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[20,21,22].

Color Measurement Techniques

Traditionally and most popularly, visual determination[23] of color is the application of Munsell color system, represented in three dimensions[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 (ΔE)[25] between the two color points corresponds to the perceptual difference between the two recorded colors (Figure 4). ΔE is defined as the color difference between two specimen and given by the equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. ΔL* is defined as $L^*_{target} - L^*_{standard}$. Similarly, $\Delta a^* = a^*_{target} - a^*_{standard}$ and $\Delta b^* = b^*_{target} - b^*_{standard}$. Essentially, ΔE gives a standardized magnitude of color difference between two specimen, taking into account all three axes of the color spectrum. A classic study defined ΔE in oral environment as 3.7ΔE 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 ΔE 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[27]. 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[23,28] to ΔE = 2.69 for all-ceramic crowns[29]. 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[89].

Current assessment of Peri-implant Mucosa

The Pink Esthetic Score (PES)[30] 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[31]. 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)[31]. 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[46,47], underlying metal of the prosthetic material[48,49], previous root canal treatment[50], and inflammation[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[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[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[56]. Other intrinsic factors that may contribute to the optical effect of the peri-implant mucosa are thickness and translucency of peri-implant mucosa.

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 *in vitro* and *in vivo*[56,33]. Using $\Delta E = 3.6$ as a clinical threshold[26], Jung investigated the effect of soft tissue thickness on masking the color of underlying prosthesis *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[33]. However, Bressan[56] showed *in vivo* that even thick tissue (i.e. >2mm)

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[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**.**

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[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.

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[59,60,61]. Other studies, however, demonstrated that some oral wounds were delayed compared to dermal wounds possibly due to inflammatory cytokine IL-1[62], saliva[63,64], or oral commensal bacteria[62].

Aside from osseointegration, the soft tissue collar called "peri-implant mucosa"[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[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[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 [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[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 *in vivo* vascular patterns may provide a better understanding of healthy peri-implant mucosa.

In vivo videocapillaroscopy has been in use in medicine for many years[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[71,72]. Oral application of such videocapillaroscopy included comparing microvasculature of gingiva in healthy patients against chemotherapy patients[70], Systemic lupus erythematosus (SLE) patients[71], and diabetic patients[72]. Most recently, an *in vivo* evaluation of the vascular pattern in oral peri-implant tissue has been studied as well[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[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 *white light emitting diode* is utilized to provide lighting for visualization of internal cavities during endoscopic procedures. *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 5±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[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[74], Barrett's Esophagus and esophageal cancer[75], chronic gastritis, gastric adenoma and gastric cancer, and ulcerative colitis[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[77,78,79] , and squamous cell carcinoma[80,81]. Specifically, Shibahara[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[82,83, 84]. 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[85]. 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[Error! Bookmark not defined.].

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[26] 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[23] to ΔE = 2.69 for all-ceramic crowns[29].

A few studies have investigated the shade of the gingiva using a spectrophotometer[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[89].

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[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[90]. Based on this study, a pink abutment was manufactured. The pink color was achieved through a proprietary anodization process (Figure 8).

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[91]. Lindeboom compared immediate and delayed implant placement, and again no difference in the level of the midfacial mucosa was observed[92]. Cooper further demonstrated that flapless surgery resulted in increased peri-implant mucosal tissue dimension[93]. According to the latest systemic review, the evidence suggests that acceptable esthetic outcomes can be achieved with immediately placed implants[94,95]. Some guidelines for successful outcomes of immediate implants include 1) placing the implant platform in the correct buccopalatal dimension[96,97,98,99,100]; 2) maintenance of the buccal bone[101,102, 103]; 3) preexisting gingival biotype[37,104,105,106]; 4) use of flapless or minimally invasive surgical implant placement[97,107, 108]; and 5) use of implant abutment or immediate abutment or provisional restoration[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[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[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[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)[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[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[119]. 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 ΔE(Test) and ΔE(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

	Peri-implant mucosa	Gingiva	Significance
L*	51.29 (0.80)	54.07 (0.82)	0.0003*
a*	13.06 (0.50)	14.29 (0.53)	0.058
b*	11.97 (0.45)	14.95 (0.51)	0.0001*

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[120]. 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 2 Mean ΔE between peri-implant mucosa and gingiva of the adjacent tooth compared to the mean ΔE between two sites of natural gingiva

ΔE (Test): between peri-implant mucosa and adjacent gingiva	**ΔE** (Control): between two gingiva at the contralateral site	Significance
7.65 (0.62)	4.98 (0.35)	0.0003*

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 (r^2= 0.03) nor thickness and translucency of the adjacent gingiva (r^2 = 0.08).

1C. Discussions and Conclusions

Normal color variance exists at the population level based on age and ethnicity[39-43]. Furthermore, even within a person, normal color variance exists[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[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 *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)[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[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[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 capillary loops were visible. They often appeared as dots or commas, with perpendicular position of the loops with respect to the surface. The interpapillary 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

	Gingiva	**Peri-implant mucosa**	***P***
Category 1	45.87 (5.63)	55.03 (5.70)	
Category 2	6.08 (1.59)	7.03 (2.15)	
Category 3	1.53 (0.58)	2.32 (0.97)	
Sum of all IPCL	53.47 (5.21)	64.37 (5.70)	0.02*

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

	IPCL and L*	IPCL and a*	IPCL and b*

Spearman's rank Correlation coefficient	0.23	-0.08	-0.001

2C. Discussions and Conclusion

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

In terms of vascular orientation, Berglundh[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[124]. More current studies have demonstrated through histologic studies that connective tissues can attach to some implant surfaces[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[53]. Likewise, in a zone close to the implant surface (i.e. 50-100um 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[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[55]. 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[127]. As demonstrated by Matsuo[128], 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[122] 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 ΔE 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 assessmet 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 ΔE =3.54. For the category scored as "moderate difference", the mean objective value was ΔE =6.99. For the category scored as "obvious difference", the mean objective value was ΔE =16.03. A full descriptive table is shown in Table 5.

Table 5 Mean objective values of ΔE, ΔL*, Δa*, Δb* for each subjective category in *ex vivo* setting

	No difference n=21	Moderate difference n=24	Obvious difference n=5
ΔE	3.54 (0.90)	6.99 (0.76)	16.03 (3.55)
ΔL*	2.72 (0.42)	3.99 (0.58)	11.89 (1.34)

Δa*	1.41 (0.22)	3.98 (0.61)	7.29 (4.21)
Δb*	1.02 (0.22)	3.06 (0.41)	7.19 (1.09)

Mean (SE)

In clinical (*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 ΔE =5.90. For the category scored as "moderate difference," the mean objective value was ΔE =6.40. For the category scored as "obvious difference," the mean objective value was ΔE =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 ΔE, ΔL*, Δa*, Δb* for each subjective category in *in vivo* setting

	No difference n=7	Moderate difference n=22	Obvious difference n=10
ΔE	5.90 (1.01)	6.40 (0.70)	10.76 (1.48)
ΔL*	3.36 (1.17)	3.90 (0.70)	5.73 (1.60)
Δa*	3.04 (0.75)	3.25 (0.47)	4.43 (1.04)
Δb*	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 ΔE, ΔL*, Δa*, and Δb*. Of all the coefficient values, the strongest correlation was noted on Δb* axis for both *ex vivo* and *in vivo*.

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

	Pearson's correlation coefficient Ex vivo	Pearson's correlation coefficient *In vivo*
ΔE	0.67	0.46
ΔL*	0.43	0.22
Δa*	0.52	0.20
Δb*	0.76	0.54

3C. Discussions and Conclusion

The literature has demonstrated significant spectrophotometric differences between the peri-implant tissue and periodontal tissue with ΔE 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 ΔE =8.5

corresponded to “good matching, but clinically distinguishable.”[89] 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 ΔE = 2.69 for all-ceramic crowns[29]. 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 ΔE= 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 (ΔE=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 ΔE values that ranged from 1.7 to 19.0. The *ex vivo* experiment pairs had ΔE 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 ΔE = 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)[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 *ex vivo*. The correlation found *in vivo* was moderate at r=0.46. The same trend of stronger correlation in *ex vivo* setting was observed in ΔL*, Δa*, and Δb*. It is notable that the correlation between Δb* and subjective perception was the strongest in both *ex vivo* and *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 ΔE with varying degrees of ΔL*, Δa*, and Δb* is needed to confirm these findings.

The limitations of this study are that the *in vivo* and *ex vivo* have different sets of samples. It may be interesting to repeat the *ex vivo* experiment with the same sample obtained from the *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 (2^{nd} premolar ~ 2^{nd} 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 (Crystaleye, 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[32](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 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

	Gray abutment n= 15	Pink abutment n=15	Significance
L*	50.42 (0.76)	50.13 (0.68)	p=0.53
a*	13.44 (0.55)	14.95 (0.41)	p=0.02*
b*	11.12 (0.42)	11.73 (0.45)	p=0.01*

Mean(SE)

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

	Thin (<2mm) n=8	Thick (≥2mm) n=7	Significance
ΔE induced by pink abutment	4.96 (0.70)	3.32 (0.89)	p=0.043*

Mean(SE)

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

Table 10 ΔE induced by pink abutment in patients with pink implant compared to those with gray implant

	Pink Implant n=7	Gray Implant n=8	Significance
ΔE induced by pink abutment	5.84 (0.68)	2.33 (0.46)	p=0.043*

Mean(SE)

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

	Pink Implant, Gray Abutment n= 7	Pink Implant, Pink Abutment n= 7	Significance
L*	51.86 (1.16)	50.54 (0.56)	p=0.04*
a*	12.16 (0.94)	15.06 (0.56)	p=0.09
b*	11.29 (0.80)	12.77 (0.80)	p=0.0002*

Mean(SE)

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

	Gray Implant, Gray Abutment n= 8	Gray Implant, Pink Abutment n= 8	Significance
L*	49.20 (0.95)	49.76 (0.97)	p=0.15
a*	14.52 (0.53)	14.85(0.59)	p=0.66
b*	10.99 (0.38)	10.83(0.40)	p=0.50

Mean(SE)

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

	GiGa and Control n= 8	PiPa and Control n=7	Significance
ΔE	7.29 (1.88)	7.42 (1.13)	p=0.46
ΔL*	-3.61	-3.59	
Δa*	-3.23	0.67	
Δb*	-3.56	-0.79	

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[56] demonstrated that the thickness of the tissue does not contribute to the peri-implant mucosa color, Jung's[33] 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

our study.

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[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.

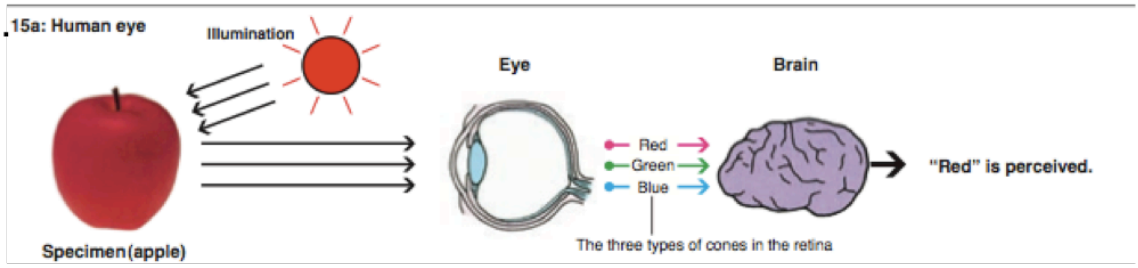
Chapter 5: Conclusions and Future Work

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.

1A



1B

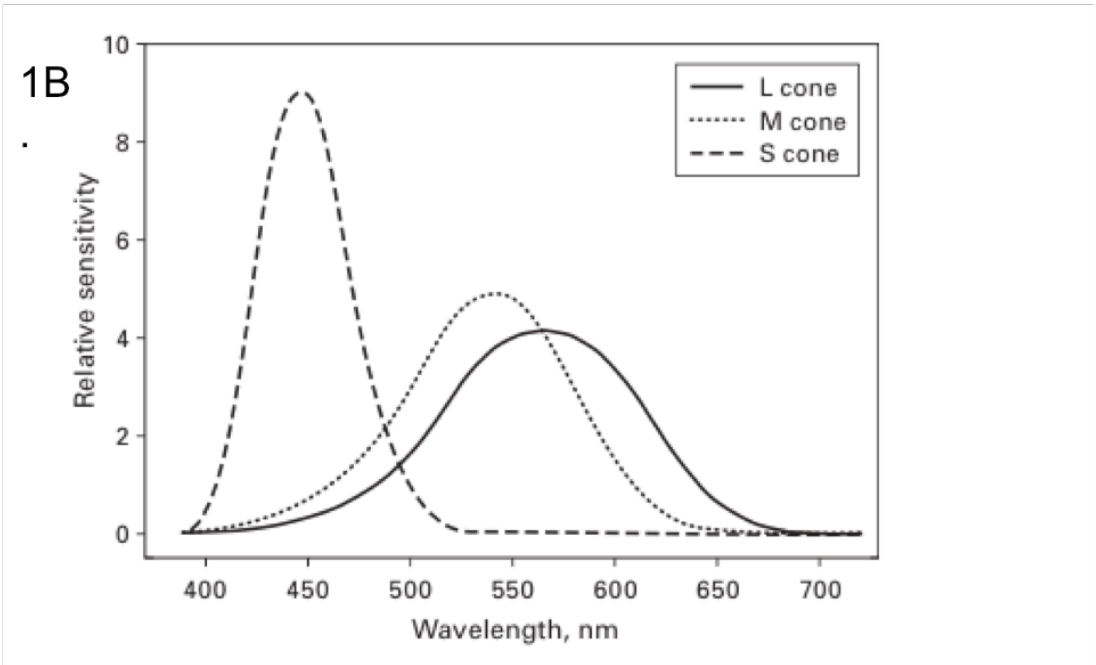


Figure 1A. Color perception by human eyes and interpretation by human brains. Picture from Precise Color Communication, Konica Minolta;

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

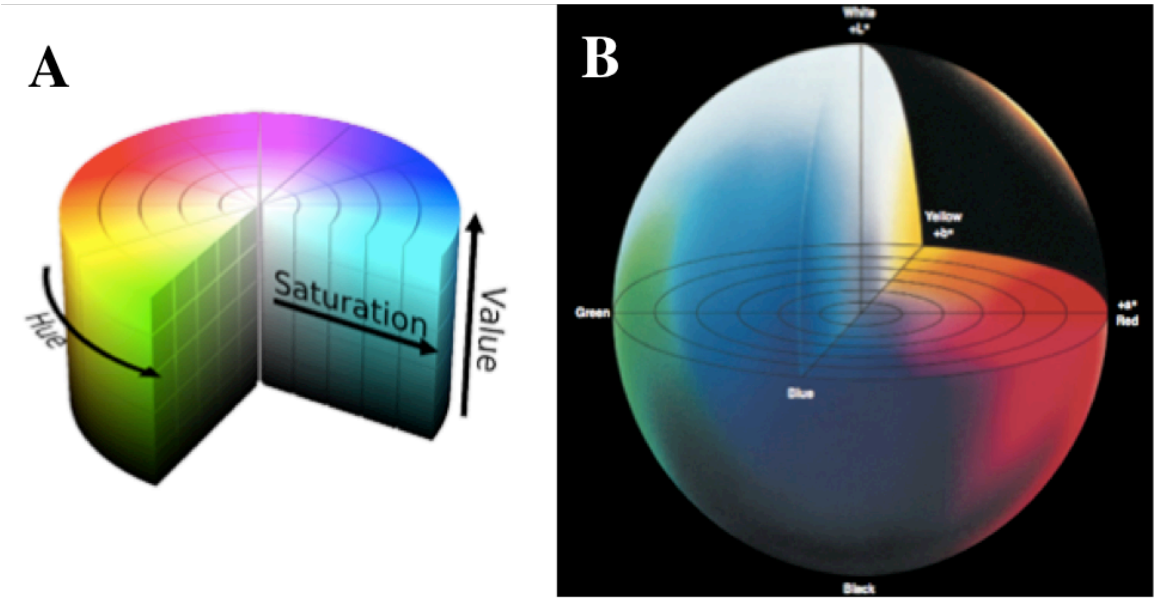


Figure 2A. Munsell Color System.

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

Picture from Precise Color Communication, Konica Minolta

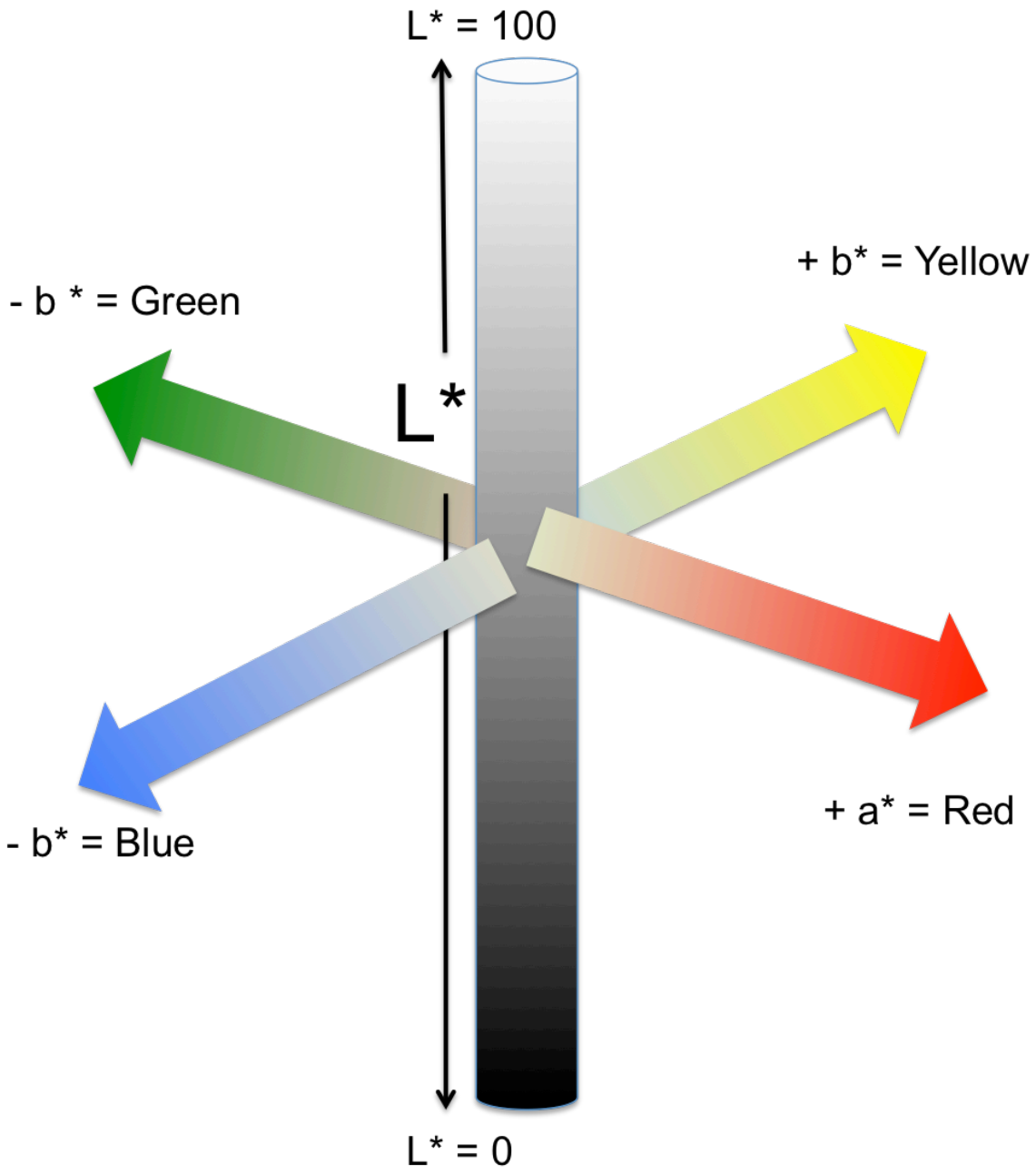
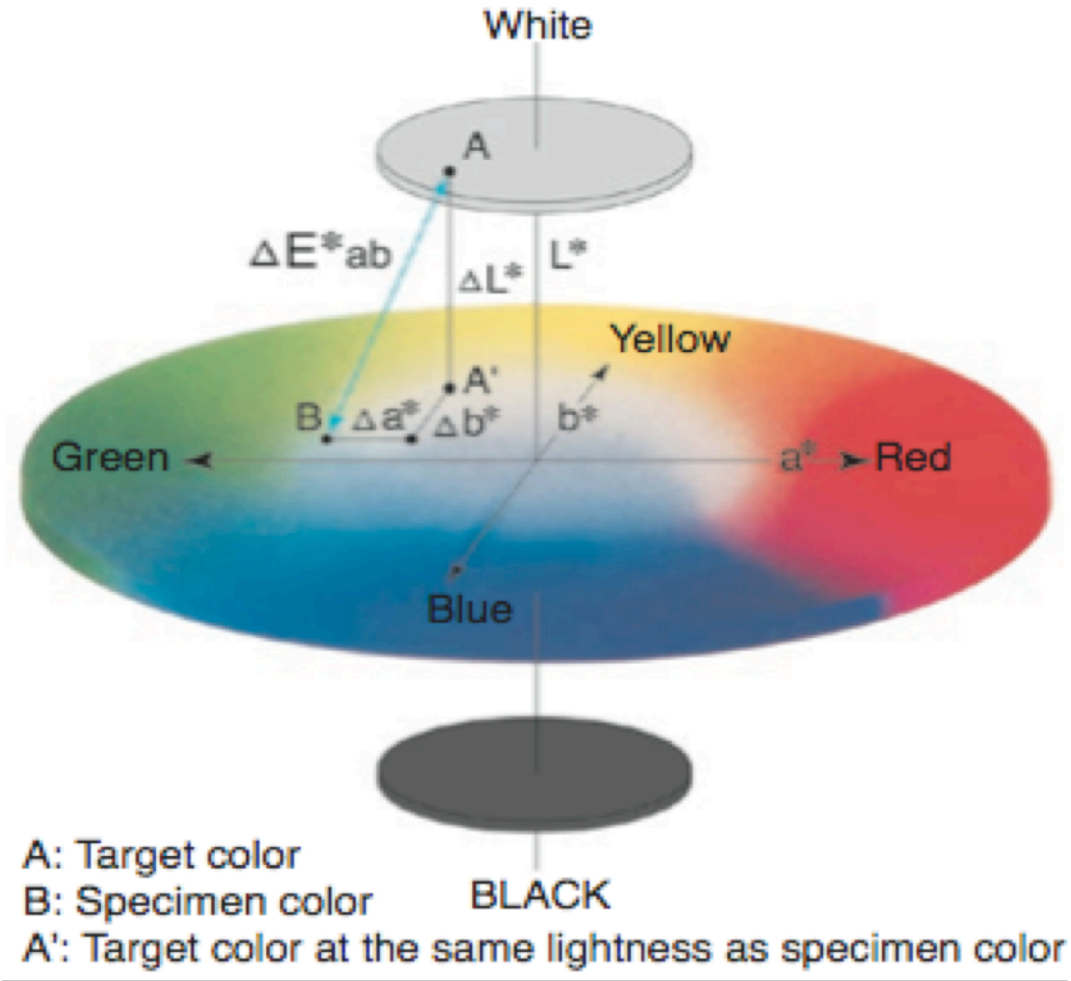
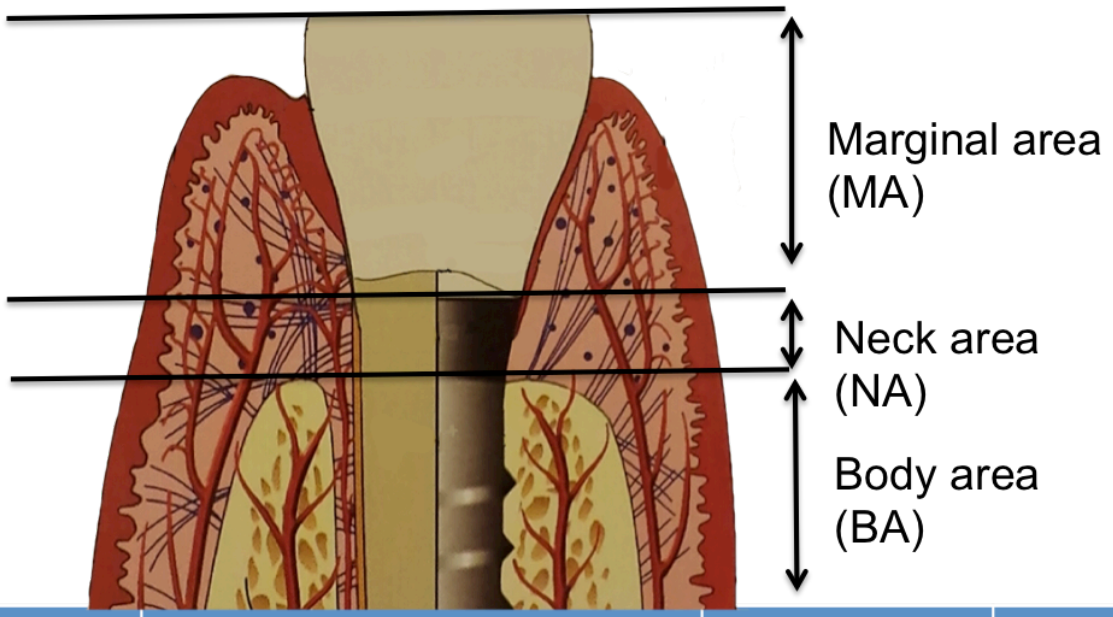


Figure 3 Commission Internationale de L'eclairage (CIE) developed L*, a*, b* space.



$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Figure 4 Euclidean Distance. Picture from precise color communication, Konica Minolta.



	Extrinsic factors that could affect optical properties	Affected area			Gray shine through effect
		MA	NA	BA	
Around a Tooth	Metal of Restoration (PFM)	✓	✓		Vivid
	Restorative Materials (MTA, Amalgam Tattoo)	✓	✓	✓	Vivid
	Contour of restoration	✓	✓		Vague
	Pharmacologic Factors (i.e. Staining)	✓			Vague
	Inflammation (Secondary Origin)	✓	✓	✓	Vivid
	Root Canal Therapy	✓	✓	✓	Vague
Around an Implant	Implant body		✓	✓	Vague
	Implant abutment	✓	✓		Vivid ~ Vague
	Metal of Restoration (PFM)	✓			Vivid

Figure 5 Extrinsic factors that could affect optical properties around a tooth and around an implant.

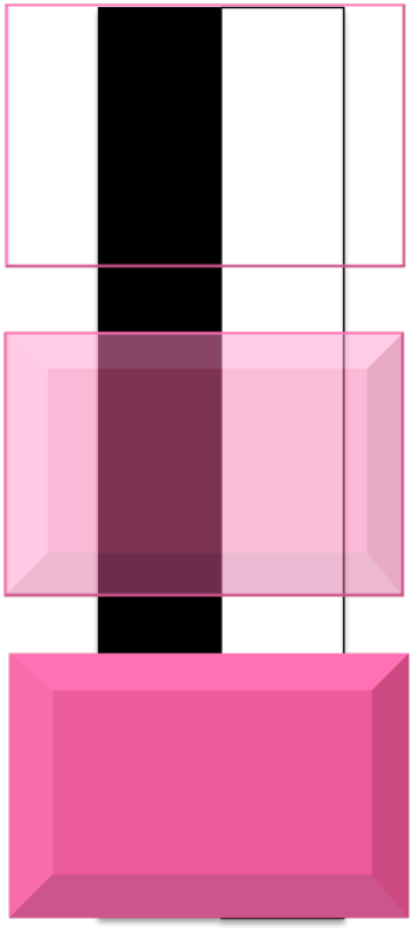


Figure 6 Translucency Parameter measurement according to Johnston's methods with a black strip and a white strip.

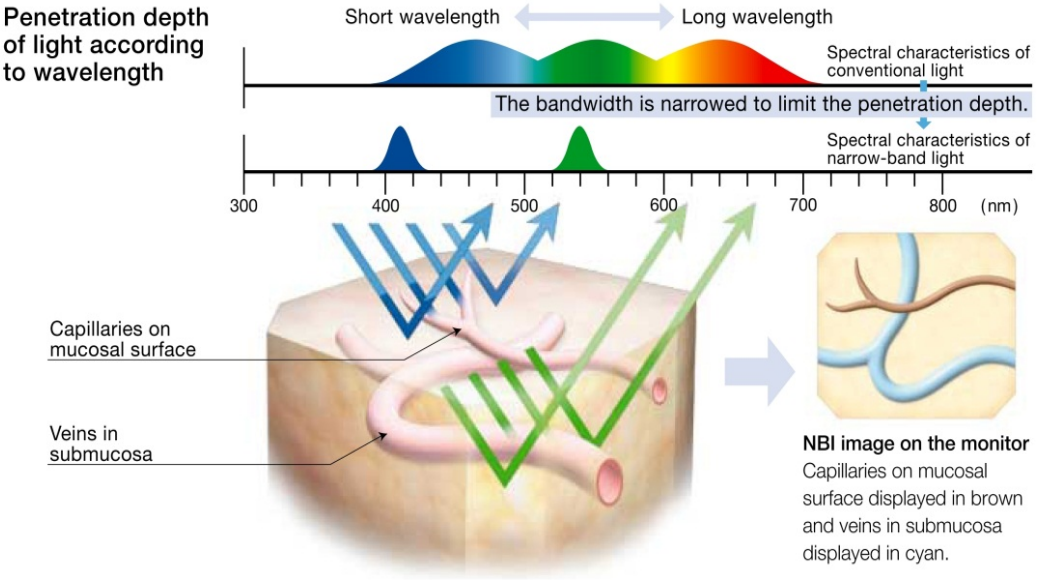


Figure 7 Narrow Band Imaging technology.

By Peter Lukes, Michal Zabrodsky, Jan Plzak, Martin Chovanec, Jaroslav Betka, Eva Foltynova and Jan Betka

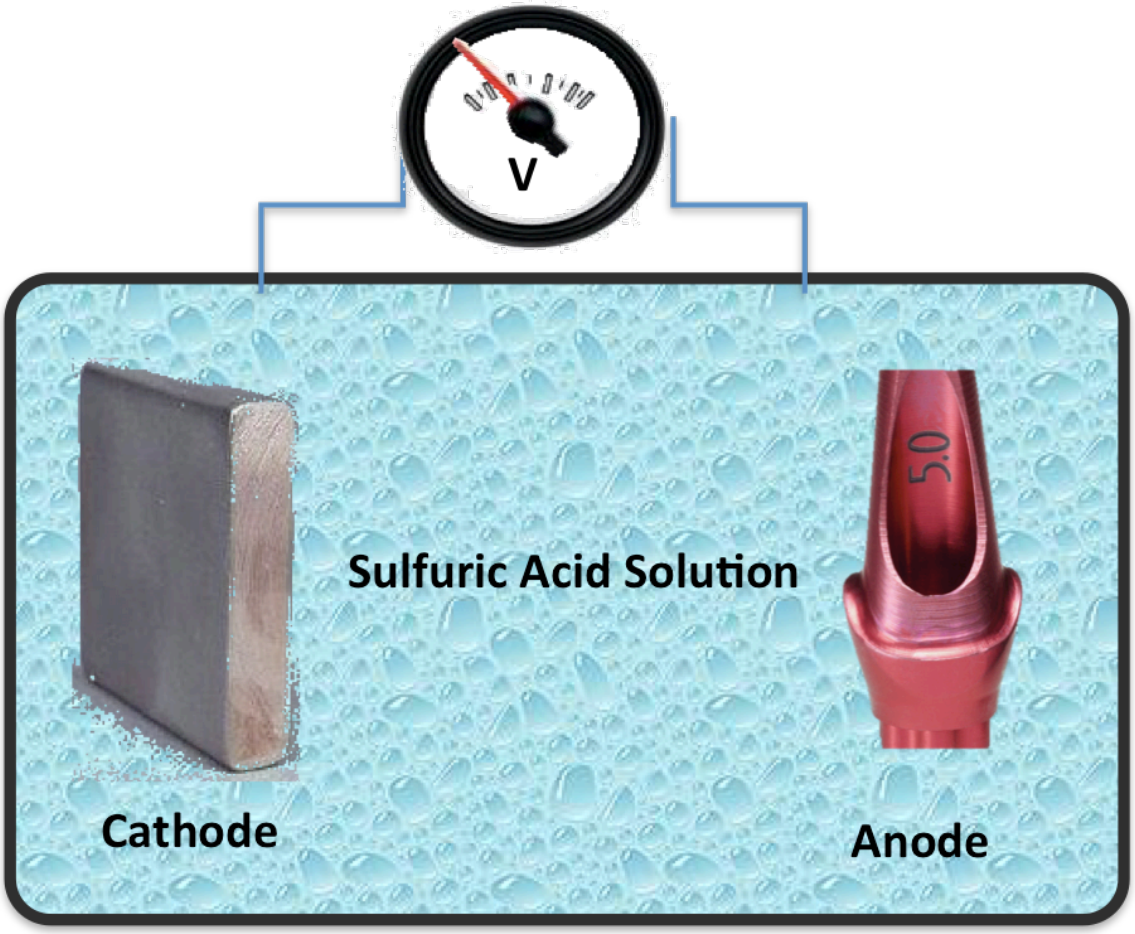


Figure 8 Proprietary anodization method allows uniform TiO_2 layer that is 200nm thick to achieve pink hue around a dental implant abutment.

Figure 9 A dental spectrophotometer (Crystaleye).

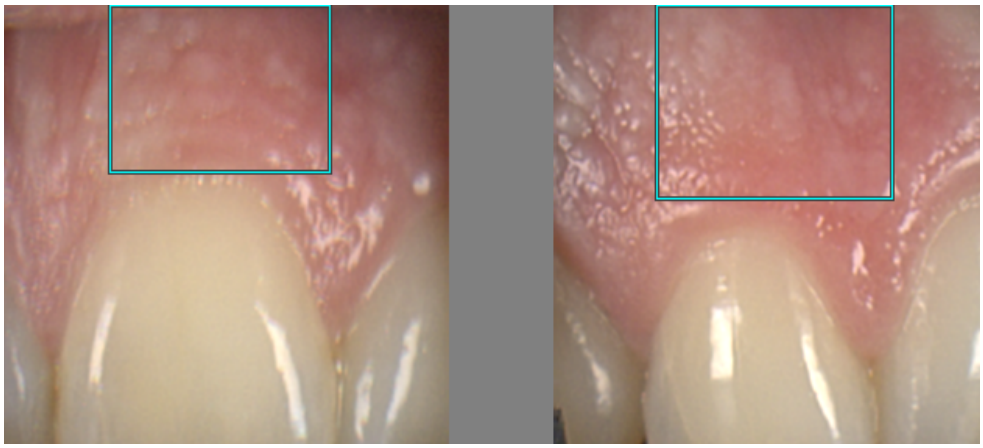


Figure 10 Screen capture of Crystaleye software to demonstrate marginal gingiva and peri-implant mucosa that are measured and quantified.

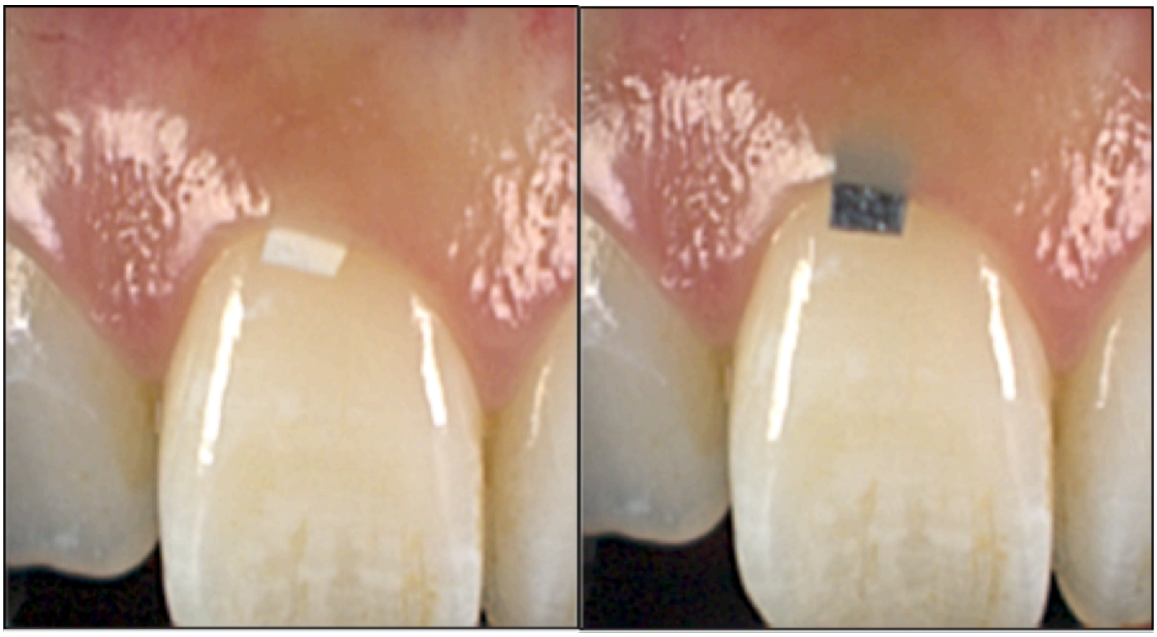


Figure 11 Translucency parameter measurement intraorally using Johnson's method.

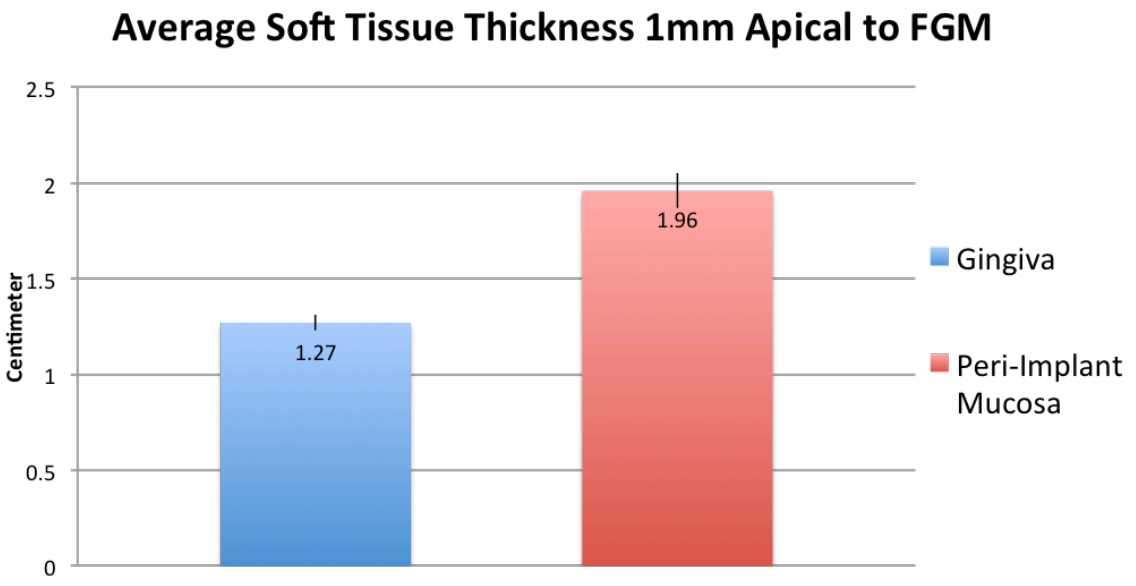


Figure 12 Average soft tissue thickness 1mm apical to free gingiva margin of gingiva and peri-implant mucosa.

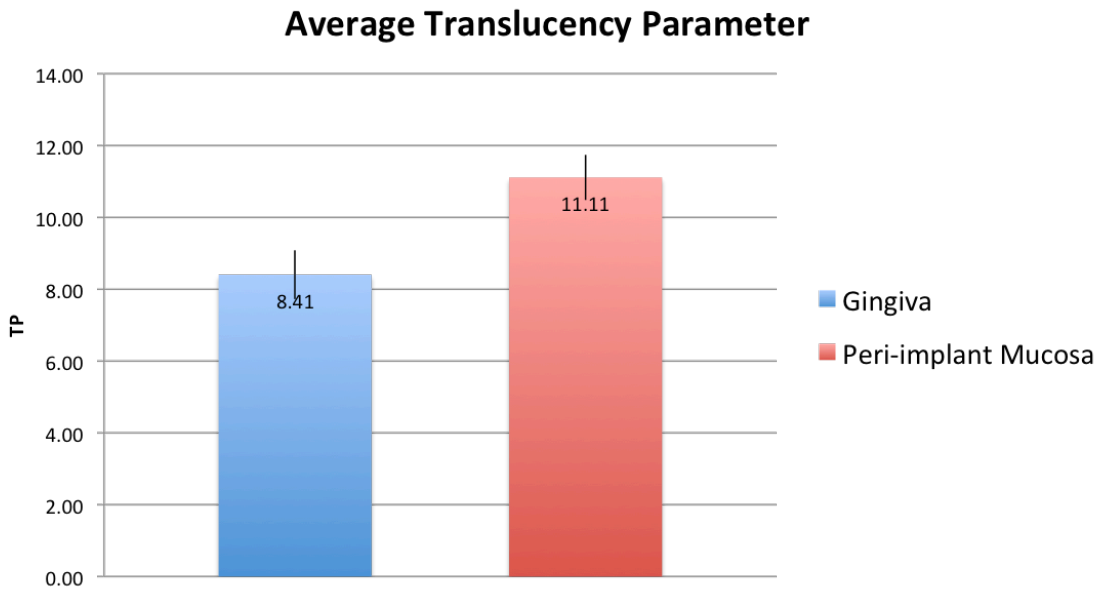


Figure 13 Average translucency parameter of gingiva and peri-implant mucosa.

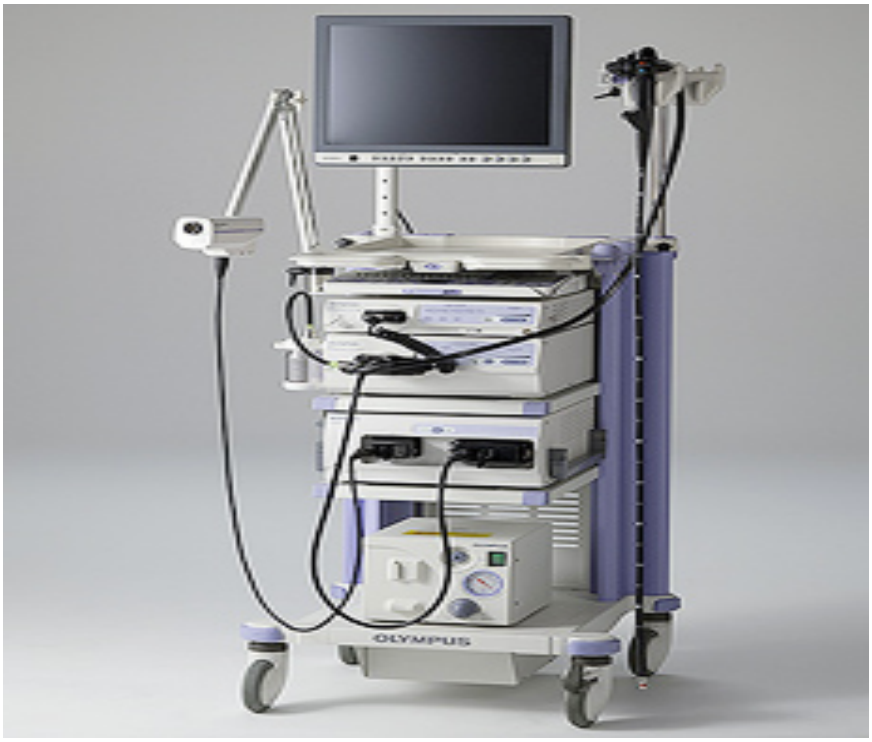


Figure 14 A narrow band imaging equipped endoscope. (Olympus)

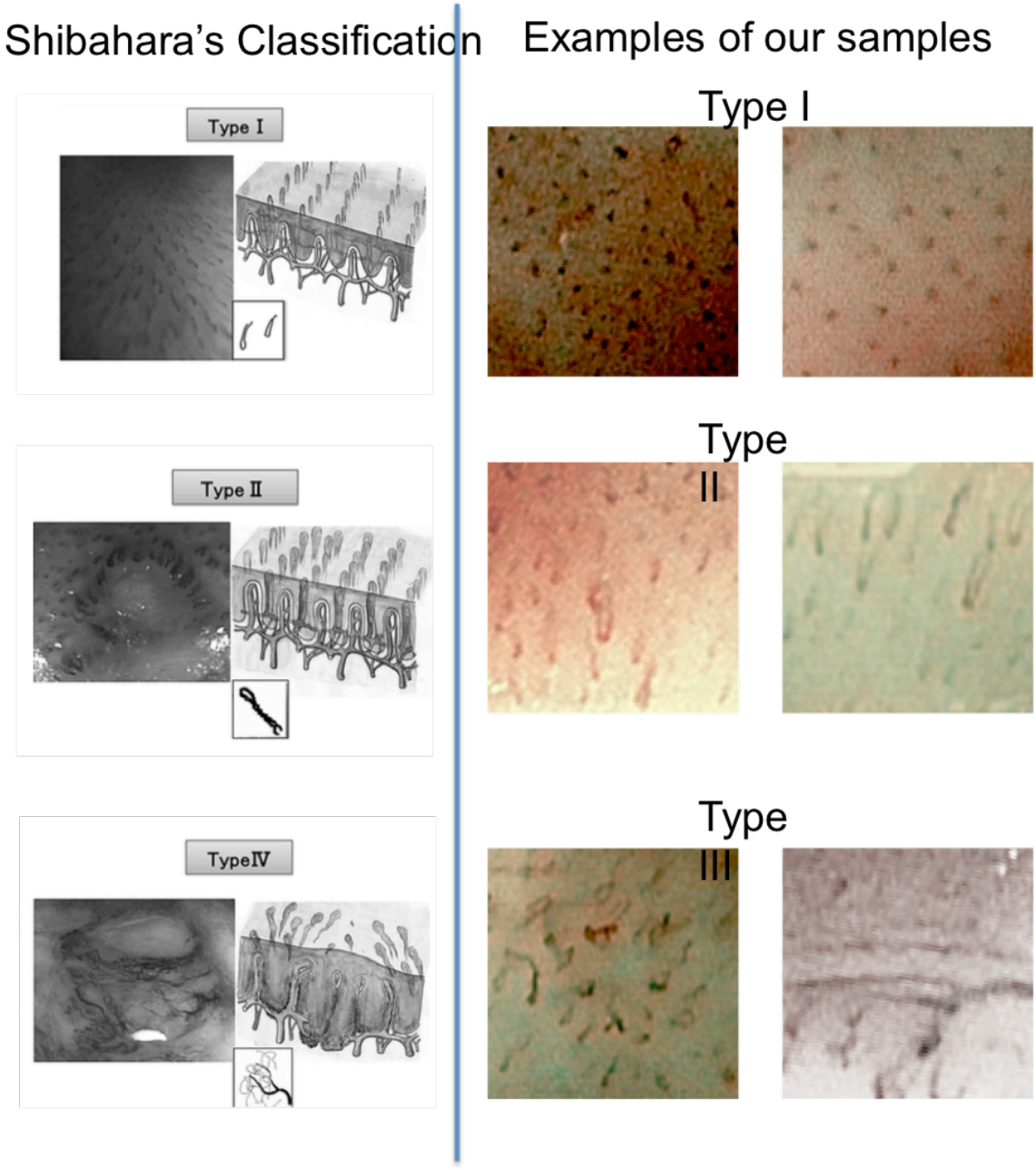


Figure 15 Shibahara's type I, II, IV classification (Left) that are modified and used to categorize our samples into type I, II, III (right).

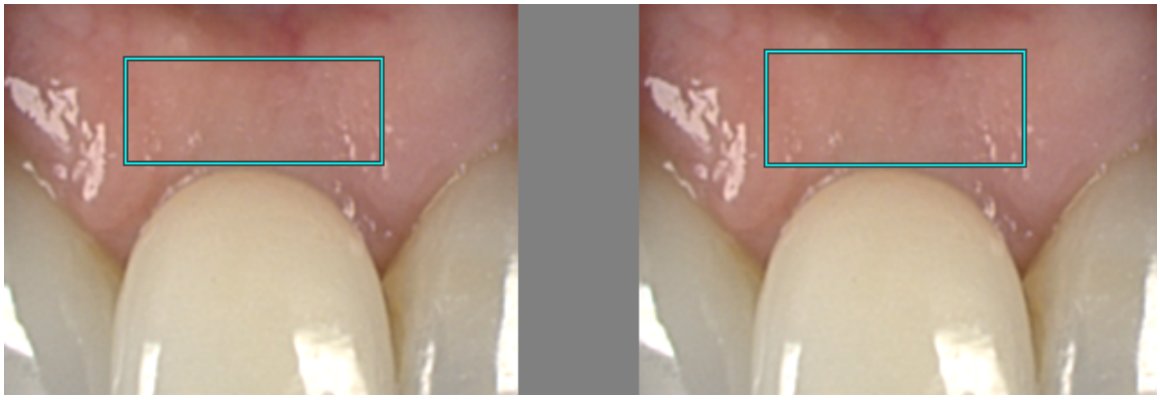


Figure 16 Example of a gingival tissue pair with specified area. Students evaluated the color difference between the two highlighted boxes.

Figure 17 Color viewing box Macbeth Judge II that allows ambient light control.

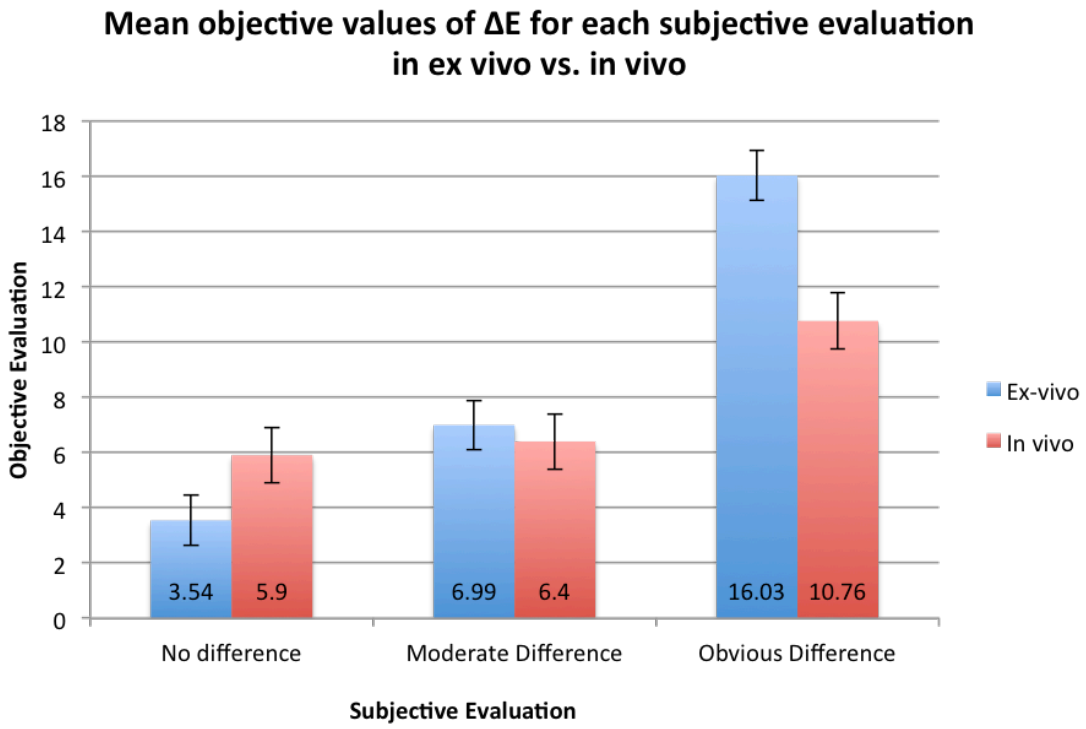


Figure 18 Mean objective values of ΔE and standard error for each subjective evaluation in ex vivo and in vivo. Clinical threshold for soft tissue lies between the range of ΔE = 6.40-6.99.

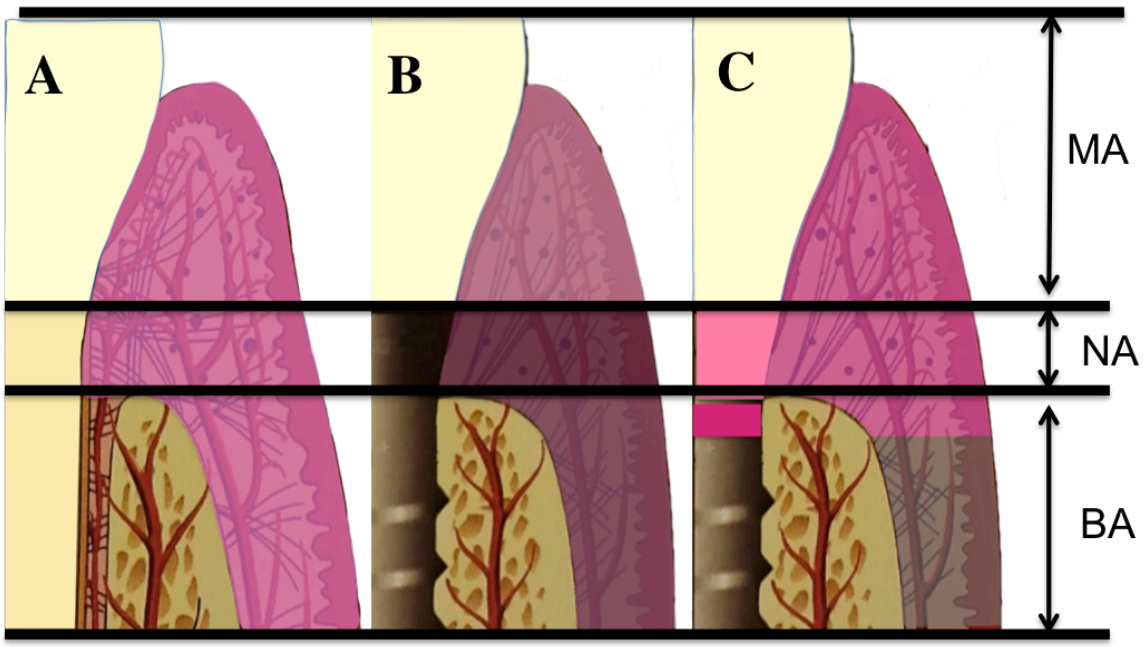


Figure 19 A. Normal periodontium around a tooth; B. Peri-implant mucosa with gray optical phenomenon contributed by titanium abutment and implant; C. Peri-implant mucosa with improved optical phenomenon with pink abutment and pink neck implant.

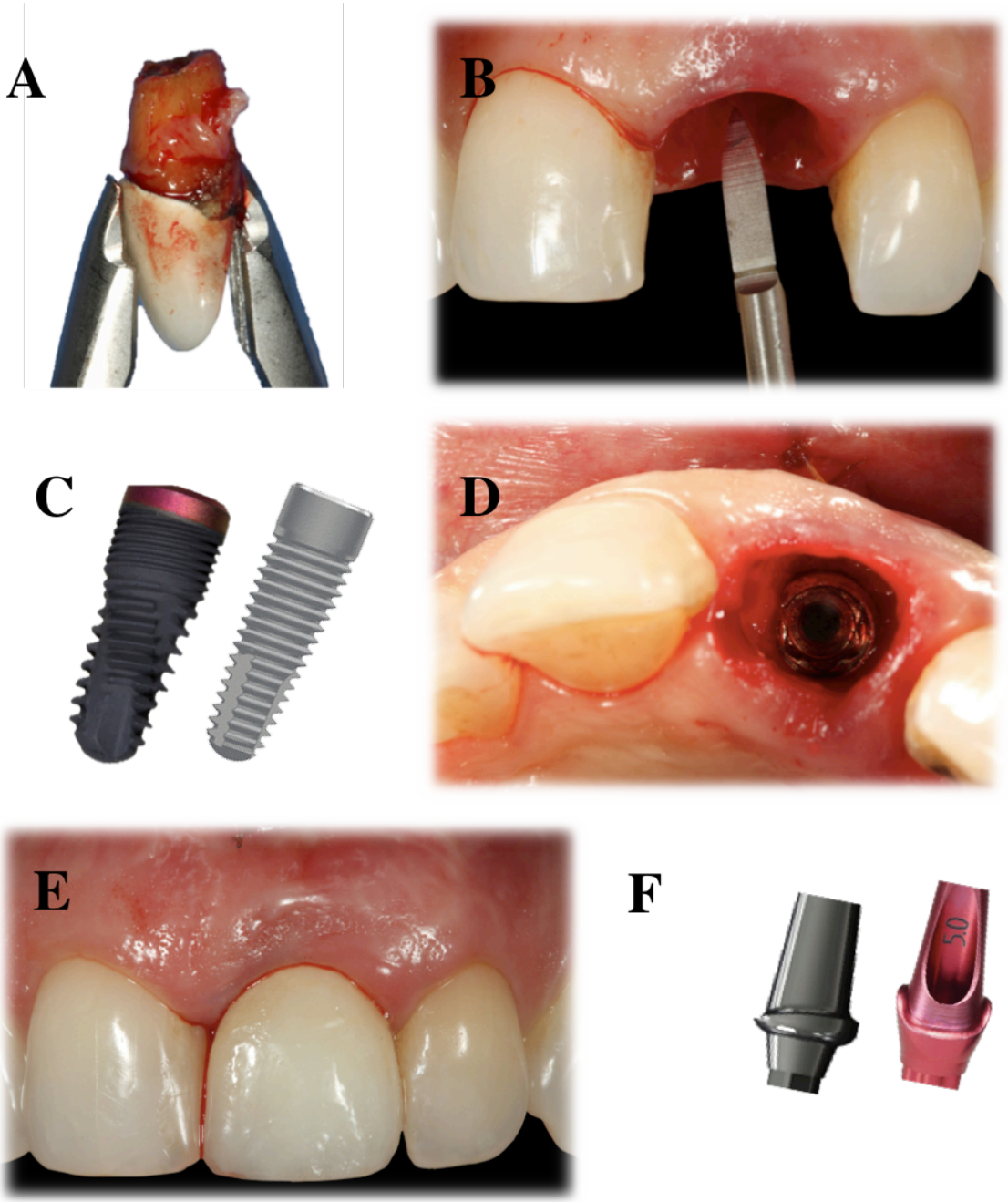


Figure 20 A. Atraumatic extraction; B. Flapless surgery; C. Genesis Implant(Left), Prima Connex (Right); D. Immediate implant placement; E. Immediate provisionalization; F. Three months following healing, each patient received both conventional gray and pink abutment.

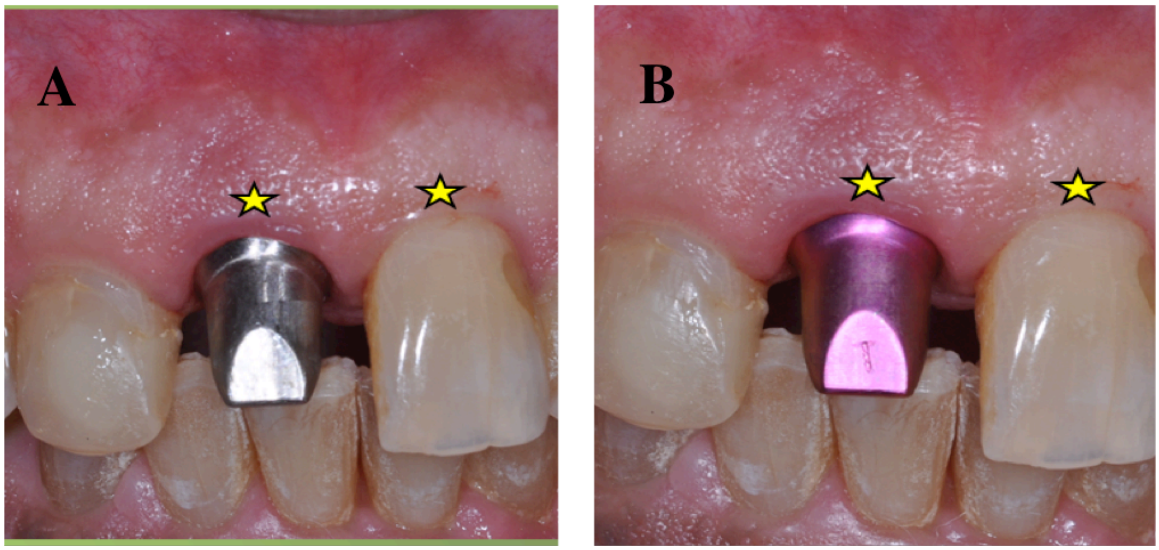


Figure 21 A. Measurements of peri-implant mucosa with a gray abutment and its adjacent gingiva are measured; B. Measurements of peri-implant mucosa in the same patient with a pink abutment and its adjacent gingiva are measured.

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

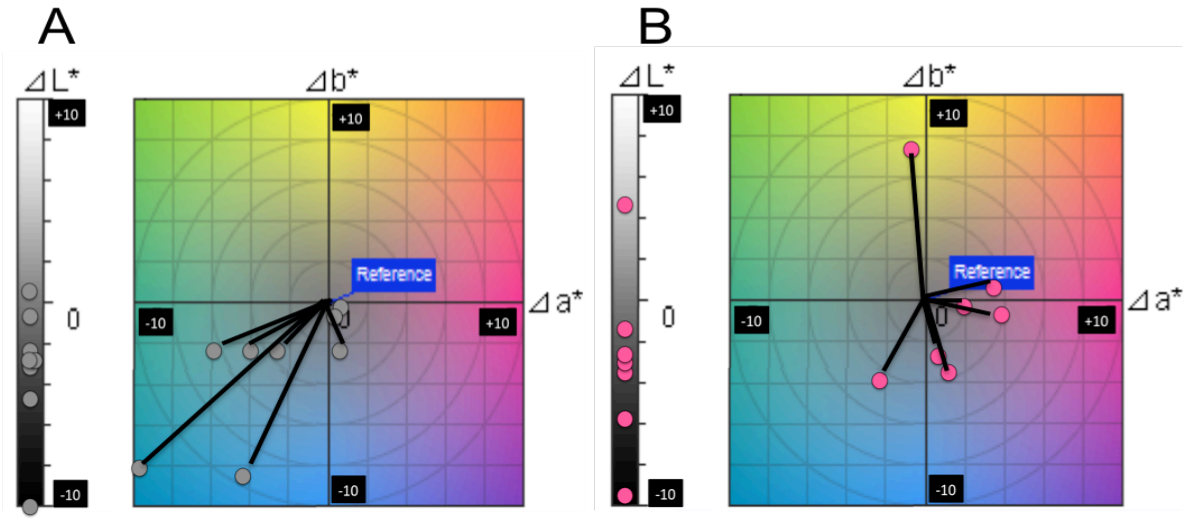


Figure 22. Scatterplot of all the measurements. A: CIELAB coordinates of GiGa peri implant mucosa with respect to the reference. B: CIELAB coordinates of PiPa implant mucosa with respect to the reference. The lines demonstrate how delta E values are calculated, irrespective of the direct ion of the color 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

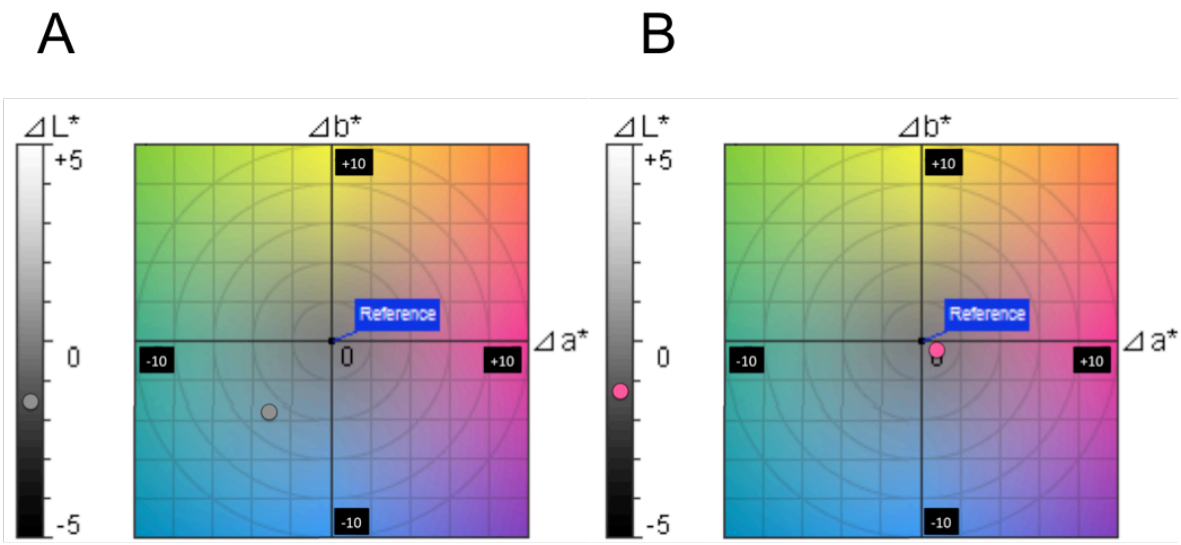


Figure 23A: Average dL*, da*, and db* values of GiGa peri-implant mucosa with respect to the reference. Figure 23B: Average dL*, da*, and db* values of PiPa peri-implant mucosa with respect to the reference.

References

[1] Lambert FE, Weber HP, Susarla SM, Belser UC, Gallucci GO. Descriptive analysis of implant and prosthodontic survival rates with fixed implant-supported rehabilitations in the edentulous maxilla. J Periodontol 2009;80:1220-1230.

[2] Prosper L, Redaelli S, Pasi M, Zarone F, Radaelli G, Gherlone EF. A randomized prospective multicenter trial evaluating the platform- switching technique for the prevention of postrestorative crestal bone loss. Int J Oral Maxillofac Implants 2009;24:299-308.

[3] Albrektsson, T., Zarb, G., Worthington, P. & Eriksson, A.R. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. The International Journal of Oral & Maxillofacial Implants 1986;1: 11–25.

[4] Smith, D.E. & Zarb, G.A. Criteria for success of osseointegrated endosseous implants. Journal of Pros- thetic Dentistry 1989;62: 567–72.

[5] Blanes, R.J., Bernard, J.P., Blanes, Z.M. & Belser, U.C. A 10-year prospective study of ITI dental implants placed in the posterior region. I: Clinical and radiographic results. Clinical Oral Implants Research 2007;18: 699–706.

[6] Jung, R.E., Pjetursson, B.E., Glauser, R., Zembic, A., Zwahlen, M. & Lang, N.P. A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clinical Oral Implants Research 2008;19: 119–30.

[7]

Da Silva JD, Kazimiroff J, Papas A, Curro FA, Thompson VP, Vena DA, Wu H, Collie D, Craig RG; Practitioners Engaged in Applied Research andLearning (PEARL) Network Group. Outcomes of implants and restorations placed in general dental practices: a

retrospective study by thePractitioners Engaged in Applied Research and Learning (PEARL) Network. J Am Dent Assoc. 2014 Jul;145(7):704-13. doi: 10.14219/jada.2014.27.

[8] Papaspyridakos P[1], Chen CJ, Singh M, Weber HP, Gallucci GO Success criteria in implant dentistry: a systematic review. J Dent Res. 2012 Mar;91(3):242-8.

[9] Vilhjálmsson VH, Klock KS, Størksen K, Bårdsen A. Aesthetics of implant-supported single anterior maxillary crowns evaluated by objective indicesand participants' perceptions. Clin Oral Implants Res. 2011 Dec;22(12):1399-403. doi: 10.1111/j.1600-0501.2010.02128.x. Epub 2011 Mar 28.

[10] Chu S, Devigus A, Paravina R, Mieleszko A. Fundamentals of Color: Shade Matching and Communication in Esthetic Dentistry. 2 ed. Chicago: Quintessence; 2010.

[11] Kuehni Rolf G. Color Spaces and Color Order Systems. A primer. Chapter 1. Figure 4.

[12] Jaju RA, Nagai S, Karimbux N, Da Silva JD. Evaluating tooth color matching ability of dental students. J Dent Educ. 2010;74(9):1002-1010.

[13] Rosenstiel SF, Land MF, Fujimoto J, Contemporary Fixed Prosthodontics: Color science, esthetics and shade selection, 3rd edition. Mosby, St Louis, Chicago, p.592-608; 1995.

[14] Joiner A. Tooth color: A review of the literature. J Dent 2004;32:3-12.

[15] Kim-Pusateri S, Brewer JD, Davis EL, Wee AG. Reliability and accuracy of four dental shade-matching devices. J Prosthet Dent. 2009;101(3):193-199.

[16] Preston JD. Current status of shade selection and color matching. Quintessence Int. 1985;16(1):47-58

[17] Miller L. Organizing color in dentistry. J Am Dent Assoc. 1987;Spec No:26E-40E.

[18] Huang JW, Chen WC, Huang TK, et al. Using a spectrophotometric study of human gingival colour distribution to develop a shade guide. J Dent. 2011;39 Suppl 3:e11-16.

[19] Heydecke G, Schnitzer S, Türp JC. The color of human gingiva and mucosa: visual measurement and description of distribution. Clin Oral Investig. 2005;9(4):257-265.

[20] Garber DA, Salama MA. The aesthetic smile: diagnosis and treatment. Periodontol 2000. 1996;11:18-28.

[21] Bayindir F, Bayindir YZ, Gozalo-Diaz DJ, Wee AG. Coverage error of gingival shade guide systems in measuring color of attached anterior gingiva. J Prosthet Dent. 2009;101(1):46-53.

[22] Schnitzer S, Türp JC, Heydecke G. Color distribution and visual color assessment of human gingiva and mucosa: a systematic review of the literature. Int J Prosthodont. 2004;17(3):327-332.

[23] Ahmad I, Protocols for predictable aesthetic dental restorations: Color and shade analysis, Blackwell Munksgaard, Oxford, UK, pp. 77-97 (2006)

[24] Bona AD, Barrett AA, Rosa V, Pinzetta C. Visual and instrumental agreement in dental shade selection; Three distinct observer populations and shade matching protocols. Dent Mater. 2009;25:276–81.

[25] Wyszecki G, Stiles WS. Color Science: Concepts and Methods, Quantitative Data and Formulae. New York, NY: John Wiley and Sons; 1982.

[26] Johnston WM, Kao EC. Assessment of appearance match by visual observation and clinical colorimetry. J Dent Res. 1989;68(5):819-822.

[27] Kuehni RG. Color-tolerance data and the tentative CIE 1976 L a b formula. J Opt Soc Am. 1976;66(5):497-500.

[28] Alghazali N1, Burnside G, Moallem M, Smith P, Preston A, Jarad FD. Assessment of perceptibility and acceptability of color difference of denture teeth. J Dent. 2012 Jul;40 Suppl 1:e10-7. doi: 10.1016/j.jdent.2012.04.023. Epub 2012 May 2.

[29] DaSilva JD, Park SE, Weber HP, Ishikawa-Nagai S. Clinical performance of a newly developed spectrophotometric system on tooth color reproduction. Journal of Prosthetic Dentistry 2008:361–1361.

[30] Furhauser R, Florescu D, Benesch T, Haas R, Mailath G, Watzek G. Evaluation of soft tissue around single-tooth implant crowns: the pink esthetic score. Clin Oral Implants Res 2005;16: 639-644.

[31] Vilhjalmsson VH, Klock KS, Storksen K, Bardsen A. Aesthetics of implant-supported single anterior maxillary crowns evaluated by objec- tive indices and participants' perceptions. Clin Oral Implants Res. 2011 Dec;22(12):1399-403

[32] Park SE1, Da Silva JD, Weber HP, Ishikawa-Nagai S. Optical phenomenon of peri-implant soft tissue. Part I. Spectrophotometric assessment of natural tooth gingiva and peri-implant mucosa. Clin Oral Implants Res. 2007 Oct;18(5):569-74. Epub 2007 Jul 26.

[33] Jung RE, Sailer I, Hämmerle CH, Attin T, Schmidlin P. In vitro color changes of soft tissues caused by restorative materials. Int J Periodontics Restorative Dent. 2007 Jun;27(3):251-7.

[34] Happe A, Stimmelmayr M, Schlee M, Rothamel D. Surgical management of peri-implant soft tissue color mismatch caused by shine-through effects of restorative materials: one-year follow-up. Int J Periodontics Restorative Dent. 2013 Jan-Feb;33(1):81-8.

[35] Small PN, Tarnow DP. Gingival recession around implants: A 1-year longitudinal prospective study. Int J Oral Maxillofac Implants 2000;15:527–532.

[36] Grunder U. Stability of the mucosal topography around single-tooth implants and adjacent teeth: 1-year results. Int J Periodontics Restorative Dent. 2000 Feb;20(1):11-7.

[37] Kan JY, Rungcharassaeng K, Umezu K, Kois JC. Dimensions of peri-implant mucosa: an evaluation of maxillary anterior single implants in humans. J Periodontol. 2003 Apr;74(4):557-62.

[38] Gargiulo AW, Wentz FM, Orban B. Dimensions and relations of the dentogingival junction in humans. J Periodontol. 1961;32:261–7.

[39] Powers JM, Capp JA, Koran A. Color of gingival tissues of blacks and whites. J Dent Res. 1977;56(2):112-116.

[40] Lindhe J, Karring TL, Niklaus P. Clinical Periodontology and Implant Dentistry. 4th ed: Blackwell Munksgaard; 2003

[41] Wilson T, Kornman KS. Fundamentals of Periodontics. Quintessence; 1996.

[42] Kolte R, Kolte A, Mahajan A. Assessment of gingival thickness with regards to age, gender and arch location. J Indian Soc Periodontol. 2014;18(4):478-481.

[43] Ibusuki M. The color of gingiva studied by visual color matching. Part II. Kind, location, and personal difference in color of gingiva. Bull Tokyo Med Dent Univ. 1975;22(4):281-292

[44] Tredwin CJ, Scully C, Bagan-Sebastian JV. Drug-induced disorders of teeth. J Dent Res. 2005;84(7):596-602

[45] Bortoluzzi EA, Araújo GS, Guerreiro Tanomaru JM, Tanomaru-Filho M. Marginal gingiva discoloration by gray MTA: a case report. J Endod. 2007;33(3):325-327

[46] Lindhe J, Karring TL, Niklaus P. Clinical Periodontology and Implant Dentistry. 4th ed: Blackwell Munksgaard; 2003

[47] McCullough MJ, Tyas MJ. Local adverse effects of amalgam restorations. Int Dent J. 2008;58(1):3-9

[48] Rosenstiel SF, Land MF, Fujimoto J. Contemporary fixed prosthodontics. 4th ed. St. Louis, Mo.: Mosby Elsevier; 2006.

[49] Shillingburg HT, Sather DA. Fundamentals of fixed prosthodontics. 4th ed. Chicago: Quintessence Pub.; 2012

[50] Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: a literature review. Dent Traumatol. 2013 Feb;29(1):2-7.

[51] Lindhe J, Karring T, Lang NP. Clinical periodontology and implant dentistry. 4th ed. Oxford, UK ; Malden, MA: Blackwell; 2003

[52] Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Biologic width around titanium implants. A histometric analysis of the implanto-gingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. Journal of Periodontology 1997; 68:186-198.

[53] Berglundh, T., Lindhe, J., Ericsson, I., Marinello, C.P., Liljenberg, B. & Thomsen, P. The soft tissue barrier at implants and teeth. Clinical Oral Implants Research 1991; 2: 81–90.

[54] Buser, D., Weber, H.P., Donath, K., Fiorellini, J.P., Paquette, D.W. & Williams, R.C. Soft tissue reactions to non-submerged unloaded tita- nium implants in beagle dogs. Journal of Periodontology 1992; 63: 225–235

[55] Moon, I.S, Berglundh, T., Abrahamsson, I., Linder, E. & Lindhe, J. The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. Journal of Clinical Periodontology 1999; 26: 658–663.

[56] Bressan E1, Paniz G, Lops D, Corazza B, Romeo E, Favero G. Influence of abutment material on the gingival color of implant-supported all-ceramic restorations: a prospective multicenter study. Clin Oral Implants Res. 2011 Jun;22(6):631-7.

[57] Johnston WM, Ma T, Kienle BH. Translucency parameter of colorants for max-illofacial prostheses. Int J Prosthodont 1995;8:79–86.

[58] Jun SH, Ahn JS, Chang BM, Lee JD, Ryu JJ, Kwon JJ. In vivo measurements of human gingival translucency parameters. Int J Periodontics Restorative Dent. 2013 Jul-Aug;33(4):427-34.

[59] Sciubba, J. J., Waterhouse, J. P. & Meyer, J. A fine structural comparison of the healing of incisional wounds of mucosa and skin. Journal of Oral Pathology 1978;7, 214–227.

[60] Szpaderska, A. M., Zuckerman, J. D. & DiPietro, L. A. Differential injury responses in oral mucosal and cutaneous wounds. Journal of Dental Research 2003b; 82, 621–626.

[61] Wong, J. W., Gallant-Behm, C., Wiebe, C., Mak, K., Hart, D. A., Larjava, H. & Hakkinen, L. Wound healing in oral mucosa results in reduced scar formation as compared with skin: evidence from the red Duroc pig model and humans. Wound Repair Regeneration 2009; 17, 717– 729.

[62] Graves, D. T., Nooh, N., Gillen, T., Davey, M., Patel, S., Cottrell, D. & Amar, S. IL-1 plays a critical role in oral, but not dermal, wound healing. Journal of Immunology 2001;167, 5316–5320.

[63] Zelles, T., Purushotham, K. R., Macauley, S. P., Oxford, G. E. & Humphreys-Beher, M. G. Saliva and growth factors: the fountain of youth resides in us all. Journal of Dental Research 1995; 74, 1826–1832.

[64] Schapher, M., Wendler, O. & Groschl, M. Salivary cytokines in cell proliferation and can- cer. Clinica Chimica Acta 2011;412, 1740–1748.

[65] Lindhe,J.,Wennstro€m,J.L.&Berglundh,T. The mucosa at teeth and implants. In: Lindhe, J., Lang, N. P. & Karring, T. (eds). Clinical Periodontology and Implant Dentistry, 5th edition, pp. 69–85. Blackwell Munksgaard; 2008

[66] Bosshardt, D. D. & Lang, N. P. The junctional epithelium: from health to disease. Jour- nal of Dental Research 2005; 84, 9–20.

[67] Berglundh T1, Lindhe J, Jonsson K, Ericsson I. The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. J Clin Periodontol. 1994 Mar;21(3):189-93.

[68] Sculean A1, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. J Clin Periodontol. 2014 Apr;41 Suppl 15:S6-22.

[69] Listgarten, M. A., Buser, D., Steinemann, S. G., Donath, K., Lang, N. P. & Weber, H. P. Light and transmission electron microscopy of the intact interfaces between non- submerged titanium-coated epoxy resin implants and bone or gingiva. Journal of Dental Research 1992; 71, 364–371.

[70] Scardina GA, Cacioppo A, Messina P.Changes of oral microcirculation in chemotherapy patients: A possible correlation with mucositis? Clin Anat. 2014 Apr;27(3):417-22.

[71] Scardina GA, Messina P.Increased gingival blood vessel density in SLE patients.

Quintessence Int. 2012 Jun;43(6):511-5.

[72] Scardina GA, Cacioppo A, Messina P.Periodontal microcirculation in diabetics: an in vivo non-invasive analysis by means of videocapillaroscopy. Med Sci Monit. 2012 Feb;18(2):CR58-64.

[73] Scardina GA, Pisano T, Messina M, Rallo A, Messina P. "In vivo" evaluation of the vascular pattern in oral peri-implant tissues. Arch Oral Biol. 2011 Feb;56(2):148-52.

[74] Danese S, Fiorino G, Angelucci E, et al. Narrow-band imaging endoscopy to assess mucosal angiogenesis in inflammatory bowel disease: a pilot study. World J Gastroenterol. 2010;16(19):2396-2400.

[75] Cho WY, Jang JY, Lee DH, Group ETaIS. Recent Advances in Image-enhanced Endoscopy. Clin Endosc. 2011;44(2):65-75.

[76] Kudo T, Matsumoto T, Esaki M, Yao T, Iida M. Mucosal vascular pattern in ulcerative colitis: observations using narrow band imaging colonoscopy with special reference to histologic inflammation. Int J Colorectal Dis. 2009;24(5):495-501

[77] Yang SW, Lee YS, Chang LC, Chien HP, Chen TA. Light sources used in evaluating oral leukoplakia: broadband white light versus narrowband imaging. Int J Oral Maxillofac Surg. 2013;42(6):693-701.

[78] Yang SW, Lee YS, Chang LC, Hwang CC, Luo CM, Chen TA. Use of endoscopy with narrow-band imaging system in evaluating oral leukoplakia. Head Neck. 2012;34(7):1015-1022.

[79] Yang SW, Lee YS, Chang LC, Hwang CC, Chen TA. Diagnostic significance of narrow-band imaging for detecting high-grade dysplasia, carcinoma in situ, and carcinoma in oral leukoplakia. Laryngoscope. 2012;122(12):2754-2761.

[80] Yang SW, Lee YS, Chang LC, Hwang CC, Chen TA. Diagnostic significance of narrow-band imaging for detecting high-grade dysplasia, carcinoma in situ, and carcinoma in oral leukoplakia. Laryngoscope. 2012;122(12):2754-2761.

[81] Yang SW, Lee YS, Chang LC, Hsieh TY, Chen TA. Implications of morphologic patterns of intraepithelial microvasculature observed by narrow-band imaging system in cases of oral squamous cell carcinoma. Oral Oncol. 2013;49(1):86-92.

[82] Belser, U.C., Gru€tter, L., Vailati, F., Bornstein, M.M., Weber, H.P. & Buser, D. Outcome evaluation of early placed maxillary anterior single-tooth implants using objective esthetic criteria: a cross- sectional, retrospective study in 45 patients with a 2- to 4-year follow-up using pink and white esthetic scores. Journal of Periodontology 2009;80: 140–151.

[83] Benic, G.I., Wolleb, K., Sancho-Puchades, M. & H€ammerle, C.H. Systematic review of parameters and methods for the professional assessment of aesthetics in dental implant research. Journal of Clinical Periodontology 2012; 39 (Suppl 12): 160–192.

[84] Lang, N.P. & Zitzmann, N.U. Working Group 3 of the VIII European Workshop on Periodontology. Clinical research in implant dentistry: evaluation of implant-supported resto- rations, aesthetic and patient-reported outcomes. Journal of Clinical Periodontology 2012; 39(Suppl 12): 133–138.

[85] Grunder, U., Gracis, S. & Capelli, M. Influence of 3-D bone-to-implant relationship on esthetics. International Journal of Periodontics and Restorative Dentistry 2005; 25: 113–119.

[86] Dummett, C.O. Oral pigmentation. Journal of Periodontology 31: 356–360; 1960

[87] Takeda, T., Ishikami, K., Shimada, A. & Ohki, K. A study of discoloration of the

gingiva by artificial crowns. The International Journal of Prosthodontics 1996; 9: 197–202.

[88] Schnitzer, S., Turp, J.S., Habil, M.D. & Heydecke, G. Color distribution and visual assessment of human gingiva and mucosa: a systematic review of the literature. The International Journal of Prosthodontics 2004; 17: 327–332.

[89] Paniz G, Bressan E, Stellini E, Romeo E, Lops D. Correlation between subjective and objective evaluation of peri-implant soft tissue color. Clin Oral Implants Res. 2013; Jun 10.

[90] Ishikawa-Nagai, S., Da Silva, J., Weber, H.P., and Park ,S.: Optical phenomenon of peri-implant soft tissue. Part II. Preferred implant neck color to improve soft tissue esthetics. Clin Oral Impl Res.18(5):575-80,2007.

[91] Palattella P, Torsello F, Cordaro L. Two-year prospective clinical comparison of immediate replacement vs. immediate restoration of single tooth in the esthetic zone. Clin Oral Implants Res 2008;19: 1148–1153.

[92] Lindeboom JA, Tjiook Y, Kroon FH. Immediate placement of im- plants in periapical infected sites: A prospective randomized study in 50 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:705–710.

[93] Cooper LF, Reside GJ, Raes F, Garriga JS, Tarrida LG, Wiltfang J, Kern M, De Bruyn H. Immediate provisionalization of dental implants placed in healed alveolar ridges and extraction sockets: a 5-year prospective evaluation. Int J Oral Maxillofac Implants. 2014 May-Jun;29(3):709-17. doi: 10.11607/jomi.3617.

[94]Chen ST, Buser D.Esthetic outcomes following immediate and early implant placement in the anterior maxilla--a systematic review. Int J Oral Maxillofac Implants. 2014;29

Suppl:186-215.

[95] Slagter KW[1], den Hartog L, Bakker NA, Vissink A, Meijer HJ, Raghoebar GM. Immediate placement of dental implants in the esthetic zone: a systematic review and pooled analysis. J Periodontol. 2014 Jul;85(7):e241-50.

[96] Buser D, Martin W, Belser UC. Optimizing esthetics for implant restorations in the anterior in the anterior maxilla: Anatomic and surgical considerations. Int J Oral Maxillofac Implants 2004;19(suppl):43–61.

[97] Chen S, Darby I, Reynolds E, Clement J. Immediate implant placement post-extraction without flap elevation: A case series. J Periodontol 2009;80:163–172.

[98] Chen ST, Darby IB, Reynolds EC. A prospective study of non-submerged implants: Clinical outcomes and esthetic results. Clin Oral Implants Res 2007;18:552–562.

[99] Evans CJD, Chen ST. Esthetic outcomes of immediate implant place- ments. Clin Oral Implants Res 2008;19:73–80.

[100] Becker W, Goldstein M. Immediate implant placement: treatment planning and surgical steps for successful outcome. Perio 2000 2009; 79 - 89.

[101] Grunder U, Gracis S, Capelli M. Influence of the 3-D bone-to-implant relationship on esthetics. Int J Periodontics Restorative Dent 2005; 25(2):113–119.

[102] Jemt T, Lekholm U. Single implants and buccal bone grafts in the anterior maxilla: measurements of buccal crestal contours in a 6-year prospective clinical study. Clin Implant Dent Relat Res 2005; 7(3):127–135.

[103] Chen ST, Darby IB, Adams GG, Reynolds EC. A prospective clinical study of bone augmentation techniques at immediate implants. Clin Oral Implants Res 2005;16:176–184.

[104] Kois JC. Predictable single tooth peri-implant esthetics: Five diag- nostic keys. Compend Contin Educ Dent 2001;22:199–206.

[105] Kan JYK, Morimoto T, Rungcharassaeng K, Roe P, Smith DH. Gingival biotype assessment in the esthetic zone: Visual versus direct mea- surement. Int J Periodontics Restorative Dent 2010;30:237–242.

[106] Schrott AR[1], Jimenez M, Hwang JW, Fiorellini J, Weber HP. Five-year evaluation of the influence of keratinized mucosa on peri-implant soft-tissue health and stability around implants supporting full-arch mandibular fixed prostheses. Clin Oral Implants Res. 2009 Oct;20(10):1170-7.

[107] Hahn J. Single stage, immediate loading, and flapless surgery. J Oral Implantol 2000;26(3):193–198.

[108] Oh TJ, Shotwell J, Billy E, et al. Flapless surgery in the esthetic region: Advantages and precautions. Int J Periodontics Restorative Dent 2007;27(1):27–33.

[109] Kan JYK, Rungcharassaeng K, Lozada JL. Immediate placement and provisionalization of maxillary anterior single implants: A surgical and prosthodontic rationale. Pract Periodontics Aesthetic Dent 2000; 12:817–824

[110] Barone A, Rispoli L, Vozza I, Quaranta A, Covani U. Immediate resto- ration of single implants placed immediately after tooth extraction. J Periodontol 2006;77:1914–1920.

[111] Jemt T. Restoring the gingival contour by means of provisional resin crowns after single implant treatment. Int J. Periodontics Restor- ative Dent 1999;19:20–29.

[112] Kois JC, Kan JYK. Predictable peri-implant gingival esthetics surgical and prosthodontic rationales. Pract Proced Aesthet Dent 2001; 13:711–715.

[113] Cooper LF. Biologic determinants of bone formation for osseointegration: clues for future clinical improvements. J Prosth Dent. 1998;80:439–49

[114] Nanci A, et al. Chemical modification of titanium surfaces for covalent attachment of biological molecules. J Biomed Mater Res. 1998;40:324–35.

[115] Gutwein LG, Webster TJ. Increased viable osteoblast density in the presence of nanophase compared to conventional alumina and titania particles. IJ Biomaterials. 2004;25:4175–83

[116] Eriksson AB, Albrektsson T. The effect of heat on bone regeneration: An experimental study in the rabbit using the bone growth chamber. *Int J Oral Maxillofac Surg*. 1984;42:705–711.

[117] American Society of Anesthesiologists (ASA) Physical Status classification system, 1941.
http://my.clevelandclinic.org/services/anesthesia/hic_asa_physical_classification_system.aspx

[118] Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986;1:11-25.

[119] O'Brien WJ, Boenke KM, Groh CL. Coverage errors of two shade guides. Int J Prosthodont. 1991;4(1):45-50.

[120] Grieco, PC et al. An in vivo analysis of the spectral properties of periodontal gingiva in the esthetic zone. (Manuscript in preparation)

[121] Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral

Maxillofac Implants 1986;1:11-25.

[122] Shibahara T1, Yamamoto N, Yakushiji T, Nomura T, Sekine R, Muramatsu K, Ohata H. Narrow-band imaging system with magnifying endoscopy for early oral cancer. Bull Tokyo Dent Coll. 2014;55(2):87-94.

[123] Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of peri-implant mucosa: an experimental study in dogs. Clin Oral Implants Res 2006;18:1–8.

[124] Listgarten MA1, Lang NP, Schroeder HE, Schroeder A. Periodontal tissues and their counterparts around endosseous implants [corrected and republished with original paging, article orginally printed in Clin Oral Implants Res 1991 Jan-Mar;2(1):1-19]. Clin Oral Implants Res. 1991 Jul-Sep;2(3):1-19.

[125] M Nevins, DM Kim, SH Jun, K Guze, P Schupbach, ML Nevins. Histologic evidence of a connective tissue attachment to laser microgrooved abutments: A canine study. Int J Periodontics Restorative Dent, Volume 30, Number 3, 2010. p. 245-255.

[126] Buser, D., Weber, H. P., Donath, K., Fiorellini, J. P., Paquette, D. W. & Williams, R. C. Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. Journal of Periodontology 1992;63, 225–235. doi:10. 1902/jop.1992.63.3.225.

[127] Schultze-Mosgau S, Blatz MB, Wehrhan F, Schlegel KA, Thorwart M, Holst S. Principles and mechanisms of peri- implant soft tissue healing. Quintessence Int 2005;36:759–69.

[128] Matsuo M, Nakamura T, Kischi Y, Takahashi K. Microvascular changes after placement of titanium implants:scanning electron microscopy Observations of machined and titanium plasma-sprayed implants dogs. J Periodontol 1999;70:1330–8.