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Evaluation of color stabilization of peri-implant mucosa in different restorative material, abutment and tissue thickness combinations: an *in vitro* study

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ABSTRACT

Aim The aim of this *in vitro* study is to determine the level of color change of the peri-implant mucosa in several combinations of parameters, including different experimentally-created peri-implant tissue thicknesses, and different prosthetic crown and abutment materials, by spectrophotometric measurements.

Materials and methods In this *in vitro* study, a sheep's head was used because it resembles human mucosa in terms of color and texture. Different experimentally-created peri-implant tissue thicknesses were determined, i.e. 1, 1.5, 2 and 3 mm, and to provide these thicknesses 0.5, 1 and 2 mm thick connective tissue grafts were harvested from the palatal mucosa of the sheep's head. These grafts were placed under the mucosal flap and fixed with tissue adhesive. Titanium and zirconia were chosen as abutment materials. Metal-porcelain crowns, zircon crowns and feldspathic porcelain crowns were selected as crown materials. Materials were represented by 5 x 5 x 1 mm blocks made of the same materials. For each study group, two measurements were made using a spectrophotometer. The first measurement determined the color of the flap in different experimentally-created tissue thicknesses, and the second measurement determined the color of the tissue after the prosthetic material and abutment material were placed under these flaps. Statistical comparison of the two measurement values was used to determine the color change.

Results Spectrophotometer measurements show that the naked eye could distinguish between all groups when the mucosa thicknesses were 1 and 1.5 mm. When the mucosa thickness was 2 mm, color change was observed in the titanium abutment and prosthesis groups, while color stability was achieved in the zircon abutment and prosthesis groups when the mucosa thickness was 3 mm.

Conclusions Within the limits of this study, peri-implant mucosa thickness is an important factor in color stabilization; mucosa thickness must be a minimum of 2 mm to achieve this stabilization.

INTRODUCTION

Today, successful implant treatments depend not only on osseointegration, but also on a good aesthetic result. For this reason, implantology research has started to focus on the evaluation of aesthetic parameters (1, 2). In evaluating the success of implant-supported restorations, it is very important that the crown is morphologically compatible with the natural dentition, as well as the soft tissue parameters such as zenith points, and color differences due to color reflection from under the mucosa (3).

The aim of this study is to determine the effect of different tissue thicknesses, abutment options and prosthetic crown materials of implant-supported restorations on peri-implant mucosal color.

MATERIALS AND METHODS

Subjects

Ten fresh sheep jaws were used for the current study, obtained from slaughterhouses with the necessary permits to be consumed as food. Therefore, this study was not classified as an animal study and the local ethics committee had no objection to the protocol. In the study, it was decided to use fresh sheep jaws because they are similar to the human gingiva in terms of color and texture.

Instrumentation/measurement

Peri-implant mucosal thickness

In order to represent different peri-implant mucosa thicknesses, connective tissue was harvested from sheep palatal mucosa. The experimentally-generated mucosal thicknesses are shown in Table 1.

	Group 1	Group 2	Group 3	Group 4
Periodontal flap thickenesses	1 mm	1 mm	1 mm	1 mm
Connective tissue thickenesses	0	0.5 mm	1 mm	2 mm
Total mucosa thickenesses	1 mm	1.5 mm	2 mm	3 mm

TABLE 1 Experimental mucosa thickeness design.

	Titanium Groups			Zirconium Groups	
Mucosa thickeness	Titanium -Metal fused porcelain	Titanium-Zirconium	Titanium- Feldspathic	Zirconium-Zirconium	Zirconium – Feldspathic
1 mm	Group 1	Group 5	Group 9	Group 13	Group 17
1.5 mm	Group 2	Group 6	Group 10	Group 14	Group 18
2 mm	Group 3	Group 7	Group 11	Group 15	Group 19
3 mm	Group 4	Group 8	Group 12	Group 16	Group 20

TABLE 2 Design of study.

Materials

Abutment material

Titanium and zirconium were selected as abutment materials, represented by 5 x 5 x 1 mm plates. Titanium and zirconium plates were specially prepared in the laboratory.

Prosthetic material

Three prosthetic crown materials were selected: metal-supported porcelain, full porcelain (feldspathic), and zirconia porcelain. Plates of the same dimensions (5 x 5 x 1 mm) were prepared to represent the porcelain crowns. A 1 mm thick metal plate and a 1 mm ceramic plate were used to represent the metal-supported crown. For the zirconium crown, 1 mm thick zirconium substructure and 1 mm thick zirconium superstructure plates were used. For the full ceramic crowns, a 1 mm thick zirconium substructure and a 1 mm thick feldspathic superstructure were used.

Study design

Sheep mucosa was used in this study because it looks like human peri-implant mucosa in terms of color and texture. To simulate different peri-implant mucosal thicknesses, connective tissue grafts of 0.5, 1 and 2 mm thickness were harvested from the palate mucosa, measured using a periodontal probe. Then, the harvested connective tissue grafts were adapted under the mucosal flap to create different soft tissue thicknesses. After blocks representing both the abutment and crown materials were placed under the flap, color analysis was performed using a spectrophotometer (Table 2).

Spectrophotometric setup

In the present study, a reflectance spectrophotometer (SpectroShade, No. LUA005, Medical High Technologies; software version 2.5, MHT) was used to objectively evaluate the color of the mucosa. Then, a mucoperiosteal flap was adjusted to 1 mm thickness and all groups were controlled with a periodontal probe.

The baseline spectrophotometric measurements were taken from the mucosa region with no blocks in place. Subsequently, the test blocks were placed one at a time under the mucosal flap and spectrophotometric measurements were taken again of the same mucosa area. Three consecutive images were captured for data analysis. Thus, three images were acquired of each mucosa thickness (1, 1.5, 2.0 and 3.0 mm) for all experimental groups. This measurement was used to calculate laboratory parameters (L, a, b) in the CIELAB color space (CIE), and then the differences (ΔL, Δa and Δb) were calculated by subtracting the baseline measurements from the test specimens (Fig. 1). To estimate the overall color difference between one of the test specimens and the baseline measurement, the following equation was used:

$$\Delta E = \sqrt{[(Ls - Lb)2 + (As - Ab)2 + (Bs - Bb)2]}$$

where s = specimen and b = baseline.

Data presentation and statistical analysis

Differences were calculated between the colorimetric values of tissues with and without block interposition for each material group. The ΔL, Δa, Δb and ΔE values of the three measurements were averaged and this value was used for further analysis. For the description of these data, mean values and corresponding 95% confidence intervals (95% CI) are given. The ΔE values were compared to the critical ΔE threshold of 3.7 for intraoral color distinction as perceived by the naked eye (4).

For statistical analysis of the differences between the L, a and b values, the one-sample t-test was used. The null hypothesis was that no visible changes occurred.

RESULTS

According to the results of spectrophotometer measurements, ΔE values were greater than 3.7 in the study groups with mucosal thicknesses of 1 and 1.5

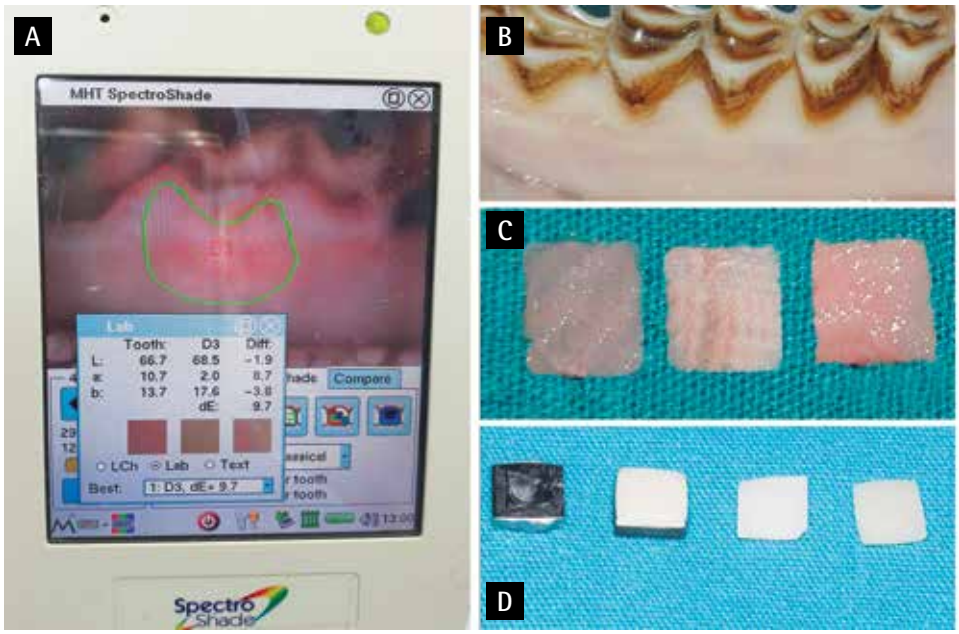

FIG 1 **A:** Spectrophotometric device.
B: Sheep jaws.
C: Different thickeness (0,5, 1,2 mm respectively) connective tissue graft.
D: 5 x 5 mm abutment and prosthetic restorations blocks.

mm. This result showed that color reflection caused by restorative materials can occur over a distance of 1 and 1.5 mm mucosal thickness. In the 2 mm mucosal groups, the ΔE values of the titanium abutment groups were greater than 3.7, but the ΔE values were smaller than 3.7 for the other groups. In the 3 mm mucosal groups, ΔE values were less than 3.7 in all groups. There were no color changes to the naked eye in any of the 3 mm groups (Table 3).
Significant changes in lightness (ΔL) were noted for the titanium groups at mucosa thicknesses of 1, 1.5 and 2.0 mm, and for the zirconium groups at mucosa thicknesses of 1 and 1.5 mm. The changes in the other groups in terms of lightness (ΔL) were not statistically significant. Significant changes in Δa values were noted for all titanium groups at mucosa thicknesses of 2 and 3 mm, whereas the zirconium groups showed significant changes in only the 1 and 1.5 mm thicknesses groups, except for 1 mm zirconium-zirconium groups. The only significant alterations in Δb values were found in the titanium groups at a mucosa thickness of 1.5 mm and the zirconium groups at mucosa thicknesses of 1 and 1.5 mm. All other groups showed no changes in Δb values at different mucosal thicknesses (Table 4).

DISCUSSION

In terms of peri-implant tissue thicknesses, 1 mm gingiva/mucosa is classified as thin gingiva, 1–2 mm as medium thickness, and more than 2 mm as thick gingiva/mucosa. As the gingival thickness increases, the amount of color reflection of the peri-implant mucosa decreases (5).
Linkhevicious et al. and Jung et al. have stated that a peri-implant mucosa thickness of 2 mm is sufficient for zirconium crowns (6, 7). In our current study, it was determined that color stability was preserved in zirconia groups at 2 mm tissue thickness, in line with these studies.
Color analysis is evaluated by computer-assisted colorimetry or spectrophotometry. Studies on these devices have shown that spectrophotometry gives more reliable results than computer-assisted colorimetry (8). For this reason, we preferred to use spectrophotometry in our study.

	Titanium abutment groups			Zirconium abutment groups	
ΔE	Metal supported (Mean±Sd)	Zirconia (Mean±Sd)	Feldspathic porcelaine (Mean±Sd)	Zirconium (Mean±Sd)	Feldspathic porcelaine (Mean±Sd)
1.0 mm	5.7 ± 3.9	6.2 ± 3.6	6.5 ± 3.2	7.2 ± 3.4	6.9 ± 3.3
1.5 mm	5.7 ± 2.5	5.9 ± 2.9	6.2 ± 2.8	5.9 ± 1.9	4.8 ± 1.2
2.0 mm	5.7 ±2.9	5.9 ± 3.2	4.4 ± 2.2	3.3 ± 1.2	3.1 ± 1.3
3.0mm	3.2 ± 1.9	3.4 ± 0.9	3.1 ± 1.2	3.0 ± 1.2	2.09 ± 1.5
*Sd :Standart deviation					

TABLE 3 Mean ΔE distribution.

	Titanium Groups			Zirconia Groups	
	Titanium -Metal fused porcelain	Titanium-Zirconium	Titanium-Feldspathic	Zirconium-Zirconium	Zirconium-Feldspathic
ΔL					
1 mm	-2.8**(-3.50 -1.89)	-2.8**(-3.50 -1.89)	-2.64**(-3.50 -1.89)	0.69 (1.84 -,35)	-2.25** (-3.2, -0.95)
1.5 mm	-2.6** (-3.48, -1.72)	-2.6** (-3.48, -1.72)	-2.45** (-3.48, -1.72)	-0.65 (-1.71,0.41)	-2.08** (-3.33, -0.82)
2 mm	-2.06* (-3.84, -0.28)	-1.5 (-1.84, -0.28)	-1.3 (-1.84, -0.28)	-0.51 (-2.50,1.48)	-1.96 (-4.35, 0.47)
3 mm	-0.55 (-1.28, 0.18)	-0.55 (-1.28, 0.18)	-0.49 (-1.28, 0.18)	-0.39 (-1.68,0.90)	-0.43 (-1.72, 0.87)
Δa					
1 mm	-0.54(-1.23,-0.34)	0.68 (1.68, -0.25)	0.408(-1.78, -013)	-1.26* (-2.55, -0.13)	-1,54 (-2.65, -0,15)
1.5 mm	-0.36 (-1.90, 0.08)	-0.54 (-1.90, 0.08)	-0.37 (-1.85, -.26)	-1.39* (-2.71, 0.07)	-1.39* (-2.71, -0.07)
2 mm	0.9* (0.12, 1.68)	0.88* (0.12, 1.68)	0.9* (0.12, 1.68)	0.03 (-0.86, 0.95)	0.03 (-0.86, 0.95)
3 mm	0.95** (0.39, 1.51)	0.90** (0.39, 1.51)	0.95** (0.39, 1.51)	-0.59 (-1.62, 0.44)	-0.59 (-1.62, 0.44)
Δb					
1 mm	-1,75(-2,76,-1,35	-1.55(-2.76, -1.55)	-1.75 (-2,57, -1,64)	-3.01* (4,85,-2,25)	-3.13* (4.85, -2.25)
1.5 mm	-1.56* (-2.97, -0.15)	-1.36* (-2.97, -0.15)	-1.29* (-3.01, -0.44)	-2.85* (-5.04, -0.44)	-2.98*(-5.04, -0.44)
2 mm	-0.71 (-3.98, 2.56)	-0.45 (-3.98, 2.56)	-0.51 (-3.98, 2.56)	-1.01 (-3.23, 1.25)	-0.95 (-3.23, 1.25)
3 mm	0.5 (-0.19, 1.19)	0.55 (-0.19, 1.19)	0.48 (-0.19, 1.19)	0.32 (-1.81, 1.38)	0.32 (-1.81, 1.38)

** Means and 95% confidence intervals (95% CI) of ΔL, Δa, and Δb values at different mucosa thicknesses;; *p < .05; **p < .01*

TABLE 4 Mean ΔL, Δa and Δb values

In their study, Paniz et al. compared the color evaluation of the spectrophotometric device and the human eye. Their study observed that the spectrophotometer devices were more accurate than the human eye evaluation, and that the human eye was affected by light, experience and age (9).

Color is evaluated using three parameters in the spectrophotometric measurements. These parameters are defined as the following values on the color axis: L black/white (black 0 white 100), a green/red (greater than 0 red ko green), and b yellow/blue (bo yellow k0 blue) (10).

In our study, the changes in the 'a' parameter in the titanium abutment groups with mucosal thicknesses of 1, 1.5 and 2 mm were statistically significant. Takeda et al. found the change in the 'a' parameter to be significant, in accordance with our study (11).

Jung et al. and Van et al. determined that when the mucosal thickness is 2 mm or less and zirconia materials are used, the tissue color shifts slightly along the yellow-blue axis of the chroma (12, 13). In this study, the change in the L parameter of color measurement was consistent with that of previous studies.

In implant restorations, peri-implant soft tissue volume can be increased as an alternative clinical approach to reducing the reflection of the abutment or prosthetic material in the buccal mucosa (14).

A systematic review by Thoma et al. reported that the use of collagen matrix in peri-implantal mucosa increased keratinized gingiva, this increase was less than in the subepithelial connective tissue graft. A subepithelial connective tissue graft is the gold standard for increasing soft tissue volume in the peri-implant mucosa (15).

Batal et al. carried out a clinical study using the subepithelial connective tissue grafting procedure at various stages of implant treatment, reporting that it can increase the thickness of the peri-implant soft tissue and that this increase will significantly contribute to aesthetics (16).

In a clinical study conducted by Vechiato et al., the effect of abutment materials on aesthetics was examined and it was concluded that the zirconium abutment had a better aesthetic effect due to its superior optical properties, in accordance with the present study (17).

A systematic review of Zembic et all. suggest that differences of survival rate of zirconium and titanium abutments have little significance, in particular the survival rate of zirconium abutments was 97.5% and of titanium abutment was 97.6%, which was not statistically significant (18).

In cases where peri-implant soft tissue thickness is insufficient, in addition to using zirconium abutments, the use of abutments created with CAD-CAM (Computer-Aided Design/Computer-Aided Manufacturing) systems can be considered as an alternative in minimizing discoloration in the peri-implant mucosa.

The present study has two major limitations. Firstly, since it could not be performed on living tissue, it could not be determined to what extent the blood flow would affect the color change in the tissue. Secondly, sheep mucosa was used for color analysis in stead of human mucosa. However, in previous studies, it has been reported that pig or sheep mucosa is similar in color and content to human mucosa. Therefore, the use of fresh sheep skulls in this study somewhat reduced this limitation.

CONCLUSIONS

Within the limits of this study, tissue maintains color stabilization when the thickness of the peri-implant mucosa is 3 mm or more, regardless of the abutment or restorative material selected. However, when the tissue thickness is less than 3 mm, aesthetic restorative materials such as zirconium or feldspathic porcelain abutments and prosthetic crown materials should be used for a more satisfactory aesthetic. More extensive clinical studies are needed to make a definitive conclusion about peri-implant mucosal color stabilization.

Source of support
There is no funding for this study.

Conflict of interest
No conflict of interest.

Ethics approval and consent to participate
Ethical approval has not been obtained due to the *in vitro* nature of the study.

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