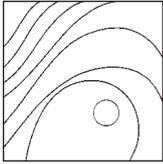


# In Vitro Color Changes of Soft Tissues Caused by Dyed Fluorescent Zirconia and Nondyed, Nonfluorescent Zirconia in Thin Mucosa



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Abutment material selection may have an effect on the color of the peri-implant soft tissue, especially in thin mucosa. The objective of this *in vitro* study was to investigate the effect of titanium, zirconia, and dyed fluorescent zirconia on the color of 1.5-mm-thick mucosa. Ten pig maxillae were used to simulate thin mucosa according to a previously published setup. Three different abutment materials were placed under the mucosa, and the color of the soft tissue was evaluated using a spectrophotometer. The test area without underlying material was used as a control. Whereas titanium induced visible color change values of  $\Delta E 7.3$ , significantly above the threshold level of  $\Delta E 3.7$  ( $P < .05$ , Student *t* test), the changes after insertion of zirconia ( $\Delta E 3.7$ ) and dyed fluorescent zirconia (3.5) were not statistically different from the visible threshold of  $\Delta E 3.7$  ( $P < .05$ , Student *t* test). The difference between the two zirconia specimens was not statistically significant, although the dyed zirconia material was darker; the color difference was  $\Delta E 10.35$  between the two. In contrast to titanium, neither nondyed zirconia nor dyed fluorescent zirconia changed the gingival coloration. Moreover, shading of white zirconia with a fluorescent dye leads to an all-ceramic abutment material that mimics the optical properties of natural teeth. (Int J Periodontics Restorative Dent 2013;33:e1–e8. doi: 10.11607/prd.1303)

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The esthetic outcome is a critical determinant of the overall success of implant therapy and a major concern for patients.<sup>1</sup> However, for many years, the esthetic result was not sufficiently documented in scientific research and not included in success criteria,<sup>2</sup> which is why indices for the documentation of the so-called white and pink esthetics have been proposed.<sup>3,4</sup> These indices include soft tissue color as a criterion for the evaluation of the esthetic outcome. In a study with 20 examiners assessing 30 single-implant cases, Fürhauser et al<sup>3</sup> reported that more than 60% of cases showed color mismatch between the soft tissue of the implant restoration and the natural tooth. This was identified as a major problem for the overall esthetics of implant restorations in the esthetic zone.<sup>3</sup>

Thin peri-implant mucosa tends to be delicate and almost translucent in appearance, contributing to an undesirable shine-through effect of the underlying material. The use of titanium might lead to a grayish appearance of the mucosal cuff (Fig 1).<sup>5</sup> To overcome this problem, all-ceramic zirconia abutments were introduced in implant dentistry and



**Fig 1** Gray shine-through effect of a titanium abutment at the maxillary right central incisor.



**Fig 2** White zirconia abutments exposed as a result of unfavorable soft tissue conditions and subsequent midfacial recession.

have demonstrated good long-term stability for the restoration of single-tooth implants for 3 to 5 years.<sup>6-9</sup> However, the clinical benefit in terms of reduced soft tissue discoloration has rarely been investigated.

Jung et al<sup>10</sup> investigated the shine-through effects of restorative materials in an in vitro study on pig jaws. Using a spectrophotometer, they found that the thickness of the soft tissue had a major influence on color changes.<sup>10</sup> Titanium caused significant color changes even at a tissue dimension of 3 mm, whereas zirconia did not affect the tissue color beyond a thickness of 2 mm.

In another spectrophotometric study, Ishikawa-Nagai et al<sup>11</sup> evaluated the ability of different colors to mask the restorative materials. They found that white materials such as pure zirconia were not ideal with regard to optical properties and caused visible color changes of the peri-implant mucosa in humans. Light orange and light pink were successful in

masking the abutment and improving the color of the peri-implant soft tissue. Moreover, changing the color of white zirconia abutments to a dentin-like color seems to be useful since midfacial recessions around implants are a common problem.<sup>12,13</sup> Patients may tolerate tooth-like abutment colors rather than clear white abutments once they become exposed (Fig 2). On the other hand, shaded ceramic abutments may decrease the brightness of the soft tissue, leading to a grayish appearance as previously seen around titanium abutments.<sup>5</sup> Until now, however, attempts to improve the optical properties of the transmucosal abutment have not addressed the optical phenomena of fluorescence found in natural teeth.<sup>14,15</sup>

Fluorescence is an optical characteristic of natural teeth that is clinically used for caries detection since application of light induces the emission of photons.<sup>14,16,17</sup> In contrast to conventional zirconia, dental porcelains have fluorescent

properties that mimic the optical appearance of natural teeth.<sup>18-20</sup> A color liquid (Color Liquid Fluorescent for Prettau, FMAA5803, Zirkozahn) has been introduced to color ready-milled and finished zirconia structures prior to sintering. This color liquid shades white zirconia and provides the material with fluorescent properties (Figs 3a and 3b).

Since ceramics with fluorescent properties are able to emit photons when stimulated, they may boost the brightness of the marginal peri-implant soft tissue as well. This phenomenon seems to be important in the marginal peri-implant soft tissue since previous studies reported a grayish appearance of the gingiva in this region.<sup>5,21</sup>

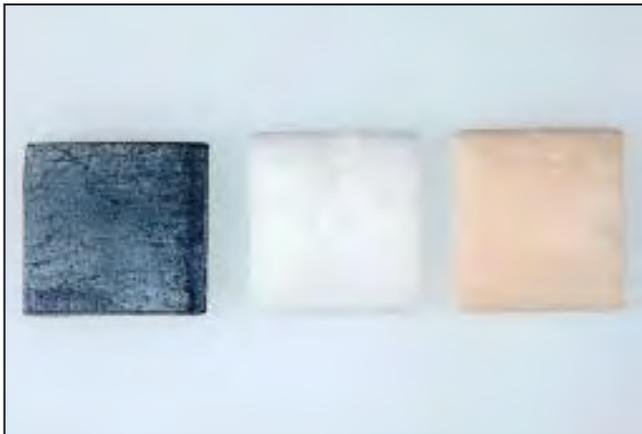
The aims of this in vitro study were to evaluate the influence of titanium and dyed and nondyed zirconia on the color of soft tissue with a thickness of 1.5 mm and to compare the color changes ( $\Delta E$ ) to the clinical perceptual threshold of 3.7.



**Fig 3a** Different abutment materials in comparison to an incisor (titanium and dyed and nondyed zirconia with and without fluorescent properties).



**Fig 3b** Same materials under ultraviolet light at a wavelength of 300 to 400 nm.



**Fig 4a** Three specimens were prepared for the experiment (left to right: titanium, zirconia, dyed fluorescent zirconia).



**Fig 4b** The three specimens under ultraviolet light at a wavelength of 300 to 400 nm. Fluorescence of the dyed zirconia specimen is apparent.

## Method and materials

### *In vitro* model

The *in vitro* model was chosen according to the setup described by Jung et al.<sup>10</sup> Ten pig maxillae were used. The *in vitro* tests were initiated no longer than 2 hours following the death of the pigs. The pigs were raised for food production according to the German standards

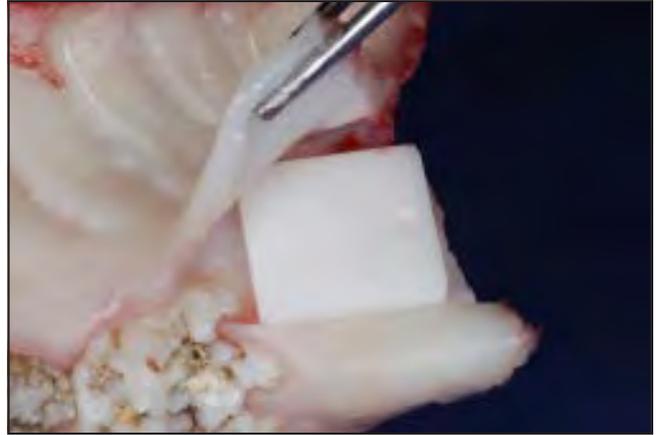
for animal care. Therefore, this investigation was not classified as an animal study, and the institutional ethics committee had no objections to the protocol. To minimize artifacts, the jaws were stored in a humidity chamber until use. Palatal pig mucosa exhibiting similarities to human keratinized mucosa with respect to color and texture was chosen as the test area.

### *Test specimens*

To evaluate the effect of the different materials, three different 14 × 12-mm specimens with a thickness of 1.5 mm were manufactured (Figs 4a and 4b): (1) titanium (grade 4; Ti), (2) plain zirconia (Znf), and (3) dyed fluorescent zirconia (Zf). To dye specimen 3, the milled and finished zirconia specimen was dipped into 100% fluorescent color



**Fig 5** Scalpel used to create the flap. Note the parallel blades.



**Fig 6** Positioning of the zirconia sample under the raised palatal flap.

liquid (Color Liquid Fluorescent for Prettau) for 10 seconds before sintering, according to the protocol recommended by the manufacturer.

#### *Data acquisition*

#### **Spectrophotometric setup**

A reflectance spectrophotometer (SpectroShade, type 71.3000, MHT Optic Research; software: version 3.01, MHT Optic Research) was used to evaluate the color of the mucosa in an objective manner. The spectrophotometer works with two basic optic systems to standardize angles of illumination and observation. The camera disposes a  $D_{65}$  light source (6,500 K), which is transformed into polarized light ( $\lambda$ , 400 to 720 nm) using a grate. With the standard lens, the light is split to illuminate the object simultaneously from two sides at a 45-degree angle. The reflected light is directed at 0 degrees onto the system detector area (18 × 13 mm)

for the measuring process. One detector area was a color charge-coupled distributor (CCD) chip responsible for the generation of the colored video image. A black and white CCD detector area recorded the spectrophotometric data. Prior to every measurement, the camera was calibrated to the white and green ceramic tiles supplied by the manufacturer. The data from each measurement were calculated and displayed based on the parameters of the Commission Internationale d'Eclairage (CIE), in which  $L^*$  = lightness,  $a^*$  = chroma along the red-green axis, and  $b^*$  = chroma along the yellow-blue axis.<sup>22</sup>

#### **Spectrophotometric assessment of specimens**

Triple measurement of the three specimens was performed using the spectrophotometer, and mean  $L^*a^*b^*$  coordinates were evaluated. The color difference between the two zirconia specimens ( $\Delta E$ ) was calculated.

#### **Spectrophotometric assessment of mucosa color**

The same palatal area of each maxilla was chosen to prepare a split-flap pouch, yielding a soft tissue thickness of 1.5 mm. A scalpel with two parallel blades at a distance of 1.5 mm was used to create a flap of equal thickness (Fig 5). Accuracy of the resulting mucosal thickness was verified by careful measurement with a modified caliper. The caliper was modified by cutting the spring to eliminate the tension of the caliper arms to avoid excessive pressure on the soft tissue.<sup>23,24</sup> To avoid trapping any air, glycerin gel was inserted into the resulting pouch.

Baseline spectrophotometric measurements were taken from the mucosa region with no test specimen in place (control group). Subsequently, the three test specimens were placed one at a time underneath the mucosa, and spectrophotometric measurements were again taken of the same mucosal region (Fig 6). For all measure-



**Fig 7** Collection of L\*a\*b\* color coordinates with the specimen in place.

ments, the adapter of the spectrophotometer was positioned perpendicular to the test site, allowing a standardized distance to the mucosa. Images were captured according to the recommendations of the manufacturer. Three consecutive images were captured to be used for data analysis.

Once the resulting video image of the area was centered orthoradially in the measuring square depicted on the computer screen, the spectrophotometric data were recorded. CIE L\*a\*b\* color coordinates were evaluated (Fig 7).

Mucosa without an underlying test specimen served as a control.

#### *Data presentation and statistical analysis*

The L\*a\*b\* values of the three measurements were averaged, and this value was used for further analysis. Color differences ( $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$ ) were calculated by subtracting the control values (mucosa

alone) from the test values. The overall color difference between the test specimens and the control was calculated using the following equation:

$$\Delta E = [(L_t^* - L_c^*)^2 + (a_t^* - a_c^*)^2 + (b_t^* - b_c^*)^2]^{1/2}$$

where t = test and c = control values.

For the description of these data, mean values and corresponding 95% confidence intervals were calculated. For statistical analysis of the differences between L\*, a\*, and b\* values in the test and control groups, the nonparametric Wilcoxon test was used.  $\Delta E$  values were compared to the critical  $\Delta E$  threshold of 3.7 using the Student t test since this value was described as the intraoral color distinction perceived by the naked eye.<sup>25</sup> The null hypothesis was that no visible changes would occur.

**Table 1** Mean L\*a\*b\* values of the three specimens

Specimen	Color coordinates (mean from triplet measurement)	
	L*	a*
Titanium	L*	6.7
	a*	1.9
	b*	-1.6
Nondyed zirconia	L*	86.4
	a*	-1.9
	b*	8.4
Dyed zirconia	L*	81.1
	a*	0.3
	b*	16.8

## Results

### *Specimens*

The mean L\*a\*b\* values of the three specimens are shown in Table 1. The results indicate that the dyed zirconia specimen appeared slightly darker (lower L\* value), more red (slightly higher a\* value, ie, shift of chroma toward red on red-green axis), and more yellow (higher b\* value, ie, shift of chroma toward yellow on yellow-blue axis). Calculation of the color difference of the two zirconia specimens revealed a  $\Delta E$  of 10.35.

### *Spectrophotometric assessment of mucosa color*

Statistical analysis of the  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$ , and  $\Delta E$  values is presented in Table 2. Significant changes in lightness ( $\Delta L^*$ ) were observed for all specimens. Titanium caused negative values, whereas the zirconia specimens induced positive

**Table 2** Mean L\*a\*b\* color coordinates for  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$ 

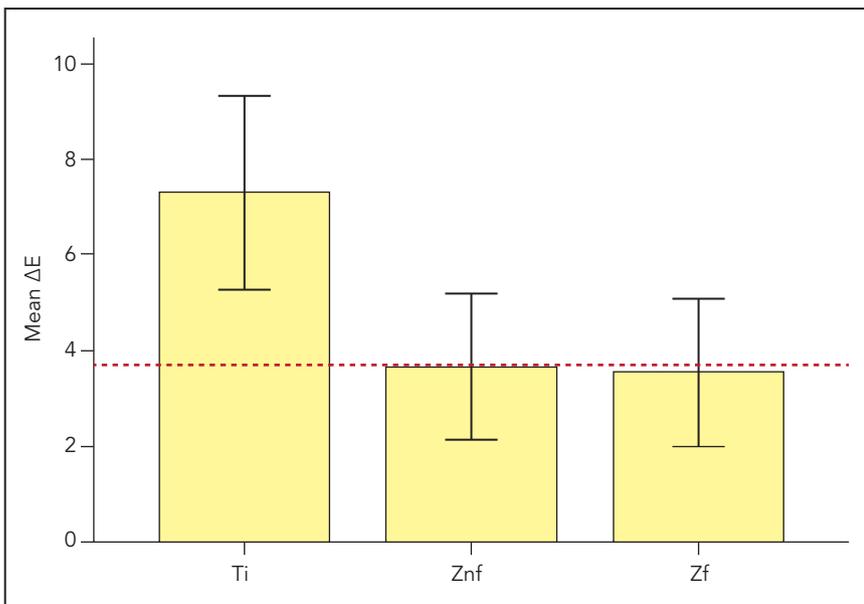
	Ti		Znf		Zf	
	Mean (95% CI)	P	Mean (95% CI)	P	Mean (95% CI)	P
$\Delta L^*$	-4.12 (-5.84 to -2.40)	.007	1.33 (0.25 to 2.41)	.005	0.98 (0.04 to 1.92)	.169
$\Delta a^*$	-3.97 (-5.12 to -2.83)	.005	1.19 (-0.49 to 2.86)	.005	1.81 (0.69 to 2.94)	.169
$\Delta b^*$	-3.56 (-5.07 to -2.04)	.009	1.61 (0.37 to 2.85)	.005	2.20 (1.22 to 3.18)	.241

Ti = titanium; Znf = nondyed zirconia; Zf = dyed zirconia; CI = confidence interval.

**Table 3** Mean  $\Delta E$  values induced by the evaluated materials

	Ti		Znf		Zf	
	Mean (95% CI)	P	Mean (95% CI)	P	Mean (95% CI)	P
$\Delta E$	7.30 (5.87 to 8.74)	.000	3.65 (2.56 to 4.75)	.924	3.53 (2.44 to 4.63)	.734

Ti = titanium; Znf = nondyed zirconia; Zf = dyed zirconia; CI = confidence interval.



**Fig 8**  $\Delta E$  values of the different materials evaluated. The red dotted line represents the critical threshold of 3.7 for intraoral color distinction as perceived by the naked eye. Ti = titanium; Znf = nondyed zirconia; Zf = dyed zirconia.

differences. Insertion of titanium under 1.5-mm-thick mucosa led to decreased brightness, a shift toward green on the red-green axis, and a shift toward blue on the yellow-blue axis.

Descriptive statistics revealed that only titanium-induced mean  $\Delta E$  values were above the critical threshold of 3.7 (Table 3). Nondyed zirconia and dyed zirconia both induced mean  $\Delta E$  values that were not

above this threshold, and dyed zirconia showed slightly lower discrepancies ( $\Delta E$ , 3.5) than the nondyed specimen ( $\Delta E$ , 3.7) (Fig 8). However,  $\Delta E$  values of the two zirconia specimens did not differ significantly.

## Discussion

The insertion of titanium under 1.5-mm-thick mucosa led to a visible color change, decreased brightness, a shift toward green on the red-green axis, and a shift toward blue on the yellow-blue axis. The two zirconia specimens showed similar color changes to a lighter, more red (higher  $a^*$  value), and more yellow coloration (higher  $b^*$  value). However, in the respective setup, visible discolorations were not induced. Interestingly, both zirconia specimens induced similar increased brightness values, although the dyed specimen was darker. This phenomenon may be explained by the fluorescent properties of the dyed specimen.

The present findings are in concordance with the results reported by Jung et al,<sup>10</sup> who found significant discolorations induced by titanium that led to a mean  $\Delta E$  of 5.06 under mucosa that was 1.5-mm thick. The mean  $\Delta E$  measured in the present experiment was even greater (7.3). This may be explained by the optical properties of the titanium specimen itself. The  $\Delta E$  values for the two zirconia specimens correlate well with the results published by Jung et al. They reported  $\Delta E$  values of 3.87 for white zirconia and 3.99 for zirconia veneered with A3 dental ceramic under 1.5-mm-thick mucosa. Unfortunately, that article does not report on the optical properties of the veneering ceramic. The results of the present experiment show only slightly different results for the zirconia specimens. Since undyed

zirconia induced a mean  $\Delta E$  of 3.7 and the dyed fluorescent specimen induced a mean  $\Delta E$  of 3.5, neither specimen caused visible color changes in the specific experimental settings. In the experiment by Jung et al,<sup>10</sup> the "darker" veneered zirconia specimen induced a mean  $\Delta L^*$  of  $-2.08$ , a brightness value that was significantly lower than the control. In the present experiment, however, the "darker" dyed fluorescent specimen induced significantly increased brightness values ( $\Delta L^*$ , 0.97).

These findings may be of importance to clinicians since pure white zirconia is not ideal in thin tissue in terms of optical considerations. A typical clinical problem in implant dentistry is the discoloration of thin marginal mucosa in the esthetic zone.<sup>3,5</sup> White zirconia does not necessarily lead to a better outcome regarding peri-implant soft tissue color<sup>11,26</sup> and does not look natural once it becomes exposed (see Fig 2). Shading the zirconia with a dye leads to a dentin-like appearance. From an esthetic point of view, this may be even more beneficial with respect to a midfacial recession at the abutment collar.

Ishikawa-Nagai et al<sup>11</sup> showed that light orange and light pink are suitable colors to mask restorative materials. This suggests that veneering zirconia abutments with light orange or light pink dental porcelain may lead to a better optical soft tissue outcome. However, the literature reports a lower biocompatibility of dental porcelain as an abutment material than zirconia.<sup>27</sup>

Since previous studies have suggested that the thickness of the soft tissue influences shine-through effects,<sup>10</sup> soft tissue thickening with connective tissue grafts may be another approach to address this issue. A case series<sup>28</sup> showed encouraging results in thickening peri-implant soft tissue over a 3-month period. In a prospective clinical cohort study, 10 patients exhibiting midfacial recession around dental implants were treated with a coronally advanced flap and connective tissue graft. Although a substantial, clinically significant improvement was reported at all treated sites, shrinkage of the initially achieved coverage of 66% at the 6-month follow-up was reported.<sup>29</sup>

The fact that pig jaws and not human tissue were used in this study and that nonvital tissues were assessed must be taken into account as limitations of this study. However, this in vitro model was chosen since it was reported in the literature as a valid model for assessing color changes induced by restorative materials under oral mucosa.<sup>10</sup> This study offers the chance to verify and compare the results of Jung et al<sup>10</sup> with the presented data and to examine the influence of dyed zirconia on oral mucosa of 1.5-mm thickness.

Although spectrophotometric measurement of the two zirconia specimens revealed a significantly high color difference of 10.35, these two specimens did not induce a significant overall color difference between them. This may be a result of the influence of the fluorescent properties of the "darker" specimen.

## Conclusions

In this experiment, shading of the zirconia with the fluorescent dye led to a tooth-colored appearance that did not induce visible color changes to mucosa of 1.5-mm thickness. Therefore, it may be concluded that shading white zirconia with a fluorescent dye leads to an all-ceramic abutment material that mimics the optical properties of natural teeth and does not induce visible color changes to thin mucosa in vitro. Further clinical studies are necessary to evaluate this effect on vital tissue in humans.

## Acknowledgment

The authors reported no conflicts of interest related to this study.

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