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Assessment of tooth wear based on autofluorescence properties measured using the QLF technology *in vitro*

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ABSTRACT

Background: The difference in autofluorescence between enamel and dentine layer has prompted recommendations to use the quantitative light-induced fluorescence (QLF) method for quantifying tooth wear (TW). This study investigated the potential of QLF for distinguishing the severity of occlusal TW based on differences in the autofluorescence intensity.

Methods: In total, 106 extracted permanent molars and premolars having suspected wear without pulp exposure were used. The severity of wear was determined by visually examining all teeth using the tooth wear index (TWI) of Smith and Knight. QLF images were captured and converted into 8-bit grayscale images. The difference in the fluorescence intensity (ΔG) was calculated by comparing mean grayscale levels between sound and worn areas. Finally, histological examination was conducted by stereomicroscope to confirm the presence of dentine exposure.

Results: 100 teeth were included in the final analysis without six teeth having enamel cracks around worn area. The ΔG values increased with the severity of TW as quantified using conventional TWI codes, and differed significantly between the sound and enamel- and dentine-wear teeth ($P < 0.001$). The histology indicated that enamel remained on 57 teeth, while 43 teeth had dentine-exposed wear and showed significant differences in ΔG compared with enamel-remained teeth.

Conclusions: The fluorescence intensity differed significantly depending on the presence of dentine exposure. ΔG could be used to distinguish between sound and enamel- and dentine-wear teeth with a significant correlation. These findings indicate that QLF could be useful for determining the severity of TW of occlusal surfaces non-invasively.

1. Introduction

Tooth wear (TW) is a physiological process associated with the loss of hard tissues on tooth surfaces due to interactions of both physical (e.g., abrasion, attrition, and abfraction) and chemical (e.g., erosion) factors [1]. The accumulation of excessive physiological wear can result in the patient experiencing discomfort, with dysfunction of dentition and abnormality of appearance being considered pathological TW [2,3]. Pathological TW results in cusps becoming rounded and the crown height shortening, thereby decreasing the vertical dimension of occlusion. Possible complications of severe TW—when the dentine or pulp become exposed—are dentinal hypersensitivity and acute pain. Another aspect of severe TW, the loss of the cusp, can also dramatically impair mastication performance [4].

The prevalence of TW increases with age due to the irreversible progression of TW throughout the life span [5,6]. However, recently there has been an increasing number of TW cases in children and adolescents. This is probably attributable to the increased intake of acidic foodstuffs, which is likely to have an effect on TW caused by erosion [7,8]. Moreover, young subjects also suffer more from bruxism or clenching caused by the emotional states such as stress and anxiety in modern societies, which can further increase the prevalence of TW [9]. Ongoing improvements in life expectancy and quality of life are resulting in people keeping their natural teeth for longer, with this

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increase in remaining teeth further leading to an increase in the number of TW cases [10,11]. This situation means that TW is now a common symptom in all age groups.

The wearing of tooth surfaces generally slowly and asymptomatically, with patients therefore not becoming aware of the presence of TW. Patients often do not recognize the need for treatment until an advanced stage due to discomfort or pain, but at that point it is often too late to manage the progressed TW [12]. Recovery from the irreversible loss of tooth tissue requires restorative treatment, and various complications caused by TW complicate the associated treatment planning. Considering this situation, providing preventive interventions through early detection can be useful for successfully managing TW [3,12], and hence distinguishing pathological TW from worn teeth via early diagnosis and screening is important [11].

A qualitative tooth wear index (TWI) based on visual examinations has been conventionally used for the diagnosis of TW. Such an index scores the severity of TW using clinical descriptions such as remaining enamel and dentine exposure [13]. However, the criteria for quantifying the severity of worn enamel and the presence of dentine exposure vary widely between the various indices, and subjective evaluations have many limitations due to the consistency and accuracy of the examiners despite the application of training and calibration [14]. While several TW indices have been developed, there is still no consensus on an ideal method for TW examinations [13,15], and so a standardized method for the objective evaluation of TW remains to be developed.

To overcome the subjectivity and limitations of conventional qualitative TWIs, various technologies for evaluating TW based on objective information about tooth structures have been suggested [16]. One representative method is optical coherence tomography (OCT), which yields cross-sectional images of internal biological structures non-invasively using near-infrared light (wavelength of 1310 nm), and it is now actively used in dentistry. This technology utilizes the different scattering properties of enamel and dentine, and internal teeth can be imaged by observing the backscattered signals of dental hard tissue [17,18]. OCT has the advantage that the enamel thickness and the presence of dentine exposure can be determined in real-time; however, the scanning range of OCT is only a few millimeters, which is insufficient for screening the entire dentition. Moreover, obtaining complete images is time-consuming, and the used of rapid image acquisition can degrade the image quality [18].

Optical methods that utilize the fluorescence properties of teeth have been used in various fields of dentistry in recent years [19–23]. One representative method is quantitative light-induced fluorescence (QLF), which involves detecting the autofluorescence emitted from teeth during illumination by near-ultraviolet light (wavelength of 405 nm) with the aid of a long-pass filter ($>$ 520 nm). This technology is typically based on previous reports that teeth emit blue fluorescence under ultraviolet illumination, and that the fluorescence intensity differs between enamel, dentine and cementum [24–26]. In particular, dentine contains much more organic material (i.e., fluorophores), which results in a much brighter fluorescence signal than that of enamel [24]. This fluorescence characteristic can be identified clinically in regions of suspected dentine exposure on occlusal surfaces when observing the entire dentition of a patient using QLF. Moreover, a previous study showed that the fluorescence intensity of worn enamel increased monotonically as the sound tooth was ground down for the artificial formation of mechanical TW [27]. However, previous studies of fluorescence differences of worn teeth have only focused on enamel wear in artificial models *in vitro*, with there being no previous investigations of the fluorescence differences of real worn human teeth. The aim of the present study was therefore to quantify natural tooth wear by using the autofluorescence intensity, measured by QLF. Moreover, the potential of QLF for distinguishing the severity of TW was investigated by comparing the fluorescence differences according to conventional TWI which is widely used as clinical gold standard.

2. Materials and methods

2.1. Selection and preparation of tooth samples

This study was conducted after obtaining ethical approval from the Institutional Review Board for Clinical Research at Yonsei Dental Hospital (IRB No. 2-2015-0032). Freshly extracted permanent teeth were collected after obtaining written informed consent from subjects who were older than 20 years. In total, 106 permanent molars and premolars without dental caries and restorations were selected from a pool of extracted human teeth without pulp exposure. The teeth were cleaned of plaque, calculus, and soft tissue using hand scalers and toothbrushes as soon as possible after being extracted. The cleaned teeth were stored at $-20\,^{\circ}C$ until being assessed [28]. All teeth were positioned perpendicularly in acrylic molds with resin (Jet^{TM} Tooth Shade, Lang dental Manufacturing, Wheeling, IL, USA) so that the height of all specimens was the same.

2.2. Visual examinations of worn teeth

After completing specimen preparation, a blinded examiner assessed all teeth visually with the naked eye according to the conventional TWI of Smith and Knight, which has been widely used for the assessment of tooth wear as clinical gold standard [11]. Two examiners were continuously trained and calibrated with respect to the TWI described in the previous studies [11,13]. When the intra- and inter-examiner reproducibility values of two examiners showed a good agreement of Kappa statistic (0.61–0.80), one examiner performed the main examination. Due to the use of teeth without pulp exposure, this study did not include any teeth classified as TWI code 4 (which represent pulp-exposed worn teeth). Descriptions of only the occlusal aspects of TWI were used as follows: no loss of enamel surface characteristics (TWI code 0), loss of enamel surface characteristics (TWI code 1), loss of enamel resulting in exposure of dentine over less than one-third of the surface (TWI code 2), and loss of enamel resulting in exposure of dentine over more than one-third of the surface (TWI code 3). In cases where two adjacent codes could not be decided upon during the examination, the lower code was assigned.

2.3. Acquisition of QLF images

The quantitative light-induced fluorescence–digital (QLF-D) Biluminator^{TM} 2+ (Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to obtain images of the teeth. The distance from the camera to the specimens was fixed, and white-light and fluorescence images were successively captured from the occlusal aspects of all tooth specimens while ambient light was blocked (Fig. 1A). Proprietary software (C3 version 1.25, Inspektor Research Systems BV) was used to acquire fluorescence and white-light images under the following conditions: shutter speeds of 1/50 s and 1/60 s, aperture values of 9.5 and 11.0, and ISO speeds of 1600 and 1600, respectively.

2.4. Quantitative image analysis

All of the QLF images were analyzed using image software (ImageJ version 1.51o, National Institutes of Health, Bethesda, MD, USA). All of the fluorescence images were converted into 8-bit grayscale images for quantifying the fluorescence intensities as grayscale levels. Suspected areas on each occlusal tooth surface were visually examined to decide the area of interest (AOI). Analysis patch of suspected worn area (AOI_{worn}) was contoured along the border of the plane surface (wear facet) in the white-light image. The patch of sound area (AOI_{sound}), meanwhile, was drawn on one adjacent areas of AOI_{worn} showing no wear. The patches were stored in a database using the AOI manager of the image software, and then applied to the respect 8-bit grayscale images to obtain the grayscale values of AOIs of all pixels (Fig. 1B).

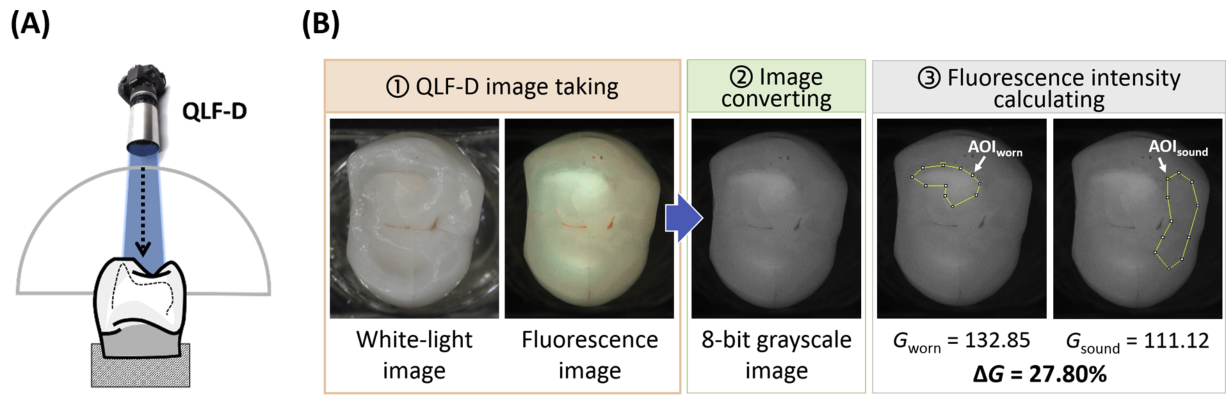

Fig. 1. (A) Image acquisition process using a quantitative light-induced fluorescence-digital (QLF-D) and (B) quantitative analysis process. AOI, area of interest; G_{worn}, the mean grayscale value in the worn area; G_{sound}, the mean grayscale value in the sound area; ΔG, the difference in the fluorescence intensity between worn and sound area.

The mean grayscale values in both AOI_{worn} and AOI_{sound} were measured to determine the changes in fluorescence intensity of each tooth. The difference in the fluorescence intensity of each tooth between worn and sound areas was calculated as:

$$\Delta G\ (\%) = \frac{G_{worn} - G_{sound}}{G_{sound}} \times 100$$

where G_{worn} is the mean grayscale value in the worn area (AOI_{worn}) and G_{sound} is the mean grayscale value in the sound area (AOI_{sound}).

The reliability of calculating ΔG was determined with the intra-class correlation coefficient and was found to be 0.724 ($P < 0.0001$).

2.5. Histology

After finishing the image analysis, the optimal cross-sectional line was determined using a swept-source optical coherence tomography (SS − OCT) system (prototype, LG Electronics, Seoul, Republic of Korea) with Santec HSL-20-100 L laser (30 mW mean power; center wavelength 1310 ± 50 nm). All cross-sectional views of the teeth was observed by moving the OCT probe from the distal to the mesial direction. When the distance between enamel surface and dentinoenamel junction (DEJ) appeared to be smallest, the target line for sectioning was established. All teeth were cut perpendicularly into two halves with a microtome (TechCut 4^{TM}, Allied High Tech Products, CA, USA). The cross-sectioned surfaces were then photographed under a stereomicroscope interlaced camera (Leica S9i stereozoom, Leica, St. Gallen, Switzerland) with dedicated image software (LAS version 4.12.0, Leica) at a magnification of 40×. The microscopic images were histologically assessed for confirming the existence of dentine exposure.

2.6. Statistical analysis

To evaluate the reproducibility of visual TWI examination before the main study, intra- and inter-examiner reliability were assessed using the Kappa statistic. The intra-class correlation coefficient (ICC) was also assessed for the quantitative image analysis. The normality of all variables was confirmed using the Kolmogorov-Smirnov test. The median values of fluorescence difference (ΔG) were compared according to the wear severity based on TWI scores using the Kruskal–Wallis test and Mann–Whitney post hoc test. Correlations between TWI scores and ΔG values were quantified using Spearman's rank correlation (ρ) test to determine the fluorescence tendency according to the severity of TW. The comparison of ΔG values between absence and presence of dentine exposure was conducted by the Mann–Whitney test. All statistical analyses were implemented using the Statistical Package for the Social Sciences (SPSS) (version 23.0, SPSS, Chicago, IL, USA), with a significance cutoff (α) of 0.05.

3. Results

As 6 damaged teeth due to enamel cracks around worn area were discarded, 100 teeth were finally analyzed. TWI codes 0, 1, 2, and 3 for distinguishing the wear severity were assigned to 13, 40, 35, and 12 teeth, respectively (Table 1).

The fluorescence intensity tended to increase significantly with the TW severity (Fig. 2). The fluorescence difference (ΔG) values versus the TWI codes are shown in the box-and-whisker plots (Fig. 3). Median values of ΔG for sound teeth (code 0) and enamel-wear teeth (code 1) were −2.80% and 4.01%, respectively ($P < 0.001$). Teeth quantified as TWI codes 2 and 3 (which were dentine-wear teeth) had ΔG values of 9.47% and 17.67%, respectively, which were significantly higher fluorescence intensities compare to groups of codes 0 and 1 ($P < 0.001$). However, ΔG did not differ significantly between codes 2 and 3 for dentine-wear teeth. A moderate positive correlation was identified between ΔG and the TW severity (ρ = 0.55, $P < 0.001$).

When all teeth were dichotomized into 2 categories according to the histological presence of dentine exposure, there were 57 enamel-remained teeth (which were sound or had some enamel wear) and 43 teeth had dentine exposure (Table 1). Histology was in discord with TWI results (clinical gold standard) in some samples assigned to TWI codes 1 (one in 40 teeth) and 2 (five of the 35 teeth). Compared to sound parts without TW, enamel-remained teeth exhibited fluorescence increases of up to 1.89% (Table 2). Meanwhile, dentine-exposed teeth showed ΔG values of up to 9.75%, which differed significantly from the enamel-remained teeth ($P < 0.001$).

4. Discussion

This study evaluated whether differences in the fluorescence intensity of teeth as quantified using the QLF technology is effective for distinguishing the severity of TW. This study evaluated extracted human teeth having questionable or real wear, and confirmed that the

Table 1
Distribution of examined teeth samples according to the tooth wear index (TWI) and the existence of dentine exposure from histology results.

		Type of wear from histology		Total
		Enamel-remained teeth	Dentine-exposed teeth	
Tooth wear index	Code 0	13	–	13
	Code 1	39	1	40
	Code 2	5	30	35
	Code 3	–	12	12
Total		57	43	100

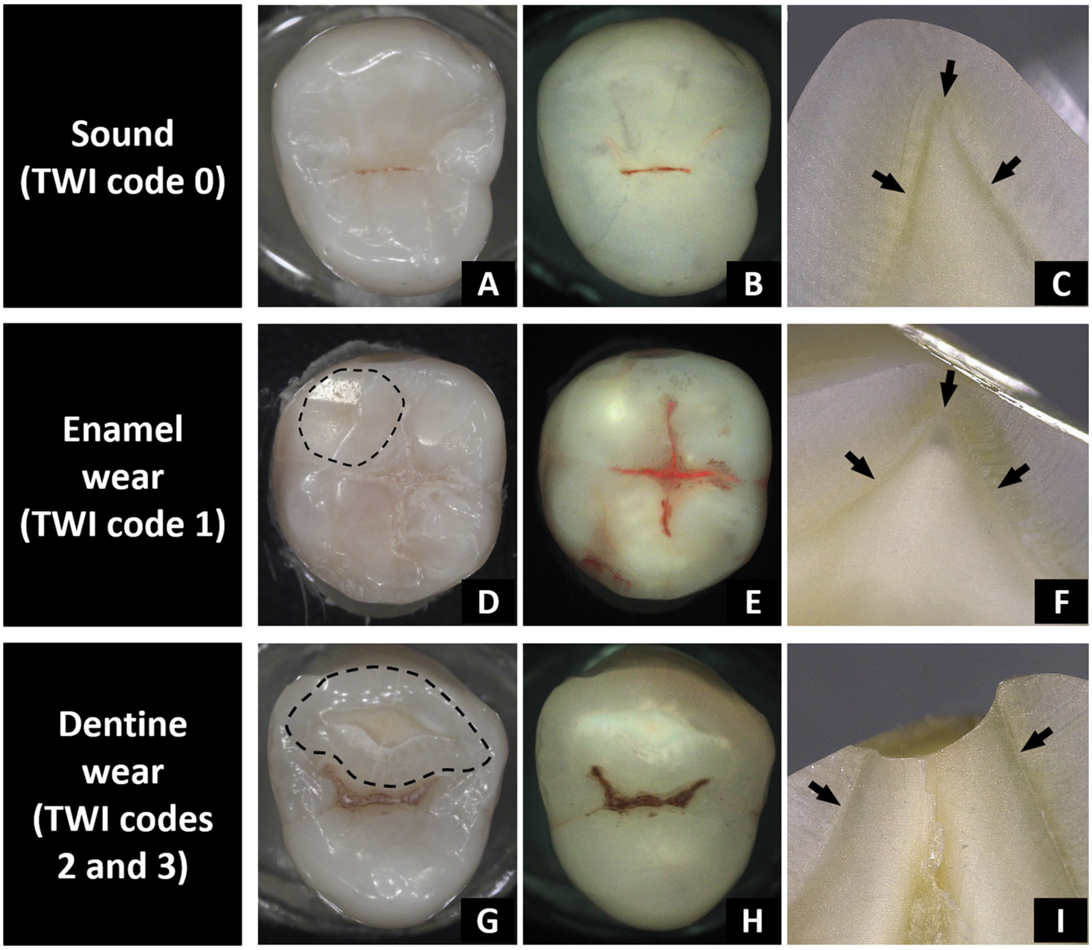

Fig. 2. Representative images of tooth wear: QLF images obtained under white-light illumination (A, D, G) and blue-light illumination (B, E, H), and the respective stereomicroscope images (C, F, I). TWI, tooth wear index. Dashed line encircles the wear facet and the AOI, and arrow represents the dentinoenamel junction (DEJ).

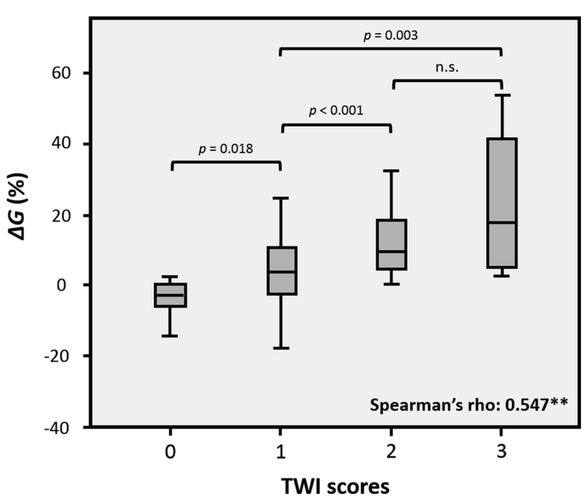

Fig. 3. Comparison of difference in fluorescence intensity (ΔG) related to the tooth wear index (TWI) scores in box-and-whisker plots. The boxes show the first quartile (lower line), median (middle line), and third quartile (upper line). The *P*-values indicate statistically significant differences between each set of scores (Kruskal–Wallis test with post hoc Mann–Whitney U tests). *, $P < 0.001$; n.s., non significant.

fluorescence intensity in worn area increased with the TW severity.

This study distinguished the severity of TW using the TWI of Smith and Knight, which has been widely used in both clinical and epidemiological fields of dentistry. The present results indicate that the difference in the fluorescence intensity increased as TW severity progressed from sound (code 0) to enamel wear (code 1) and dentine wear (code 2 and 3), with a moderate correlation coefficient of 0.55 and a significant difference between the groups ($P < 0.001$, Fig. 3). However, there was no significant difference between dentine wear coded as 2 and 3. The reason may be the high variation of the ΔG with code 3 samples. Code 3 samples sometimes have a discolored area which may affect to the results. Another reason of the small difference of ΔG between code 2 and 3 samples could be due to the criteria of Smith and Knight TWI scoring system differentiating code 2 and 3, which considers only the extent of the exposed area of the dentine. A previous study also found that the Smith and Knight TWI does not differentiate dentine wear (code 2 and 3) mainly due to the wide variation of the area of the exposed dentine surface [16].

The presence of dentine exposure is commonly used as an indicator for evaluating TW, and the exposed dentine visually appears as

Table 2
Difference in fluorescence intensity (ΔG) according to the existence of dentine exposure from histology results.

	Type of wear from histology (*N*)		*P
	Enamel-remained teeth (57)	Dentine-exposed teeth (39)	
ΔG (%)	1.89 (−4.36–8.75)	9.75 (4.64–20.03)	< 0.001

Data are median (first quartile to third quartile) values.
* Mann–Whitney test.

yellowish or brownish [13,14]. Using this feature for the TWI indicated that 47 of the 100 teeth exhibited exposed dentine wear, which was more than the 43 real dentine-exposed teeth when observing the cross sections of teeth by stereomicroscope. This outcome is consistent with a previous report that visual TW examination and histological results different depending on how exposed dentine is distinguished [14]. Although TWI was originally developed for identifying dentine exposure, its accuracy has been restricted by the subjectivity of different examiners [16,29]. On the other hand, QLF data showed clear significant differences in the fluorescence intensity depending on the presence of dentine exposure, with the fluorescence in the exposed dentine area being increased by up to 9.75% compared with the sound area, and also significantly higher than that in the worn enamel area (1.89%) ($P < 0.001$, Table 2). These results are supported by those of a previous study that constructed a TW model artificially *in vitro* and confirmed that the fluorescence intensity increased dramatically at the point where the dentine layer was exposed. The QLF technology as used in the present study may be effective at detecting the presence of dentine exposure because the dentine layer fluoresces up to 4 times more brightly than the enamel layer due to its high fluorophore content [24,25].

This study calculated the fluorescence intensity as a grayscale level in order to facilitate quantifications of the TW severity, and found that small fluorescence changes according to TW severity could be presented by comparing grayscale values between TW regions and sound areas. When the fluorescence images obtained using the QLF technology were converted into 8-bit grayscale images, the fluorescence intensity of teeth as quantified from 0 (black) to 255 (white) was effective for distinguishing between sound enamel, worn surfaces, and restorative and adhesive resin materials [26,27,30]. Previous studies calculated only the absolute value in the AOI and confirmed simple comparisons or trends, whereas this study quantified the TW severity as percentages by calculating the ratio of the brightness difference between sound and worn areas. The obtained results indicate that both patients and dental professionals can understand the presence and severity of TW intuitively using only fluorescence images in the clinical situation.

The present study used OCT before performing histological evaluations of cut teeth. Our approach made real-time observations of all cross-sectional surfaces possible, and we could readily determine the location where the enamel was thinnest. As a result, we could minimize errors from selection bias because selected tissue slides cannot represent an entire tooth. In the case of enamel wear, however, it was difficult to distinguish between physiological and pathological wear through cross-sectional observations. In order to distinguish pathological from physiological wear, a longitudinal observational study is needed to examine pathological wear based on the wear rate, which is a key factor for evaluating TW. In a previous study we generated artificial TW that ranged from a sound baseline to dentine exposure in a step-by-step manner, and confirmed that the fluorescence intensity increased by an average of 4.00% when the thickness of the enamel layer was reduced by 100 μm [27]. Considering that the annual rate of progression of physiological TW is about 15 μm for premolars and 29 μm for molars [31], the present results indicate that changes in fluorescence intensity as detected using a QLF device can be used to identify the wear rate and pathological status of worn teeth. Moreover, the conventional TWI has the limitation that it cannot be used for the diagnosis and monitoring of early TW [29], while the early stage of wear can be evaluated using QLF since subtle fluorescence changes according to enamel thickness can be quantified [32].

In this study, for relative calculating of fluorescence changes, the contours around the worn and sound areas were manually determined. The examiners had to be fully trained and calibrated to produce consistent analysis outcomes. As a result, the calculated ΔG between the two examiners showed a good agreement (intra-class correlation coefficient = 0.724, $P < 0.0001$). In the future, automatic fluorescence analysis algorithms (e.g., auto calculation of the sound and/or worn area of fluorescence intensity) will be needed to reduce errors due to manually contouring introduced by the human factor.

To our knowledge this is the first study to apply the QLF technology to investigate human teeth exhibiting various patterns of real TW. The present study assessed the occlusal aspects of wear firstly, therefore TW existing in other surfaces such as cervical and incisal/buccal sites should be investigated in the next steps. Particularly cervical area is vulnerable to wear and strongly related to dentine hypersensitivity [33]. As the paradigm of restorative treatment for managing TW has shifted to minimum-intervention approaches, recognition of TW at an early stage and preventing progression to the advanced stage are the most effective approaches [34]. All of the results obtained in the present study support that the fluorescence intensity of QLF can be used to determine TW of all tooth surfaces and quantitatively monitor TW from early to advanced stage. The QLF technology uses harmless visible light and noninvasively acquires real-time fluorescence images of teeth within the oral cavity. It also has advantages in reducing the cost and time for image acquisition, allowing direct examination of patients, and facilitating objective comparative analyses of TW severity by quantifying QLF images acquired over a long timescale. Considering these strengths, QLF may be an effective diagnostic tool for TW that augment the conventional TWI and evaluation methods. Future studies should attempt to compare worn teeth with various characteristics based on their etiologies and ages in order to clinically validate the efficacy of the QLF technology in determining and monitoring TW.

5. Conclusions

This study found that differences in fluorescence intensity between sound and worn areas gradually increased with the progression of wear. The fluorescence intensity differed significantly depending on the TW severity, with QLF being demonstrably useful for distinguishing between worn enamel and dentine.

It can be concluded that QLF is a useful tool for the quantitative diagnosis of TW. Future clinical validations may reveal that QLF technology is a superior method for objectively evaluating and monitoring TW.

Conflicts of interest

The authors declare that there are no conflicts of interest related to the present study.

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