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ORIGINAL ARTICLE

Multiple sleep bruxism data collected using a self-contained EMG detector/analyzer system in asymptomatic healthy subjects

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Abstract
Purpose Small, self-contained electromyographic (EMG) detector/analyzer (D/A) devices have become available for the detection of jaw muscle activity events above threshold. These devices claim to be less intrusive to the subjects sleep so it is less prone to induce disturbed sleep. The objective of this study was to evaluate for night-to-night variability and examine for a systematic alteration on the first night in EMG levels.
Methods Ten asymptomatic healthy volunteers (mean age, 26.8±3.78) were recorded for six sequential nights in their home environment using EMG D/A system. The device yields a nightly EMG level above threshold score on a 0–4 level. Because the data are categorical and nonparametric, the data of the ten subjects across six nights were submitted to a Friedman repeated measures ANOVA. The significant level was set as alpha equal to 0.05.
Results The median and mode values of the subjects were tabulated and analyzed and we did not find a significant difference in EMG D/A level across the six nights (p=0.287, Kendall's coefficient of concordance=0.124, Friedman two-way repeated measures ANOVA). The data did show clear and substantial night-to-night variability.
Conclusion Substantial night-to-night variability in masseter EMG activity levels was clearly observed in our subjects. There was no evidence of a suppressed or elevated first-night effect-like variability on masseter muscle EMG level seen in these subjects using a small portable self-contained EMG detector/analyzer. These data suggest that recordings should be at least 5–6-nights duration to establish a reasonable measure of an individual's average nightly masseter EMG level.

Keywords Sleep bruxism · Portable device · Electromyography · First-night effect-like variability · Validation study · Clinical assessment

Introduction

Polysomnography (PSG) measures a variety of physiologic signals from patients throughout the night at a sleep laboratory and is commonly used to record bruxism levels in subjects [1]. One unavoidable drawback with a polysomnographic recording is that there are multiple physiologic sensors being attached to the subject. All these sensors have wires attached to them thus increasing the potential that they would interfere with the subject's sleep pattern. Moreover, these recordings usually are done in a foreign sleeping environment which also disturbs the subjects sleep. Some subjects are unable to achieve a natural sleep pattern during a polysomnographic recording and thus if sleep is achieved it results in what has been called the first-night effect (FNE) [2]. The FNE has been

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described in several studies and it includes the following characteristics: less total sleep time and rapid eye movement (REM) sleep, lower sleep efficiency index, more intermittent wake time, and longer REM latency [3–14]. These FNE studies have generally looked at how intrusive polysomnograph is on parameters of sleep other than periodic motor events such as leg or jaw contractions. It is generally presumed that the pattern of sleep bruxism would be considerable altered by this phenomena also, but little actual data exist to substantiate or refute this assumption.

Another drawback of polysomnography as it relates to the recording of bruxism is that even if a patient has two nights of sleep recorded in a sleep laboratory, it may not capture the typical bruxism level of an individual subject, largely because sleep bruxism has a substantial night-to-night variability [15, 16]. For example, 1 study examined the variability of sleep bruxism (SB) oromotor, masseter, and temporalis activities examining 37 nights of recording collected from 9 subjects by using audio-polysomnographic recordings. Unfortunately these data did not provide consecutive night recordings and the interval between these recording varied from weeks to months.

Several portable wireless-based devices have been developed to record EMG data that are used in the subject home sleeping environment [17–21]. One such system did report masseter muscle EMG recording for six consecutive nights in the subjects' home [22]. This study collected data from 103 healthy adult subjects (age range, 22 to 32 years), but unfortunately, the authors did not examine the data for, or report on the level of night-to-night variability. Using this same equipment, another report examined the reproducibility of the system, but again did not analyze the night-to-night variability or examine if a true FNE could be seen [23].

Portable, in-home recording devices are logical when polysomnography is not possible (due to expense or availability) or not needed for the question being asked. In theory, these systems should be less intrusive to the subjects thus less likely to induce a substantial sleep disturbance than a full polysomnographic recording is. This study was designed to (1) verify the presence of FNE-like variability in SB assessment by means of a portable EMG miniature device and (2) examine the degree of night-to-night variability across six sequential nights in asymptomatic healthy subjects. The null hypothesis for the study was that there was no systematic FNE-like variability in the recording series.

Materials and methods

Study subjects

Ten asymptomatic healthy volunteers (five females and five males; mean age, 25.3±1.32 years) were randomly selected from the pooled subjects who had been recorded previously using an ambulatory telemeter nocturnal masseter muscle electromyography pulse video recording system. The details of these subjects were four mild bruxisers (one male, three female), four moderate bruxisers (two male, two female), and two severe bruxisers (two males). The criterion of bruxiser was based on the number of rhythmic masseter muscle activity (RMMA) which was set as the 10% of maximum voluntary clenching, and the phasic type of bruxism episode consisted of at least three EMG bursts of 0.25 to 2.0-s duration with less than 3.0 s separation, and the tonic type to a single burst lasting more than 2.0 s. The criteria of mild/moderate/severe bruxiser as based on the number of RMMA per hour (less than 2.3, 2.3–5.3, and more than 5.3, respectively). All subjects had had a full clinical examination of orofacial region and magnetic resonance imaging (MRI) of their temporomandibular joints (TMJ). Inclusion criteria were those: (1) willing to participate in this study and (2) with neither palpation pain nor disk displacement on at least one side of their TMJ by MRI findings. Exclusion criteria were: (1) using any oral device, (2) taking one or more medications, (3) any medically diagnosed sleep disorders, or (4) compromised mental or physical ability. The study and its requirements were explained to all subjects and subjects were asked to sign the consent form. This study protocol was approved by the Ethical Committee for Human Research in Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (#69, 173).

EMG D/A system

The device used in this study was a self-contained EMG D/A system that consisted of EMG electrodes, an amplifier to acquire masticatory muscle signals, a small central processing unit with real-time software that analyzes the EMG patterns (S.L.P. Ltd, Israel). This device counted the number of masseter muscle EMG signal elevations above threshold. Previous reports demonstrated that this device had sufficient validity to detect SB event, equivalently to PSG assessment [24]. Furthermore, this device automatically started to measure the muscle hyperactivities after 30 min when the sensor had detected the cheek skin. Thus, the measurement period was set as 4.5 h exactly, and the accumulated number of SB events was not affected by the sleeping period variation. This device indicates the total number of SB events on a four-grade scale: L, 1, 2, and 3 (less than 30 events, 30–59 events, 60–99 events, and more than 100 events per 4.5 h, respectively). These outcome ratings have been translated as 0, 1, 2, and 3 for descriptive and analytic purposes in this study. All participants were handed this device directly and explained the usage in their home environment by using a mirror and an instruction

manual over 15 min by a pre-trained instructors. The eligible ten subjects were asked to apply the EMG D/A on their left cheek skin surface for six sequential nights. They were also asked to sleep more than 5 h per night, as well as to avoid irregular life events such as alcohol ingestion.

Data analytics

The median and mode value from the six sequential SB scores were tabulated and the median and mode values were calculated for each subject. Because the outcome data of our recording system is categorical (0–3), the data were considered nonparametric, and we used the Friedman repeated measures ANOVA to compare for any consistent effect across the six recording nights [25]. The significant level was set as alpha equal to 0.05.

Results

The ten participating subjects in this experiment included five females and five male. All subjects were healthy and the mean age of these subjects was 26.8±3.8 years. In Table 1 are the night-to-night EMG D/A data scores collected for our ten subjects and the median and mode values for each consecutive night of recording. Our analysis for a FNE-like variability showed no significant score difference in score levels across the recording period (p=0.287, Kendall coefficient of concordance=0.124, Friedman two-way repeated measures ANOVA).

Discussion

This study examined and did not find a consistent alteration (higher or lower) in bruxism levels during the first night when using a portable bruxism detection device for six sequential nights on ten subjects. While this study is not the first study to record six nights consecutively with portable equipment, it is the first to examine the data for any systematic suppression of the bruxism level on the first or second night. Our data suggests that no systematic lower (or higher) level of sleep bruxism exists on the first or second night in our subjects. This could be due to two reasons. First, the level of night-to-night bruxism variation is much larger than any suppressive effect the recording process has on it during the first or second night so it is not seen. Second, in this study we utilized a small wireless, self-contained EMG-based detector/analyzer, and we had our subjects sleep in their own home sleep environment (i.e., their own bed). The relative intrusiveness of this method of recording is undoubtedly much lower than a polysomnographic study performed in a sleep laboratory.

The main weakness of our own study is that the recorder we used is a relatively low-resolution recorder in that the EMG data it collects is reported only using a four-point scale (0–3). Obviously a more detailed reported of all EMG events above threshold would be helpful. Given the clearly evident night-to-night variability of the sleep bruxism behavior, daily diary data need to be collected to see if any association is evident between the behavior and the recorded level. For example, alcohol, caffeine, and nicotine intake was not monitored and these agents are widely recognized as potential confounders to the EMG duration of masticatory muscle hyperactivity [26].

Another weak point of this study and of a self-contained EMG D/A system was that it could not measure the EEG by itself. Generally, FNE was defined by the alteration of brain wave (EEG)-based phenomenon; therefore, the result of this study would not indicate the FNE, strictly. Thus, we applied the term of "first-night effect-like variability" instead of FNE in this report.

In summary, we did not see a first or second night suppression or elevation of the pattern of sleep bruxism in

Table 1 Results of night-to-night EMG D/A data scores

PSG assessment			1st	2nd	3rd	4th	5th	6th
Mild	Subject #1	Male	3	3	3	2	3	3
Mild	Subject #2	Female	2	2	3	2	2	3
Mild	Subject #3	Female	3	1	0	3	3	3
Mild	Subject #4	Female	0	0	1	1	1	3
Moderate	Subject #5	Male	1	2	1	1	0	0
Moderate	Subject #6	Male	0	0	1	1	1	2
Moderate	Subject #7	Female	2	3	3	3	2	2
Moderate	Subject #8	Female	3	0	0	3	3	3
Severe	Subject #9	Male	3	3	3	3	3	3
Severe	Subject #10	Male	0	1	2	2	1	3
		Mode	3	3	3	2	3	3
		Median	2	1.5	1.5	2	2	3

p=0.287, Kendall's coefficient of concordance=0.124, Friedman two-way repeated measures ANOVA

our study of six consecutive nights of sleep. We did see a substantial night-to-night variability of or scores suggesting that multiple night of sleep recording might be needed to capture the true level of this behavior on a single individual.

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Real time assessment of surface interactions with a titanium passivation layer by surface plasmon resonance

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ABSTRACT

Due to the high corrosion resistance and strength to density ratio titanium is widely used in industry, and also in a gamut of medical applications. Here we report for the first time on our development of a titanium passivation layer sensor that makes use of surface plasmon resonance (SPR). The deposited titanium metal layer on the sensor was passivated in air, similarly to titanium medical devices. Our "Ti-SPR sensor" enables analysis of biomolecule interactions with the passivated surface of titanium in real time. As a proof of concept, corrosion of a titanium passivation layer exposed to acid was monitored in real time. The Ti-SPR sensor can also accurately measure the time-dependence of protein adsorption onto the titanium passivation layer at sub-nanogram per square millimeter accuracy. Besides such SPR analyses, SPR imaging (SPRI) enables real time assessment of chemical surface processes that occur simultaneously at "multiple independent spots" on the Ti-SPR sensor, such as acid corrosion or adhesion of cells. Our Ti-SPR sensor will therefore be very useful to study titanium corrosion phenomena and biomolecular titanium–surface interactions with application in a broad range of industrial and biomedical fields.

1. Introduction

Thanks to its high strength to density, excellent corrosion resistance, decreasing cost, and increasing availability, titanium and its alloys enjoy widespread industrial applications in a wide variety of highly corrosive environments, including sea water, bleaches, alkaline solutions, oxidizing agents, and organic acids [1]. These excellent properties mean that titanium is widely used in industries including aerospace, marine, power generation, and desalination plants, for instance [2–5]. Its extremely high corrosion resistance results from the formation of a very stable, continuous, highly adherent, and protective oxide film on the titanium surface, formed spontaneously and instantly once fresh metal surfaces are exposed to air or moisture.

Titanium is also commonly used to fabricate a variety of medical devices such as hip and knee joints, bone screws and plates, dental implants, stents, pacemaker cases and centrifugal pumps in artificial hearts [6–8]. Due to rapidly aging populations, especially in developed countries, national health care costs are escalating. In particular, the increased incidence of hard tissue and cardiovascular diseases such as periodontitis, osteoarthritis, and arteriostenosis is strongly correlated with the rapidly growing elderly population. Therefore, the development of innovative treatment techniques for functional repair or complete cure of these diseases is highly desirable. In attempts to improve the healing potential of such medical device, much research has been devoted to titanium surface modification methods that enable controlled adsorption of biomolecules and ions or regulated drug release [9–12]. In biomaterial sciences the strategic importance of fundamental research in nanobiotechnology has recently been acknowledged [13]. The development of highly sensitive methods that can monitor the interaction of biomolecules at titanium surfaces are therefore needed.

Surface plasmon resonance (SPR) can offer real time and label-free analysis of the interfacial events that occur on the surface of a metal layer under physiological conditions [14,15]. Recently, the technique of SPR imaging (SPRI) has been developed and applied to monitor the adsorption of organic materials and biomolecules at multiple independent spots [16]. In this study we report for the first time on our development of a titanium passivation surface sensor chip for SPR [17]. There are few reports of titanium SPR (Ti-SPR) sensors in which the titanium metal layer was passivated

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in air. Although many studies of a TiO_2-coated sensor for SPR have been reported [18–23], their sensors were directly coated with TiO_2 and titanium metal was not used. In medicine and dentistry the titanium metal surface of dental implants and artificial bones oxidize in air. Our Ti-SPR sensor has a titanium passivation layer, closely resembling the conditions under which titanium medical devices are normally used during clinical treatment.

2. Experimental section

2.1. Materials

Bovine serum albumin was purchased from Sigma–Aldrich Japan K.K. (Tokyo, Japan). γ-Globulin was purchased from Nacalai Tesque Inc. (Kyoto, Japan). bFGF (recombinant human basic growth factor, KCB-1) was kindly donated by Kaken Pharmaceutical Co. Ltd. (Kyoto, Japan). Dulbecco's phosphate buffered saline without calcium and magnesium (pH 7.4) (PBS) was purchased from Nissui Pharmaceutial Co. Ltd. (Tokyo, Japan). Dodecylphosphate (DDP) was purchased from Alfa Aesar (Ward Hill, MA). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were used as received without any additional purification. Glass plates made of S-LAL10 (refractive index 1.72, diameter 15 mm, thickness 1 mm) were purchased from Arteglass Associates Co. (Kyoto, Japan).

2.2. Surface plasmon resonance instruments

We constructed a SPR instrument which determines the SPR spectrum and the SPR angle shift [24,25]. The SPR instrument, constructed with reference to Knoll's method, utilized the Kretschmann configuration in which the metal was in the form of a thin film mounted directly onto a S-LAL10 glass plate coupled to an S-LAL10 dispersing prism with an index matching fluid [14,26]. An SPR chip was attached to the SPR flow cell, which was 10 mm in length, 1 mm in width, and 1 mm in thickness. Solutions were allowed to pass through the flow cell [25]. The He–Ne laser light ($\lambda = 632.8$ nm) was linearly *p*-polarized using a Gran–Thomson prism and then passed through a non-polarizing cube beam splitter. The sample surface was exposed to the *p*-polarized light through the prism. The intensity of the reflected light was determined by a photodiode detector. A computer was used to control a biaxial rotation stage and to process the intensities of the incident and reflected light as a SPR spectrum. At the angle at which there is a dip in the spectrum the light resonated surface plasmons on the metal layer. This angle is called the SPR angle [14,15].

The SPR-1000 SPRI apparatus (UBM, Kyoto, Japan) employed in this study was developed with reference to our SPRI apparatus [16]. The SPR chip with arrayed spots was mounted on an S-LAL10 dispersing prism with index matching fluid. The flow cell was constructed using a washer made of silicone and a vinyl chloride lid with an inlet and outlet. The back of the chip was illuminated by *p*-polarized, collimated, and polychromatic white light through the prism. The reflected light was passed through an interference filter and collected by a CCD camera. The data was acquired using our in-house designed software.

The Ti-SPR sensor chip can be used in both instruments. All experiments were carried out at 25 °C.

2.3. Development and evaluation of the titanium SPR sensor

2.3.1. Design of the titanium SPR sensor

General SPR sensor chips are based on gold-coated glass substrates. The Ti-SPR sensor chips were prepared by depositing titanium metal on the contamination-free gold surface of the SPR sensor. To design an optimal Ti-SPR sensor it was necessary to consider the thickness and oxidation of the deposited titanium layer and the detection of protein adsorption in water solutions. So the SPR spectra of the Ti-SPR sensor were simulated using the Fresnel equation of reflection and transmission using a prism, glass plate, Cr, Au, Ti, TiO_2, protein, and water multilayer (Fig. 1) to estimate the optimal layer thickness of the deposited titanium [26,27]. When the amount of protein adsorption or the thickness of the TiO_2 layer is changed, the SPR angle is shifted. So the shift in the SPR angle (degree) can be calculated as the amount of protein ($4.02\ ng\ mm^{-2}\ degree^{-1}$) or the thickness of the etched TiO_2 layer ($0.77\ nm\ degree^{-1}$).

The designed Ti-SPR sensor chips were prepared by Osaka Vacuum Industrial Co. Ltd. (Osaka, Japan). These chips were prepared by depositing Cr, Au, and Ti on S-LAL10 glass plates under 2.0×10^{-2} Pa using an electron beam evaporation method.

2.3.2. Characterization of the titanium layer on the Ti-SPR sensor

A transmission electron microscopy (TEM) cross-section of the developed Ti-SPR sensor chip embedded in epoxy resin was prepared using an Ion Slicer (EM-09100IS, JEOL), and imaged using a JEM-3010 (JEOL) microscope operated at 300 keV. The surface elemental composition of the Ti-SPR sensor was determined using X-ray photoelectron spectroscopy (XPS) (AXIS-HS, Kratos, Manchester, UK) in vacuo at less than 10^{-7} Pa. We used AlK_α monochromatic X-rays with a source power of 150 W (acceleration voltage of 15 kV and filament current of 10 mA) and measured the elemental composition ratio of Au, Ti, and O at photo-electron take-off angles of 90°, 60°, 45°, 30°, and 15°. The layer thicknesses of the titanium and the oxidized titanium on the Ti-SPR sensor were estimated from the SPR spectrum of this sensor and the relationship between the amount of protein adsorbed and the SPR angle shift was determined.

2.4. SPR measurements of acid etching and biomolecule adsorption

2.4.1. Effect of acid etching on the titanium surface

The effect of acid etching the titanium surface was determined by SPR. Phosphoric acid solutions were prepared by dropping 85 wt.% phosphoric acid into pure water to obtain pH values of 1.8, 1.9, 2.0, and 3.0. The running solution used was pure water.

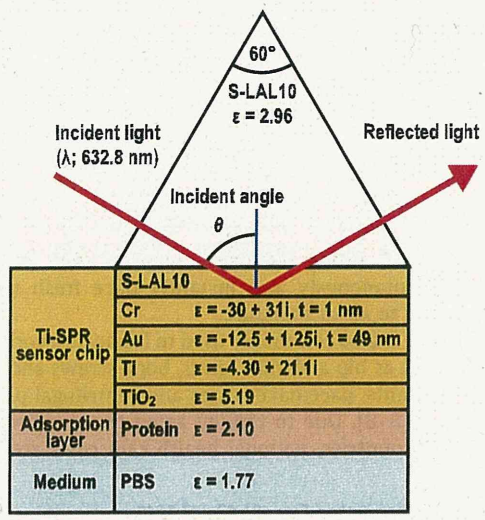

Fig. 1. Schematic illustrating the Ti-SPR sensor with the coupled prism for SPR. The deposited titanium metal layer is easily oxidized by air and its thickness changed. The thickness and dielectric constant are indicated for each coated/adsorbed substance (S-LAL10 glass, Cr, Au, Ti, TiO_2, protein, and PBS).

The Ti-SPR sensor chip was attached to an SPR flow cell. Pure water was allowed to pass through the flow cell until the SPR angle was stable. The SPR spectrum for the Ti-SPR sensor exposed to pure water was recorded and then the incident light angle was fixed at 1.0° lower than the minimum of reflectance, which was the SPR angle. Subsequently the intensity of the reflected light was followed during exposure to phosphoric acid solution flow for 20 min. Then this solution was washed out with water for 19 min and the SPR spectrum again recorded. The SPR angle shift was determined from the minima of the two resonance profiles.

The effect of acid etching the titanium was also determined using a quartz crystal micro-balance (QCM) (Q-Sense D300). A QSX 310 (titanium QCM sensor) was attached to a QCM flow cell (QWiC301). All QCM apparatus and chips were purchased from Q-Sense AB (Västra Frölunda, Sweden). Pure water was allowed to pass through the flow cell until the frequency of the quartz crystal was stable. Subsequently phosphoric acid solution was allowed to flow for 20 min. Finally, this solution was washed out of the flow cell with pure water for 19 min. The phosphoric acid solutions used were either pH 1.8 or 2.0.

All solutions flowed at 3.3 ml min^{-1} in both experiments.

2.4.2. Protein and polymer adsorption on the titanium surface

The amount of protein and polymer adsorbed onto the titanium surface was determined by the Ti-SPR sensor. The Ti-SPR sensor chip was attached to an SPR flow cell and then PBS was allowed to pass through the flow cell at 3.3 ml min^{-1}. The SPR spectrum was recorded when the SPR angle had become stable. Subsequently the intensity of the reflected light was followed during exposure to a protein or polymer solution under flow for 10 min. Then this solution was washed out with PBS for 9 min and the SPR spectrum was again recorded. The protein solutions used were 2 μg ml^{-1} albumin, γ-globulin, and bFGF in PBS. The polymer solutions used were 100 μg ml^{-1} polyethyleneimine (PEI) and gelatin in PBS, and 1 wt.% poly(phosphoric acid) (PPAc) in water.

2.5. SPRI measurements of cell response and corrosion resistance

2.5.1. Preparation of the titanium array for SPRI

The titanium surface of the Ti-SPR sensor chip was cleaned by argon plasma irradiation (electrode current of 23 mA for 90 s), and then immersed in acetone and toluene for 5 min each. This washed chip was then immersed in 2 mM octadecyltrichlorosilane (ODTCS) dissolved into toluene for 24 h at 60 °C. After silane treatment this chip was washed successively with toluene, acetone, a 1:1 (v/v) acetone–water mixture, and, finally, again with acetone. This OTDCS layer adsorbed onto the titanium surface was then irradiated/etched with argon plasma (electrode current of 10 mA for 10 min) through a stainless steel mask (5 × 5 pore arrays with a circular pore size of 1 mm, and an inter-pore interval of 1 mm) in order to produce 1 mm diameter titanium spots on the OTDCS-coated Ti-SPR sensor chip. This patterned array chip was immersed in acetone.

2.5.2. Preparation of the polymer-coated titanium array

The polymer-coated titanium array chip was prepared by dropping 1 μl of PBS onto the titanium spots of the titanium array chip, after which the chip was kept in saturated water vapor for 20 min. Then the PBS drops were removed and 0.5 μl of PEI, gelatin, PPAc, and PBS were dropped onto these spots, after which the chip was again kept in saturated water vapor for 20 min. Finally, each spot was washed five times with PBS.

2.5.3. Cells interactions with titanium assessed using SPRI

The interaction of cells with the polymer-coated titanium array chip was determined by SPRI. An albumin-free PIPES buffered medium (25 mM PIPES (pH 7.2), 159 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 5.6 mM glucose) [28] was prepared as solvent and the sample solution used was a MC3T3-E1 cell suspension (500,000 cells in 1 ml of this medium). The chip was attached to an SPRI batch cell. 300 μl of the medium was dropped onto the batch cell and the SPRI image was allowed to stabilize. Then 100 μl of the cell suspension was dropped onto the batch cell and mixed in situ, while the SPRI image data were recorded for 1 h. Phase contrast microscopy images of cell attachment to the polymer-spotted array chip were obtained under the above conditions.

2.5.4. Preparation of the DDP-treated titanium array

The DDP-treated array chip was prepared by dropping 0.3 μl of 1 wt.% DDP dissolved in 1:1 (w/w) water–ethanol mixture onto the titanium spots of the titanium array chip, after which the chip was kept in saturated water vapor for 30 min. Then the chip was washed three times with acetone.

2.5.5. Observation of the corrosion resistance of DDP-treated titanium by SPRI

The process of acid etching the DDP-coated titanium array chip was observed by SPRI for 10 min. An HCl/KCl buffered solution (41.4 mM HCl, 50.0 mM KCl, pH 1.5) [29] and a KCl solution (63.1 mM) were prepared as the "running" and "sample" solutions, respectively. The chip was attached to an SPRI flow cell. The running solution was allowed to pass through the flow cell and the SPRI image was allowed to stabilize. Then the SPRI data were recorded simultaneously at different spots during exposure to the sample solution at a flow rate of 3.3 ml min^{-1}.

3. Results and discussion

3.1. Development of the titanium SPR sensor

To design the Ti-SPR sensor the SPR spectrum needed to be simulated. Fig. 2 shows the simulated SPR spectra in relation to the thickness and the oxidation rates of the titanium layers. The titanium layer deposited on the SPR sensor chip easily oxidizes in air (leading to increased corrosion resistance). When the titanium layer (atomic weight 47.9, density 4.5 g cm^{-3}) is oxidized to a titanium dioxide layer (molecular weight 79.9) the density is reduced and the thickness increased [30]. As the density of titanium dioxide crystals is 3.8 (antase) or 4.2 (rutile) g cm^{-3} and the oxidized titanium layer on the Ti-SPR sensor is expected to show low crystallinity and to contain many hydroxyl groups [30–32], we assumed that the density of the oxidized titanium layer was 4.0 g cm^{-3}, that it grew in the uniaxial direction and that the thickness of this layer increased 1.9 times as a result of oxidization. In the case of a 5 nm thick titanium surface layer the peak of the SPR spectrum became sharper with increasing oxidation. In case of a 10 nm titanium layer, however, the peak of the SPR spectrum did not become sharper and the SPR angle, which is a minimum reflectance angle in the SPR spectrum, occurred at a high incident light angle. The latter is disadvantageous because the amount of protein adsorbed is related to the increase in SPR angle. In the case of a 20 nm Ti layer no peak occurred in the SPR spectrum. These simulations suggest that the thinner the titanium layer is the more accurate ("sharper") the SPR spectrum is. Therefore, a Ti-SPR sensor with an optimal 5 nm thick titanium layer was employed.

3.2. Evaluation of the Ti-SPR sensor surface

The simulation was insufficient to determine the actual thickness of the surface oxide layer. So we measured the Ti-SPR sensor chip, as shown in Figs. 3 and 4, using TEM and SPR. The thinnest

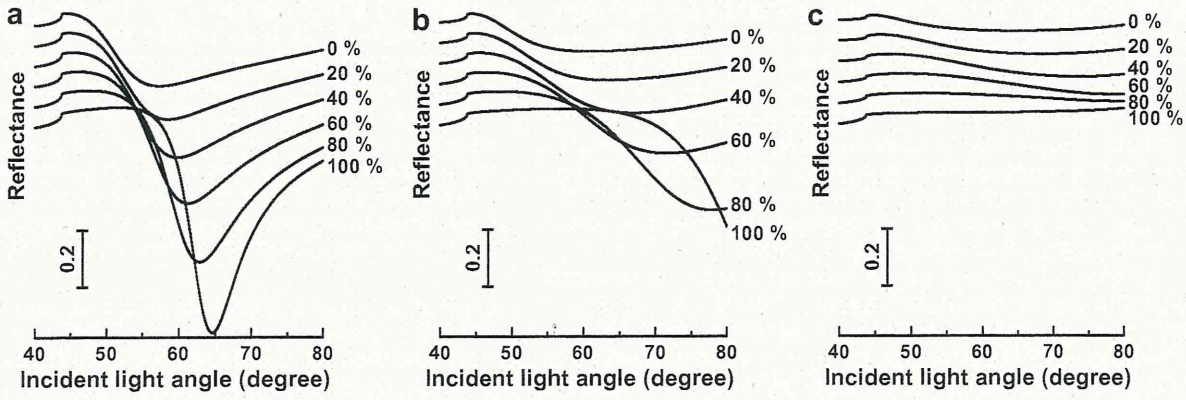

Fig. 2. Simulation of SPR spectra for 0, 20, 40, 60, 80, and 100% oxidized titanium metal layers in air. The thicknesses of the vapor deposited titanium metal layer were (a) 5, (b) 10, and (c) 20 nm.

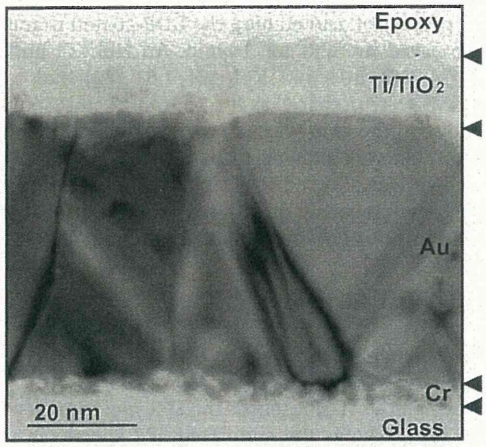

Fig. 3. High magnification cross-sectional TEM image of the Ti-SPR sensor.

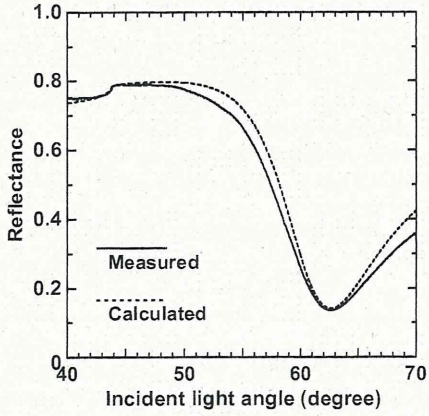

Fig. 4. SPR spectrum of the developed Ti-SPR sensor. A 5 nm titanium metal layer was deposited on this sensor. The continuous and dotted lines represent the measured and theoretically calculated spectra (titanium, 0.7 nm; titanium dioxide, 7.6 nm), respectively.

achievable titanium layer that could be sputter coated was 5 nm. High magnification cross-sectional TEM images of the Ti-SPR sensor (Fig. 3) showed a 49 nm Au layer with an about 10 nm Ti/TiO_2 layer on top and a Cr layer underneath on top of the glass substrate. The spectrum of the Ti-SPR sensor was detected in the SPR

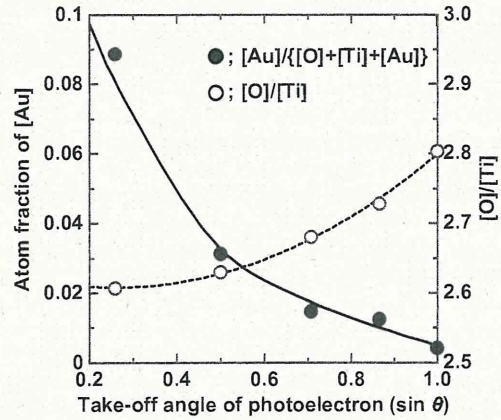

Fig. 5. Atom fraction of Au (●) and O/Ti (○) ratio on the surface of the Ti-SPR sensor as measured by angle-dependent XPS.

results (Fig. 4), and the thickness of the oxide layer was obtained by comparing the simulated and measured SPR spectra. The dotted line in Fig. 4 shows the simulated SPR spectrum for a 0.7 nm thick titanium layer and a 7.6 nm titanium dioxide layer. Good agreement was found with the actual and simulated SPR spectra.

Fig. 5 shows the results for the atom fraction of Au and the O/Ti ratio at the Ti-SPR sensor surface as measured by angle-dependent XPS, confirming that the gold layer was uniformly coated with an ultrathin oxidized titanium layer, and that the O/Ti ratio at the top was larger than at the bottom. The non-stoichiometric O/Ti ratio may be due to the fact that the outer surface contained titanium hydroxide ($TiO(OH)_2$ with an O/Ti ratio of 3, while the O/Ti ratio of TiO_2 is 2), and because the oxygen atoms in these hydroxyl groups seemed to mainly exist in the top layer. From these results it was concluded that the external layer of the titanium surface SPR sensor was titania.

3.3. Detection of acid etching on the titanium surface

Titanium is very corrosion resistant, thanks to the presence of the passivating film on the surface. When we exposed the surface of the Ti-SPR sensor to phosphoric acid solutions of different pH (Fig. 6a) the SPR angles shifted to lower values, except for the pH 3.0 solution. This indicates that the oxidized titanium surface was etched when exposed to phosphoric acid solutions with a pH of 2.0 or below. The average thickness changes at pH 2.0, 1.9, and1.8 were about 20, 50, and 120 pm, respectively.

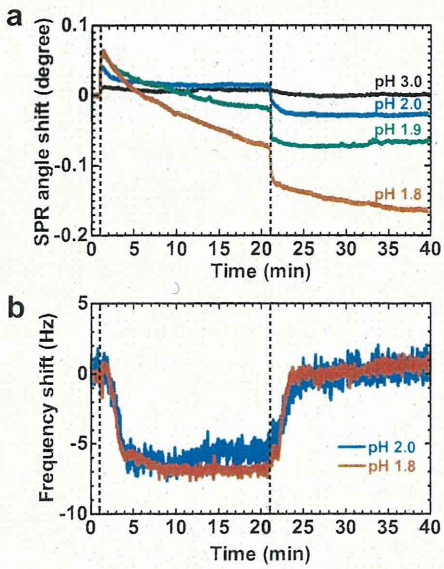

Fig. 6. Effect of etching the titanium passivation layer with phosphoric acid at different pH, as measured by (a) SPR and (b) QCM. The acid solutions were flowed for 20 min and then were washed out with pure water for 19 min.

Using QCM, the effect of etching titanium was also determined under the same conditions (Fig. 6b), but the relatively low signal-to-noise ratio obscured the QCM spectrum. Pressure fluctuations in the flow solution strongly affect the frequency of the quartz crystal, whereas the optical performance of the Ti-SPR sensor is largely unaffected under similar conditions. The Ti-SPR sensor has high sensitivity when measuring titanium etching, which is achievable because TiO_2 has a higher dielectric constant ($\varepsilon = 5.19$) than phosphoric acid ($\varepsilon = 2.12$) [33] in water and, consequently, the SPR angle shift due to changes in layer thickness is about 5 times more sensitive for TiO_2 (0.77 nm degree^{-1}) than for phosphoric acid (4 nm degree^{-1}).

The above mentioned experiments confirm the real time high resolution acid etching measuring capability of the Ti-SPR sensor.

3.4. Measurement of biomolecule adsorption on the titanium surface

Thanks to high biocompatibility, titanium is commonly used in a variety of medical devices. However, chemical and biological reactions of non-treated titanium surfaces are poor because of its chemical stability. Therefore, many methods of modifying titanium surfaces have been studied to increasing the bioactivity, bone conductivity, and biocompatibility [32,34,35].

In this study the Ti-SPR sensor was used to measure the interaction between biomolecules and the titanium passivation layer in real time. Fig. 7 shows increasing protein absorption on Ti with time for the three proteins studied. It was assumed that the difference in pI and molecular weight of the proteins would influence the amount of protein absorbed. At pH 7.4 the Ti surface is negatively charged [36], γ-globulin is slightly negatively charged, albumin is negatively charged, and bFGF is positively charged. Therefore, we hypothesized that γ-globulin (158 kDa, pI 5.8–7.3) would be most adsorbed on Ti (2.85 ng mm^{-2}) because of its greater molecular weight, albumin (69 kDa, pI 4.9) would be least adsorbed (0.85 ng mm^{-2}) because of its negative charge, and bFGF (17 kDa, pI 10.1), although having a molecular weight 10 times less than that of γ-globulin, was adsorbed to a greater extent (2.08 ng mm^{-2}) than albumin because of its positive charge.

The Ti-SPR sensor also enabled us to measure changes in adsorption of polymers to surfaces such as, for instance, titanium

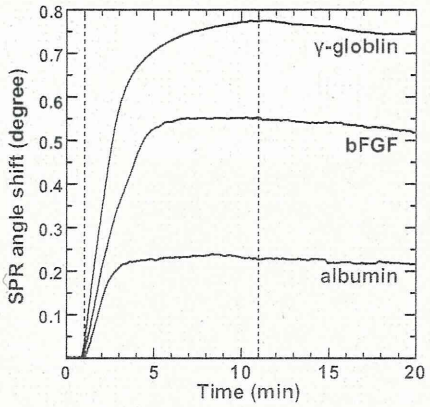

Fig. 7. Time evolution of protein adsorption on the titanium passivation layer by SPR. Protein solutions in PBS were 2 μg ml^{-1} γ-globulin, 2 μg ml^{-1} albumin, and 2 μg ml^{-1} bFGF. The protein solutions were flowed for 10 min and then were washed out with PBS for 9 min.

medical devices coated with different polymers in order to achieve slow release of drugs and cytokines [37]. Real time changes in the SPR angle were measured when titanium was exposed to PEI, gelatin, and PPAc suspensions (Fig. 8). While PEI and gelatin were rapidly adsorbed onto the titanium surface and resisted removal by washing, PPAc significantly etched the surface due to its low pH (pH 1.5), but deposited a thin PPAc layer upon washing [38].

These results indicate that the Ti-SPR sensor developed can accurately measure time-dependent protein and polymer adsorption onto a titanium passivation layer.

3.5. SPRI observation of cell response on the titanium surface

A variant of the sensor enables SPRI mapping of biological surface processes. Using SPRI the cell response on a polymer-coated titanium array chip could be studied in real time (Fig. 9a and b). The response of MC3T3-E1 cells was found to increase on the spots coated with PPAc, titanium, PEI, and gelatin, in that order. Unfortunately, the cell response on the surface reflects not only cell adhesives but also cell reactions [39]. This differential cell adhesion was confirmed by phase contrast microscopy (Fig. 9c), which showed that cells adherent on the PPAc spot and the gelatin spot developed filopodia along with spread cell bodies, while cells on the PEI spot

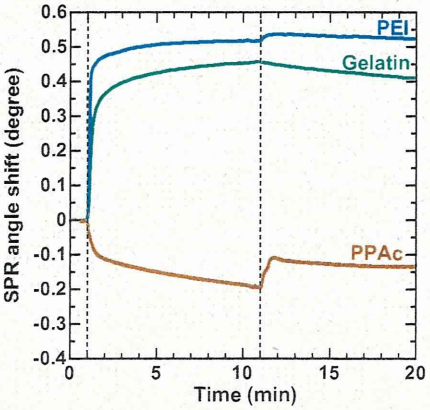

Fig. 8. Time evolution of polymer adsorption on the titanium passivation layer by SPR. Polymer solutions were 100 μg ml^{-1} PEI and 100 μg ml^{-1} gelatin in PBS, and 1 wt.% PPAc in water. The polymer solutions were flowed for 10 min and then were washed out with PBS for 9 min.

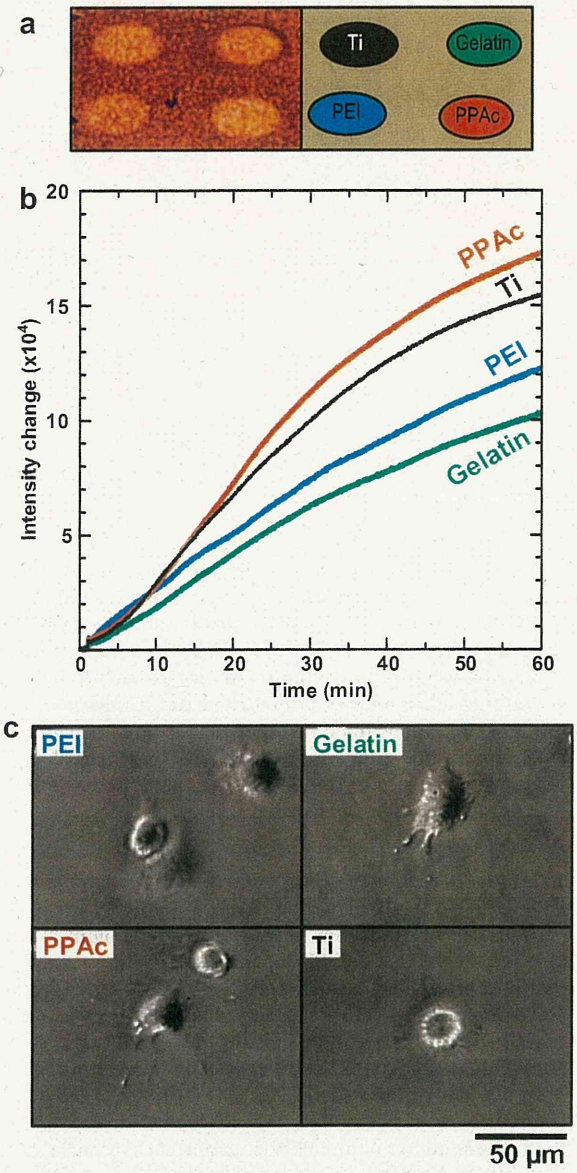

Fig. 9. MC3T3-E1 cell adhesion on the polymer-coated titanium array. (a) SPRI image of the array chip after 1 h incubation with cells. The four spots were titanium (passivation surface), PEI, gelatin, and PPAc, as shown in the schematic. (b) Real time assessment of MC3T3-E1 cell response on the polymer-coated titanium array by SPRI. (c) Phase contrast microscopy of MC3T3-E1 cell attachment.

and Ti spot clearly showed fewer filopodia without spread cell bodies. These results indicate that the cell response determined by SPRI did not correlate with the area over which the cells spread, but was at least in part due to some actions of the living cell. Although another measurement method to detect cell reactions is needed in combination, we propose that the study of early cell responses to titanium using SPRI could contribute to a better understanding of the early phases of osseointegration of titanium implants, leading to improved osseointegration therapy.

3.6. SPRI observation of the corrosion resistance of the titanium surface

The imaging potential of the Ti-SPR sensor was illustrated in a surface corrosion experiment involving exposure to a HCl/KCl buffered solution. DDP and ODTCS can be strongly immobilized on the

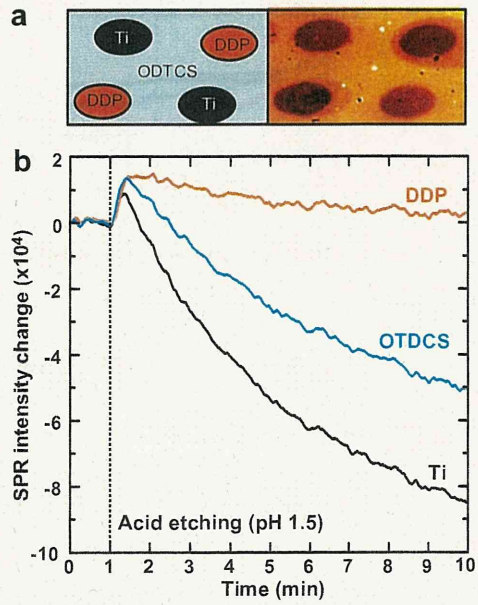

Fig. 10. SPRI imaging of acid etching of the DDP treated titanium array chip. (a) The array chip of four 1 mm spots, of which two spots were titanium (passivation surface, black spots in the schematic), and the two other spots were treated with DDP (red spots in the schematic), amidst the ODTCS-treated titanium (blue surrounding area in the schematic). The SPRI image revealed that the DDP solution etched the ODTCS-treated titanium near the DDP spots. (b) The whole area, including the four spots and the surrounding area, were exposed to HCl/KCl buffer (pH 1.5), during which the SPR reflected light intensity was recorded in real time using SPRI.

surface as a self-assembled monolayer because they comprise long alkyl chains and surface-active head groups [40–41], and these immobilized layers are supposed to protect the titanium surface from acid. In Fig. 10a the SPRI image of a DDP-treated titanium array revealed that the DDP solution, which is acidic, etched the ODTCS-coated titanium around the DDP spots. The acid etching of titanium with HCl leads to the greatest corrosion at the titanium spot, slightly less of the OTDCS-coated surface, while the DDP spot appeared most resistant to HCl acid attack (Fig. 10b). This result suggests that these monolayers coated on a titanium surface are corrosion resistant, especially the DDP-treated surface.

4. Conclusions

The surfaces of titanium medical devices oxidize in air, the oxide forming a passivation layer. Our Ti-SPR sensor has a similar passivation layer to these devices because the titanium metal layer on the sensor oxides in a similar way. The Ti-SPR sensor could detect acid etching, biomolecule adsorption, cell reactions, and corrosion resistance of the titanium surface in real time.

The Ti-SPR sensor shows a broad applicability to study surface interactions with a titanium passivation layer. Within biomaterial sciences this instrument most obviously enables investigations of the surface chemistry and biomolecular aspects of the integration of titanium implants in human bone, especially to gain an insight into the earliest processes of protein adsorption and cell attachment, a scientifically important enigma that needs to be clarified in order to further ameliorate implant therapy in dental medicine and orthopedics. This sensor may also prove useful in studying the interactions of biomolecules and cells with other titanium-based medical devices used within the human body. Within materials sciences the process of corrosion could be studied in more depth to further improve the corrosion resistance of titanium-based

machines/equipment used in the marine and aerospace industries. Thus this innovative high resolution surface analytical tool could not only be employed to investigate the surface properties of titanium, but also to develop new materials with better surface treatment methods for titanium devices.

Acknowledgements

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figure 1, is difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2011.11.025.

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Response shift in oral health-related quality of life measurement in patients with partial edentulism

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SUMMARY **The purposes of this study were to determine whether a response shift was observable after partial denture treatment and to identify the predictors that influenced the response shift magnitude and direction. A total of 173 consecutive patients with no more than eight missing teeth who received implant-supported, fixed or removable partial dentures at Okayama University Dental Hospital were asked to complete a full-version Oral Health-Related Quality of Life (OHRQoL) questionnaire before (pre-test) and after treatment (post-test). Additionally, a short form (then-test) consisting of seven questions selected from the full version had its reliability verified and was utilised to retrospectively assess the pre-treatment OHRQoL status. The difference between the summary scores of the then-test and the pre-test determined the response shift magnitude and direction. The then-test mean score (22·9 ± 6·6) was significantly lower (worse OHRQoL) than that of the pre-test (26·4 ± 5·2). The response shift effect size was of moderate magnitude and negative direction ($d = -0{\cdot}78$). A multiple regression analysis showed that age (younger patients) ($P < 0{\cdot}01$), number of replaced teeth (fewer) ($P < 0{\cdot}01$) and pre-test scores (lower) ($P < 0{\cdot}01$) were the significant predictors for response shift. In conclusion, a response shift phenomenon with negative and moderate effect size was observed after partial denture treatment. The significant predictor variables were young age, fewer numbers of replaced teeth and lower pre-test scores**

KEYWORDS: **quality of life, oral health-related quality of life, response shift, predictor variables, treatment efficacy, dental prosthesis**

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Introduction

Patients' perceived Health-Related Quality of Life (HRQoL) may be defined as a multidimensional construct incorporating at least three broad domains: physical, psychological and social functioning (1, 2). In the dental field, assessment of an individual's Oral Health-Related Quality of Life (OHRQoL) has been exponentially increasing during the last decade to evaluate the impact of a disease or of treatment efficacy (3–5). Particularly in prosthodontics, several studies and systematic reviews have demonstrated that partial (implant-supported, fixed or removable) and conventional complete dentures significantly improved OHRQoL, especially concerning aesthetics and function (4–9).

However, numerous studies in the medical field have reported that simple comparison between pre- and post-treatment scores of the HRQoL appraisal might show ambiguous or paradoxical findings because of fluctuations in patient's internal standards or values (1, 2, 10–12). For instance, patients with cancer or people with spinal cord injuries reported higher levels of HRQoL after treatment compared with the general population (13, 14). Such a phenomenon, called response shift, refers to a change in the meaning of HRQoL over time and can be due to: (i) a redefinition of the HRQoL concept (reconceptualization), (ii) a change

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in the importance of the component domains that constitute HRQoL (reprioritization) or (iii) a change in the respondent's internal standards of measurement (recalibration) (1, 2, 10–12, 15). The most common approach to assess and quantify the response shift is by the application of a *then-test* at the same time of the post-test to retrospectively assess the pre-treatment HRQoL status based on the same post-test internal standards (1, 2, 10–12, 15–18). The difference between the then-test and pre-test scores indicates the response shift magnitude and direction (1, 2, 9–18).

In the dental field, little is known about response shift. Only one study described response shift reconceptualization phenomenon in 81% of 117 patients after complete denture treatment (19). In that study, however, the magnitude and direction of response shift effect size were not quantified. Additionally, an individualised HRQoL questionnaire was utilised rather than a more field-specific OHRQoL instrument. Therefore, the results could also contain appraisal bias from other subjective, emotional or social aspects of life likely to influence the subject's perception of HRQoL (11).

By utilising an OHRQoL questionnaire, this follow-up study aimed: (i) to determine whether a response shift was observable after partial denture treatment in few-missing-teeth patients and if so, (ii) to identify the significant predictor variables affecting the response shift magnitude and direction.

Methods

Subjects

The intended sample was gathered from consecutive partially edentulous patients who received implant-supported fixed partial dentures (IFD), conventional fixed partial dentures (FPD) or removable partial dentures (RPD) at the Fixed Prosthodontic Clinic of Okayama University Hospital. The IFD group was enrolled from April 2003 to March 2007, while the FPD and RPD groups were enrolled from January 2005 to March 2007.

Inclusion criteria were those subjects with edentulous spaces corresponding to a loss of no more than four teeth, however, limited to a sum of at most eight missing teeth, and, who answered the full-length OHRQoL questionnaire at baseline (pre-test), before dental treatment onset. Exclusion criterion was those patients who received any other dental treatment apart from oral hygiene and prosthesis adjustments during the whole-study period.

According to the selection criteria, 173 subjects were initially eligible to participate (Fig. 1). They were provided with the study guidelines and asked to sign the informed consent. The Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences approved the research protocol (#96). After treatment completion, post-tests were sent to all eligible subjects by mail in February 2008. However, among the 173 subjects, 35 were excluded because six of them did not provide a correct address, 12 did not return the questionnaire and an additional 17 subjects did not answer the test questions completely (Fig. 1). Therefore, the actual sample consisted of 138 subjects (IFD: 78, FPD: 37, RPD: 23).

Questionnaires

A previous validated OHRQoL questionnaire (20) in its full length was utilised for pre- and post-test assessments. This questionnaire consisted of 28 items subdivided into two major groups: 'oral health condition' (16 items) and 'psychological health condition' (12 items). Additionally, patient's satisfaction level with their oral and general health condition was assessed by two 100-mm visual analogue scales (VAS) for interconsistency analysis. In contrast to the Oral Health Impact Profile (OHIP) (21), this questionnaire had reversed rating

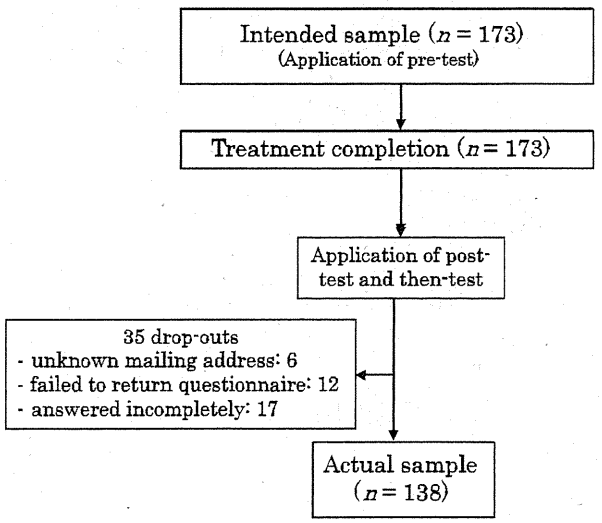

Fig. 1. Study design. Pre-test was applied before the treatment onset, while the post-test and then-test were mailed after the patient had no complaint with the newly installed partial denture.

scales, i.e. scores varied from 0 = very often to 4 = never, indicating that the higher the score, the higher was the patient's reported OHRQoL. The pre-test was handed directly to the patients at the initial visit by the treating dentists who had been previously calibrated. After treatment completion, the post-test was sent by post-mail because many patients were living far or even in other cities.

To retrospectively assess patient's OHRQoL, a then-test was mailed to all patients at the same time as the post-test. Therefore, it was necessary to have two clearly distinct questionnaires to enable the patients to recognise them more easily as being separate tests. Moreover, based on some prior reports showing that a few questions are sufficient to significantly measure an individual's OHRQoL changes, a short version of this questionnaire was developed and utilised as the then-test (22, 23). The then-test was comprised of seven questions selected from the full version that were previously reported to have the highest test–retest reliability and internal consistency (24) (Table 1). It contained five items in the subgroup 'oral health condition' and two items in the other subgroup concerning 'psychological health condition'. Similarly to the full-length OHRQoL questionnaire, this short form also included two VAS scales to assess patient's satisfaction with oral and psychological health conditions (Table 1). Moreover, it had a clear header note instructing the patient to 'retrospectively assess your oral health condition before you had received the dental treatment'. The title of the questionnaire was 'How often (had you) (had you had) (X)...before you received dental treatment?'. To verify the test–retest reliability of the then-test, 35 subjects (IFD: 21, FPD: 9, RPD: 5) randomly selected from the initial intended sample of 173 patients, completed the short-form questionnaire in two surveys within a 2-week interval. Among these selected patients, six of them were excluded (first survey: two subjects; second survey: four

Table 1. Full-version OHRQoL questionnaire* (English translation)

How often (have you) (has) ... during the last 1 week, due to problems with your teeth, mouth or denture?
Rate as (very often = 0; fairy often = 1; occasionally = 2; hardly ever =3; never = 4)

Oral health condition

(A) Mastication and oral pain/discomfort
1. *Had to avoid eating some foods*†
2. *Had painful regions in your mouth*†
3. Had painful teeth
4. Had painful gums
5. *Had an uncomfortable sensation in your mouth*†
6. Felt that clenching your teeth has worsened
7. Had a feeling of something wrong in your mouth
8. Has difficulty chewing hard foods
9. Found it uncomfortable to eat any foods

(B) Pronunciation
1. People misunderstood some of your words
2. *Had trouble pronouncing words*†

(C) Aesthetics
1. *Felt that your teeth don't took right*†
2. Been incapable of showing your teeth
3. Avoided smiling

(D) Swallowing
1. Had difficulty in swallowing any foods

(E) Oral cleaning
1. Been unable to brush your teeth properly

Psychological health condition

(A) Physical function
1. Felt depressed
2. Found it difficult to relax
3. Has your concentration being affected
4. Had difficulty doing your job
5. *Felt under stress*†
6. Felt tired easily
7. Felt you had no time for yourself

(B) Psychological state
1. *Felt uneasy about the future*†
2. Been nervous within a group
3. Envied another person
4. Your sleep been interrupted
5. Felt that your health had worsened

Satisfaction with oral and general health condition

(1) *Are you satisfied with your oral health condition?*†

Not satisfied — Completely satisfied

(2) *Are you satisfied with your general health condition?*†

Not satisfied — Completely satisfied

*Questionnaire provided in Japanese.
†Questions with highest test–retest reliability and internal consistency selected for the short-form questionnaire.
Italic text were used to highlight the questions/items which were selected for the short-form questionnaire.

subjects) because they failed to return the questionnaire. Consequently, the final subsample for assessment of the then-test reliability was comprised of 29 subjects (IFD: 19, FPD: 6, RPD: 4).

Measures

To assess response shift and treatment efficacy, the total scores of the pre-test and the post-test were initially reduced to the corresponding score of the same seven questions of the then-test. The summary score of the questionnaires was calculated by the sum of the seven items except for the VAS scales assessing patient's satisfaction levels.

The response shift was measured by the difference between the then-test and the pre-test (then-test minus pre-test) (Fig. 2). The analysed predictor variables likely to influence response shift were age, gender, number of replaced teeth, position of the replaced teeth (anterior/posterior/both), functional duration of the prosthesis, treatment modality (IFD/FPD/RPD), time interval between pre-test and post-test delivery and the pre-test reduced scores. All variables were gathered from patient's hospital records by the study coordinator (A.K).

The treatment efficacy of the three partial dentures (IFD/FPD/RPD) was initially calculated by the conventional difference between the post-test and the pre-test scores (post-test minus pre-test) and subsequently, by the difference between the post-test and the then-test scores (post-test minus then-test) to identify the retrospectively assessed treatment efficacy (Fig. 2).

Statistical analysis

Baseline data comparison between the intended and actual samples, as well as between the actual sample and the subsample for the then-test reliability, was analysed by *t*-test and chi-square test.

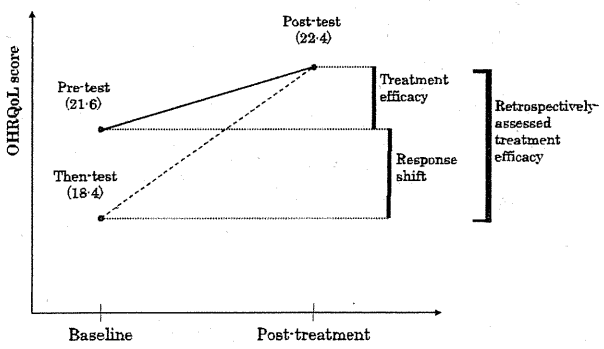

Fig. 2. Measurement of the response shift and the retrospectively assessed treatment efficacy.

Construct validity to evaluate the interconsistency levels between the selected seven items and the respective full version of the pre-test and the post-test were analysed by Spearman's correlation coefficient (25).

Wilcoxon analysis was utilised for response shift and treatment effect size measurements. The response shift effect size was calculated according to Cohen's criteria, by the difference between the then-test and the pre-test scores divided by the standard deviation of the pre-test score (26). It was categorised as small ($d < 0{\cdot}5$), moderate ($0{\cdot}5 \leq d < 0{\cdot}8$) or large ($d \geq 0{\cdot}8$) (26). The inter-treatment group (IFD/FPD/RPD) differences were analysed by the Kruskal–Wallis test. Predictor variables influencing the response shift magnitude and direction were assessed by multiple linear regression analysis.

The mean differences with 95% confidence intervals were computed as the representative values. The significance level was defined as $P < 0{\cdot}05$. All calculations were carried out using SPSS version 16.0*.

Results

Demographic baseline data

The actual sample had an age range from 24 to 87 years, with a mean age of 61·4 ± 11·8 years and gender ratio of 1:2·73 (male/female) (Table 2). The number of free-end edentulous patients allocated according to the amount of tooth loss was one missing tooth: two subjects; two missing teeth: 21 subjects; three missing teeth: 17 subjects; and four missing teeth: 26 subjects, while the bounded edentulous patients were distributed as follows: one missing tooth: 37 subjects, two missing teeth: 16 subjects, three missing teeth: 15 subjects and four missing teeth: four subjects. Regarding the position of the replaced teeth, no patient was classified as having both anterior and posterior tooth loss; therefore, only those presenting either of the conditions were included in the statistical analyses. The return rate of the post-test was 79·7%, and the average interval between pre-test and post-test delivery was of 2·0 ± 1·7 years (ranging from 0·1 to 4·2 years).

With regard to the subsample for the then-test reliability, the mean age was 66·2 ± 9·8 years and the gender ratio was 1/1·9 (male/female). This subsample

*SPSS Inc., Tokyo, Japan.

presented a questionnaire return rate of 82·8%. No significant difference was observed either between this subsample used for the then-test reliability and the actual sample, or between the actual and the intended samples concerning any of the analysed predictor variables (Table 2).

Reliability of the short form

The mean weighted kappa values (test–retest consistency) for each question of the then-test were calculated according to Cohen's method (26) and are shown in Table 3. The overall mean kappa value was 0·59 ± 0·11 with most values clustered within the range of 0·62–0·64. The highest kappa value was for the question concerning the presence of a painful site inside the mouth (k = 0·78), while the lowest value was for the item regarding an uncomfortable sensation in the mouth (k = 0·34).

The results of the VAS intra-class correlation coefficients (ICCs) assessing subjects' satisfaction with their oral and general health conditions were 0·72 and 0·88, respectively (Table 3).

The Cronbach's alpha coefficient (internal consistency) was 0·75 between the five items and the VAS scores measuring the oral health condition, and 0·72 between the two questions about psychological health condition and the VAS measuring general health satisfaction level.

Table 3. The reliability and internal consistency of the short-form questionnaire

Had to avoid eating some foods	0·64*
Had painful regions in your mouth	0·78*
Had an uncomfortable sensation in your mouth	0·34*
Had trouble pronouncing words	0·62*
Felt that your teeth don't look right	0·63*
Felt under stress	0·64*
Felt uneasy about your future	0·62*
VAS – satisfaction with oral health condition	0·72†
VAS – satisfaction with general health condition	0·88†

*Weighted kappa value.
†Intra-class correlation coefficient (ICC).

Construct validity of the short form

Spearman's correlation coefficient showed a significant interconsistency between the total scores of the selected seven items and the respective full version of the pre-test ($r_{Spearman}$ = 0·430, $P < 0·001$) and the post-test ($r_{Spearman}$ = 0·728, $P < 0·001$).

Response shift measurement and related predictor variables

The overall mean value of the then-test (22·9 ± 6·6) was significantly lower than that of the pre-test (26·4 ± 5·2) ($P < 0·01$) (Table 4). The overall magnitude of the response shift was −3·5 ± 7·4, with a moderate effect size (d = −0·78). A statistically significant

Table 2. Baseline demographic data

	Intended sample	Actual sample	Subsample for then-test validity	Intended sample vs. actual sample	Actual sample vs. then-test subsample
				P-value	*P*-value
Number of patients	173	138	29		
Mean age (years)	62·1 ± 13·0	61·4 ± 11·8	66·2 ± 9·8	0·24*	0·19*
Gender (male/female)	54/119	37/101	10/19	0·14†	0·17†
Treatment modality (IFD/FPD/RPD)	88/46/39	78/37/23	19/6/4	0·09†	0·13†
Position of the replaced teeth (anterior/posterior/both)	45/128	29/109	8/21	0·15†	0·37†
Number of replaced teeth	3·3 ± 3·5	3·1 ± 3·4	2·9 ± 1·8	0·28*	0·52*
Functional duration (years)	1·7 ± 1·8	1·6 ± 1·8	1·7 ± 1·4	0·18*	0·12*
Interval between pre- and post-tests delivery (years)	2·2 ± 1·5	2·0 ± 1·7	2·3 ± 1·9	0·41*	0·36*
Reduced pre-test score of the selected 7 items	26·4 ± 6·5	26·4 ± 5·2	NA	0·11*	NA

Mean ± s.d.
NA, not applicable; IFD, implant-supported fixed partial dentures; FPD, fixed partial dentures; RPD, removable partial denture.
*t-Test.
†χ^2 test.

Table 4. The response shift magnitude and effect size

	Pre-test	Then-test	Response shift (then-test–pre-test)	Response shift	
				Effect size	*P*-value
IFD	26·2 ± 5·3	22·4 ± 6·7	−3·8 ± 7·8	−0·76	<0·01††
FPD	27·2 ± 5·1	23·4 ± 6·1	−3·8 ± 7·3	−0·74	0·02†
RPD	26·0 ± 5·0	23·7 ± 7·0	−2·3 ± 6·1	−0·43	0·052
All subjects	26·4 ± 5·2	22·9 ± 6·6	−3·5 ± 7·4	−0·78	<0·01††

Mean ± s.d.
The difference between the then-test and pre-test scores was calculated by Wilcoxon analysis (†$P < 0·05$, ††$P < 0·01$).
Score range: 1–35.
IFD, implant-supported fixed partial dentures; FPD, fixed partial dentures; RPD, removable partial denture.

Table 5. Multiple linear regression analysis of the predictors of the response shift

Predictor variables	SRC	*P*-value
Age	0·209	<0·01*
Gender (male/female)	−1·192	0·32
Treatment modality (IFD/FPD/RPD)	−0·908	0·14
Position of the replaced teeth (anterior/posterior/both)	−1·011	0·45
Number of replaced teeth	−0·737	<0·01*
Functional duration (years)	−0·367	0·44
Interval between pre- and post-tests delivery (years)	−0·063	0·89
Reduced pre-test scores of the selected 7 items	−0·798	<0·01*

$n = 138$, $R^2 = 0·361$.
SRC, standard regression coefficient (*$P < 0·01$); IFD, implant-supported fixed partial dentures; FPD, fixed partial dentures; RPD, removable partial denture.

response shift effect size was observed for the IFD and the FPD groups ($P < 0·05$); however, the value was only marginally significant ($P = 0·052$) for the RPD group (Table 4). Multiple regression analysis revealed that age (younger subjects) ($P < 0·01$), the number of replaced teeth (fewer) ($P < 0·01$) and pre-test scores (lower) ($P < 0·01$) were the significant predictor variables of the response shift (Table 5).

Treatment efficacy

The results of the conventional calculation by the difference between post-test minus pre-test scores showed a partial denture treatment efficacy of 1·1 ± 6·2 with a significant positive effect size of small magnitude ($d = 0·22$, $P < 0·01$). On the other hand, subtraction of post-test minus then-test scores revealed a retrospectively assessed treatment efficacy of 4·6 ± 7·4, with a positive and moderate effect size ($d = 0·71$, $P < 0·01$). Wilcoxon analysis revealed that IFD was the only group presenting a significant effect size both in the conventional ($d = 0·43$, $P = 0·01$) and in the retrospectively assessed treatment measurements ($d = 0·91$, $P < 0·01$) (Tables 6 and 7).

Discussion

Recent studies in quality-of-life research have led to a consensus that the response shift is a crucial component in treatment outcomes and in longitudinal observations of HRQoL (10–12). However, assessment of HRQoL appraisal and response shift phenomenon still presents some unresolved questions because of the complexity of obtaining empirical data to explain controversial paths of theoretical models of HRQoL measurement (12). In this study, the response shift was assessed by application of the then-test method, which has been extensively utilised in the medical field for detection of response shift recalibration changes, because of its proven effectiveness, as well as its feasibility of numerical effect size calculation (11). A recent systematic review, however, suggested that the then-test could underestimate the true HRQoL changes because of its susceptibility to patient's recall bias (11). In other words, collected data could contain both real recalibration effects and some error variance because of memory distortion or cognitive effects (10, 11, 27). Therefore, Schwartz *et al.* (27). suggested that other instruments, such as individualised QoL, interviews, direct assessments of values or preferences and ordering tasks, should be concomitantly applied with the then-test to

Table 6. Conventional assessment of treatment efficacy and effect size

	Pre-test	Post-test	Treatment efficacy (post-test–pre-test)	Treatment efficacy Effect size	*P*-value
IFD	26·2 ± 5·3	28·5 ± 4·6†	2·2 ± 6·7	0·43	0·01†
FPD	27·2 ± 5·1	26·9 ± 4·6†	−0·3 ± 4·6	−0·07	0·26
RPD	26·0 ± 5·0	25·6 ± 4·1	−0·4 ± 6·1	−0·10	0·53
All subjects	26·4 ± 5·2	27·6 ± 4·6	1·1 ± 6·2	0·22	<0·01††

Mean ± s.d.
Inter-group analysis by Kruskal–Wallis test (*$P < 0·01$).
The difference between the post-test and pre-test scores was calculated by Wilcoxon analysis (†$P < 0·05$, ††$P < 0·01$).
Score range: 1–35.
IFD, implant-supported fixed partial dentures; FPD, fixed partial dentures; RPD, removable partial denture.

Table 7. Retrospectively-assessed treatment efficacy and effect size

	Then-test	Post-test	Retrospectively-assessed treatment efficacy (post-test–then-test)	Retrospectively-assessed treatment efficacy Effect size	*P*-value
IFD	22·4 ± 6·7	28·5 ± 4·6*	6·0 ± 7·3	0·91	<0·01†
FPD	23·4 ± 6·1	26·9 ± 4·6*	3·5 ± 7·0	0·57	0·31
RPD	23·7 ± 7·0	25·6 ± 4·1	1·9 ± 7·3	0·27	0·87
All subjects	22·9 ± 6·6	27·6 ± 4·6	4·6 ± 7·4	0·71	<0·01†

Mean ± s.d.
Inter-group analysis of the pre-test and post-test scores was analysed by Kruskal–Wallis test (*$P < 0·01$).
The difference between the post-test and then-test scores was calculated by Wilcoxon analysis (†$P < 0·01$).
Score range: 1–35.
IFD, implant-supported fixed partial dentures; FPD, fixed partial dentures; RPD, removable partial denture.

evaluate the response shift reconceptualisation or reprioritization changes. Unfortunately, these additional assessment methods could not be performed during this present investigation.

The results obtained herein showed that patient's retrospective perception of the pre-treatment OHRQoL (then-test) was worse than the actual pre-test, i.e. the overall mean score of the then-test was significantly lower than that of the pre-test (Table 4). Consequently, a significant response shift (−3·5 ± 7·4) was observed after partial denture rehabilitation with an effect size of negative direction and moderate magnitude ($d = −0·78$). Intertreatment group analysis revealed a statistically significant response shift with a moderate effect size for the IFD ($d = −0·76$) and FPD ($d = −0·74$) groups. On the other hand, the RPD group demonstrated a response shift with a small effect size ($d = −0·43$), however, with a marginally significant result ($P = 0·052$), probably due to the notably smaller sample size gathered in this group.

Only one previous study in the dental field demonstrated the occurrence of a change in nomination of at least one HRQoL domain (reconceptualization) in 81% of the 117 gathered patients (19). That study utilised the Schedule for the Evaluation of Individual Quality of Life – Direct Weighting (SEIQoL-DW) instrument in which the subjects nominated the five most important life domains constructing their HRQoL. The advantage of such individualised instruments is the detectability of changes in the importance of life domains before and after treatment (reconceptualization). On the other hand, the main drawbacks of the SEIQoL-DW instrument are the limited number of only five cues for one's HRQoL nomination and the complexity to convert the results into numerical values of response shift effect size, because the five cues nominated after treatment

are not necessarily the same as those at baseline (11, 19). Moreover, previous medical reports have emphasised the importance of using more field-specific questionnaires to minimise bias from other subjective or affective aspects likely to influence subject's internal values and standards and the perception of his/her QoL (10, 11).

In the dental field, the widely known and applied OHRQoL questionnaire is the OHIP. However, at the study onset, the Japanese version of the OHIP had not been officially published; therefore, the authors utilised an instrument that had already been reported in both Japanese and English languages and presented substantial reliability and validity (20, 24). Nevertheless, due to the fact that this questionnaire has been reported in a limited number of studies, the generalizability of the results obtained herein could be somehow affected by the questionnaire itself; therefore, future studies are necessary to verify whether the same observations in response shift can also be measured by other OHRQoL instruments, such as the OHIP. Another factor that could affect the external validity of this study to all prosthodontic patients is the inclusion of only those subjects with edentulous spaces corresponding to no more than four teeth but limited to a total of no more than eight missing teeth. The reasons why the authors focused on such particular patients were because they can be considered a homogeneous sample in terms of OHRQoL appraisal (28, 29) with a demonstrated improvement in OHRQoL status after prosthodontic treatment (6, 9, 20, 30–32). Additionally, these patients could receive any of the three treatment modalities (IFD, FPD and RPD) that could enable a stratified investigation of the effect of each intervention in the response shift phenomenon. Future studies with different populations are necessary to also evaluate the presence of a response shift phenomenon in OHRQoL appraisal.

Regarding the predictors that influenced response shift phenomenon, a multiple regression analysis revealed that age, the number of replaced teeth and pre-test reduced scores were the significant predictors (Table 5). Younger subjects presented larger response shift effect sizes than did the elderly. Based on the Schwartz and Sprangers' response shift model (1, 10), it was assumed that the improvement in OHRQoL (*well-being*) promoted by partial denture rehabilitation (*catalyst*, e.g. change in health status or treatment interventions) was superior in younger subjects because of their higher social demand and self-concern with facial aesthetics and oral function (33, 34) (*antecedent factors*, e.g. characteristics of the person, culture and social environment) compared with the elderly. Additionally, OHRQoL appraisal could also have been higher in younger individuals because of their emotional and psychological statuses for coping with tooth loss and to accommodate the benefits of the prosthetic treatment (*mechanisms*, e.g. behavioural, cognitive or affective processes).

The present results also demonstrated that the number of replaced teeth was another significant predictor for response shift. The larger number of tooth loss (*disability*), the higher was the impact of partial prosthetic treatment (*catalyst*) in promoting patients' OHRQoL (*well-being*). Nevertheless, stratified comparison by the number of missing teeth could not be performed in this study because of the relatively small number of subjects and uneven intertreatment group distribution. Therefore, the actual impact of the loss of a specific number of teeth (one, two, three or four missing teeth) on the response shift phenomenon could not be explored in detail.

The last important predictor variable was the pre-test score. It was speculated that there could have a *ceiling effect* in this short-form OHRQoL questionnaire, i.e. those patients who had scored a high OHRQoL level in the pre-test could have been eventually unable to indicate a higher score in the post-test assessment because it had a ceiling. Therefore, they could have rated the then-test purposely lower to express their perceived benefit and satisfaction with the treatment. Additionally, some subjects could have been concerned with their treating doctors; therefore, rating the then-test purposely lower than the post-test to return a 'good' answer (reporting bias). To minimise this bias, care was taken to inform the patients in the cover letter sent with the mailed questionnaires that their treating doctors would not read their answers.

Finally, it was also assumed that sample non-randomization could have influenced the overall results by possible bias from unknown confounding variables. Moreover, as some patients had not received the post-test and the then-test immediately after the completion of treatment, both questionnaires were simultaneously mailed at a later time point (February 2008). This difference in the method of administration between the pre-test and post- and then-tests as well as the time gap could also be expected to influence the response shift.

However, recent reports on quality of life research have shown no significant difference among methods of questionnaire application or interview (35, 36).Additionally, statistical analyses revealed that the pre-test and post-test delivery interval was not a significant factor accounting for response shift phenomenon. In corroboration, prosthesis' functional duration (1·6 ± 1·8 years) did not significantly influence the overall response shift either (Table 5).

Treatment efficacy

The difference between the post-test and the pre-test scores (conventional measurement) showed an overall partial denture treatment efficacy of 1·1 ± 6·2 with a positive and small effect size ($d = 0{\cdot}22$, $P < 0{\cdot}01$) (Table 6). This result is in agreement with previous reports that general partial denture rehabilitation is an important treatment modality to restore individual's oral motor function, aesthetics and chewing abilities, consequently improving one's perceived OHRQoL (4–6, 9, 20, 31, 37). However, comparisons between the post-test and the then-test scores demonstrated that the retrospectively assessed treatment efficacy of partial dentures was more than four times higher (4·6 ± 7·4) than the conventional measurement, with a larger effect size of moderate magnitude ($d = 0{\cdot}71$, $P < 0{\cdot}01$) (Table 7). Therefore, it could be assumed that the conventional OHRQoL assessment was less sensitive or distorted for detection of the actual treatment effect size because of the response shift phenomenon (11). In agreement with this hypothesis, Ring *et al.* (19) reported that unless the response shift was accounted for in the measurement of treatment efficacy, conventional complete denture rehabilitation had not significantly improved patients' reported OHRQoL in that study.

Interestingly, intergroup comparison with regard to the treatment efficacy either by the conventional method (2·2 ± 6·7) or including the response shift measurement (6·0 ± 7·3) revealed that IFD was the only treatment modality that significantly improved the patient's perceived OHRQoL (Table 6). The clinical interpretability of these results could indicate that IFD was the best treatment for rehabilitation of patients missing only a few teeth, while FPD and RPD treatments would not significantly improve patients' OHRQoL. In corroboration, a previous well-controlled study that compared IFD, RPD and no treatment groups showed that patients who received RPD or no treatment reported similar and significantly lower OHRQoL levels compared with the scores of those patients who had received IFD (31). On the other hand, Sonoyama *et al.* (20). reported that both IFD and resin-bounded FPD treatments promoted a similar increase in OHRQoL. This apparent contradiction with the present results could be partially explained by the distinct patient sample characteristics, i.e. that study assembled only subjects with a limited number of missing teeth (one or two teeth) mostly in the anterior region, while in this present investigation, many patients were missing several teeth, and even patients with unilateral free-end edentulism were included. Therefore, this study's findings are more similar to those reported by Kuboki *et al.* (31).

Finally, although the retrospective assessment of partial denture treatment efficacy showed an effect size of moderate magnitude, its clinical significance should be considered with reservations because the extent of one's HRQoL appraisal depends on the impact of a disease *(disability)* or treatment *(catalyst)* on the individual's internal standards and coping strategies. For instance, in terminal conditions such as cancer or acquired immune deficiency syndrome, patients may report HRQoL similar to or higher than that of healthy controls, and small detectable improvements in HRQoL are very meaningful to those who are eager for definitive treatment responses (38). In partial denture rehabilitation, although such life-threatening conditions are not observable, the impact of anterior tooth loss, for example, could be expected to be more dramatic in the individual's sociability and self-esteem than that of posterior tooth loss. Therefore, the actual clinical relevance should be further analysed in more detail, such as according to the position and the number of replaced teeth, by means of psychological assessments and/or by application of a minimal clinically important difference in the measurement of prosthodontic treatment efficacy (38).

Conclusion

This study could not only ratify that partial denture treatment significantly improved individual's OHRQoL (with better results obtained with implant-supported fixed dentures), but also could demonstrate the occurrence of a response shift with moderate magnitude and