Effect of two bleaching regimens on enamel bonding performance

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This study evaluated the post-bleaching bonding performance of a one-step and two-step self-etch adhesive (SEA) to bleached enamel using in-office or at-home bleaching. Bovine enamel surfaces were treated with an in-office or at-home bleaching agent and divided into four post-bleach time periods: immediate, one day (1d), one week (1w) and one month (1m) before application of SEA systems. Enamel was bonded using the following procedures: Group 1: one-step SEA application (BeautiBond Multi, BBM), Group 2: two-step SEA application (FL-Bond II, FLBII). Microshear bond strengths (µSBS) were measured 24-h after bonding for each of the post-bleaching time periods. Acid-base resistant zones (ABRZs) were observed under SEM. Data were analyzed using three-way ANOVA. Bleaching caused a significant decrease of the immediate µSBSs for both adhesives (p<0.005) then increased. Thick ABRZs were observed in BBM and FLBII after bleaching. Enamel µSBSs were adversely affected by bleaching. Thick ABRZ was created with bleaching.

Keywords: Bleaching, Enamel, Bonding performance, Microshear bond strength, Acid-base resistance zone

INTRODUCTION

Bleaching with carbamide peroxide or hydrogen peroxide is a safe, evidence-based and conservative alternative to more invasive esthetic treatments, such as the placement of veneers or full crowns on discolored teeth.

Different regimens for bleaching teeth are available (at-home or in-office). These regimens use various concentrations of bleaching agents. In general, in-office bleaching is done with high concentrations of hydrogen peroxide (25–35%), whilst at-home bleaching products, with a few exceptions, contain carbamide peroxide in varying concentrations, ranging from 5 to 30%⁶. The in-office technique has emerged as more popular than home use because highly concentrated products usually provide a faster tooth whitening outcome (the higher the concentration of bleaching solution, the more quickly a shade change will occur)².

One of the adverse effects of tooth bleaching has been the reported decrease in bond strengths for resin-based materials to enamel immediately after the bleaching procedure. The reduction in bond strength has been ascribed to the oxygen released from bleaching agents into tooth structure that inhibits resin polymerization⁹.

According to the clinical application steps, self-etch adhesive (SEA) systems have been classified as one-step or two-step adhesives. One-step SEA systems, combine acidic functional monomers, hydrophilic and hydrophobic monomers, water and organic solvents in a single solution. However, two-step SEA systems contain an acidic monomer that simultaneously etches and primes the tooth substrate, prior to the application of a separate bonding resin. The acidic functional monomer is believed to play several important roles during the etching and priming steps to form a chemical bond to the calcium of the tooth substrate.

A new version of a fluoride-releasing adhesive system has been introduced in order to inhibit the initiation of recurrent caries at the tooth-restoration interface⁵. Some researchers demonstrated significant cariostatic and antibacterial effects of fluoride-releasing restorative materials⁶,⁷.

When a resin-based adhesive is bonded to the enamel and dentin, an acid-base resistant zone (ABRZ) is formed at the adhesive-tooth substrate interface. This was first reported by Tsuchiya et al.⁸. The ABRZ is characterized as a structure at the resin-tooth substrate interface which remains after an acid-base challenge. The first report was based on the resin-dentin interface, but recent studies have also demonstrated the formation of ABRZ at enamel interfaces with one-, two- and three-step etch and rinse adhesive systems as well as SEA systems⁹–¹². The ABRZ seems to be associated with sealing of restoration margins and therefore enhancing restoration durability⁶,⁸,¹⁰,¹³,¹⁴, Shinohara et al. reported that the thickness of the ABRZ was increased by fluoride ion release from the adhesive⁵. It has been reported that bleaching agents can affect the morphology of the enamel ABRZ and create thicker ABRZs when an in-office bleaching system was used⁶. However, the effect of at-home bleaching on ABRZ morphology has yet to be reported.
Therefore, the present study aimed to evaluate the effect of different bleaching regimens on enamel bonding performance using a one- and two-step SEA systems at various time intervals after completion of the bleaching process and before adhesive application. In-office and at-home bleaching materials were used prior to adhesive application and tested by means of a microshear bond strength (µSBS) test. Micro-morphological features of the ABRZ were assessed using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials used in this study

The materials used in this study are described in Table 1. A one-step SEA (BeautiBond Multi, BBM, Shofu Dental, Kyoto, Japan) and a two-step SEA (FL-Bond II, FLBII, Shofu Dental) were used. BBM is a fluoride-free adhesive which contains a carboxylic acid monomer with water and organic solvents in the one bottle adhesive. FLBII is also composed of the same functional carboxylic acid monomer in the self-etching primer and has fluoride-releasing components of F-PRG r and S-PRG fillers. These filler particles were created using pre-reacted-glass-ionomer (PRG) technology that has been previously described[16]. A resin composite, Beautifil II (Shofu Dental), is a fluoride-containing hybrid resin composite and used as part of manufacture of specimens. An in-office bleaching agent (Hi-Lite office bleaching, Shofu Dental) containing 35% hydrogen peroxide (pH=3.9–4.1) and an at-home bleaching agent (Hi-Lite shade up home bleaching, Shofu Dental) containing 10% carbamide peroxide gel (pH=6–7) were the bleaching agents selected.

Specimen preparations

Specimen preparations are illustrated in Fig. 1. One hundred and twenty-six bovine incisors, free from structural cracks and defects, were used in this study. Roots were resected 1 mm above the cemento-enamel junction using a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water-cooling. Each tooth was sectioned to obtain one fragment from the labial surface per tooth (10×10 mm). The enamel surfaces were wet-ground with 600-grit silicon carbide abrasive papers to create a flat surface and standardized smear layer[3,17], as well as to ensure a flat surface which is parallel to the applied shear stress during the µSBS test.

The specimens were divided into three main groups according to bleaching regimen: 1: no bleaching (control), 2: in-office bleaching (Hi-Lite office), and 3: at-home bleaching (Hi-Lite shade up). The control group was subdivided into the one-step (BBM) and two-step (FLBII) self-etching adhesive groups. The two bleaching

| Material                  | Composition                                                                 | Application procedures                                                                 |
|---------------------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| FL-Bond II (FLBII)        | Primer: Carboxylic acid monomer, 6-MHPA, Water, Solvent, Photo-initiator   | Apply primer to tooth surface, and leave in place for 10 s. Dry with air stream. Create a uniform film with gentle air stream. Light cure for 10 s |
|                           | Bond: HEMA, UDMA, TEGDMA, S-PRG filler, Photo-initiator                    |                                                                                         |
| BeautiBond Multi (BBM)    | Bis-GMA, carboxylic acid monomer, TEGDMA, phosphonic acid-based monomers, acetone, purified water | Apply using a microbrush. Leave undisturbed for 10 s after application. Dry thoroughly for 3 s with maximum air pressure. Light cure for 10 s |
|                           | Filler: 83.3 wt% (68.6 vol%) Multi-functional glass and S-PRG filler based on fluoroboraluminosilicate glass particle size range: 0.01–4.0 μm (mean 0.8 μm) |                                                                                         |
|                           | Base resin: Bis-GMA, TEGDMA                                               |                                                                                         |
| Beautifil II              | Powder: Potassium persulphinate, manganese sulfate, monohydrate, hydrated amorphous silica, mixed sodium and calcium salt of poly (MVE/MA), pigment | One scoop of powder and three drops of liquid were mixed for 30 s. Apply the paste in 1–2 mm thickness and left on the surface for 6 min following light curing for 3 min, and left for another 2 min then wiped off by cotton. Repeated 3 times then washed with water and dried gently. |
|                           | Liquid: 35% hydrogen peroxide, water                                      |                                                                                         |
| Hi-Lite shade up (Home bleaching) | 10% carbamide peroxide gel                                                 | apply using brush and left for 2 hours then washed with water. Repeated on 14 consecutive days. Stored in distilled water at 37°C between bleaching cycles. |
groups were further divided randomly into 4 equal-sized subgroups according to storage period after completion of the bleaching process, namely, immediate, 1d, 1w and 1m. Specimens were stored in distilled water at 37°C before the adhesive was applied. Each subgroup based on time was treated with either the one-step self-etch or two-step SEAs (n=28/group) (Fig. 2).

In-office bleaching was performed on 56 specimens with Hi-Lite bleaching agent. One scoop of powder and three drops of liquid were mixed for 30 s using a spatula until a uniform paste was formed. The paste was applied 1–2 mm thick on the enamel surface of the specimens. The paste was left on the surface for 6 min and then irradiated with a quartz-halogen light curing unit (Optilux 501, Demetron, Danbury, CT, USA) with a power density of 550 mW/cm² for 3 min. The paste was left on the surface for another 2 min and then removed with a damp cotton roll. This procedure was repeated three times, and the specimen was then rinsed under water and dried gently. After bleaching, the teeth were stored in distilled water at 37°C for various times 1d, 1w and 1m.

At-home bleaching was performed on 56 specimens with Hi-Lite shade up bleaching. The bleaching material was applied to the enamel surfaces with a brush and left for 2 h. The procedure was repeated over 14 consecutive days, as recommended by the manufacturer. The specimens were stored in distilled water at 37°C between bleaching cycles. The bleaching agent was changed every day after the bleaching cycle was completed and stored in distilled water at different times 1d, 1w and 1m.

**µSBS testing**

After bonding resin application, tygon tubes (R-3603, Norton Performance Plastic, Cleveland, OH, USA), internal diameter of 0.79 mm and height of 0.5 mm (n=5 teeth, each tooth bonded with 9 tubes), were placed on the bleached enamel surfaces and light-cured for 10 s. A resin composite (Beautifil II, shade A2, Shofu Dental) was inserted into the tygon tubes and light polymerized for 40 s using the quartz-halogen light curing unit. After polymerization of the resin composite, the specimens were stored in distilled water at 37°C for 1-day.

After the storage for 1-day in water, the tygon tubes were removed using a scalpel blade. Each specimen was attached to a jig (Bencor- Multi-T, Danville Engineering, San Ramon, CA, USA) using a cyanoacrylate glue (Model Repair, Dentsply-Sankin, Ohtawara, Japan) and placed in a universal testing machine (EZ-Test-500N, Shimadzu, Kyoto, Japan) to measure the µSBS. A thin wire, with a 0.25 mm cross-section, was looped around the base of the resin composite cylinder bonded to the enamel surface and connected to a metal rod of the bond testing jig on the universal testing machine. A shear stress was applied at a crosshead speed of 1.0 mm/min until bond failure occurred. 
SEM observation
1. Failure mode analysis
After the µSBS testing, debonded areas of all specimens were gold sputter-coated to evaluate the failure patterns on the enamel surface using SEM (JSM- IT100LV, JEOL, Tokyo, Japan) at ×200 magnification. Failure modes were classified into three types: Type A: adhesive failure at the interface between the bonding resin and enamel; Type B: cohesive failure in the bonding resin; and Type M: mixed failure including adhesive failure and cohesive failure in the bonding resins.

2. ABRZ
Thirty-six specimens were used for ABRZ observation in each experimental group (n=2) as illustrated in Fig. 3. The specimens were prepared in the same manner as for the bond test. Additionally, a thin coating of resin composite (Clearfil AP-X, shade A2, Kuraray Noritake Dental, Tokyo, Japan) was applied on top of the bonded specimens and cured for 40 s. After storage in distilled water at 37°C for 1-day, each specimen was sectioned into two halves perpendicular to the bonded interface then embedded in slow-curing epoxy resin (Epoxicure Resin, Buehler) overnight. Specimens were wet-polished with SiC paper to 1200-grit to standardize the surface and then subjected to an acid-base challenge with a demineralizing solution (pH 4.5) for 2 h and 5% NaClO for 20 min. A self-cure resin, Super-Bond C&B (Sun Medical, Moriyama, Japan) was then applied to the treated surface without acid etching to prevent wear of the interface. The specimens were then cut perpendicular to the interface, polished with SiC paper to 2000-grit, and finished with diamond pastes to 0.25 µm followed by argon-ion beam etching (EIS-IE, Elionix, Tokyo, Japan) of the polished surfaces to bring the adhesive/enamel interface into sharp relief, with an accelerating voltage of 1 kV and an ion current density of 1.5 mA/cm² for 30 s in order to distinguish the different zones of the interface that were observed using SEM (JSM- IT100LV, JEOL) at ×3,500 magnification.

Statistical analysis
Data were analyzed for normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. µSBS data were analysed using three-way ANOVA followed by pairwise comparison with Bonferroni correction (IBM Released 2015. IBM SPSS Statistics for Windows, Version 23.0. IBM, Armonk, NY, USA) (α=0.05).

RESULTS
µSBS
The µSBS values are listed in Table 2. Three-way ANOVA showed that “bleaching” did not have a significant effect (p=0.221), while, “adhesive” and “post-bleaching period” showed significant effects on the µSBS (p<0.001). The interaction between the 3 variables showed a significant effect (p=0.018).

The µSBS of BBM was 16.6 MPa in the control groups which was significantly lower than that of FLB II 27.6 MPa (p<0.01). The µSBS of in-office bleaching, for BBM, significantly decreased immediately after bleaching (p<0.001) and increased 1d after bleaching. The bond strength recovered to the original value 1w and 1m after bleaching. For FLB II, the µSBS was significantly lower in the immediate group and increased slightly 1d after bleaching, and recovered 1w after bleaching but did not achieve the same strength as the original value (27.6 MPa) 1m after bleaching.

The µSBS of in-office bleaching, in both adhesive materials showed a significant decrease in immediate groups and slightly increased after 1d and had recovered by 1w and 1m after bleaching. For the at-home bleaching, both adhesive materials showed a significant decrease in the immediate groups and slightly increased after 1d and remained stable for BBM and FLBII but did not achieve the same bond strength as in the control groups for FLBII.

The µSBS of at-home bleaching, for BBM, significantly decreased immediately after bleaching (p<0.001). However, the µSBS recovered to the original value by 1d, 1w and 1m after bleaching. For FLB II, the µSBS significantly decreased in the immediately after bleaching group (p<0.001) which was not significantly different from that of FLB II after 1d, 1w and 1m of bleaching.

Fig. 3  Methodology of specimen preparation for SEM observation of ABRZ.
Table 2  Effect of time after bleaching of in-office bleaching and at-home bleaching on μSBS to enamel

| Time        | BBM       | FLBII      | p-value       |
|-------------|-----------|------------|---------------|
| Control     | 16.6±3.8ab | 27.6±5.0a  | ≤0.001*       |
| Immediate   | 4.5±1.9*  | 6.6±4.0*   | 0.096 NS      |
| 1d          | 12.7±3.3d | 19.8±4.4bc | ≤0.001*       |
| 1w          | 18.0±5.3c | 25.7±5.1a  | ≤0.001*       |
| 1m          | 16.3±4.7abc| 22.0±4.6b  | ≤0.001*       |
| Immediate   | 13.7±2.9cd | 16.3±3.1d  | ≤0.001*       |
| 1d          | 14.8±4.2bcd| 19.7±4.9bc | ≤0.001*       |
| 1w          | 15.6±4.2bcd| 17.5±5.6cd | ≤0.059 NS     |
| 1m          | 15.0±4.8bcd| 16.7±3.8cd | 0.131 NS      |

Mean±Standard deviation with the same letters (a, b, c or d) within each column indicate no significant difference (NS) at (p>0.001). * = significant difference.
Number of specimens: 45 for each group.

Failure mode analysis

The failure mode results are shown in Table 3.

In the control group, for both BBM and FLB II, Type A (adhesive between enamel and resin) was the main failure mode, however, Type B (cohesive failure in bonding resin) was observed in FLB II in approximately 5% of specimens.

In-office bleaching, for BBM, Type B cohesive failure in the bonding resin were observed in all groups except at 1w which showed 60% adhesive failures (Type A) and 40% mixed failures (Type M). For FLB II, 8–13% Type B cohesive failures in bonding resin were observed in all groups.

At-home bleaching, for BBM, the main failure mode was Type A in all groups however, 7% Type B (cohesive failure in bonding resin) was only observed at 1d. For FLB II, the main failure mode was Type A in all groups except for 1m, which exhibited 40% for mixed failure. Type B was observed in the 1w and 1m groups.

The μSBS of in-office and at-home bleaching, for both adhesive materials showed the main failure was adhesive (Type A). Mixed failure (Type M) increased at 1m for FLB II and at 1d, 1w and 1m for in-office bleaching with BBM.

ABRZ

Typical SEM images of the enamel-adhesive interface for each group after acid-base challenge are shown in Figs. 4 (control), 5 (in-office bleaching), and 6 (at-home bleaching), respectively. The outer lesion (OL), created by dissolution of enamel due to the acid-base challenge was observed in each group and the depth of the OL ranged from 10 to 15 µm. ABRZ was observed at the demineralization front of OL in all groups.

In the control groups, for BBM, the ABRZ was 0.5 µm thick (Fig. 4a). A funnel-shaped lesion (lightening symbol) formed at the base of the OL in BBM (Fig. 4a). In FLB II (Fig. 4b), the ABRZ was approximately 1 µm thick, sloped and increased from the top up to the end of OL without a funnel-shaped lesion.

In office bleaching, in the BBM groups, the ABRZ increased to a thickness of about 3 µm in the immediate and 1d after bleaching groups and returned back to approximately 0.5 µm thick with a funnel-shaped lesion in the 1w and 1m after bleaching group (Figs. 5a–d). For the FLB II groups (Fig. 5e–h), the ABRZ increased to a
Fig. 4  SEM image of enamel-adhesive interface after acid-base challenge in the control group. OL: outer lesion; B: bonding; E: Enamel; R: resin; ABRZ: acid-base resistant zone. a- BBM, and b- FLBII. ABRZ was detectable in all groups.

Fig. 5  SEM image of enamel-adhesive interface after acid-base challenge of in-office bleaching groups. OL: outer lesion; B: bonding; E: Enamel; R: resin; ABRZ: acid-base resistant zone. a- BBM with bleaching immediate, b- BBM with bleaching one day, c- BBM with bleaching one week, d- BBM with bleaching one month, e- FLBII with bleaching immediate, f- FLBII with bleaching one day, g- FLBII with bleaching one week, and h- FLBII with bleaching one month. ABRZ was detectable in all groups.

Fig. 6  SEM image of enamel-adhesive interface after acid-base challenge of at-home bleaching groups. OL: outer lesion; B: bonding; E: Enamel; R: resin; ABRZ: acid-base resistant zone. a- BBM with bleaching immediate, b- BBM with bleaching one day, c- BBM with bleaching one week, d- BBM with bleaching one month, e- FLBII with bleaching immediate, f- FLBII with bleaching one day, g- FLBII with bleaching one week, and h- FLBII with bleaching one month. ABRZ was detectable in all groups.

thickness of about 3 µm in the immediate, 1d, 1w and 1m after bleaching groups. For at-home bleaching, in the BBM groups, the ABRZ was 3 µm thick in the immediate and 1d groups with the presence of a funnel-shaped lesion in the immediate group. The 1w and 1m after bleaching groups showed an ABRZ approximately 3 to 5 µm thick, however, the funnel-shaped lesion could not be observed (Figs. 6a–d). For the FLB II groups, the ABRZ increased to a thickness of about 3 to 5 µm in the immediate, 1d, 1w and 1m after bleaching groups (Figs. 6e–h).
DISCUSSION

Many bleaching products and techniques are now available for professional and non-professional use. Patients might be scheduled for orthodontic or restorative treatment shortly after bleaching.

Bovine teeth resemble human teeth in many characteristics and have been used to compare the bond strength of bleached enamel with that of unbleached enamel. In order to simulate the clinical situation, an intact enamel surface should be used. But for better standardization and to understand the effect of the bleaching agent on bond strength and ABRZ enamel surfaces a flat surface were created.

For in-office bleaching, the µSBS for the one-step and two-step SEA systems decreased immediately after bleaching followed by a gradual increase 1d after bleaching and completely recovered to bond strengths the same as the control group after 1w. The immediate decrease in bond strength after bleaching may be due to the presence of residual oxygen from the bleaching agent, which inhibits resin polymerization. The delayed application of bonding by 1w seems to eliminate the effect of residual oxygen. Spyrides et al., also reported decreased bond strengths when bleached teeth were immediately bonded, while, there was a significant increase in bond strength when bonding was delayed for a week. Other studies reported delaying bonding after bleaching for 2 to 4 weeks, to eliminate the detrimental effects of residual oxygen from the bleaching agent which inhibited resin polymerization and interfered with resin attachment.

For at-home bleaching, the µSBS of BBM slightly decreased immediately after bleaching and returned to the original level by 1w. On the other hand, for FLB II, the µSBS decreased immediately after bleaching, and also 1d and 1w. BBM is an acetone-solvated adhesive which might minimize the inhibitory effects of oxygen due to the interaction between acetone and oxygen. FLB II has multi-ion-releasing components, which might be the reason the µSBS did not recover until 1m after at-home bleaching, but this still remains unclear. One study reported that ethanol or acetone-based adhesive systems counteract the effect of whitening because of their water-clearing effect. Gungor et al. reported that at-home bleaching affected the µSBS more adversely. They explained that the longer application periods associated with the at-home bleaching method caused alterations in the organic substance in the enamel and the loss of calcium may also increase over time. Another study reported that the normal µSBS values attained in an at-home bleaching group were probably attributable to the lower concentration of the hydrogen peroxide (10%) which was mainly because, after each daily bleaching, the teeth were stored in distilled water. This might have eliminated the residual peroxide absorbed by the enamel.

The majority of failures in all groups were adhesive (Type A). However, for at-home bleaching at 1m there was an increase in cohesive failure in the bonding resin for both adhesive systems. The cohesive failures of the adhesive materials were often associated with high bond strength values, indicating effective bonding. In this case, an increase in cohesive failure of the bonding resin probably means lower mechanical properties of the adhesive possibly due to poorer polymerization.

The characteristics of the ABRZs for FLBII were thicker than that of BBM. A thicker ABRZ was found in the FLBII groups, because FLBII contains a multi-ion-releasing component and S-PRG filler in the adhesive. Fluoride release from adhesives was reported to be a key factor to create thick ABRZs. The ABRZ of FLBII sloped and increased from the top to the end of the OL, while the ABRZs for BBM were parallel and homogeneous. The slope formation of FLBII is believed to be due to fluoride-release from the adhesive. ABRZ formation may be due to the potential for monomer penetration and fluoride release in the adhesive systems. However, a funnel-shaped lesion adjacent to the ABRZ was observed in BBM, which is similar to that found in previous studies, suggesting a weak area vulnerable to acid-base attack beneath the bonding interface.

On the other hand, when enamel was bleached using either the at-home or in-office bleaching, the thickness of the ABRZ was obviously increased and showed an irregular shape. This may be due to an increase in enamel porosity after bleaching which led to deeper penetration of the primer. A previous study suggested that bleaching enamel may increase enamel porosity, leading to deeper penetration of the self-etching primer, however, residual oxygen from the bleaching agent may hinder polymerization of the adhesive, thus causing a decrease in the µSBS. In a previous study, SEM examination confirmed that the application of a bleaching agent produced an aggressive etching pattern. The resin monomers were able to penetrate more deeply into the demineralized layer, resulting in thicker ABRZ formation. One study reported that the change of storage water may have also contributed to mineral loss from dentin surfaces due to deionized water lacking calcium and phosphate ions and therefore possessing a higher chemical potential for dissolution of the mineral leaving unprotected collagen fibrils. In the current study, the loss of mineral from the enamel was also reflected by a gradual increase in OL depth, which was significantly correlated with storage time. In-office bleaching showed an ABRZ thickness that decreased by 1w and 1m in both adhesive and lesion formation for BBM. This was presumably because enamel porosity decreased, so primer penetration was not as deep as in the immediate and 1d after bleaching groups which led to a thinner ABRZ.

CONCLUSION

Based on the results of this study, the following conclusions could be made.

In-office and at-home bleaching adversely affected the µSBS to enamel when bonded immediately and after 1 day, however, the µSBS values had recovered by 1
week after cessation of the bleaching process. Delaying bonding for at least 1 week after in-office or at-home bleaching is recommended.

Thick ABRZs were created with in-office bleaching and at-home bleaching for all storage times except for BBM which had a thin ABRZ at 1w and 1m after in-office bleaching application.

**CLINICAL RELEVANCE**

It is suggested that the present study regimen be carried out under clinical conditions and both adhesive systems undergo delayed bonding at different intervals so that the minimum delay in bonding can be determined for the two bonding systems.

**ACKNOWLEDGMENTS**

This study was partly supported by a grant from the Japan Society for the Promotion of Science (16H05515).

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