Evaluation of Bending Properties and Adherence of *Candida Albicans* to Antibacterial Glass-added Polyetheretherketone as a Denture Base Material

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**Introduction**

Polyetheretherketone (PEEK) is an insoluble thermoplastic polymer with high mechanical properties and biocompatibility. In the dental field, implant superstructures, abutment built post-core materials and bridges, orthodontic wires, and local denture applications, such as clasps, have been extensively examined (1–6). In our previous study, although PEEK showed high bending properties, *C. albicans* adherence to PEEK remained unchanged from that on existing denture base material (7). To apply the clinical situation for PEEK, PEEK may have the potentials to reduce the adhesion of *Candida albicans*. However, no study has yet investigated the bending properties of PEEK combined with antibacterial glass.

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**Abstract**

Inhibition in the adhesion of *Candida albicans* to the polyetheretherketone (PEEK) surface may be useful for preventing denture stomatitis and reducing the risk of systemic disease when PEEK is used as a denture base material. The present study examined the three-point bending test according to ISO standards for denture polymer materials and the inhibition of *C. albicans* on the surface of a new PEEK with antifungal effects.

PEEK only (VK) and PEEK combined with antibacterial glass added at various concentrations (new polymers) were evaluated as experimental materials. In the three-point bending test, all new polymers satisfied ISO standards. *C. albicans* adhesion rates were lower on all new polymers than on VK. Scanning electron microscopy was used to examine the effects of antibacterial glass and an antifungal drug (micafungin) on the adherence of *C. albicans*. The results obtained revealed that *C. albicans* cells treated with micafungin were deformed and their surface layer was rougher than that of untreated control cells. Furthermore, the surface layer of *C. albicans* cells treated with antibacterial glass was similar to that of cells treated with micafungin. Collectively, these results demonstrated that all new polymers satisfied ISO standards and were suitable as denture base materials.

The bending properties of all new polymers were equal to conventional PEEK. The antibacterial glass used herein exerted the same effects as MCFG on the *C. albicans* surface. These new polymers possess higher bending strength than conventional denture base materials, suggesting their efficacy at preventing denture stomatitis.

**Keywords:** polyetheretherketone, denture base material, flexural properties, *Candida albicans*, adhesive ability
mucosal surface. *C. albicans* has also been implicated in the development of respiratory infections, such as pulmonary candidiasis and aspiration pneumonia (8). The prevention of oral candidiasis may improve the oral environment and prevent systemic diseases. Inhibition in the adhesion of *C. albicans* to the PEEK surface may be useful for preventing denture stomatitis and reducing the risk of systemic disease when PEEK is used as a denture base material. PEEK, which has excellent bending properties, may improve *C. albicans* adhesion without adding to the strength required for denture base materials when an antifungal agent is added.

Antibacterial agents are classified as organic and inorganic. Organic antibacterial agents have a number of disadvantages, such as transient antibacterial effects, alterations by processing operations, and biological irritation (9). Inorganic antibacterial agents are attracting increasing attention as a solution to the shortcomings of organic antibacterial agents and are now beginning to be applied in many fields. Among inorganic antibacterial agents, antibacterial glass is an inorganic material with antibacterial properties that are recognized by ISO standards; these antibacterial properties are associated with the elution of silver ions in water (10). We speculate that the addition of antibacterial glass to PEEK may improve the adherence of *C. albicans* to material surfaces. Therefore, the present study performed the three-point bending test according to ISO standards for denture polymer materials and the *C. albicans* adherence test on material surfaces using a new PEEK with antifungal effects. The possibility of antibacterial glass application to PEEK was examined.

**Materials and Methods**

**Materials**

The three-point bending test materials used were PEEK only (VESTAKEEP® DC4470, Daicel-Evonik, Tokyo, Japan; hereafter referred to as VK). In addition, 5 types of PEEK (VESTAKEEP® D4, Daicel-Evonik, Tokyo, Japan) with antibacterial glass (DL-7900, Nippon Electric Glass, Shiga) with different concentrations were used (VK+DL polymer, hereafter referred to as VK+DL). Five types of VK+DL polymers with DL-7900 addition rates of 3, 5, 7.5, 10, and 15% were manufactured: VK+DL1 (DL-7900 addition rate 3%), VK+DL2 (DL-7900 addition rate 5%), VK+DL3 (DL-7900 addition rate 7.5%), VK+DL4 (DL-7900 addition rate 10%), and VK+DL5 (DL-7900 addition rate 15%) (Table 1). Two acrylic resins were used as comparative materials for the VK+DL group. The acrylic resin used was a heat-curing resin (Shofu Urban®, Shofu, Kyoto, Japan; hereafter referred to as UR) and an injection-type multipurpose resin (Procast DSP®, GC, Tokyo, Japan; hereafter referred to as PC). The VK and VK+DL groups were evaluated in the *C. albicans* adherence test. In a comparative study of an antifungal agent and DL-7900 against *C. albicans*, the Cell Desk (Celtight PL Cell Desk LF, Sumitomo Bakelite, Tokyo, Japan) and the antifungal micafungin (Funguard® 25 mg for Infusion, Astellas Pharma, Tokyo, Japan; hereafter referred to as MCFG) were used.

**Specimens**

The VK and VK+DL groups were manufactured by injection molding, UR by heating polymerization, and PC by room temperature polymerization with pouring. Specimen dimensions for the three-point bending test were a length of 64 mm, width of 1.0 mm, and thickness of 3.3 mm according to ISO 20795-1. Ten test specimens were manufactured for each test material (n=10). Each specimen was polished with #600 SiC water-resistant abrasive paper under running water and immersed in distilled water at 37°C for 48 h before testing (11). The dimensions of the *C. albicans* adherence test were a length of 1.0 mm, width of 1.0 mm, and thickness of 2.0 mm. Three test specimens were prepared for each test material (n=3). Each specimen was polished with #800 SiC water-resistant abrasive paper under running water (12).

**Test strain and culture medium**

*C. albicans* used in the present study was ATCC 90029. The culture medium used was Bacto™ Brain Heart Infusion (BHI, Becton, Dickinson)/liquid medium and liquid medium (0.5% bouillon, 1% peptone, and 0.5% sodium chloride) obtained by removing agar from the composition of normal agar medium was used as normal liquid medium. Candida GE agar plates (manufactured by Nissui) were used to calculate *C. albicans* viable counts. In the *C. albicans* adherence test and effect comparison test of MCFG and DL-7900 on *C. albicans*, *C. albicans* was added to BHI liquid medium and precultured at 37°C for 24 h. A total of 1.0×10⁸ colony-forming units (CFU)/ml were prepared as a preculture and used in experiments. Culture conditions were shaking at 120 r/min at 37°C using
a constant temperature shaking incubator (Bio-Shaker® BR-30LF, Taitec Co., Ltd., Saitama, Japan).

Test method
1 Three-point bending test
A precision universal testing machine (AG-100kNG X/R, Shimadzu Corporation, Kyoto, Japan) was used for the three-point bending test. The distance between fulcrums was 50 mm and the crosshead speed was 5 mm/min (11). Stress at 0.05% plastic deformation (0.05% proof stress) was obtained from the three-point bending test as a measure of bending strength, the flexural modulus, and elastic limit. Fractured specimens were measured for bending strength, the flexural modulus, and 0.05% proof stress at the load at breaking point. Specimens that did not break were measured for flexural strength, the flexural modulus, and 0.05% proof stress at the maximum load. Bending strength was calculated using the following formula:

\[ \text{Bending strength} = \frac{3PL}{2bh^2} \]

Where \( P \) is the maximum load (N), \( L \) is the distance between fulcrums (mm), \( b \) is the sample width (mm), and \( h \) is the sample thickness (mm).

The flexural modulus was calculated using the following formula:

\[ \text{Flexural modulus} = \frac{FL^2}{4dbh^3} \]

Where \( F \) is the load amount at a point in the proportional relationship on the load deformation curve (N), \( L \) is the distance between fulcrums (mm), \( d \) is the deformation amount at load \( F \) (mm), \( b \) is the sample width (mm), and \( h \) is the sample thickness (mm).

2 Adhesion test of C. albicans
Each test specimen was immersed in a normal liquid medium, autoclaved, and 50µl from the pre-cultured bacterial solution was added and cultured with shaking for 24 hours. After the incubation, the test specimen was removed from the test tube and washed with sterilized physiological saline (0.9% NaCl) to remove mildly attached bacteria. After immersing the specimen in 0.9% NaCl, firmly attached bacteria were dispersed by ultrasound.

| Abbreviation | Material | Manufacture |
|--------------|----------|-------------|
| VK           | VESTAKEEP® DC4470 | Daicel-Evonik, Tokyo, Japan |
| VK+DL1       | VESTAKEEP®D4 | Daicel-Evonik, Tokyo, Japan |
|              | DL-7900 addition rate 3% | Nippon Electric Glass, Shiga |
| VK+DL2       | VESTAKEEP®D4 | Daicel-Evonik, Tokyo, Japan |
|              | DL-7900 addition rate 5% | Nippon Electric Glass, Shiga |
| VK+DL3       | VESTAKEEP®D4 | Daicel-Evonik, Tokyo, Japan |
|              | DL-7900 addition rate 7.5% | Nippon Electric Glass, Shiga |
| VK+DL4       | VESTAKEEP®D4 | Daicel-Evonik, Tokyo, Japan |
|              | DL-7900 addition rate 10% | Nippon Electric Glass, Shiga |
| VK+DL5       | VESTAKEEP®D4 | Daicel-Evonik, Tokyo, Japan |
|              | DL-7900 addition rate 15% | Nippon Electric Glass, Shiga |
(Sonicator®, 50 W, 20 sec, 20 kHZ, Otake Manufacturing Co., Ltd., Tokyo). Those was used as a biofilm solution. The bacterial solution was diluted 10-fold with 0.05 M Tris-HCl buffer (pH 7.2), seeded on Candida GE medium, and cultured aerobically at 37°C for 48 hours. The amount of bacteria (CFU/test piece) adhering to each specimen was calculated from the colonies that grew after the culture. Experiments were performed in triplicate for each specimen type.

### 3 Comparison of antifungal effects between MCFG and DL-7900 on C. albicans

The cell desk was immersed in normal liquid medium that had been autoclaved and divided into three groups: a control group, DL-7900 action group, and MCFG action group. Fifty microliters of precultured bacterial solution was added to each test tube and cultured with shaking for 24 hours. After culturing, the control group the cell desk from the test tube, fixed with 4% glutaraldehyde for 1 hour, and dehydrated with an ascending ethanol series. After replacing with t-butyl alcohol, the specimen was freeze-dried with a freeze vacuum dryer (ES-2030, Hitachi, Tokyo) and deposited with Pt-Pd using an ion coater (IB-5, Eiko, Tokyo) (7 mA, 3 minutes). In the MCFG action group, after the culture, 50µl of MCFG stock solution was added and allowed to act for 5 minutes, and the same fixation as that described for the control group was performed. In the DL-7900 action group, 50µg of DL-7900 was added after the culture, the culture was shaken for 24 hours, and then fixed in the same manner as that described for the control group. The sample was observed with a scanning electron microscope (S-4300, Hitachi, Tokyo, hereinafter SEM) at an acceleration voltage of 7 kV.

#### Statistical analysis

1. **Three-point bending test**

   The results for bending strength, the flexural modulus, and 0.05% proof stress obtained in the three-point bending test were divided into each specimen thickness and each material. Statistical analysis of each test piece was carried out using one-way analysis of variance (ANOVA), with Tukey’s method for multiple comparisons, with the level of significance set at p=0.05.

#### Results

### Three-point bending test

#### 1 Bending strength

Fig. 1 shows a comparison of the bending strength of each test material. VK+DL4 and VK+DL5 showed significantly lower values than VK (p < 0.05). In contrast, VK+DL1, VK+DL2, and VK+DL3 showed no significant difference from VK (p > 0.05). UR and PC showed significantly lower values than the VK and VK+DL groups (p < 0.05).

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3 0.05% proof stress

Fig. 3 shows a comparison of the 0.05% proof stress of each test material. VK+DL4 and VK+DL5 showed significantly lower values than VK (p < 0.05). In contrast, VK+DL1, VK+DL2, and VK+DL3 showed no significant difference from VK (p > 0.05). UR and PC showed significantly lower values than the VK and VK+DL groups (p < 0.05), except for VK+DL5 (p > 0.05).

4 Material breakage

VK, VK+DL1, VK+DL2, and VK+DL3 did not break during the study. In contrast, VK+DL4 and VK+DL5 specimens partially fractured during the test, while three VK+DL4 and seven VK+DL5 specimens completely fractured. All UR and PC specimens fractured during the test.

Adhesion test of C. albicans

Fig. 4 shows C. albicans adhesion rates in the VK and VK+DL groups as a scatter diagram. The regression analysis showed a negative correlation between the antibacterial glass addition rate and C. albicans adhesion rate (R² = 0.7048).

Comparison of antifungal effects between MCFG and DL-7900 on C. albicans

Fig. 5 shows SEM images of the adherence of C. albicans to the Cell Desk of the control, DL-7900, and MCFG.
groups. In the control group, *C. albicans* cells maintained a spherical shape similar to a lemon, with smooth surface layers. In contrast, *C. albicans* cells on the Cell Desk of the DL-7900 group were circular or elliptical and exhibited rough surface layers. The cells of the MCFG group showed surface deformation similar to that observed in the DL-7900 group.

**Discussion**

In the present study, antibacterial glass was added to PEEK, and five new polymers were developed (VK+DL). The possibility of applying VK+DL as a denture base material was examined by conducting the three-point bending test and *C. albicans* adhesion ability test. The results obtained revealed that all VK+DL groups satisfied ISO standards in the three-point bending test, and the *C. albicans* adhesion test showed a lower adhesion rate of *C. albicans* than that for VK only. DL-7900, an antibacterial glass, is currently used for clothes, such as lab coats, and interior wall paint materials in hospitals in the medical field. DL-7900 is a powdery inorganic material with an average particle size of 10µm and composition of ZnO-B$_2$O$_3$-Ag$_2$O. It is a chemically stable oxide and has excellent heat resistance (13). Since the melting temperature at the time of injection molding of PEEK is approximately 300°C, any antibacterial material added to PEEK must have high heat resistance. DL-7900 is suitable as an antibacterial agent to be added to PEEK because it has the excellent heat resistance characteristic of inorganic materials and retains its antibacterial effects at the melting temperature during PEEK injection molding. However, several disadvantages are associated with the application of inorganic materials to dental materials, particularly a high flexural modulus, low bending strength, fracture toughness, and an increased likelihood of breakage when plastic deformation occurs (14). Therefore, when DL-7900 was added to VK, a criterion for the addition rate that did not affect mechanical strength was examined in the three-point bending test. In the three-point bending test, the VK+DL group showed higher values than acrylic resin in evaluation items of bending strength, the elastic modulus, and 0.05% proof stress (p < 0.05). These results suggest that the VK+DL group has better bending properties than acrylic resin. Comparisons among the VK-DL group indicated that VK+DL1, VK+DL2, and VK+DL3 showed no significant difference from VK in the evaluation items of bending strength, the elastic modulus, and 0.05% proof stress (p > 0.05) (Figs. 1–3). However, some VK+DL4 and VK+DL5 specimens broke during testing. Furthermore, VK+DL4 and VK+DL5 had significantly lower flexural strength and 0.05% proof stress than VK, and a significantly higher flexural modulus (p < 0.05) (Figs. 1–3). These results were attributed to the characteristics of inorganic materials, such as a higher flexural modulus and lower flexural strength and fracture toughness value, affecting the VK+DL group with antibacterial glass added. The above results indicate that the addition of more than 10% DL-7900 to VK has a negative impact on mechanical strength.

The results of the *C. albicans* adhesion test showed that the addition of DL-7900 to VK decreased *C. albicans* adhesion rates. A negative correlation was observed between the antibacterial glass addition rate and *C. albicans* adhesion rate. Thus, DL-7900 appears to prevent *C. albicans* adherence in the DL-7900 effect confirmation test.

Control group: The Cell Desk was cultured in culture medium.
MCFG group: MCFG was added to the Cell Desk after culturing with culture solution.
DL-7900 group: The Cell Desk was cultured in culture medium with DL-7900 added.
MCFG: Funguard® 25 mg for infusion.

**Fig. 5.** Representative SEM images of *C. albicans* adherence in the DL-7900 effect confirmation test.
albicans adherence or exerts antifungal effects against C. albicans. DL-7900 contains silver oxide and zinc oxide and exerts antibacterial effects by eluting metal ions in water (13). In addition, DL-7900 shows antibacterial activity value according to ISO 22196: 2007 standard (10). Heavy metal ions possess antibacterial properties, and silver ions exhibit strong antibacterial activity. Silver ions are taken into cells, bind to proteins, and inhibit their functions (14). Silver is also instantaneously reduced even if it is oxidized. The surface of silver constantly fluctuates between ionic and metallic states, thereby generating electrical energy. Previous studies reported that this electrical energy exerted antifungal effects by affecting fungal cell walls (15).

In the C. albicans adhesion test, the VK+DL group showed decreases in C. albicans adherence with increases in the antimicrobial glass addition rate (Fig. 4). SEM showed that C. albicans cells in the control group had a spherical shape with smooth surface layers, whereas fungal cells in the DL-7900 and MCFG groups had a deformed surface morphology with rough surface layers (Fig. 5). MCFG exhibits fungicidal activity and causes cell wall dysplasia (16). C. albicans cell deformation was similar in the DL-7900 and MCFG groups, indicating that DL-7900 exhibits fungicidal activity. These results suggest that silver ions eluted from DL-7900 are useful for preventing denture stomatitis by killing C. albicans. Furthermore, the application of DL-7900 may help to prevent aspiration pneumonia caused by C. albicans, which has recently been reported (8).

One major limitation of the present study is that tests were performed in a static environment, which does not mimic the self-cleaning action of human saliva and its effects on fungal cell adhesion. Therefore, additional investigations under dynamic conditions, such as in a flow chamber, are needed to confirm the present results.

**Conclusion**

Antibacterial glass-added PEEK showed bending properties applicable as a denture base material by satisfying the ISO20795-1 standard. Furthermore, the addition of antibacterial glass appears to be useful for inhibiting C. albicans adhesion to the denture base. VK+DL3 showed no significant difference from VK in each evaluation item of the three-point bending test. Therefore, the antibacterial glass addition rate of 7.5% may be the maximum addition rate that most effectively suppresses the growth of adherent C. albicans cells without impairing the bending properties of VK.

VK+DL produced by adding antibacterial glass in the present study had the same high bending properties as conventional PEEK and exerted the same effects as MCFG on the surface of C. albicans. When used, it has higher bending strength than conventional denture base materials, suggesting its potential efficacy at preventing denture stomatitis.

**Conflict of interest**

We declare that we have no competing financial interests.

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