From Production Skills to Scientific Knowledge,
and Onward to Fusion with Medical Science

Dental Adhesive

# Safety Evaluation Report

## Physical Properties and Safety of the Dental Adhesive, “Multi Primer”

Vol.11 in a Series on Safety Reports

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INTERNATIONAL20190208

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# Physical Properties and Safety of the Dental Adhesive, “Multi Primer”

## 1. Introduction

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The resin material used for repairing a dental crown is built up on a frame and it can reproduce natural teeth easily with excellent operability; as a result, hard resin is widely used. Also, for the implant superstructure, hybrid hard resin— which has the convenience of hard resin, and strength and aesthetics superior to hard resin—is often used. On the other hand, cast precious metal alloy is generally used for as a material in the frame for repairing a dental crown. However, with the advance of digitization, it is now possible to process dental materials with CAD/CAM, and zirconia, which suitable for cutting work, is now widely used. It is expected that in the future there will be an increase in the number of cases using non-precious metal alloys such as titanium or cobalt chromium alloy, which are difficult to process.

In frames produced by casting, a strong bond between the resin and frame is mechanically maintained by applying retention beads. On the other hand, in the cutting process, unlike in the casting process, retention beads cannot be used. Therefore, a strong bond between resin and cutting frame cannot be obtained; the chemical adhesion effect of the primer is therefore important. However, the chemical adhesion of the adherend has a different optimum compound depending on the material of the adherend; as a result, it is necessary to use primers appropriately for each frame material, resulting in complicated technical operations.

Given the problem above, we have investigated a new primer with the development concepts of: (1) high adhesion strength; (2) long-term durability; and (3) excellent versatility (i.e., that it can be used for various materials in a more simpler manner). We then succeeded in developing the "Multi Primer" series.

The "Multi Primer" series is made up of three products. All of them can be used for building up our "Luna wing" and "TWiNY" hybrid resin for crowns.

The liquid-type primer "Multi Primer LIQUID" can also be applied to frames made of precious metal alloys, non-precious metal alloys or zirconia. The paste-type primer "Multi Primer PASTE" is a primer for metals (precious metal alloys and non-precious metal alloys) and also achieves the opaquer function of Invisible Opaque of "Luna-Wing" and "TWiNY." The liquid-type primer "Multi Primer REPAIR LIQUID ONE" is a pretreatment liquid for additional layering by hard resin, when shape modification is carried out. Each product has achieved greatly improved versatility by expanding and developing the functions of primers and the additional layering solutions adopted for "Luna Wing" and "TWiNY."

In these tests, we examined adhesion between "Multi Primer" and precious metal alloy, non-precious metal alloy, and zirconia; their durability and biocompatibility were also evaluated.
We then compiled the results in "Safety Test Report Vol. 11, Physical Properties and Safety of the Dental Adhesive ‘Multi Primer.’"
We hope that this test report will be of help as technical information for dental practitioners, technicians, and patients.



Ritaro Matsuura, Ph. D. in Agriculture, Senior Chief Researcher, Biological Science and Safety Laboratory

Dental materials are medical devices used in the oral cavity. Therefore, excellent biocompatibility is required for dental materials. However, the oral cavity, in which dental materials are used for long periods of time, features harsh environmental conditions such as temperature swings, fluctuations in pH, and chewing force. Thus, there is concern about adverse effect of poor-quality dental materials on the human body, since intraoral abrasion and dissolution of such products could cause human tissue to be exposed to the components.

In order to resolve such concerns, our Biological Science and Safety Laboratory has established a collaborative research program with the Department of Oral and Maxillofacial Surgery, Kochi Medical School, Kochi University to provide evidence about the biocompatibility of dental materials. For example, we have conducted evaluations according to ISO 10993 "Biological evaluation of medical devices" [1] along with original in-house experiments and evaluations using cells, tissues and genetic engineering. We have subsequently published the findings of our investigation as Safety Test Reports. Our Safety Test Reports (Vol. 4, “Biological Evaluation of Luna-Wing” and Vol. 8, “Biological Evaluation of Hybrid Resin, TWiNY”), academic conference presentations[2], and academic papers[3] have shown excellent biocompatibility of dental resin materials.

Resin material for dental crown restoration is built up on a frame, and reproduces natural teeth. Dental primers are used to adhere the resin material to the frame tightly. Direct contact between dental primer and human tissue in the oral cavity is unlikely but not impossible. Therefore, the biocompatibility of dental primers should be evaluated.

In order to evaluate the biocompatibility of dental primers, we prepared clinical-mimetic test specimens in which TWiNY was additionally built up on TWiNY using the newly developed "Multi Primer REPAIR LIQUID ONE." We then conducted two cytotoxicity tests (Trypan blue-exclusion test and WST-8 cytotoxicity test) to investigate the effect of test specimens on the human monocytic leukemia cell line THP.1.

Our series of Safety Test Reports has been issued in line with our mission to provide Safety and Soundness. We hope that this test report will deepen your interest in the biocompatibility of dental materials, and resolve any concerns you may have about them.

## 2. Material Composition

· Multi Primer LIQUID

The main component of “Multi Primer LIQUID” is a volatile solvent (ethanol); small amounts of thiol compound and silane coupling agent are included as priming components. The thiol compound is for precious metals, and the silane coupling agent is effective for pretreatment of adhesion to non-precious metals and ceramics.

· Multi Primer PASTE

“Multi Primer PASTE” is mainly composed of monomers (urethane dimethacrylate and triethylene glycol dimethacrylate) and silica fillers. It also contains small amounts of polymerization initiator, polymerization co-initiator, pigments and priming components. The thiol compound and silane coupling agent are employed for priming; a the thiol compound is for precious metals, and the silane coupling agent is effective for adhesion to non-precious metals and ceramics. It also functions as an opaquer corresponding to Invisible Opaque for “Luna-Wing” and “TWiNY.”

· Multi Primer REPAIR LIQUID ONE

“Multi Primer REPAIR LIQUID ONE” is mainly composed of monomers (urethane dimethacrylate and triethylene glycol dimethacrylate). It also contains small amounts of polymerization initiator, polymerization co-initiator, and a silane coupling agent as a priming component. Since hard resin is mainly composed of inorganic fillers and monomers, ceramics derived from the fillers is abundantly exposed on the surface. We have designed the “Multi Primer” series so that a molecule of silane coupling agent attaches effectively to a reaction site on the surface of the ceramics.

Table 1 Application of Primers

| Product | | Name | State | Application | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Non-precious metal | | | Precious metal | | Ceramics |
| | | | | Ti and Ti Alloy | Ni-Cr Alloy | Co-Cr Alloy | Au Alloy | Au-Ag-Pd Alloy | Zirconia $ZrO_2$ |
| Conventional Product | Luna-Wing | Primer | Liquid | – | – | – | ✓ | ✓ | – |
| | TWiNY | Primer | Liquid | – | – | – | ✓ | ✓ | – |
| New Product | Multi Primer | Multi Primer LIQUID | Liquid | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | | Multi Primer PASTE | Paste | ✓ | ✓ | ✓ | ✓ | ✓ | – |

## 3. Adhesion

In the field of dentistry, it has been known that thiols are suitable for precious metals, phosphates for non-precious metals [4] and silane coupling agents for resin materials [5] for priming. Recently, we have found that not only phosphate compounds but also silane coupling agents have an effect of improving resin adhesion to non-precious metals [6]. As a results, we have established that both thiol and silane coupling agent can be applied in the priming of various materials [7].



In the case "Multi Primer LIQUID", test specimens were prepared by the following steps. After application of Multi Primer LIQUID on the adherends, opaque resin was applied on the masked surface, the surface was irradiated by light to polymerize it, and a stainless steel forming rod for tension was fixed on the cured surface with resin cement.

In the case of "Multi Primer PASTE", test specimens were prepared by the following steps. Multi Primer PASTE was applied on the surface of the masked adherends, the surface of the adherend was irradiated by light to polymerize it, and a stainless steel forming rod for tension was fixed on the cured surface with resin cement (Figure 1). The test specimens were kept in water at 37 °C for 1 day and then subjected to a test to pull the stainless steel molded rods vertically to the adhesion surface at a speed of 0.5 mm/min, and the stress at fracture was taken as the tensile bond strength.

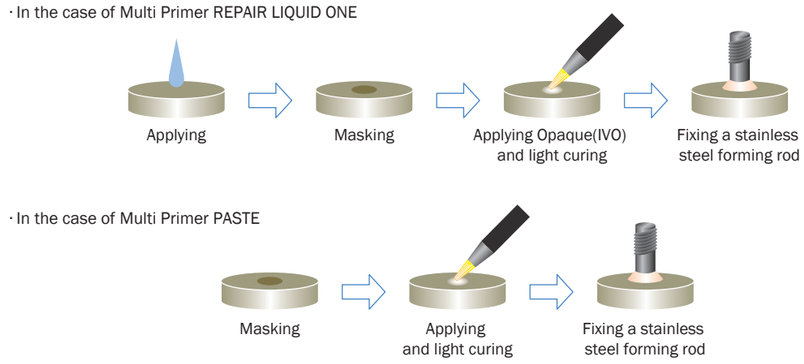



Figure 1 Preparing test specimens for tensile bond strength test

As shown in Figure 2, it can be confirmed that the adhesive strength of the resin improves for each metal by use of "Multi Primer LIQUID" and "Multi Primer PASTE". We also confirmed that "Multi Primer LIQUID" is effective for zirconia.

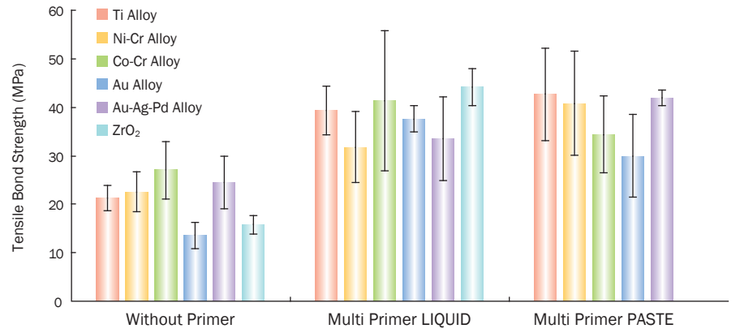



Figure 2 Tensile bond strength between resin and each adherend

Table 2 shows the compositions of metals (alloys) as adherend for the test.

Table 2 Compositions of metals (alloys) as adherend for the test

| Adherend | Composition |
|---|---|
| Ti (ASTM standard Grade 4) | Ti 99.0%, Cmax 0.08%, Hmax 0.015%, Omax 0.40%, Nmax 0.05%, Femax 0.50% |
| Ni-Cr Alloy | Ni 65%, Cr 22.5%, Mo 9.5%, Nb 1%, Si 1%, Fe 0.5%, Ce 0.5%, Cmax 0.02% |
| Co-Cr Alloy | Co 61%, Cr 26%, Mo 6%, W 6%, Si 1%, Fe 0.5%, Ce 0.5%, Cmax 0.02% |
| Au Alloy | Au 83%, Ag 11.97%, Cu 5%, Ir 0.03% |
| Au-Ag-Pd Alloy | Au 12%, Ag 49.5%, Pd 20%, Cu 16.85%, (Zn, In, Ga, Ir) 1.65% |

In the additional layering of "TWiNY," the amount of ceramics filler contained in the product is large. Therefore, it is necessary to restore the surface of the unpolymerized layer with the additional layer solution “TWiNY” Repair Primer after the silane coupling treatment. (On the other hand, "Luna-Wing" uses organic-inorganic composite filler, so "Luna-Wing REPAIR LIQUID" alone is sufficient.) Therefore, "TWiNY" has one more step of additional layering than "Luna-Wing."

In the case of "Multi Primer REPAIR LIQUID ONE," test specimens were prepared by the following steps. Multi Primer REPAIR LIQUID ONE was applied on the surface of masked adherend (resin), and the flowable resin TWiNY FLOW was applied and cured by light. A stainless steel forming rod for tension was then fixed on the cured surface with resin cement (Figure 3). The test specimens were kept in water at 37 °C for 1 day and then subjected to a test to pull the stainless steel molded rods vertically to the adhesion surface at a speed of 0.5 mm/min, and the stress at fracture was taken as the tensile bond strength.

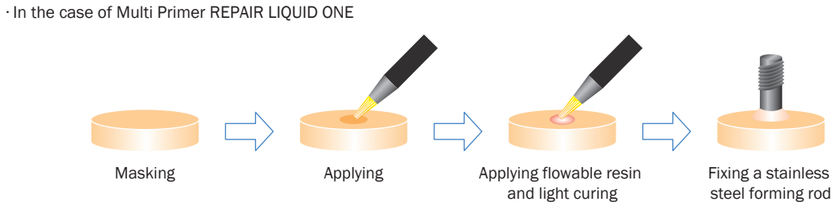



Figure 3 Preparing test specimens for tensile bond strength test

Application of "Multi Primer REPAIR LIQUID ONE" can simultaneously perform silane coupling treatment and recovery of the unpolymerized layer on the surface. However, as shown in Fig. 4, it was confirmed that the adhesive strength was equivalent to that of the conventional products[8]. By using "Multi Primer REPAIR LIQUID ONE," it will be possible to simplify the additional layering operation of "TWiNY" from 2 steps to 1 step. Of course, it can also be used for "Luna-Wing."



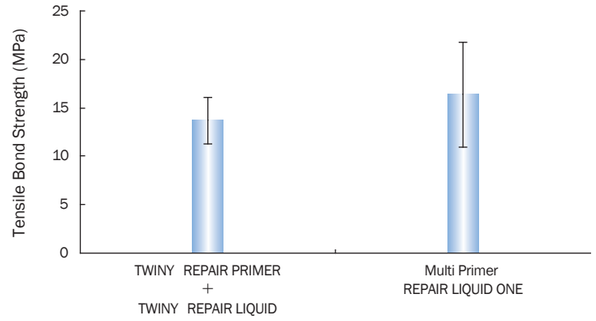



Figure 4 Tensile bond strength between resin materials

## 4. Durability

When evaluating the durability of composite materials, a thermal cycle test is commonly performed in which alternate immersion in hot water and cold water damages the bonding interface between different materials due to the difference in thermal expansion coefficient. In the ISO standard (ISO 10477: 2018) for hard resin for dental crowns, when adhesion to the lower structure without mechanical maintenance such as retention beads is indicated, the standard value of the shear bond strength after 5,000 thermal cycles is 5 MPa or more[9].

For shear tests, a frame with cylindrical holes was used to polymerize body resin in a columnar shape on the cured surface of opaque resin (Figure 5). The test specimens were kept in water at 37 °C for 1 day, and testing for 5,000 thermal cycles (5-55 °C, 30 sec. each) was carried out. A load was applied to the cylinder of the resin along the adhesion surface at a speed of 1 mm/min, and the stress at break was defined as the shear bond strength.

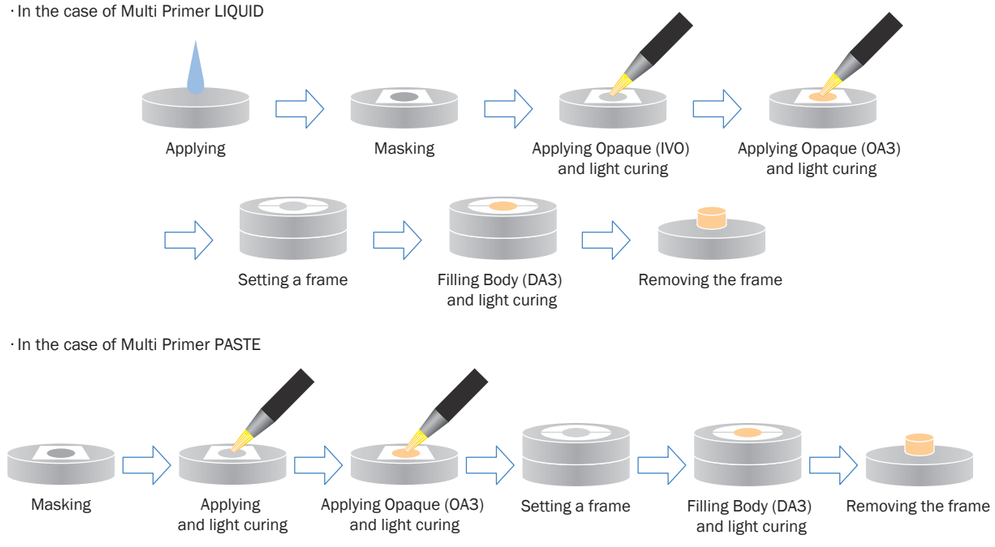



Figure 5 Preparing test specimens for shear test

As shown in Figure 6 and 7, both "Multi Primer LIQUID" and "Multi Primer PASTE" have a longitudinal shear strength exceeding the standard value of 5 MPa even after the thermal cycle, and resin ("TWiNY") was fixed to each adherend. Sufficient durability was thus confirmed. In "Multi Primer REPAIR LIQUID ONE," the adhesive strength was not measurable because the resin on the adhered side cracked while the bonded resin remained fixed, before the bond site was broken, but it was confirmed that the durability is sufficient.

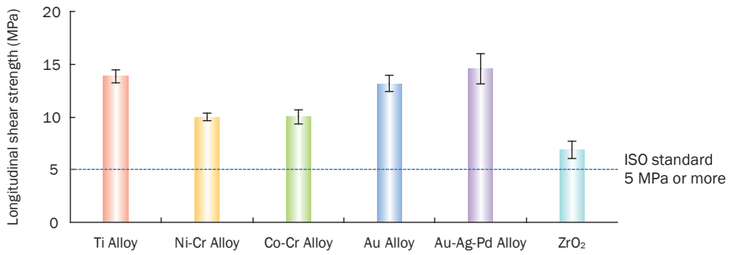



Figure 6 Longitudinal shear strength of resin after thermal cycle (Multi Primer LIQUID)

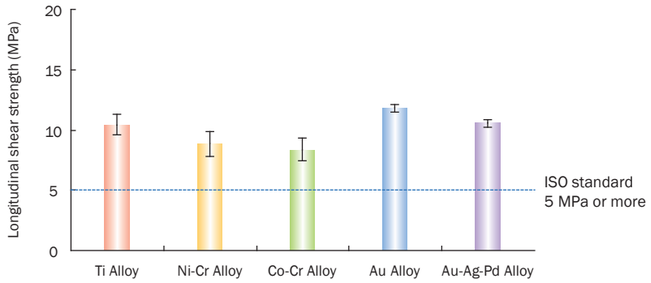



Figure 7 Longitudinal shear strength of resin after thermal cycle (Multi Primer PASTE)



## 5. Biocompatibility

Cytotoxicity could cause toxicity in tissues, organs, and organisms. Therefore, essential requirements in the evaluation of biological safety of dental materials include the evaluation of cytotoxicity. In order to evaluate the biocompatibility of "Multi Primer," we conducted cytotoxicity tests using the human monocytic leukemia cell line THP.1 cell (distributed by the Department of Oral and Maxillofacial Surgery, Kochi Medical School, Kochi University).

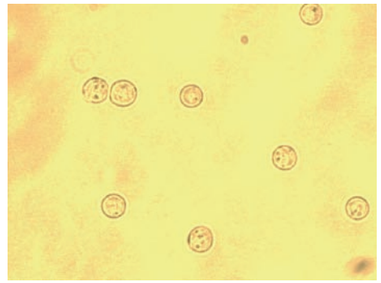


Figure 8 Human monocytic leukemia cell line THP.1 cell

Test specimens were prepared as follows. Hybrid resin “TWiNY” was cured to a diameter of 12 mm × a thickness of 1 mm. Subsequently, another layer of "TWiNY" was additionally built up on it, using "Multi Primer REPAIR LIQUID ONE." As a reference, a test specimen of “TWiNY" was superposed on another layer of "TWiNY" without "Multi Primer REPAIR LIQUID ONE."

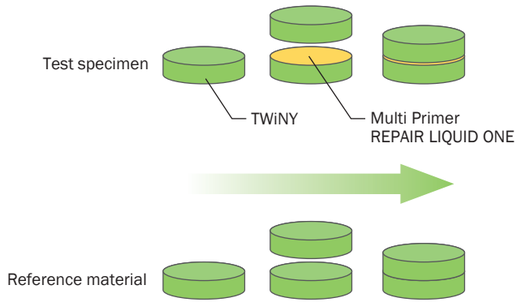
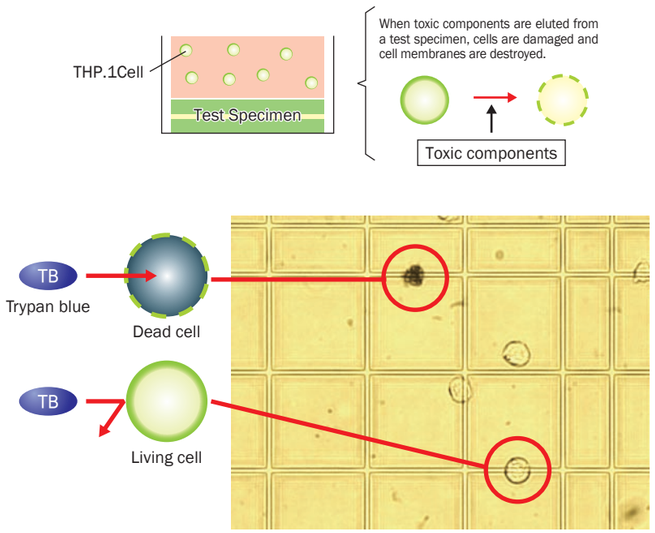



Figure 9 Preparation of specimens for cytotoxicity test

Each test specimen was placed in a well of a24-well plate, and 100,000 THP.1 cells were seeded on the specimen. THP.1 cells were cultured at 37℃ for 3 days under 5% $CO_2$, and then harvested for the following cytotoxicity tests.

Trypan blue dye exclusion test [10)]

When cells are damaged by the toxic components, the cell membrane are destroyed. Since cell membrane exclude dye compounds such as trypan blue, living cells with cell membrane could not be stained, whereas dead cells without membranes could be stained blue. Therefore, counting living cells and dead cells with a hematocytometer enabled evaluation of the effect of the test specimen on cell proliferation and cell viability.




Trypan blue are taken up to the cell with destroyed cell membrane, and stains the cytoplasm.

Figure 10 Principle of Trypan blue dye exclusion test

Cells were mixed with Trypan blue, and living cells and dead cells were then individually counted with a hematocytometer. Cell viability was calculated from the ratio of living cell number to total cell number.

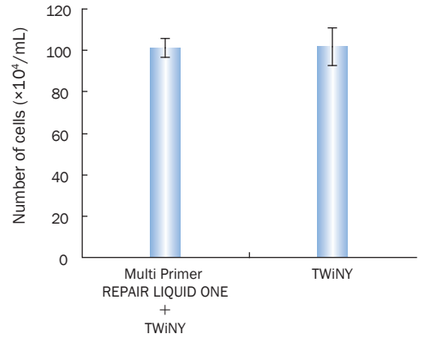


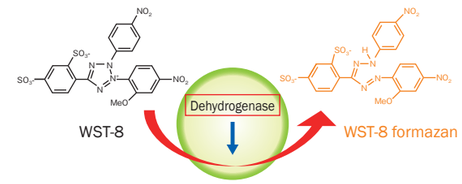

Figure 11 Proliferation of THP.1 cells cultured on resin

We have reported excellent biocompatibility of the reference material "TWiNY" [2]. There were no significant differences in cell proliferation and cell viability between the test specimen and reference material.



WST-8 Cytotoxicity test [11, 12]

In this experiment, the indicator WST-8 was metabolized by living cells to orange-colored WST-8 formazan ($\lambda$max = 450 nm). Therefore, quantification of WST-8 formazan by measuring the absorbance at 450 nm could evaluate influence of the test specimen on the metabolic activity of the cell. The value of absorbance increased with the increase in the number of living cells.




WST-8 is reduced to orange-colored WST-8 formazan by the activity of dehydrogenase in cells.

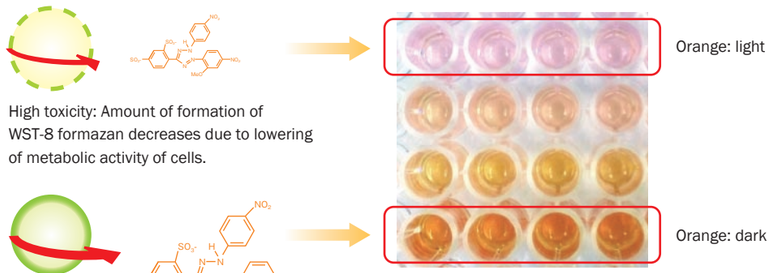



Figure 12 Principle of WST-8 cytotoxicity test

Cells cultured on a test specimen were transferred to wells of a 96-well culture plate, and WST-8 reagent was then added. After incubation at 37 °C. for 2 hours, the absorbance of WST-8 formazan was measured at 450 nm.

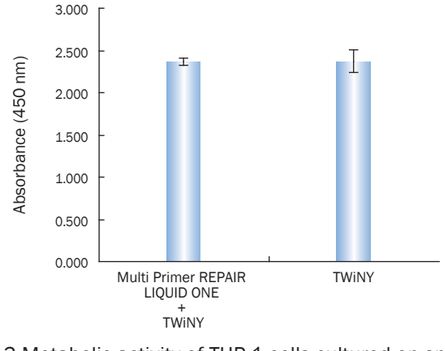



Figure 13 Metabolic activity of THP.1 cells cultured on specimen

In this assay, the test specimen showed equivalent absorbance to the reference material. Therefore, we considered that there is no adverse effect of "Multi Primer REPAIR LIQUID ONE" on the metabolic activity of THP.1 cells.

## 6. Conclusion

· Adhesion
Each component of "Multi Primer" series was confirmed to have adhesion to the material to be applied.

· Durability
It was confirmed that adhesion of each component of the "Multi Primer" series to the object of application was maintained over the long term. The use of "Multi Primer" is thought to greatly reduce the risk of resin falling out of the frame in the oral cavity.

· Biocompatibility
A test specimen, in which "TWiNY" was additionally built-up on another layer of "TWiNY" using "Multi Primer REPAIR LIQUID ONE," showed no cytotoxicity to THP.1 cells. Therefore, we considered that Multi Primer has excellent biocompatibility.

The cytotoxicity test was carried out in collaboration with the Department of Oral and Maxillofacial Surgery, Kochi Medical School, Kochi University.

## Body of related technological presentations

### Monographs submitted

Kato T, Kimura H, Saigo K, Yamada B, Yamauchi J, Anraku T：Effects of a silane coupling agent on the tensile adhesive strength between resin and titanium. *J. Appl. Polym. Sci.*, 129(5)： 2922-2930, 2013.

### Academic conference presentations

· 58th Japanese society of dental materials and devices ( 22-23 Oct. 2011)
Kimura H, Kato T, Saigo K, Yamada B, Yamauchi J, Yamamoto S: Evaluation of adhesive strength between resin and titanium with a primer containing a silane coupling agent (Poster presentation)

· 60th Japanese society of dental materials and devices ( 13-14 Oct. 2012)
Kimura H, Kato T, Saigo K, Yamada B, Yamauchi J, Anraku T: Development of a repairing liquid containing a silane coupling agent for hybrid resins (Poster presentation)

· 61th Japanese society of dental materials and devices ( 13-14 Apr. 2013)
Kimura H, Kato T, Saigo K, Yamada B, Yamauchi J, Anraku T: Development of primer for zirconia using silane coupling agent (Poster presentation)

· 62th Japanese society of dental materials and devices ( 19-20 Oct. 2013)
Kimura H, Kato T, Saigo K, Anraku T: Evaluation of the adhesive strength of resins with a newly developed primer against precious metals, non-precious metals and ceramics (Poster presentation)



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## Previously Published Safety Test Reports

Vol. 1 Pursuing International Standards in Quality and Safety (Dec. 2004)
Vol. 2 ZEO METAL Series Elution Test and in Vitro Cytotoxicity Test (Jun. 2005)
Vol. 3 Elution Test and in Vitro Cytotoxicity Test of Precious-Metal Alloys and Gold Alloys for Metal Ceramic Restoration Use (Dec. 2005)
Vol. 4 Biological Evaluation of Luna-Wing (Jun. 2006)
Vol. 5 Report on Physical Properties and Safety of High-Carat Gold Alloys (Oct. 2007)
Vol. 6 Examination of the Biological Impact of the Physical Properties of Dental-Material Alloys and Gold Alloys for Hard Resin and Metal Ceramic Restoration Use (May 2008)
Vol. 7 Report on the Physical Properties and Safety of the Gold Alloy Nexo-Cast (Oct. 2008)
Vol. 8 Biological Evaluation of the Hybrid Composite Resin, TWiNY (Jun. 2010)
Vol. 9 Chemical and Biological Characteristics of Precious-Metal Alloys: Elution Characteristics Produced Through Mixture with Titanium (Feb. 2011)
Vol. 10 Physical Properties and Safety of the Precious-Metal Alloy for Metal Ceramic Restoration Use Brightis (Oct. 2011)
Vol. 11 Physical Properties and Safety of the Dental Adhesive, Multi Primer (Mar. 2014)
Vol. 12 Safety of the Dental Pulp Capping Material, TMR-MTA CEMENT (Jan. 2018)

Editor: Teruo Anraku
Publisher: Takahiko Yamamoto
Date of publication: 20 Mar. 2014