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Preparation and Characterization of Antibacterial Dental Resin Cement Material

Duck-Hyun Kim[†], Hwi-Su Jung[‡], Sun-Hun Kim[§], and A-Young Sung[†,*]

[†]*Department of Optometry & Vision Science, Catholic University of Daegu, Gyeongsan 38430, Korea.*
[*]*Email: sayy@cu.ac.kr*
[‡]*Korea Optics Technology Institute, Gwangju 61008, Korea.*
[§]*Department of Oral Anatomy, College of Dentistry, Chonnam National University, Gwangju 61186, Korea.*
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ABSTRACT. Bis-GMA, TEGDMA, and camphorquinone were used as the main material, cross-linking agent, and photoinitiator, respectively. In addition, 2-isocyanatoethyl methacrylate was used as an additive for high strength, while the 3-hydroxypyridine was used as an additive for antibacterial activity. Photopolymerization was also carried out at a 440-480 nm wavelength and at about 1000 mW/cm^2 intensity for about 40 seconds. The breaking strength measurement of the samples showed that the breaking strength increased along with increasing the addition ratio of IEM, while it took less time until the polymerization was complete, thereby suggesting that the degree of polymerization has the tendency to increase. And also, compared to the size of the clear zone formed by ampicillin, the 3-hydroxypyridine group exhibited antimicrobial activity induced by ampicillin. The results of this study suggest that the use of 2-isocyanatoethyl methacrylate as an additive for high strength and 3-hydroxypyridine as an additive for improved antibacterial activity would improve the usability of the fabricated polymer as a dental resin cement material with high functionality.

Key words: 3-hydroxypyridine, 2-isocyanatoethyl methacrylate, Antibacterial, Higher strength, Dental resin cement

INTRODUCTION

Amalgam has been used as a dental restorative material for over 100 years due to its malleability and low cost.[1] However, amalgam is an alloy that contains tin, mercury, copper, and silver. In particular, mercury, which accounts for 50% of the total content, can cause headaches, nervous system disorders, and vomiting when a person is exposed to it for an extended period of time.[2–4]

In order to reduce such side effects, zirconia-based dental restoration has recently been introduced mainly for its biocompatibility, strength, and pleasing aesthetics in the end result. Resin cement is also being used to maintain the aesthetics and mechanical strength during the zirconia cementation process.[5,6]

Bisphenol A glycerolate dimethacrylate (Bis-GMA), which is one of the key dental materials, has multiple advantages in that methacrylate is located at both ends in order to form a three-dimensional cross-linking structure, it is not volatile in the oral cavity, and it hardens rapidly. However, its high viscosity limits its potential use as a dental resin cement material. Triethylene glycol dimethacrylate (TEGDMA), which is a cross-linking agent, is used as an additive in the process in order to compensate for this shortcoming. Meanwhile, camphorquinone, which is a photoinitiator that absorbs light in a visible light range of 440 nm wavelength, is mainly used as a photoinitiator of dental resin cement.[7–10]

The 2-isocyanatoethyl methacrylate used in this experiment is an isocyanate family substance, which is used to make adhesives, curing agents, and urethane elastomers.[11] When used as an additive for an ophthalmic lens, it increases the tensile strength of the lens.[12] In addition, the 2-isocyanatoethyl methacrylate is a monomer of a newly synthesized inorganic polymer with a high ceramic yield.[13] The dental material, which is a monomer created with a new macro method,[14] is used to impart bonding property to dentin, thereby making it a popular material for various industrial and dental applications.[15]

The 3-hydroxypyridine, which is used as an additive in this experiment, is a pyridine family material. Pyridine is being used in various fields for its antibacterial property, which makes it an ideal material for ion exchange resin, microfiltration membrane, biosensor,[16,17] or ophthalmic lenses.[18,19]

In this study, Bis-GMA, TEGDMA, and camphorquinone were used as the main material, cross-linking agent, and photoinitiator, respectively. In addition, 2-isocyanatoethyl methacrylate was used as an additive for high strength, while the 3-hydroxypyridine was used as an additive for antibacterial activity. Finally, dental resin cement was manufactured in order to evaluate its physical and antibacterial properties, as well as its applicability as a dental resin cement material.

EXPERIMENTAL METHOD

Materials and Reagents

In this study, bisphenol A glycerol dimethacrylate (Bis-GMA), which is a dental resin cement, and hydroxypropyl methacrylate (HPMA), is a dilution agent, were used as the main materials. In addition, benzoyl peroxide (BPO) and camphorquinone (Cam) were used as photoinitiators, whereas butylated hydroxytoluene (BHT) was used as a photo catalyst, and triethylene glycol dimethacrylate (TEGDMA) was used as a cross-linking agent. Finally, 2-isocyanatoethyl methacrylate (IEM) was used as an additive for high strength, while 3-hydroxypyridine (3HP) was used as an additive for antibacterial property. All reagents used in this study were made by Sigma-Aldrich.

Experimental Method

The main dental materials (Bis-GMA and HPMA) and a cross-linking agent (TEGDMA) were mixed together, according to their recommended proportions, before being stirred for approximately 24 hours by using an agitator (Vortex GENIE 2, Scientific Industries, USA) to prepare the desired mixture. In addition, BPO, BHT, and Cam were used as photoinitiators and photocatalyst, and IEM and 3-HP were used as additives to impart high strength and antibacterial properties to the mixture, respectively. The mixture was then stirred for approximately 5 hours by using an agitator. In regard to the conditions for photo-polymerization, the mixture was polymerized for 40 seconds in a 460 nm wavelength range. The fabricated samples were then evaluated for various physical properties, such as flexural strength, flexural bonding strength, antibacterial activity, breaking strength, and viscosity. The surface of the samples was also evaluated via SEM analysis. The groups, to which 3DHP was added according to their respective proportions, were each named 3HP-1, 3HP-2, 3HP-3, and 3HP-4, whereas the groups, to which IEM was added according to their respective proportions, were named Iso-1, Iso-2, Iso-3, and Iso-4, respectively. *Table* 1 shows the mixing proportion of the dental resin cement used in the experiment.

Measuring Instruments and Analysis

Flexural Strength: The sample was filled into a mold measuring (25 ± 2) mm × (2 ± 0.1) mm × (2 ± 0.1) mm, and then it was polymerized by radiating light beams, so that their energy sources would be overlapping with each other at the center. After the specimen hardened at (37 ± 1) °C, it was stored in a thermostat for 15 minutes before it was removed from the mold. The specimens were stored in (37 ± 1) °C distilled water until the load test was performed after 24 hours.

The specimen was measured with a caliper before it was tested for its flexural strength, and the load was applied at a rate of (1 ± 0.3) until the specimen was fractured in the flexural strength measuring device before recording the maximum load at the time of fracture of the specimen. At this time, the diameter of the support roller and the load roller were 2 mm each, and the distance between the centers of the three-point bending support rollers was 20 mm. The flexural strength (σB) of each specimen was calculated in MPa units.

$$\text{Flexural strength} = \frac{3Fl}{2bh^2}$$

F: Maximum load to the specimen (N)
I: Distance between the centers of support rollers (20 mm)
b: Width of the specimen (mm)
h: Thickness of the specimen (mm)

Breaking Strength: The casting/molding method was used in the experiment. The specimen, which was fabricated by irradiating light with a thickness of approximately 100 μm was subject to a loading force at a rate of 10 mm/

***Table* 1.** Percent compositions of samples (unit: %)

Sample	DT	TEGDMA	BPO	BHT	Camphor	IEM	3HP	Total
Ref	89.69	4.48	2.69	0.45	2.69	0.00	0.00	100.00
Iso 1%	88.89	4.44	2.67	0.44	2.67	0.89	0.00	100.00
Iso 5%	85.84	4.29	2.58	0.43	2.58	4.29	0.00	100.00
Iso 10%	82.30	4.12	2.47	0.41	2.47	8.23	0.00	100.00
Iso 20%	76.05	3.80	2.28	0.38	2.28	15.21	0.00	100.00
3HP 1%	88.89	4.44	2.67	0.44	2.67	0.00	0.89	100.00
3HP 3%	87.34	4.37	2.62	0.44	2.62	0.00	2.62	100.00
3HP 5%	85.84	4.29	2.58	0.43	2.58	0.00	4.29	100.00
3HP 7%	84.39	4.22	2.53	0.42	2.53	0.00	5.91	100.00

min by using a universal testing machine (AGS-X 20N, SHIMADZU, Japan), where the maximum load, at which the specimen was broken, was measured in N units.

Viscosity measurement: After the temperature setting of the thermostat was completed, 500 μg of the sample was applied to the center of the cup and an appropriate torque was set for the sample. The viscosity of the sample was then measured at the stabilization time.

Antibacterial Property: *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli* FMF were used as bacterial colonies. The frozen bacteria were cultured in a solid medium by using a platinum wire to form colonies of single cell clusters. Then, a single colony was inoculated to a 5 mL liquid medium and cultured at 37 ℃ for 16 hours. For the preparation of the solid medium, 6 g of brain heart infusion, 6 g of peptic digest of animal tissue, 5 g of sodium chloride, 3 g of dextrose, 14.5 g of pancreatic digest of gelatin, 2.5 g of disodium phosphate, and 15 g of agar, which altogether add up to 37 g, were mixed in a flask, and then added with distilled water to make a 1 L solution. The prepared solution was then heated for 1 minute until it has completely dissolved. Thereafter, it was autoclaved at 121 °C for 15 minutes and cooled down to 50 °C before it was dispensed to 90 mm petri dishes and cured. The BHI liquid medium was prepared in the same manner by extracting agar from the solid medium. Furthermore, the absorbance of the bacterial culture was measured at 600 nm by using an absorbance meter to calculate the number of bacteria (the number of bacteria is 1×10^9 CFU/ml when the absorbance is 1 at OD 600). In order to measure the antibacterial activity of the specimen, 2 g of beef extract, 17.5 g of acid hydrolysate of casein starch, 1.5 g of starch, and 17 g of agar, which altogether add up to 38 g, were mixed in a flask, and then added with distilled water to make a 1 L solution. The prepared solution was then heated for 1 minute until it has completely dissolved. The mixture was then autoclaved at 121 °C for 15 minutes and cooled down to 50 °C before it was dispensed to 90 mm petri dishes and cured. A total of 3×10^6 CFU/ml bacteria were rubbed onto Mueller Hinton (BM) agar medium by using a cotton swab until they spread uniformly across the medium. Sterile paper disks (2 mm in thickness and 6 mm in diameter) were placed on the bacteria-bearing media, and samples 1, 2, and 3 (each measuring 20 μl) were applied on each paper disc under dark condition. The sterilized water was used as a negative control and the antibiotic ampicillin (20 μg), which is currently available in the market, was used for Gram-negative bacteria as a positive control, whereas penicillin-streptomycin was used for Gram-positive bacteria. *E. coli* and *S. aureus* were cultured for 20 hours at 37 °C in the culture medium, and *S. mutans* with a slow growth rate for 72 hours until clear zones were formed. It is believed that the clear zone was formed due to the zero growth of bacteria in the culture medium. In addition, 0.04, 0.4, and 4 μg of ampicillin were each added to *E. coli* and *S. aureus* in order to compare the antibacterial activity of the samples to that of ampicillin. The size of the clear zone, which was formed as a result of the slow growth rate of the specimen, was measured because it implies that the larger the clear zone, the greater the antibacterial activity. The experiment was repeated over 5 times.

RESULTS AND DISCUSSION

Polymerization and Fabrication of Polymer

The specimens that were polymerized by adding Ref., 3-hydroxypyridine and 2-isocyanatoethyl methacrylate glowed with a clear yellow light, while the polymerization tendency was identified by measuring the time required for the entire polymerization process. The polymerized samples and matching polymerization times are shown in *Fig.* 1 and *Table* 2, respectively.

Viscosity Measurement

Viscosity is also an important factor in the development of dental resin cements that promote adhesion between dental ceramics and damaged section of the tooth. The measurements show that the viscosity has the tendency to increase along with increasing the addition ratio of IEM

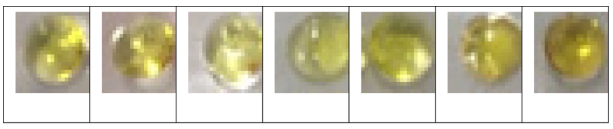

***Figure* 1.** Polymerization of Samples.

***Table* 2.** Polymerization time (degree) of samples

Sample	Polymerization time (sec)
Ref.	30
Iso 1%	30
Iso 5%	30
Iso 10%	20
Iso 20%	15
3HP 1%	120
3HP 3%	120
3HP 5%	X
3HP 7%	X

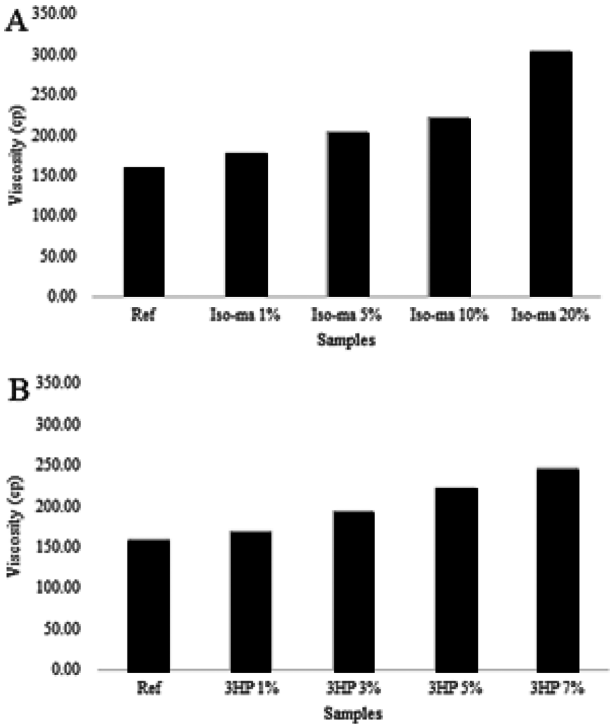

***Figure* 2.** Viscosity of samples (A: IEM group, B: 3HP group).

and 3-hydroxypyridine, respectively, whereas such increase was more pronounced when 3-hydorxypyridine, rather than 2-isocyanatoethyl methacrylate, was added to the mixture. The viscosity measurement results of the samples are shown in *Fig.* 2.

Measurement of Breaking Strength

The measurements of the breaking strength of the samples showed that the breaking strength increased along with increasing the addition ratio of IEM, while it took less time until the polymerization was complete, thereby suggesting that the degree of polymerization has the tendency to increase. In addition, when 3-HP was added to the mixture, the breaking strength decreased, while it took longer until the polymerization was complete, thereby suggesting that the degree of polymerization has the tendency to decrease as a whole. The breaking strength of each sample is shown in *Fig*. 3 and *Table* 3, respectively.

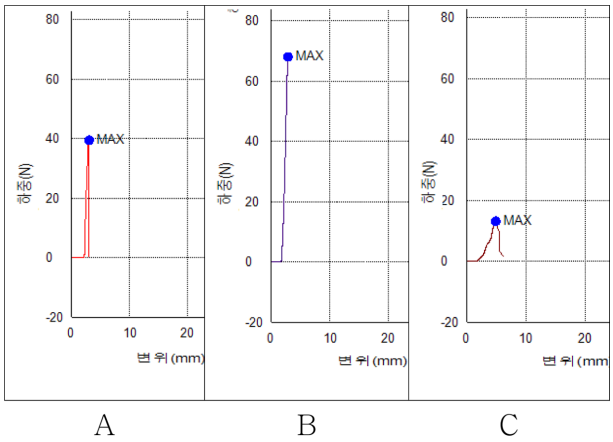

***Figure* 3.** The diagram of breaking strength of samples (A: Ref., B: Iso-3, C: 3HP-1).

***Table* 3.** Breaking strength test of samples

Sample	Breaking strength (N)
Ref.	39.25
Iso-1	49.13
Iso-2	63.5
Iso-4	69.46
3HP-1	13

Flexural Strength

The control group was prepared by fabricating a resin, which was prepared by adding approximately 10% of commercial resin cement and IEM. The average flexural strength of Iso 3 was 82.642 MPa, which was higher than that of the conventional resin cement. The measurements of flexural bond strength of each sample are shown in *Table* 4.

Antibacterial Experiment

The specimen was absorbed in the absorbing disk and cultured for 20 to 72 hours before it was inspected for any changes. The specimen demonstrated strong antibacterial activity against all three bacteria, thereby forming the widest clear zone when treated with ampicillin or penicillin-streptomycin, which was used as a positive control. When it was treated with sterilized water, which was used as a negative

***Table* 4.** Flexural Strength Test of samples (Result Summary)

Results	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1.1 F(N)	19.67	21.02	21.11	17.98	19.48
1.2 b(mm)	1.99	2.00	2.00	1.99	2.00
1.3 h(mm)	1.90	1.90	1.90	1.90	1.90
1.4 I(mm)	20	20	20	20	20
1.5 Flexural Strength	82.14	87.34	87.71	75.08	80.94
1.6 Average	82.642 MPa (SD: 5.198 MPa, CV: 6.29 %)				

control, no antibacterial activity was observed at all in the specimen. In the case of *S. mutans*, which is a Gram-positive bacterium, no apparent clear zone was formed, as it took a long time until the clear zone was formed due to the slow growth rate and insufficient number of germs in the vicinity. However, a relatively wider clear zone was formed in sample colonies 1, 2, and 3. In the case of *S. mutans*, there was no significant difference in the antibacterial activity among samples 1, 2, and 3. In the case of *S. aureus*, which is another Gram-positive bacterium, sample 3 showed the highest antibacterial activity, while samples 1 and 2 showed similar results. In the case of *E. coli*, which is a Gram-negative bacterium, a similar antibacterial activity was observed among samples 1, 2, and 3. However, samples 1, 2 and 3 all showed a higher antibacterial activity when *E. coli*, rather than *S. aureus*, was applied to them (*Fig*. 4). Ampicillin was treated according to different concentration levels in order to compare their antibacterial activity to that of ampicillin, which was used as a positive control. The results showed that a clear zone was formed in the case of *S. aureus* with 0.2, 0.4, 0.5, 1, and 2 μg of ampicillin, respectively. When compared to the size of the clear zone formed by ampicillin, samples 1 and 2 showed 0.2 μg of antibacterial activity, whereas sample 3 showed 0.4 μg of antibacterial activity. In the case of *E. coli*, a clear zone was formed by at least 1 μg of ampicillin, and when compared to the size of the clear zone formed by ampicillin, samples 1, 2, and 3 all showed an antibacterial activity that was triggered by ampicillin of 10 μg or more (*Fig*. 5).

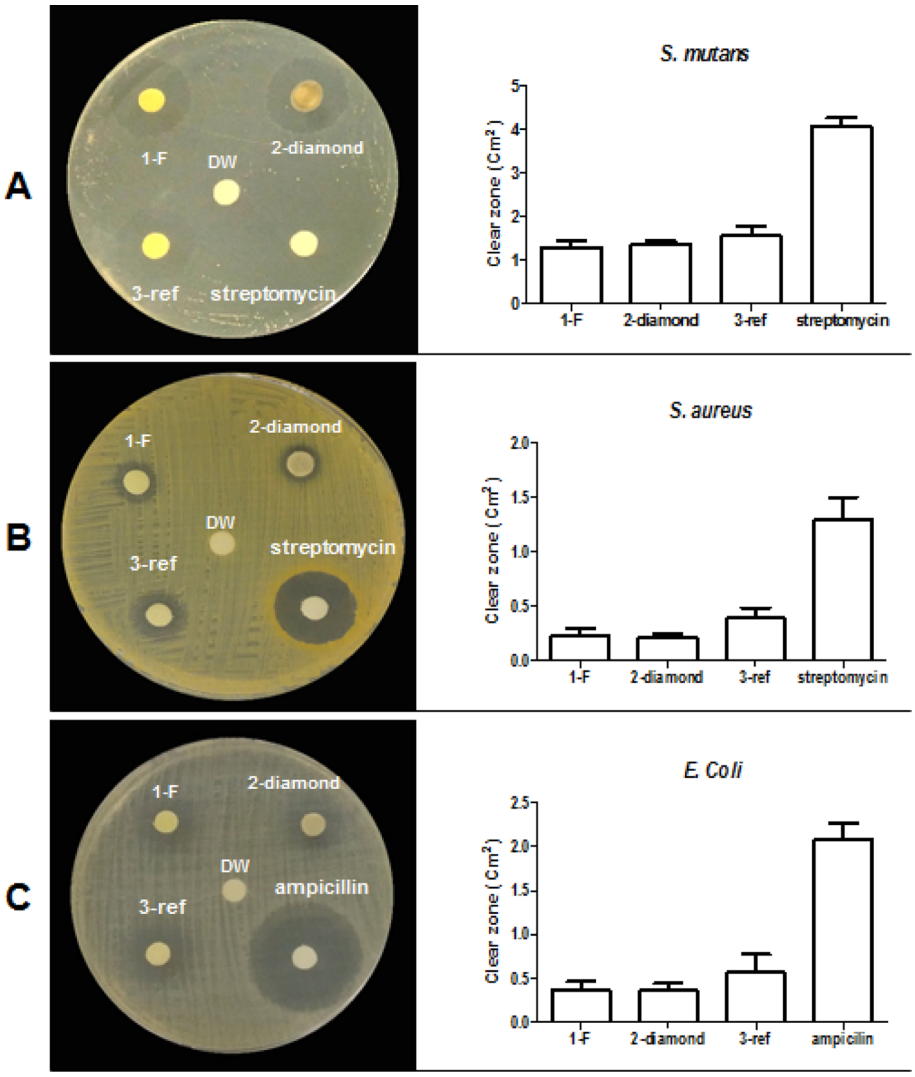

***Figure* 4.** Test of antibacterial activity of samples using absorbing disk. A is *S. mutans*, B is *S. aureus*, C is *E. coli*. (On the left side is the medium showing the antibacterial ability using the absorbing disk. The right side shows the size of the clear zone by numerical value. n = 5).

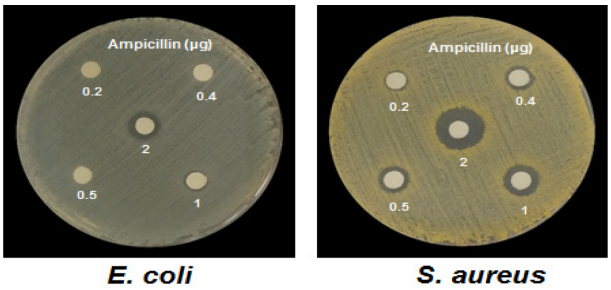

***Figure* 5.** Test for antibacterial activity of ampicillin using absorbing disk.

CONCLUSION

A functional dental resin cement was photopolymerized by mixing the following ingredients in proportion: Bisphenol A glycerol dimethacrylate (Bis-GMA) and hydroxypropyl methacrylate (HPMA) as the main ingredients; benzoyl peroxide (BPO), camphorquinone (Cam), and butylated hydroxytoluene (BHT) as the photoinitiator and photocatalyst; triethylene glycol dimethacrylate (TEGDMA) as the cross-linking agent; 2-isocyanatoethyl methacrylate as an additive for higher strength; and 3-hydroxypyridine as an additive for antibacterial activity. Transparent polymer was formed in all combinations as a result of polymerization. The measurements of polymerization time and breaking strength performed to investigate the degree of polymerization of each sample showed that the polymerization time decreased along with the rising ratio of 2-iscyanatoethyl methacrylate, whereas the degrees of high strength and polymerization have the tendency to increase along with the increasing value of breaking strength. In the case of the sample, to which 5% of 2-iscyanatoethyl methacrylate was added, the increase of breaking strength was the most appropriate level. In the case of the sample, to which 3-hydroxypyridine was added, less than 1% seemed to be the appropriate ratio. In the case of the sample, to which 10% of 2-isocyanatoethyl methacrylate was added, the flexural strength was higher than that of the conventional resin cement, thereby proving that it is appropriate as a high-strength material. The results of the antibacterial experiment, in which 3-hydroxypyridine was added to the samples in proportion, showed that ampicillin was effective against Gram-positive bacterium, but showed low antibacterial activity against Gram-negative bacterium in the test of antibacterial activity by using an absorbent disk and a solid medium with relatively limited diffusion of samples. In addition, samples 1, 2, and 3 showed high antibacterial activity against Gram-positive and Gram-negative bacteria. In particular, they were effective toward negative bacteria. In addition, water absorption and solubility of the resin cement was about 29 ug/mm^3 in absorbance and 1.4 ug/mm^3 in solubility. These results imply that this material is suitable for use as a dental resin cement. The results of this study suggest that the use of 2-isocyanatoethyl methacrylate as an additive for high strength and 3-hydroxypyridine as an additive for improved antibacterial activity would improve the usability of the fabricated polymer as a dental resin cement material with high functionality.

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