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ORIGINAL ARTICLE

Liquid crystalline epoxy nanocomposite material for dental application

Yun-Yuan Tai [a], Sheng-Hao Hsu [b], Rung-Shu Chen [c], Wei-Fang Su [b,d,**], Min-Huey Chen [e,f,g,*]

[a] *Dental Department, National Taiwan University Hospital, Taipei 10002, Taiwan*
[b] *Institute of Polymer Science and Engineering, National Taiwan University, Taipei 10002, Taiwan*
[c] *Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei 10002, Taiwan*
[d] *Department of Materials Science and Engineering, National Taiwan University, Taipei 10002, Taiwan*
[e] *Research Center of Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei 10002, Taiwan*
[f] *Graduate Institute, School of Dentistry, National Taiwan University, Taipei 10002, Taiwan*
[g] *Director, Visiting Staff, Division of Esthetic Restoration, Department of Dentistry, National Taiwan University Hospital, Taipei 10002, Taiwan*

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KEYWORDS
biocompatibility;
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liquid crystalline epoxy nanocomposite;
microhardness

Background/Purpose: Novel liquid crystalline epoxy nanocomposites, which exhibit reduced polymerization shrinkage and effectively bond to tooth structures, can be applied in esthetic dentistry, including core and post systems, direct and indirect restorations, and dental brackets. The purposes of this study were to investigate the properties of liquid crystalline epoxy nanocomposites including biocompatibility, microhardness, and frictional forces of bracket-like blocks with different filler contents for further clinical applications.
Methods: In this study, we evaluated liquid crystalline epoxy nanocomposite materials that exhibited various filler contents, by assessing their cell activity performance using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and their microhardness with or without thermocycling. We also evaluated the frictional force between bracket-like duplicates and commercially available esthetic bracket systems using Instron 5566.
Results: The liquid crystalline epoxy nanocomposite materials showed good biocompatibility. The materials having high filler content demonstrated greater microhardness compared with commercially available bracket materials, before and after the thermocycling treatment. Thus, manufacturing processes are important to reduce frictional force experienced by orthodontic brackets.
Conclusion: The microhardness of the bracket-like blocks made by our new material is superior to the commercially available brackets, even after thermocycling. Our results indicate that the

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei 10002, Taiwan.
** Corresponding author. Institute of Polymer Science and Engineering, National Taiwan University, Taipei 10002, Taiwan.
E-mail addresses: suwf@ntu.edu.tw (W.-F. Su), minhueychen@ntu.edu.tw (M.-H. Chen).

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evaluated liquid crystalline epoxy nanocomposite materials are of an appropriate quality for application in dental core and post systems and in various restorations. By applying technology to refine manufacturing processes, these new materials could also be used to fabricate esthetic brackets for orthodontic treatment.
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Introduction

Social progress and development have increasing demands on personal beauty. In cosmetic dentistry, materials are selected for their esthetic qualities as well as their function in the oral cavity. Anterior alloy restorations are no longer applied in contemporary dentistry because the darkened alloy core and post system affect the appearance of the full ceramic anterior crown. In addition, the difference in the elastic coefficient between the alloy and the residual tooth structure is considerably large, potentially risking failure of the adhesive, rupture of the post, or rupture of the root. Metal brackets can also prompt allergies in orthodontic patients because of their slight nickel composition.[1,2] These factors can cause patients to refuse orthodontic treatment to resolve oral hygiene problems caused by chewing disorders and crowded teeth.

Cosmetic brackets have been successfully produced using various materials; however, problems remain.[3] Although the esthetic quality of the porcelain bracket is appropriate, its fragility can cause tooth wear and bracket breakage during clinical manipulation.[4,5] If the porcelain bracket is fractured, it must be immediately replaced. Polycarbonate has recently been used in cosmetic brackets because of their light weight and thinness. However, if the metal sheet is not appropriately installed in the slot of the polycarbonate bracket (potentially affecting the esthetics), the slot can lose its original shape because of grinding of the orthodontic wire; thus, the bracket can no longer be used.[6,7]

Nanocomposite materials are the primary dental filling materials because they can be conveniently applied and yield a high esthetic quality. Nanocomposite materials are used to fabricate core and post systems and dental brackets, and applied in various restorations (inlays, onlays, veneers, and crowns). However, the volume of conventional nanocomposites is reduced during the curing process. This phenomenon introduces a gap between the restoration and the restored tooth, potentially causing the recurrence of secondary caries.[8,9] Nanocomposites used in major restorations can be susceptible to wear caused by heavy chewing, and their hardness decreases over time when water and bacteria are absorbed. Further development is required to eliminate the disadvantages of nanocomposite materials and expand their dental applications.

Liquid crystalline epoxy nanocomposite materials can be applied in direct or indirect clinical restorations and used to fabricate dental core and post systems and dental brackets.[10,11] Epoxy nanocomposite can be polymerized using various curing agents to form a polymer with low shrinkage. In this study, we fabricated material blocks and bracket-like models using new epoxy nanocomposites to evaluate the potential of these materials in dental applications.

Methods

Material block fabrication

The material blocks were fabricated in cylinders (height, 2 mm; diameter, 5 mm). Primisa (Kerr Co., Orange, CA, USA) and EZ350 (3M ESPE, St. Paul, MN, USA) were fabricated according to the manufacturer's instructions and used as the control groups. In the experimental groups, the liquid crystalline epoxy nanocomposite resin was used and its compositions were provided by the Institute of Polymer Science and Engineering, National Taiwan University[10]; the major component is epoxy resin ERL 4221 that contains 0%, 5%, 10%, 20%, and 30% liquid crystalline biphenol epoxy resin, respectively. The material blocks were fabricated using a heat-curing method. The nonsticky silicon mold was preheated in an oven at 70 °C for 60 minutes. The material ingredients were then mixed and preheated at 70 °C for 10 minutes. The material was poured into the silicon mold at 70 °C and left for 30 minutes. The temperature was then increased at a rate of 10 °C/minute until 160 °C, and the material blocks were heat cured for 3 hours, before being gradually cooled to room temperature. More than 40 blocks were fabricated for each material.

Spirit MB plastic brackets (Ormco Co., Orange) and Rave resin brackets (Ortho Technology, Tampa, FL, USA) were selected for comparisons of microhardness with the bracket-like material blocks before and after thermocycling. In this study, we used nonsticky silicon mold to copy the Rave brackets, and duplicate more than 40 bracket-like blocks for further examination according to the aforementioned heat-curing procedures.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The cellular viabilities of cells on the studied biomaterials were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) assay. The MTT assay is based on the reduction of yellow tetrazolium bromide to a purple formazan product by mitochondrial dehydrogenases activity in active cells.[12] Crystals of MTT (Sigma-Aldrich) were mixed with 30—40 mL of phosphate-buffered saline to form a 5 mg/mL solution. The solution was filtered using a 0.22-mm filter (Merck Millipore, Billerica, MA, USA), covered with aluminum foil, and stored at 4 °C. A dimethyl sulfoxide (DMSO) solution was also filtered using a 0.22-mm filter (Merck Millipore, Billerica, MA, USA).

Three blocks of each material were chosen for the MTT test. The surface area of each material block (comprising 0%, 10%, 20%, or 30% liquid crystal biphenol epoxy resin, respectively) was calculated to determine the required volume of Dulbecco's modified Eagle's medium (DMEM; 1 mL

of DMEM/cm^2). Primisa and EZ350 material blocks were fabricated and their surface areas were calculated as the control groups as mentioned earlier. After autoclaving, the appropriate volumes of DMEM were added to the blocks, which were subsequently incubated at 37 °C for 3 days. After 3 days of incubation, the test medium was collected from each block. The MG-63 cells were cultured in DMEM and approximately 2 × 10^5/mL cells were used for experiment as cell solution. In the experimental groups, approximately 100 μL cell solution was collected and 100 μL test medium was added into each well of a 96-well culture plate, with 18 wells repeated for each sample (6 wells were used for each time point). Add 100 μL cell solution and 100 μL pure DMEM into each well of the 96-well culture plate, with 18 wells repeated as the control group (TCPs, tissue culture polystyrenes). The solutions were incubated at 37 °C for 1, 3, or 7 days, and media were not changed to keep the same concentration of the test media from the different groups.

After incubation, test media were removed. Approximately 30 μL of the MTT solution was added to each well in darkness, and incubated for 5 hours at less than 37 °C. Subsequently, the MTT solution was removed and 50 μL of DMSO was added and agitated for 15 minutes until the blue–purple crystal is dissolved. An enzyme-linked immunosorbent assay was then performed at an optical density of 570 nm (n = 6 for each time point of the MTT test).

Microhardness test before and after thermocycling

The microhardness test was performed using an HMV2 (Hardness test machine; Hardness Ark-600, Mitutoyo Co., Kawasaki, Japan) at 98.07 mN and 0.01 Vickers hardness for 10 seconds using a Vickers' indenter (Fig. 1). The microhardness of the epoxy nanocomposite cylinder blocks comprising 0%, 5%, and 10% liquid crystalline biphenol epoxy resin content was tested before thermocycling. Ten blocks from each material were chosen, and each block was hit with 10 strikes. The material that yielded the optimal performance was selected to fabricate the bracket-like blocks, and a microhardness test was performed on the fixed bracket wing area before and after thermocycling. Ten blocks from each material were used for test, and each block was hit with 10 strikes. The results were compared with those of microhardness tests performed on the Spirit MB and Rave brackets. We used a custom-made thermocycling machine for thermocycling, maintaining the material blocks between 5 and 55 °C for 6000 cycles at 30 seconds/cycle.[13]

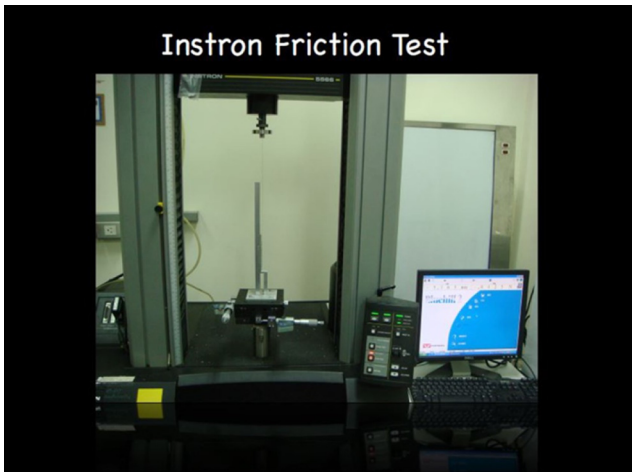

Figure 2 Friction test. Instron 5566 (Instron Co., Norwood, MA, USA) and 10-bracket model for friction test.

Friction test

A composite bracket that exhibited a lower microhardness compared with the epoxy nanocomposite, Rave (Ortho Technology), was selected to test the frictional force. The upper/lower 5–5 (Roth prescription) Rave brackets were set on an aluminum plate with a 5-mm space between each bracket.[14] The aluminum plate was set into the Instron 5566 (Instron Co., Norwood, MA, USA) and the static and dynamic frictional forces were tested (Fig. 2). The pulling speed was set at 0.5 mm/minute, within a 4000-cN force magnitude. The straight wire (014/016/016-016/017-025 stainless steel wire) was fixed on the brackets using elastic O rings.[13] Five sets for each wire group were tested. The wire was pulled for 2 mm followed by a 3-minute interval, and then pulled to another 2 mm to determine the static frictional force. A tightened clamp was used to hold the wire, which was returned to its original position and pulled for another 2 mm to determine the dynamic frictional force. A wire moving a distance of at least 0.5 mm was required to obtain significant data, and each test was continued until 2 mm of movement could be attained. The frictional force was calculated using the integration average statistical function of the Instron 5566 Bluehill Lite software program. To determine how the manufacturing procedures affected the frictional force, we evaluated the frictional forces of the proposed bracket-like

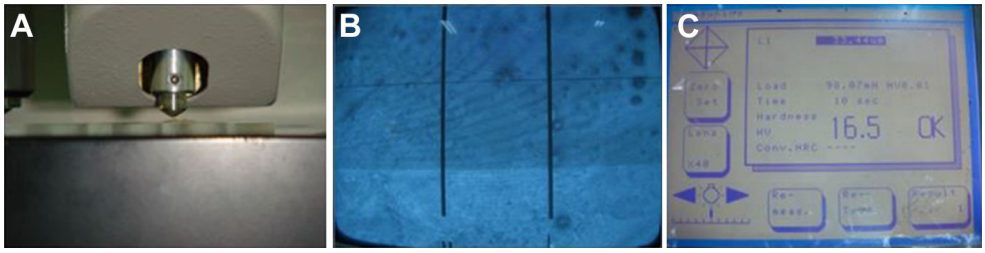

Figure 1 Microhardness test model. (A) HMV2 (hardness test machine; Hardness Ark-600, Mitutoyo Co., Kawasaki, Japan) under 98.07 mN, Vickers hardness 0.01, 10 seconds with Vickers' indenter. (B) The indentation upon epoxy nanocomposite block is calculated on the screen. (C) The result of the microhardness test is shown on the monitor and recorded. Here, the epoxy nanocomposite-duplicated bracket-like block is fixed and tested upon the bracket wing area before thermocycling.

material blocks, which were fabricated using 014 stainless steel round wire, with five sets being tested.

Statistical analysis

The standard deviations of the data were collected from every group at each time point. Statistical significance was calculated using one-way analysis of variance followed by a *post hoc* procedure (Bonferroni analysis). The data were entered into SigmaPlot version 10.0 (SYSTAT Software Inc., San Jose, CA, USA) and plots were constructed for the results of the MTT, microhardness, and frictional force tests.

Results

Biocompatibility of the new material is equivalent to that of the commercially available restorative composites

The MTT assay results indicated that the new materials with various liquid crystalline epoxy resin contents are biocompatible (Fig. 3). In the MTT tests, we used the medium cocultured with different material blocks for 3 days, to test whether the released toxicity from the material can inhibit cell activity significantly.

After the 1st day of cell culture, cytotoxicity of the new materials demonstrated nonsignificant differences compared with the Z350 and Primisa samples. After 3 days of culture, all materials seem to have good cell viability than TCPS, and the F20 (20% liquid crystalline epoxy resin content) and Primisa displayed more cell activity compared with the other samples and TCPS. After 7 days of culture, the F10 (10% liquid crystalline epoxy resin content) still maintained good cell activity compared with the other samples and TCPS (Fig. 3). During the incubation, media were not changed to maintain the same concentration of the test media, and the total cell viability was decreased in all groups after 3 and 7 days of incubation. There is no significance between the 3- and 7-day incubation of TCPS. The results indicated that F10 (10% liquid crystalline epoxy resin content) could provide good cell growth environment after long incubation periods, which means that F10 is the best percentage of liquid crystalline epoxy resin content formula in this new material. However, irrespective of the percentage of liquid crystalline epoxy resin content, the biocompatibilities of the new materials were equivalent to those of the Z350 and Primisa samples.

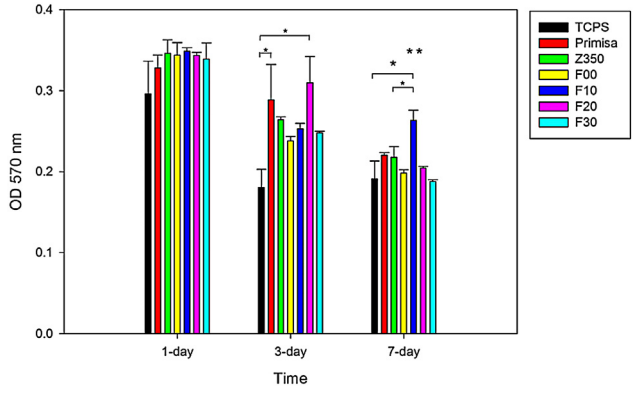

Figure 3 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The result of MTT reveals good biocompatibility of the new liquid crystalline epoxy nanocomposite—F00: 0% liquid crystalline resin; F10: 10% liquid crystalline resin; F20: 20% liquid crystalline resin; and F30: 30% liquid crystalline resin. Biphenol epoxy resin is the liquid crystalline resin we used here. During the incubation, media were not changed to keep the same concentration of the test media, and the total cell viability was decreased after 3 and 7 days of incubation. F10 is the best group after 7-day incubation. Asterisks (* and **) denote significant differences ($n = 6$; $p < 0.005$).

Microhardness increases with increasing liquid crystalline epoxy resin content

The microhardness test results (collected after 100 strikes to the new material blocks fabricated using epoxy nanocomposites at 0%, 5%, and 10% liquid crystalline epoxy resin content) indicated that the material with 10% liquid crystalline epoxy resin content exhibited the highest microhardness (Fig. 4). Our results suggested that the microhardness of the new materials increases as liquid crystalline epoxy resin content increases.

Greater microhardness of the new materials in comparison with the bracket materials on the market with or without thermocycling

We compared the microhardness of the bracket-like blocks fabricated using the new epoxy nanocomposites with those of the Spirit MB plastic brackets and Rave resin brackets before and after thermocycling (Fig. 5). Our results indicated that before thermocycling, the Spirit MB and Rave displayed nonsignificant differences in microhardness. However, after 6000 thermocycling cycles, the Rave brackets were weaker

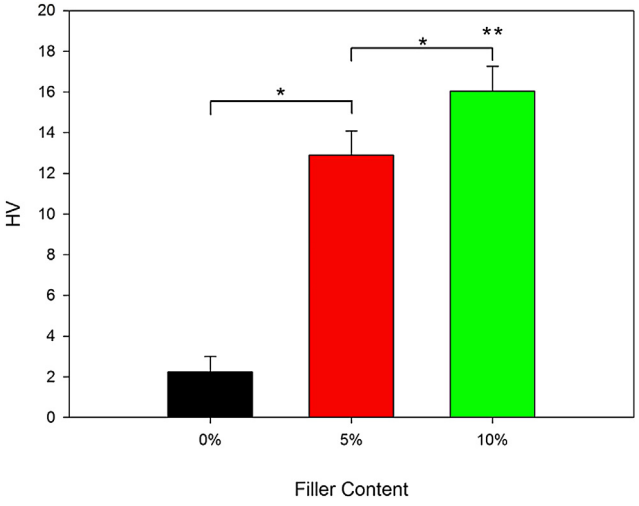

Figure 4 Microhardness test for different percentages of biphenol filler. Higher percentage of the biphenol liquid crystalline epoxy resin can increase microhardness of the new epoxy nanocomposite. Asterisks (* and **) denote significant differences ($n = 100$; $p < 0.01$).

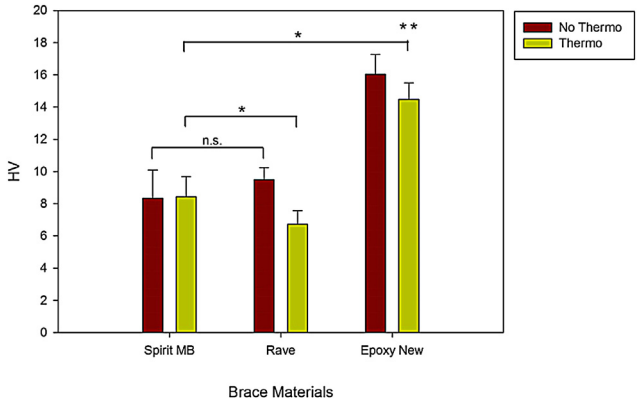

Figure 5 Microhardness test for Spirit MB plastic bracket, Rave resin bracket, and epoxy nanocomposite-duplicated bracket-like material block, with and without 6000 times thermocycling. Before thermocycling, the well-fabricated Rave and Spirit MB reveal almost the same strength, but inferior to the new epoxy nanocomposite bracket. After thermocycling, the new epoxy bracket still holds the strongest microhardness than Rave and Spirit MB. Asterisks (* and **) denote significant differences ($n = 100$; $p < 0.01$).

than the Spirit MB plastic brackets. The microhardness of the epoxy nanocomposite comprising 10% liquid crystalline epoxy resin content was 50% higher than those of the Rave and Spirit MB, irrespective of thermocycling.

Frictional force is associated with the manufacturing method

The frictional force of the Rave resin brackets was less than 450 cN (Fig. 6A). We used the new epoxy nanocomposite to fabricate bracket-like blocks, and evaluated their frictional force using a 014 stainless steel round wire. Our results indicated that the frictional force of the epoxy nanocomposite was higher than that of Rave resin because of the manufacturing method used, and that the slot of the bracket-like block was too rough to yield optimal function (Fig. 6B). This suggests that the frictional force of a material is associated with its method of manufacturing.

Discussion

Liquid crystalline epoxy nanocomposites can be produced using various methods including heat-curing and light-curing processes.[12] Liquid crystalline polymers exhibit increased fracture toughness, high elasticity, strong barrier properties, a low thermal expansion coefficient, and specific optical, electrical, and thermal properties. They can be used as matrices in composites that are used in demanding applications in the electronics and aerospace industries. The advantages of such materials are reduced polymerization shrinkage, sufficient strength for restoration, strong biocompatibility, and high esthetic quality for dental applications.[15–18]

The MTT assay results indicated that the epoxy nanocomposites exhibit nearly the same biocompatibility as commercially available restorative resins after the 1st day of culture, and that the material comprising 10% liquid crystalline epoxy resin content provides a superior cell culture environment to other materials after 7 days of culture. All tested materials, containing various liquid crystalline epoxy resin contents, displayed similar or even superior biocompatibility compared with the Primisa and Z350 samples (Fig. 3).

High microhardness was associated with high liquid crystalline epoxy resin content in the tested materials (Fig. 4). After 6000 cycles of thermocycling, the epoxy nanocomposite comprising 10% liquid crystalline epoxy resin content displayed microhardness approximately double than that of the Rave resin bracket. The microhardness of the plastic bracket, Spirit MB, was at least 75% lower compared with that of the new epoxy nanocomposite (Fig. 5). The results are expected due to the rigid rod structure of liquid crystalline epoxy resin.[12] Because of the

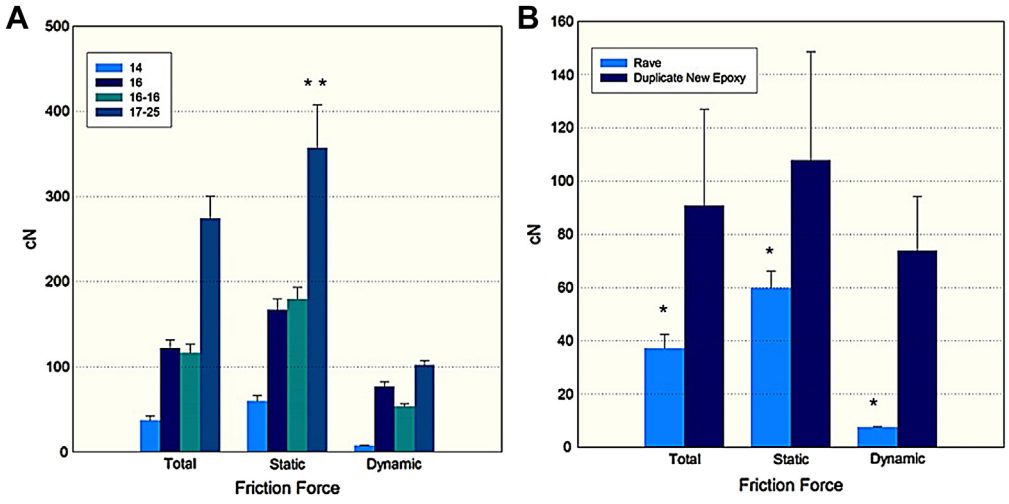

Figure 6 Friction test. (A) The more inferior but well-fabricated high-tech Rave resin brackets can provide very low frictional resistance than lots of esthetic bracket systems, lower than 450 cN. (B) Owing to the lack of manufacturing technology, the duplicated bracket-like blocks present higher frictional force than Rave. However, the frictional force of the duplicated bracket (less than 150 cN) is still lower than the conventional metal brackets (approximately 490 cN). Asterisks (* and **) denote significant differences ($n = 5$; $p < 0.05$).

similar biocompatibility and superior microhardness compared with the commercially available materials, this new liquid crystalline epoxy nanocomposite can be used to fabricate core—post systems with excellent performance.

Rave resin brackets are manufactured using technology that creates a smooth surface slot with sufficient strength to reduce frictional force during orthodontic wire sliding and increase orthodontic treatment efficiency. Because of limitations to the proposed manufacturing method regarding the injection-molded brackets, we tested the frictional force of the Rave resin brackets. The Rave resin brackets displayed lower microhardness levels than did the new epoxy nanocomposite materials (Fig. 5). The frictional force of the Rave resin brackets with stainless steel 017-025 wire was less than 450 cN (Fig. 6A), which was lower compared with several commercially available esthetic brackets, such as the Allure (GAC International), Image (Gestenco International), Inspire (Ormco), and Transcend (3M Unitek) models, and two self-ligating esthetic brackets, namely, the Opal (Ultradent Products) and Oyster (Gestenco Int.) models.[19] The frictional forces of these esthetic brackets were higher than 3 N when tested using a stainless steel 017-025 rectangular wire.[19] Therefore, the quality of resin brackets is not inferior to that of brackets fabricated from ceramic or plastic materials.

However, the bracket-like material block displayed higher frictional forces compared with the Rave bracket when tested using stainless steel 014 round wires because of the rough slot surface (Fig. 6B). The manufacturing method can determine the frictional force of a bracket. As shown in a previous study using stainless steel 014 round wires, the average frictional force of conventional metal brackets was more than 490 cN,[14] which was higher compared with the highest frictional force exhibited by our proposed bracket-like blocks (less than 150 cN; Fig. 6B). Our results indicate that our new epoxy nanocomposite materials have potential applications in the field of orthodontic bracket fabrication.

In contemporary cosmetic dentistry, materials are required to fulfill functional and esthetic demands. Liquid crystalline epoxy nanocomposite materials yield high microhardness levels and can be used for long-term direct restorations. Reducing polymerization shrinkage can downgrade postoperational sensitivity and the occurrence of secondary caries after completing a direct restoration. By using the same material to fabricate core and post systems and full-coverage crowns or onlays, the plasticities of the post and crown are equivalent, generating a monoblock effect. If orthodontic brackets can be easily bonded, repaired, and removed without majorly damaging the tooth surface, and retaining esthetic quality, adult patients may be open to accepting orthodontic treatment, allowing them to smile confidently. The proposed innovative liquid crystalline epoxy nanocomposite material can meet all of these requirements for orthodontic brackets.

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