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The self-etching two-bottled dental adhesive system containing 10-methacryloyloxydecyl dihydrogen phosphate monomer

Jiyeon Roh ¹, Min-Ho Hong ^{2,*}

¹ Research Planning and Coordination Division, National Forensic Service, Wonju-si, Republic of Korea; hindhorn@gmail.com

² Nature Inspired Materials Processing Research Center, Department of Energy Science, Sungkyunkwan University, Suwon-si, Republic of Korea; mhong@skku.edu

* Correspondence: mhong@skke.edu; Tel.: +82-31-299-4266

Abstract: Dentin bonding is a key in restorative dentistry. Herein, we developed self-etching two-bottle adhesive system containing 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) and the physical, mechanical, and biocompatible properties were evaluated. The characteristics of MDP were analyzed using nuclear magnetic resonance (NMR). The water sorption and solubility, the shear-bond strengths to dentin and enamel, and cytotoxicity tests were performed. The newly blended experimental group showed the lowest thickness and water sorption and solubility values. The shear bond strength to enamel and dentin were comparable to control groups (the three other products) all test groups showed 60% of cell viability. In this study, the properties of the newly synthesized adhesive are comparable with the others. The fundamental goal of this study is to get the MDP patent released, as it is intended for domestic production. For this purpose, this dentin adhesive was developed and compared with the commercial product.

Keywords: self-etching two bottled dental adhesive; 10-methacryloyloxydecyl dihydrogen phosphate monomer; biocompatibility; shear bond strength

1. Introduction

The dental adhesive market has been increasing over the last half of the century, with dentin adhesives being developed along with other restorative materials in dentistry. Many new adhesives have been developed in recent times. With better understanding of the smear layer and composition of dentin, the formulation of dentine adhesives changed over time [1]. Before the 4th generation, all adhesives were required to have total etching such as a phosphoric acid for improvement of bond strength. In the 4th generation, the wet bonding system was introduced, and enamel and dentin were treated differently [2]. The acidic functional self-etching monomer was also introduced, and self-etching adhesives were developed. Therefore, the paradigm was shifted to the 5th, 6th, and one-bottle system, with the self-etching system being developed variously.

Nowadays 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) may be considered as a gold-standard functional monomer in any self-etching system [3]. From its development, this monomer requires royalties to be paid due to the patent period [4]. However, after the patent period ended, synthesis and use of the monomer were allowed. Although dentin adhesives have improved tremendously over the past decade, the properties of the new generation have not been proven yet. In addition, its dependence on import is high, and few companies and research centers focus on and invest in dental adhesives.

Among the various adhesives, self-etching adhesive systems have been widely used because of their shorter application time. Self-etching systems are generally less technique sensitive compared to employing separate acid conditioning and rinsing steps [5]. In addition, the collapse of dentin

collagen and the production of demineralized collagen are prevented. Despite the presence of short resin tags and intact smear plugs, a good seal has been reported. Kijasamanmith *et al.* reported that there was an improvement in the regional tensile strength test over the pulp horn regions when compared with systems that utilize a total etch technique [6].

Several self-etching and self-priming adhesives have been introduced recently. Among the products, CLEARFIL™ SE BOND (Kuraray, Okayama, Japan) is a multi-purpose system containing an unsaturated methacrylate phosphate ester and MDP as the acidic resin monomer. AdheSE® (Ivoclar Vivadent, Schaan, Liechtenstein) is a two-bottle self-etching system which contains dimethacrylate phosphoric acid acrylate initiators and hydrophilic monomer. Moreover, UniFil® (GC Corp., Tokyo, Japan) is also a two-bottle self-etching system with 4-MET and hydrophilic monomer [7].

In this study, we developed a two-bottle self-etching adhesive system containing MDP and hydrophilic monomer. In addition, the physical, mechanical, and biocompatible properties were evaluated. Commercial two-bottle products on the market were used as control groups. The null hypothesis of this study was that there were no significant differences between the experimental and control groups.

2. Materials and Methods

2.1. Materials

Using the monomer, the primer and bonding were blended, and brand-named products such as Nexo. Clearfil™, UniFil®, and AdheSE® were purchased and used as control groups. L929 (Korean Cell Line Bank, Seoul, Korea), RPMI 1640 Medium (Gibco, Grand Island, NY), FBS (Gibco), Methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma-Aldrich, San Diego, CA), dimethyl sulfoxide (DMSO, Amresco, Solon, OH).

2.2. Physical and mechanical properties

The characteristics of MDP were analyzed using ¹H nuclear magnetic resonance (NMR; Avance II, Bruker Biospin, Rheinstetten, Germany). In short, the monomer was diluted in acetone. The accumulation and repetition times were 1000 times and 15 seconds, respectively.

The bonding agent (20 µg) was placed on the center of a glass plate and covered with another glass plate. Each glass had a contact surface area of 200 ± 25 mm². With a loading device, the bonding agent was loaded (150 ± 2 N) on the glasses for 180 ± 10 sec. Then, it was light cured as recommended by the manufacturer. The combined thickness of the two glass plates and the specimen film were recorded using a micrometer. The film thickness test was carried out five times.

The water sorption (W_{sp}), solubility (W_{sl}), and thickness were evaluated according to ISO 4049 [8]. The mold (d 15 ± 0.1 mm × h 1.0 ± 0.1 mm) was then filled with bonding agent and covered with a polyethylene film and slide glass. After removing the specimen from the mold, its volume (V) was measured. It was then weighed (m_1), and the samples were placed in a 6-well plate and immersed in 10 mL of distilled water. The immersed samples were placed in a 37°C water bath for 7 days, after which they were removed. The moisture on the surfaces was removed and measured (m_2) and the specimens were placed in a new 6-well plate and stored in a desiccator maintained at room temperature. Each disk was weighed daily until a constant dry mass (m_3) was obtained. Water sorption and solubility were calculated using the following equations.

$$W_{sp} (\mu\text{g}/\text{mm}^3) = (m_2 - m_3)/V \quad (1)$$

$$W_{sl} (\mu\text{g}/\text{mm}^3) = (m_1 - m_3)/V \quad (2)$$

The shear bond strength to enamel or dentin was tested according to ISO 29022 [9]. The embedded teeth in acrylic resin were polished with a 600-grit SiC paper until the enamel or dentin surface was exposed. The prepared tooth specimen was placed in an apparatus for notched shear bond strength test. The primer was applied immediately after removing it from water after which the bonding agent

was applied and light cured according to the manufacturer's protocol. The composite resin was condensed in the mold ($d\ 2.38 \pm 0.03$ mm), and the sample composite button was placed and aligned to the notched blade. Loading was done with a universal tensile machine with a crosshead speed of 1.0 ± 0.1 mm/min until failure. The maximum force (N) prior to failure of the bond was recorded and the bond strength was calculated using the formula.

$$\sigma \text{ (MPa)} = F \text{ (N)} / A_b \text{ (mm}^2\text{)} \quad (3)$$

2.3. Biocompatibility

The MTT assay was performed to evaluate the biocompatibility according to ISO 10993-5 [10]. In brief, the specimens with 10 ± 0.1 mm in diameter and 1 ± 0.01 mm in height were prepared. The sample was sterilized under UV light for 30 min and immersed in serum-free media ($3.0 \text{ cm}^2/\text{mL}$) according to ISO 10993-12 [11]. After a day, the supernatant was decanted into another well and diluted with serum-free media. Mouse fibroblast cell line, L929 was seeded at 1×10^4 /cells in a 96-well plate, in 100 μL of cell culture medium RPMI 1640 with 10% of FBS. After a day, the medium was changed to extractions from each type of material (1:1) or dilutions of extractions with serum-free media (1:2). After another a day, the extractions were removed and 50 μL of MTT solution (1 mg/mL) were added. After two hours, the solutions were changed to 100 μL of DMSO to dissolve purple formazan. After that, the microplates were read using a microplate reader (Epoch microplate spectrophotometer, Synergy-BIOTEK, Winooski, VT). The tests were repeated three times and all results were calculated as relative value by dividing to negative control group.

2.4. Statistical analysis

In all experiments, statistical analyses including one-way ANOVA tests were performed for all data using SPSS 23 statistical software (IBM, Armonk, NY), and a post hoc analysis was performed using Tukey's method. The significant difference between each group was determined at a p -value < 0.05 .

3. Results

The monomer was dissolved in acetone and ^1H NMR spectrum analysis was performed. There was an acetone peak in Fig. 1(c). Each hydrogen network was written in the chemical structure. The peak patterns were matched with the chemical structure of MDP. The boxes were labelled with alphabets and the upper boxes were magnified peaks to clarify the peaks. The simplified molecular-input line-entry system (SMILE) and chemical abstracts service (CAS) number are described in Table 1.

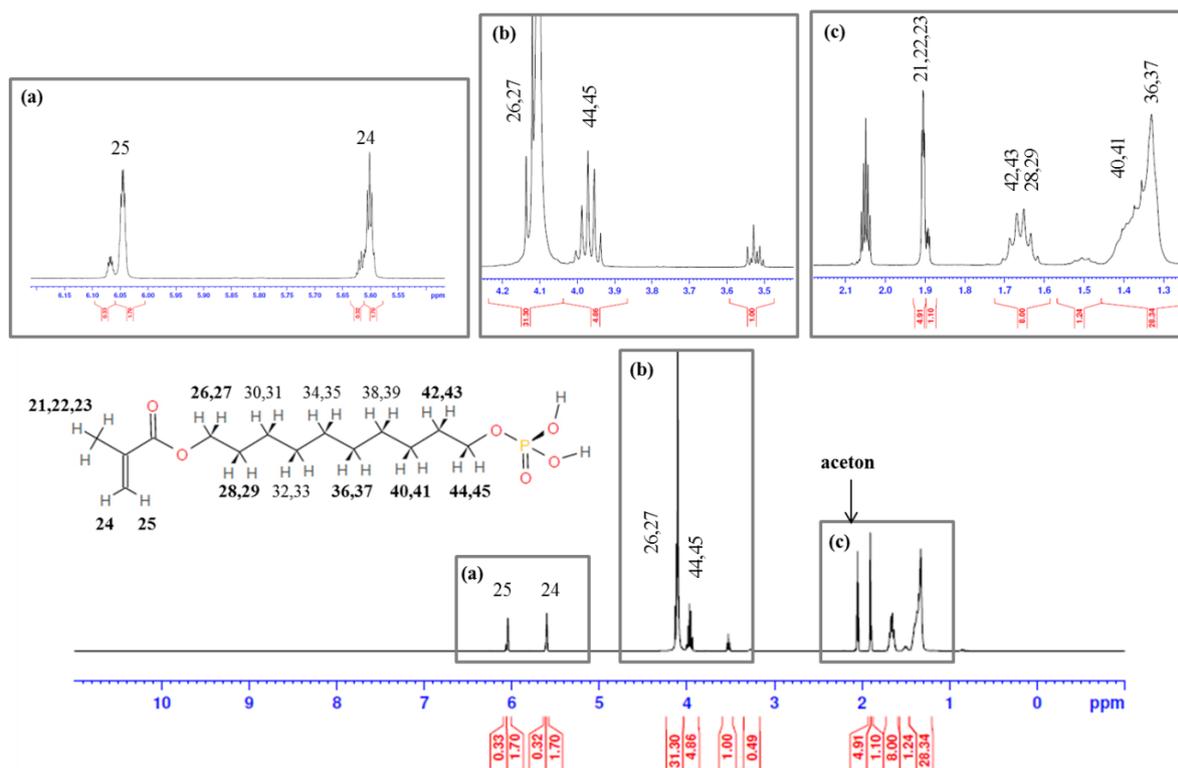


Figure 1. ^1H NMR spectrum of MDP.

Table 1. The SMILE of organic compound with associated CAS number.

| | |
|----------------------|--|
| Compound name | 10-Methacryloyloxydecyl dihydrogen phosphate |
| SMILE | <chem>C=C(C)C(=O)OCCCCCCCCCOP(=O)(O)O</chem> |
| CAS number | 85590-00-7 |

The film thickness was measured to confirm the validity for other tests. The results of film thickness are shown in Fig. 2. In this test, the Nexco and Unifil groups showed the lowest thickness. The results of water sorption and solubility are shown in Fig. 3. The requirements of ISO 4049 are below the value of $40 \mu\text{g}/\text{mm}^3$ in W_{sp} and $7.5 \mu\text{g}/\text{mm}^3$ in W_{sl} . The AdheSE had the highest value in W_{sp} and Clearfil SE has the highest value in W_{sl} . The results of shear bond strength on enamel and dentin are shown in Fig. 4. The same letters mean that there are no significant differences in that graph. The shear bond stress to enamel of Clearfil SE was significantly higher than others.

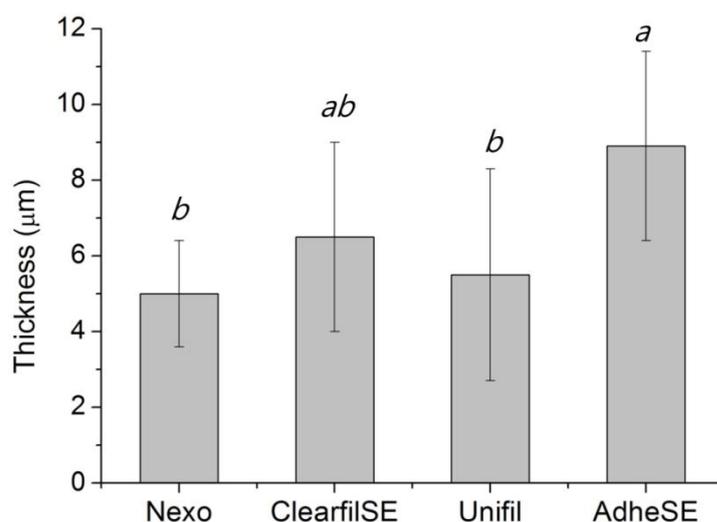


Figure 2. Thickness of bonding agent (µm). The same letters mean no significant differences.

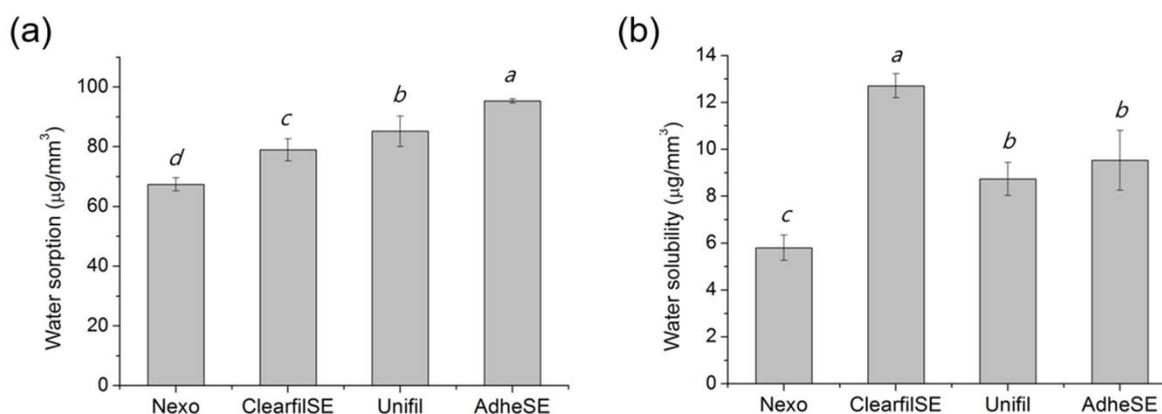


Figure 3. Water sorption (a) and solubility (b). The same letters in one graph mean no significant differences.

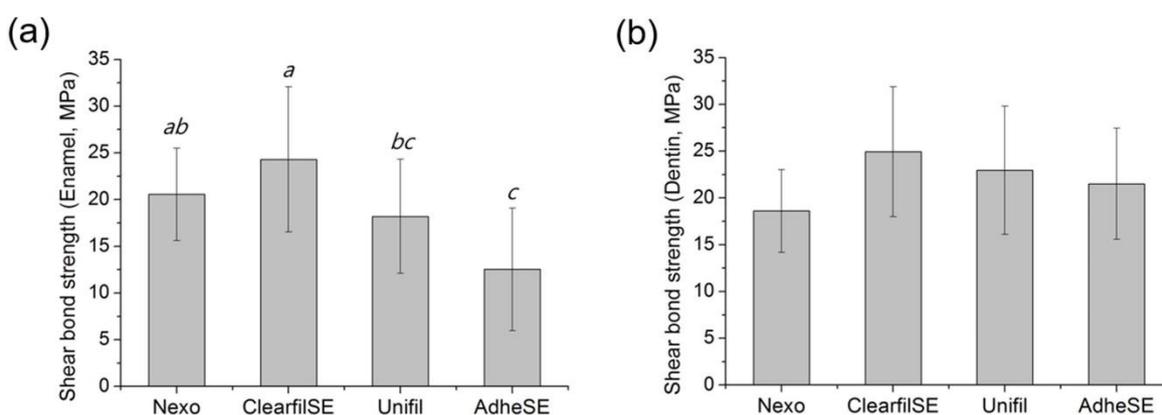


Figure 4. Shear-bond strength to enamel (a) and to dentin (b). The same letters in one graph mean no significant differences.

To confirm the biocompatibility of each group, the MTT assay was performed according to ISO 10993-5. The results of the MTT assay are shown in Fig. 5. The ratio in blanks, 1:1 means 100% of extraction, and 1:2 means extraction and non-serum media ratio. The MTT assay of all groups was shown to be over 60 % of cell viability in 1:1 except AdheSE.

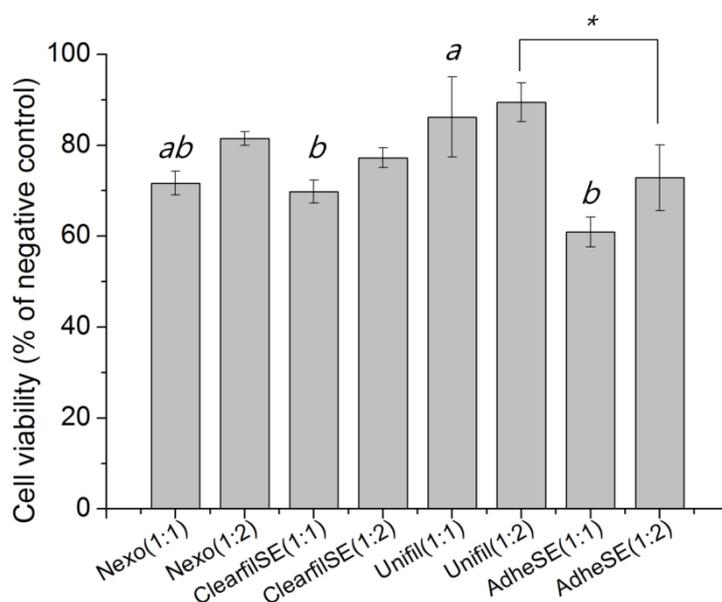


Figure 5. MTT assay (% of negative control). The same letters and marks in the same concentration mean no significant differences.

4. Discussion

Dentin bonding is a key in many restorative clinical procedures and is fundamental in aesthetic dentistry. The basic mechanism of dentin adhesive is the resin bonding tag in dentin holes that connects the dentin and resin composites. To satisfy this mechanism, many generations of dentin adhesives have been developed for more than half a century [12].

The total etching system was the only method; however, acidic functional monomer is incorporated into dental adhesive. The functional monomers play the essential role on the bonding and physicochemical properties. The mechanism of functional monomer, such as MDP is calcium in enamel and dentin chemically bond and the MDP-Ca salts on the dental hard tissue. Therefore, they create a particular nano-layered structure with the remaining dentin hydroxyapatite [3].

The dentin adhesives require proper characters in the clinic and among the various characters of a dentin adhesive, a bonding strength to enamel and dentin, a water sorption and solubility, and biocompatibility are considered as important properties. However still few standards were applicable for the test dentin adhesives. In this study, we evaluated the materials according to ISO 4049 polymer based dental materials.

After releasing the patent, MDP has been modified in many self-etching adhesives, and we also added the MDP for a self-etching system. The synthesized monomer was confirmed by ^1H NMR, and hydrogen was detected at the same peaks. Commonly used acid monomers in dental adhesives are usually constituted in an acidic group, a polymerizable group, and a spacer like an aliphatic chain [13]. In addition, MDP and N,N' -diethyl-1,3-bis(acrylamido)propane (DEBAAP) have been mainly used in a self-etching system. To evaluate and compare new products, we purchased three products which were classified as same generation. Only ClearfilSE contained MDP, and the new Nexo was comparable in the aspect of monomer. AdheSE contained acidic monomer, DEBAAP, which could show high mechanical properties with low quantity [13]. The Unifil contains 4-methacryloxyethyl trimellitic acid (4-MET) with ethanol or acetone; however, it was reported that methacryloxy ester portion in the 4-MET resulted in the hydrolysis reaction [14].

We measured the film thickness according to ISO 4049, and it is one of the important properties of resin-based cements. Compared to the resin cement, the dentin adhesives did not contain filler for strength, but only monomers. Although the values of all test results were compiled, we assumed that other test methods or other standard for its flowability were required.

The water sorption and solubility tests have been conducted in many dental adhesives, and generally, the extent and rate of these properties increased with the hydrophilicity of the resin compositions [15]. Among the monomers, hydroxyethyl methacrylate (HEMA) has well known as hydrophilic monomer. Malacarne *et al.* reported that lower rate of water uptake showed less hydrophilic adhesive [15]. All test groups contained HEMA in primer and bonding, and the Nexo group showed the lowest values in both results. The water solubility was under $7.5 \mu\text{g}/\text{mm}^3$ which is acceptable requirement in ISO 4049. On the other hand, of all data, the water sorption was higher than its requirement. Yoshida *et al.* reported that addition of the HEMA also influenced not only water sorption, but also the chemical bonding of MDP by inhibited interfacial nano-layering [16], but we could not result its character with bonding strength related to MDP and HEMA in this study.

The most important characteristic is bonding strength to enamel or/and dentin. In this study, we conducted shear bond strength test according to ISO 29022. There were three kinds of bond strength, and among them, we conducted notched-shear bond strength test. All high values of shear bond strength required various factors such as tooth surface, adhesion surface area, parallel specimen, and composite resin used in the study. Therefore, the results are not comparable with previous tests. In this study, the enamel and dentin shear bond strength in the same groups were similar, and the Nexo group showed no significantly different result to Clearfil SE. Even though we conducted at least fifteen teeth according the test method, the range of standard deviation was huge in enamel bonding strength. Previous enamel etching resulted in fewer marginal defects and marginal discoloration, compared with using the SE approach alone. For restoration retention, the differences between the two groups were not significant [17]. In the previous study, they mentioned that self-etching showed promising results in superficial and deep dentin [18], and when properly handled, self-etching is more effective to dentin than total etching [19,20].

Understanding the mechanism of cytotoxicity is necessary for selection of a strategy to protect the dentine-pulp complex. In the ISO 7405 for biocompatibility of dental materials [21], there is special test method for dentin-pulp structure connective applications. Schmalz *et al.* and Ulker *et al.* conducted the test in dentin barrier cell culture structure. They reported that this test method was very similar to the *in vivo* situation, where the materials covers the dentinal walls and only indirectly interacts with the pulp, by means of the tubular fluid [22-24]. The cytotoxicity of dentin adhesives was affected by its ingredients, especially uncured monomers [25]. In this study, the cytotoxicity test was conducted only with a bonding agent due to its characteristics. The primer contains acidic monomer, and the product showed pH lower 2.0. In this study, the limitation was that we just evaluated the cytotoxicity of the adhesive, which showed mild cytotoxicity. In addition, cytotoxicity tests using extractions of dental adhesives were severe method than commonly used method by indirect contact dentin barrier.

In this study, the hypothesis of this paper was rejected, and the new blended two-bottle self-etching adhesive system was comparable to the products in the market. Although the various dentin adhesive systems have been developed and evaluated in many previous researches, more researches are needed to prove their properties *in vitro* and *in vivo*. Moreover, more long-term clinical case reports are needed.

To approve the product on the market, more experiments need to be conducted. Compared to the development of generation, the guideline may not fully cover. Appraisal criteria should be developed by reputable organizations such as the International Organization for Standardization (ISO) to guide manufacturers, or scientists who are interested in patenting their own inventions. Finally, the cautiously forecasting of domestic production in self-etching adhesive is expectable.

5. Conclusions

The newly developed two-bottled self-etching adhesive containing MDP showed significant results compared to other products on the market and more formal research (case reports and case series) is needed to be conducted to promote its production for the market. In addition, the appraisal criteria for dental adhesives need to be developed.

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Conflicts of Interest: The authors declare no conflict of interest.

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