

Guinea Pig Maximization Test of Tri-ethylene Glycol Mono-methacrylate

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The aim of this study was to evaluate tri-ethylene glycol mono-methacrylate (TEGMA) in terms of dermatological allergic reaction using a Guinea Pig Maximization Test. Skin reaction was evaluated according to the criteria of International Contact Dermatitis Research Group. TEGMA, as a constituent in new primers, has been reported to contribute to a priming ability similar to that of highly purified glyceryl mono-methacrylate (GM). In this study, it was found that its adverse effect was less than that of 2-hydroxy ethyl methacrylate (2-HEMA), but similar to that of highly purified GM. In conclusion, it was anticipated that TEGMA would hereafter replace 2-HEMA as a primer, as it posed a lower risk of triggering dermatological allergic reaction.

Keywords: tri-ethylene glycol mono-methacrylate, Dentin primer, Allergic reaction

INTRODUCTION

With the advent of minimal intervention (MI), dental caries treatment has undergone a drastic change from that of invasive dentistry to one of adhesive dentistry. With this shift in treatment method, several manufacturers have since produced bonding materials with due consideration for adhesiveness to dentin. Beginning with GLUMA in 1984, it is now widely recognized that primers are indispensable to forming a strong bond between composite resins and dentin¹⁾. Predictably and inevitably, primers are now recognized by dental healthcare workers as a commonplace component in composite resin restorations.

However, it has been reported^{2,3)} that 2-hydroxy ethyl methacrylate (2-HEMA) — which is often used as a primer as it is readily available — induces an inflammatory reaction. The latter is manifested in the form of severe itching due to direct and repeated contact of 2-HEMA with skin. Following such an allergic reaction, momentary skin contact with even a very small amount of the compound results in severe dermatitis. In addition, Jolanki *et al.* reported that bisphenol — which is present in composite resins — also induced contact allergy⁴⁾. In the same vein, Pegum and Medhurst reported that an acrylic monomer could permeate latex gloves⁵⁾, while Katsuno *et al.* warned that dental healthcare workers should use primers with care⁶⁻¹⁰⁾.

In 1989, Chigira *et al.* reported that glyceryl mono-methacrylate (GM) exhibited a better priming effect than 2-HEMA¹¹⁾. However, allergic reactions cannot be averted with the use of GM because it has a structural similarity with 2-HEMA. Nonetheless, GM poses a lower risk of allergic reaction than

2-HEMA because the amount of methacrylate, which is considered to induce allergic reaction, is relatively less in GM. Subsequently, in a report by Takahashi *et al.* on the priming effect and allergic reaction of highly purified GM, it was concluded that the use of highly purified GM was very useful for the adhesion of composite resins with dentin, and that it could avert the occurrence of delayed-type hypersensitivity¹²⁾.

In 2003, M. Kusunoki, a fellow researcher at our laboratory, reported on a new primer — tri-ethylene glycol mono-methacrylate (tri-ethylene glycol mono-methacrylate, NOF Corporation, Tokyo, Japan) (TEGMA) — with a priming effect similar to that of GM. The aim of this study was to evaluate TEGMA in terms of dermatological allergic reaction using a Guinea Pig Maximization Test (GPMT).

MATERIALS AND METHODS

Materials used

Four types of primers — 2-HEMA, TEGMA, polyethylene glycol mono-methacrylate (polyethylene glycol mono-methacrylate, NOF Corporation, Tokyo, Japan) (PEGMA), and highly purified GM (highly purified GM, NOF Corporation, Tokyo, Japan) (p-GM) — were used in this study. The positive control was 3,4-dichloronitrobenzene, as its ability to induce severe inflammatory reaction in skin has been clearly demonstrated.

Guinea pig maximization test

GPMT, a protocol established and published by Magnusson and Kligman (1969), was used to assess the potential adverse effects of 2-HEMA, TEGMA, PEGMA, and p-GM on skin¹³⁾. The backs of 25

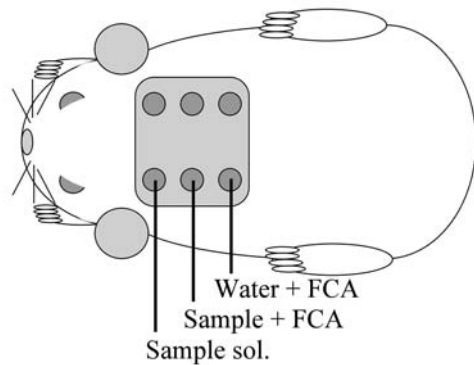


Fig. 1 Primary sensitization with a primer, an emulsion of Freund's Complete Adjuvant (FCA) with primer, or an emulsion of FCA with distilled water, by injecting percutaneously on both sides of the back of a guinea pig.

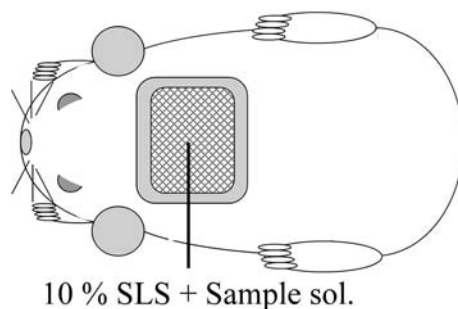


Fig. 2 Topical sensitization using a filter-paper patch soaked in 10% sodium lauryl sulfate (SLS) and primer.

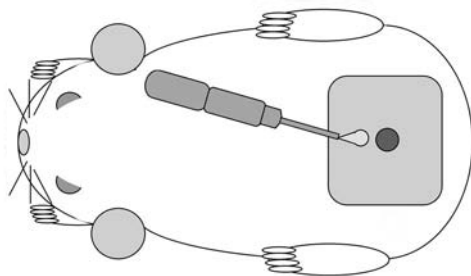


Fig. 3 Primer (100 μ l of each primer at a concentration of 100%) was applied to the skin, and the induced delayed-type hypersensitivity was estimated after 24 and 48 hours.

Table 1 International Contact Dermatitis Research Group Index

- | |
|---|
| (1) Erythema formation |
| 0 : No erythema |
| 1 : Very slight erythema (barely perceptible) |
| 2 : Well-defined erythema |
| 3 : Moderate to severe erythema |
| 4 : Severe erythema (deep redness) to slight eschar formation (injuries in depth) |
| (2) Edema formation |
| 0 : No edema |
| 1 : Slight edema |
| 2 : Moderate edema |
| 3 : Severe edema (raised more than 1mm and extending beyond area of exposure) |
| verall maximum score |

$$\text{Mean response} = \frac{\sum_{i=1}^n \text{score}_i}{\text{Total number of animals (n=5)}}$$

albino guinea pigs weighing 300 to 500 g were shaved just below the shoulder blade. For primary sensitization, 50 μ l of each of the three reagents (primer alone, emulsion of Freund's Complete Adjuvant (FCA) with primer, and emulsion of FCA with distilled water) was percutaneously injected on both the left and right sides of the back of each guinea pig (Fig. 1). At seven days after primary sensitization, the back of each guinea pig, including the six injection sites, was shaven. Then, 10% sodium lauryl sulfate (SLS) solution was applied to the shaved area to increase skin permeability.

For secondary sensitization, a filter-paper patch soaked in 10% SLS and 200 μ l of 100% 2-HEMA, TEGMA, PEGMA, p-GM, or 3,4-dichloronitrobenzene was affixed to the shaved area with cohesive stretch bandage for 48 hours (Fig. 2). At two weeks after secondary sensitization, the back of the guinea pig was shaven. For the challenge test, 100 μ l of 100% 2-HEMA, TEGMA, PEGMA, p-GM, and 3,4-dichloronitrobenzene was directly applied to the shaved skin to induce delayed-type hypersensitivity, and the skin was again covered with cohesive stretch bandage (Fig. 3). After 24 hours, the bandage was removed, and the disappearance of the nonspecific flare-up caused by the cohesive stretch bandage was recognized.

Skin reactions at 24 and 48 hours were observed and evaluated according to the International Contact Dermatitis Research Group Index (ICDRG) (Table 1). This experiment followed the guidelines of the Practice of Animal Care and Experiments in Showa

University and was supervised by the University's Committee on Animal Experiments.

RESULTS

All signs of inflammation, such as flare-ups, caused by the test samples were evaluated. In the case of 2-HEMA, inflammatory reaction was observed in four among five guinea pigs, and the mean response was 1.4. This was the highest value among the four test samples.

With p-GM, inflammatory reaction was observed in one among five guinea pigs, and the mean response was 0.2. With TEGMA, inflammatory reaction was observed in three among five guinea pigs, and the mean response was 0.6. With PEGMA, inflammatory reaction was observed in three among five guinea pigs, and the mean response was 1.0. With 3,4-dichloronitrobenzene, inflammatory reaction was observed in five among five guinea pigs, and the mean response was 3.6.

In comparison with p-GM, the inflammatory reaction caused by PEGMA was relatively severe. On the overall, for the entire observation period from 24 hours to 48 hours, reductions in mean response and positive response values were observed for TEGMA, PEGMA, and 3,4-dichloronitrobenzene (Table 2).

DISCUSSION

The use of a primer is essential to establishing solid, durable composite resin-dentin bonds. In this

connection, methacrylate derivatives have been used as bonding materials in dental clinics. Amongst which, 2-HEMA was widely used as a major component in primers. This was mainly because several available derivatives of GLUMA, which was first reported by Munksgaard and Asmussen, contained 2-HEMA¹⁾.

Presently, restraint has been urged in the clinical use of methacrylate derivatives, including the representative 2-HEMA. This is because their contact with soft tissues, such as skin, induces adverse effects and delayed-type hypersensitivity. To date, dental researchers have reported that an allergic reaction was observed in patients who came into repeated contact with 2-HEMA, thereby developing delayed-type hypersensitivity due to exposure to this compound during work¹⁴⁻¹⁶⁾. Latex gloves cannot protect the skin against the contact of 2-HEMA, because the latter permeates through rubber gloves¹⁷⁾. Furthermore, another impetus to replace 2-HEMA is that it does not completely prevent contraction gap formation.

In an urgent quest to completely prevent contraction gap formation, we proposed GM as the potential dentin primer to replace 2-HEMA in 1989. Additionally, Takahashi *et al.* reported that the allergic reaction caused by GM was not as critical as that caused by 2-HEMA, and that purified GM could avert the reaction^{11,12)}. However, GM being a methacrylate derivative causes adverse effects such as delayed-type hypersensitivity. GM is synthesized from glycidyl methacrylate, which is considered to trigger adverse reactions on skin. Glycidyl

Table 2 Guinea Pig Maximization Test results

	Sensitize	Challenge	Positive response	Mean response
(after 24h)	2 - HEMA	2 - HEMA	4/5	1.4
	P - GM	P - GM	1/5	0.2
	TEGMA	TEGMA	3/5	0.6
	PEGMA	PEGMA	3/5	1.0
	3,4-dichloro-nitrobenzene	3,4-dichloro-nitrobenzene	5/5	3.6
(after 48h)	2 - HEMA	2 - HEMA	4/5	1.4
	P - GM	P - GM	1/5	0.2
	TEGMA	TEGMA	2/5	0.4
	PEGMA	PEGMA	2/5	0.6
	3,4-dichloro-nitrobenzene	3,4-dichloro-nitrobenzene	5/5	3.4

n = five guinea pigs

methacrylate is present as an impurity in GM of a low degree of purity, and it cannot be eliminated by purification. Presently, the GM purification method to eliminate glycidyl methacrylate is not established on a commercial scale. As such, the current complicated purification method results in low recovery of highly purified GM. Due to these given reasons, TEGMA and PEGMA were thus proposed as potential candidates to replace highly purified GM primer¹⁸⁾.

TEGMA and PEGMA, as new dentin primers, have been reported to have a priming ability similar to that of highly purified GM¹⁸⁾. In the present study, allergic reaction caused by TEGMA and PEGMA was lower compared to 2-HEMA, but similar to that of highly purified GM. In conclusion, it was anticipated that TEGMA, PEGMA, and highly purified GM would hereafter replace 2-HEMA as primers for dentin bonding by virtue of their lower risk of triggering allergic reactions.

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