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Histologic observation of Microleakage of Four Kinds of Resin Materials by Using the Artificial Caries Technique

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ABSTRACT

Four kinds of resin materials including self-curing and light-curing resins were tested for their microleakage potential after they had been used to restore the experimental teeth. The artificial caries-like lesion was used to detect microleakage potential. The specimens were imbibed in water and examined under a polarized light microscope. The lesion was composed of two parts: one was the outer lesion and the other was the wall lesion. The latter was used to measure the extent of microleakage. The purpose of this study is to observe the various phenomena of caries-like lesion formation after the teeth had been restored with 4 kinds of resin materials under different filling techniques. From the reults, self-curing resin material without etchant and bonding agent application received a significantly higher perecentage and greater depth of wall lesion than other reins filled with tags formation ($p < 0.01$). Many methods can be used to detect the existence of micoleakage. From this study, the artificial caries technique and the polarized light microscopic examination can be regarded as one of the best ways to make such observation.

In operative dentistry, microleakage is a recurring problem that results in the failure of tooth restoration. Microleakage occurs through a micropathway. Such leakage is undetectable in clinical examination. In the process, bacteria, fluids, and ions can enter the gap between the cavity wall and restorative material[1]. It has been proved that microleakage may lead to many adverse effects, such as recurrent caries, tooth discoloration, hypersensitivity, pulp damage, and breakdown of filling material, etc[2].

Previous studies of microleakage had also proved that the use of composite resin often results in microleakage in the microspace between the filling and the tooth[3-6]. The presence and the size of the microspace are related to several factors: initial adapation, subsequent dimensional change of the material due to polymerization shrinkage, thermal

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contraction, absorption of water, mechanical stress, strength of the bond beween restorative material and tooth structure, and the abbillity of the bond to resist strain[5]. The leakage and the subsequent secondary caries formation around exisiting restoration are the primary reason for the replacement of composite resin restorative material[7].

Many efforts were made to reduce the shortcomings of the restorative material. Such efforts included varnish application[3], an acid etching technique for making surface irregularity, and the bonding of chemical materials to the tooth surface. Many techniques were developed to check the microleakage of the restorative material both in vitro and in vivo. Such techniques included dye penetration, the use of radioactive isotopes, eletron microscope scanning, and the creation of artificial caries[4,8-12].

In the artificial caries technique which uses an acidified gelatin gel, the hydrogen ion from the medium attacks the microspace between tooth and restorative material and also the tooth surface by diffusion[6]. This attack to the tooth surface appears as two caries-like lesions, the outer lesion and the wall lesion. Both can be observed through examination with a polarized light microscope when the specimen is imbibed in water. The benefit of using the artificial caries technique for detecting microleakage is that this technique creates a medium most similar to the real oral environment.

The purpose of this study is to observe the degree of the microleakage by means of wall lesion formation with the artificial caries technique. Caries-like lesion was detected by its characteristic histologic appearance under a polarized light microscope.

MATERIALS AND METHODS

Forty caries-free human molars and premolars were selected in this study. They were stored in the normal saline solution after extraction and kept in a freezer at -15°C. The experimental teeth used in this study were all erupted teeth from patients over a wide age span. Subject selection was thus because the lesion fromation in unerupted teeth may progress at a faster rate than in erupted teeth[13].

All of the teeth were carefully cleaned with the flouride-free pumice powder and soft bristle toothbrush to remove tooth stain and plaque. The buccal and lingual smooth surfaces were chosen out to perform the experimental procedure. The buccal cavities were the experimental sites and the lingual cavities served as control sites to be a guide for selecting the proper tooth that could create caries-like lesion. All cavity preparations were carried out in the middle third of the anatomical crown by the same operator. The cavity was designed to be rectangular in shape about 2mm occlusogingivally and 5mm mesiodistally with butt joint margin and rounded internal line angle(Fig.1). The cavity depth was made to be 1mm or less by using a round carbide bur 1mm in diameter. Each cavity was made within the enamel portion.

The acid etching procedures were performed with the etchant by applying it on the cavity for 60 sec. The specimens were washed with water for 20 sec and dried with oil-free air.

Four dental restorative composite resins, Heliosit (Vivadent Co. Schaan Liechtenstein),

Photoclearfil (Kuraray Co. LTD Japan), Microrest, jar type, (G-C International Corp. Japan), and Bosto (Sud-Dental Co. West Germany) were used to fill the cavity according to the manufacturers' instructions. The material was carried into the cavity with a resin applicator, and then pressed firmly and held in position with a tightly adapted celluloid strip until resin set. The first three materials had unfiller bonding agent added. The last was filled without any acid etching procedure, and no bonding agent was added.

After all the restorative materials had been used, the teeth were stored in normal saline solution for 24 hours before finishing and polishing the surface of the restored areas. A 1mm rim surrounding the filling was marked. An acid resistant varnish, Copalite (Bosworth Co.USA), was applied to the whole tooth except the area within the marking . Then inlay wax was also applied over the varnish to be a reinforcement (Fig.2). All the specimens preparation procedures in this study were done very carefully in the same way by one operator.

Artificial caries medium was prepared with 10% gelatin gel adjusted to pH 4.0 by titrating with lactic acid under the pH meter assessment[14]. 0.1% Thymol was added as a disinfectant. The acid environment produced in this way was reported very similar to the condition in oral cavity where caries is formed by diffusion of acidic products or hydrogen ions from dental plaque[6]. Therefore, The presence of the gel medium in vitro functioned as an analogue of the plaque in vivo.

The restored teeth were put in four separately labelled bottles according to the material used. All were then exposed to the medium for a period of 15 weeks. Over this time it is possible to produce caries-like lesion that penetrates through the four classical zones of enamel caries and has distinctive characteristics in each zone[14]. After exposure period, the restored teeth were longitudinally sectioned into two halves along a plane in the buccal-lingual direction. The low speed saw of Isomet (Buehler Co. U.S.A.) was used to dissect the specimen into sections 1mm in thickness (Fig.3). Ground sections were prepared to about 100μm, then they were imbibed in water and ready to be examined under a polarized light microscope.

The depth of both the outer and wall lesions were measured with a micrometer. The outer lesion depth was measured from the tooth surface to the most advanced front of the lesion. The wall lesion penetration depth was measured from the junction between the outer lesion and the wall lesion to its point of greatest depth.

Statistical Analysis:

All the data obtained were treated statistically by two methods. The data of outer lesion was analyzed by one way ANOVA test. Kruskal-Wallis one way analysis of variance by rand method was used to treat the data of wall lesion[15].

RESULTS

The 40 experimental teeth contained a total of 160 potential caries attack sites that consisted of the occlusal and cervical margins at both buccal and lingual sites. Some of these sites were abraded away carelessly during ground section preparation. They were not

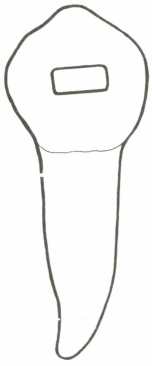

Fig 1. cavity located in the middle third of the anatomical crown, buccal view.

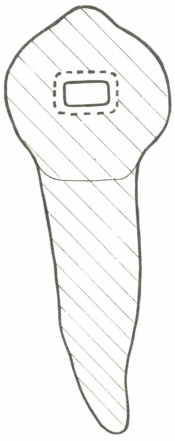

Fig 2. Areas of acid resistant varnish and inlay wax application. (shadow area).

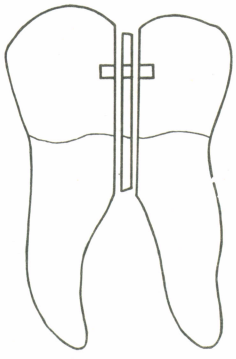

Fig 3. Specimen was dissected to 1 mm thicknes in the buccal-lingual direction.

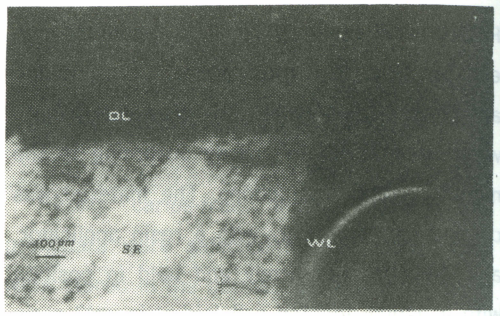

Fig 4. Outer lesion of control site. Surface zone as a negative birefringence was at the top layer. Beneath it was the body of the lesion. The wall lesion involved the entire cavity. x 40 OL=outer lesion, WL=wall lesion, SE=sound enamel.

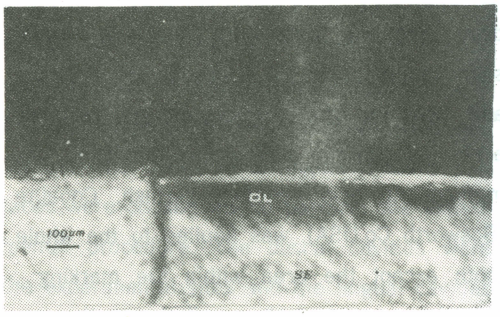

Fig 5. Longitudinal ground section of Heliosit restored teeth only outer lesion presented, no wall lesion was created. x 40 OL=outer lesion, SE =sound enamel.

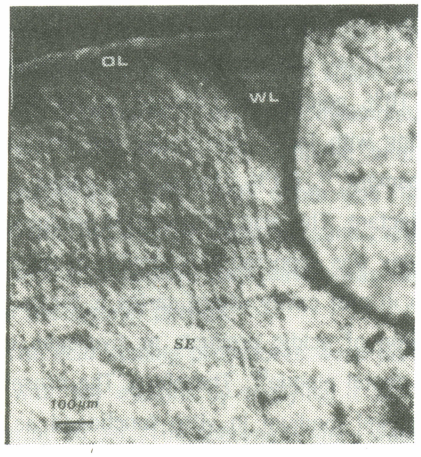

Fig 6. Longitudinal ground section of Microrest treated cavity. The wall lesion is present adjacent to the intact enamel restoration interface. x 40 OL=outer lesion, WL=wall lwsion, SE= sound enamel.

counted and data from them was not recorded. Excluded lingual sites, 64 satisfactory caries risk sites of both occlusal and cervical margins were obtained to be examined for microleakage existence.

after water imbibition and polarized light microscope examination, the specimen revealed caries-like lesion. All the test caries risk sites had the outer lesion formation (Table 1), but wall lesion formed only at 17 of 64 caries risk sites, about 27%. Of all those with wall lesions, the Bosto accounted for the majority, that is 7/17, about 41%. The self-curing resin material without etching technique had a significantly greater mean depth of wall lesion than those with bonding agent added (Table 2).

The lingual sites:

The unfilled cavities were completely exposed to the acidified gelatin gel medium. When imbibed in water and examined under a polarized light microscope, we found that throughout all the cavities, down to the pulpal floor, there was caries-like lesion. The surface zone, as a negative birefringence, contained the points of origination for all outer lesions. the second layer, the body of the lesion, showed a positive birefringence (Fig.4).

Heliosit:

Heliosit filled cavities developed wall lesions only at 4 of 16 caries risk sites. The mean depth was 300±106μm, and the mean depth of outer lesion was 263±120μm.

The histologic picture under the polarized light microscope also showed a negative birefringence surface zone and an underlying positive birefringence body of the lesion (Fig. 5).

Microrest:

Only 3 of the 17 caries risk sites had wall lesion formation in this group. The mean depth of wall lesion was 167±72μm, and that of outer lesions was 248±107μm. Only about 18% of the specimens had wall lesion (Fig.6).

Clearfil:

Only 3 of the 18 caries risk sites developed wall lesion. The mean depth of wall lesion was 83±38μm, and that of outer lesions was 273±131μm. In this study, this material had the lowest rate of wall lesion formation.

Bosto:

This is the only resin material used without etching technique and bonding agent added in the study. The mean depth of outer lesion was 194±62μm, and that of wall lesions was 675±192μm. Seven wall lesions were developed out of a possible 13 caries risk sites. The wall lesions penetrated deeper in this group than in any of the others. Some even penetrated to the pulpal floor.

One way ANOVA test revealed no significant differences among the outer lesions of the four groups ($p > 0.05$). Since the total number of wall lesion was only 17 and a slightly higher standard error was obtained from this study, it is not proper to treat the data of wall lesion with ANOVA[15]. Using the Kruskal-Wallis one way analysis of variance by rank method, we found that there is no difference in wall lesion formation among Heliosit, Microrest, and Clearfil, however, significant

Table 1. The Depth of Outer Lesion (OL) Diffusion Adjacent to Four Kinds of Composite Resins.

Filling materials	No. of OL / No. of site examined	Incidence of OL (%)	Mean depth of OL (μm)
* Heliosit	16/16	100	263±120
* Clearfil	18/18	100	273±131
+Microrest	17/17	100	248±107
++Bosto	13/13	100	194± 62

*light-curing resin, using acid etching technique.
+self-curing resin, using acid etching technique.
++self-curing resin, without using acid etching technique.

Table 2. The Depth of Wall Lesion (WL) Penetration adjacent to Four Kinds of Composite Resins

Filling materials	No.of positive WL / No.of site examined	Incidence positive WL (%)	Mean depth of WL (μm)
* Heliosit	4/16	25	300±106
* Clearfil	3/18	17	83± 38
+Microrest	3/17	18	167± 72
++Bosto	7/13	54	675±192

*light-curing resin, using acid etching technique.
+self-curing resin, using acid etching technique.
++self-curing resin, without using acid etching technique.

differences existed between Bosto and the other three resin materials ($p<0.01$).

DISCUSSION

The lingual site of each tooth was chosen to serve as a guide for selecting the proper tooth in this study. After cavity preparation and artificial caries medium immersion for a definite period of time, each tooth was examined under polarized light microscope. If the caries-like lesion occured in the lingual site, it meant that this tooth had no problem in creating artificilal caries. In other words, if microleakage existed between restorative material and tooth substance, it should also have the potential to create caries-like lesion due to hydrogen ion penetration under the same environmental factor. The role of the lingual site in this study was to act as an individual guidance for selecting the proper tooth . From the results, we found that all of the teeth we collected were up to the standard.

Summarizing all the informations obtained in this study, we can see very clearly that resin material without etching technique, when filled in the prepared cavity, creates the greatest chance of microleakage (54%). The other three resin materials had a mean

chance of producing microleakage ranging from 17 to 25%. Compared with other three resin materials, the resin material Bosto was responsible for wall lesions of greatest mean depth. Of the four tested groups, Clearfil created the lowest rate and smallest mean depth of wall lesion. A study from Artemis and Leonidas also found that Clearfil was highly satisfactory, not showing any marginal leakage for a period of 90 days[16].

The findings of this study were consistent with the previous findings that when restoration is performed in a conventionally prepared butt joint cavity, severe marginal leakage often results[4]. Leakage can be reduced when the restoration is extended onto etched enamel periphery.

From many studies about resin materials, we know that the use of etching technique and bonding agent added lead to less problems in marginal adapation[4,17]. There is limited microleakage formation because the bonding agent can penetrate the etched enamel prisms fully.

When using the acidified gelatin technique in vitro, the artificial caries-like lesion in enamel appeared indistinguishable from natural caries[13]. When the specimen is imbibed in water and examined under a polarized light microscope, four layers can be seen. The top layer is surface zone. Caries-like invasion of the surface zone was only remineralization rather than the loss of tissue because calcium and phosphorus ions are released by subsurface dissolution and precipitates into the surface enamel in the form of calcium phosphate[13]. Therefore, it appeared as an intact surface and showd a negative birefringence. The greater resistance of the surface enamel to caries attack may be due to its high degree of calcification.

A study done by Silverstone[13] found that when the original surface enamel was removed intentionally prior to the experiment, lesions were created that still demonstrated the existence of a surface zone. This phenomenon was due to the fact that surface zone is a remineralization site, in spite of its being on a surface that was previously subsurface in position. This zone still retained a negative birefringence when examined under a polarized light microscope after imbibed with water. But the surface zone would form at a faster rate than if the surface enamel was not removed beforehand.

The second layer, body of the lesion, is a positive birefringence layer. In other words, there is a larger than 5% pore volume in this area[13]. When the incidence of lesion has progressed to an advanced stage where the area has a pore volume larger than 5%, new lesion begins to form in this layer. The border between the second layer and the surface zone is very well demarcated.

The next two layers are the dark zone and the translucent zone. If the specimen is imbibed with quinoline (refractive index 1.62), then only the dark zone will have a positive birefringence[12].

The acidified gelatin gel provides the hydrogen ions to attack the enamel surface and forms the caries-like lesion. If the microspace between tooth and filling is large enough for hydrogen ions to penetrate, then a wall lesion will be formed along the filling wall, possibly down to the pulpal floor. In other words, if tags are not present or are ineffective in preventing microleakage, diffu-

sion of hydrogen ions into the resulting microspaces will result in wall lesion formation[18]. If the junction of enamel with resin material is intact, no microleakage will occur, and no wall lesion will develop. The depth of outer lesions of the four tested groups were not consistent. This inconsistency can possibly be explained by the fact that the depth of outer lesion may be affected slightly by the amout of fluoride in the enamel surface[13]. The higher standard error of outer lesions in this study can also be explained by the various flouride content in the experimental teeth.

From this study, the resin tags projected into the enamel pores created by the acid etching technique probably give us a reasonable explanation for the ability of enamel to resist acid dissolution.

There are many methods that can detect microleakage between the enamel and filling material, including the dye penetration, the use of radioactive isotope, and so forth. Studies have shown that the artificial caries technique in vitro is a way to detect microleakage because this technique creates an environment similar to the real oral cavity[6,13]. In addition, this method produces artificial caries lesions that are histologically indistinguishable from naturally occurring enamel caries. Therefore it is possible to see whether the wall lesion (recurrent caries) will occur or not by hydrogen ions attacking from the acidified gelatin gel (like natural caries formation by bacteria within the plaque in the oral cacity). Imbibition in water and viewing under a polarized light microscope allows one to easily differentiate in all specimens whether microleakage exists or not by checking for the presence of wall lesion. With respect to imitation of oral environment, the artificial caries technique is also easier and better than conventional methods.

Using the artificial carise technique, Hicks et al pointed out that when cavities were filled with glass ionomer or glass ionomer mingled with silver, there was no microscopic evidence of wall lesion formation in the enamel or dentin composing the cavity wall adjacent to the restoration[6]. When examined under the electron microscope, he also showed there was very little microleakage. In other words, the results gained by using the artificial caries technique were consistent with the observations using the electron microscope. Anyhow, artificial caries technique, in detecting microleakage, offers a method easier and more effective than older, or conventional methods.

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以人造齲齒技術作填補樹脂微滲漏之顯微觀測

張瑞昌　郭倍榮　林哲堂

40 顆無蛀洞的牙齒，在經過窩洞修形後，以四種複合樹脂填補材料，包括自動聚合及光聚合類複合樹脂，將其填補於窩洞內，並籍觀察壁病灶的形成與否來得知這些材料有無微滲漏的存在。本實驗對於充填物於牙齒窩洞內微滲漏的鑑定，主要是利用人造齲齒形成之技術而達成。人造齲齒的技術可提供一個類似於口內的環境，由經酸化明膠凝膠中釋出的氫離子可滲透入微滲漏中形成類齲齒之病灶，就如同於口內牙菌斑中的產酸細菌可對呈微滲漏的修復齒產生再發性齲齒一般。將此標本浸於水中，經由偏光顯微鏡的觀察，可呈現出二部份的病灶，一爲外病灶，一爲壁病灶。後者的形成與否及其深淺可當作微滲漏程度大小測量的準則。經本實驗觀察類齲齒病灶形成之結果，經由統計分析得知未採酸蝕術及黏著劑的樹脂比利用此類技術的樹脂產生更高的壁病灶百分比，於深度方面也呈有意義之差別（$P>0.01$）。微滲漏的探測方法衆多，但以人造齲齒的技術提供的鑑定法，不失爲一方便而精確的觀察方式。

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