Original

Studies on Enamel Solubility and Acid Resistance by Laser

Mamoru KUMAZAKI, Takashi MORIMOTO, Yafumi TSUCHIHASHI, Masaharu MORIKAWA, Takumi ARAI and Yasuhiko UEDA

Department of Operative Dentistry Osaka Dental University
(Chief: Prof. Benji FUJI)

(Accepted for Publication February 1, 1993)

レーザーによるエナメル質溶解性と耐酸性の研究

熊崎 護 森本 哲司 土橋 弥文
森川 正治 新井 巧 上田 裕彦

大阪歯科大学歯科保存学講座

要旨: レーザー光を歯科に応用しようとしたのは、菌斑部分の除去や削合などであったが、1965年にSognnaes, 1972年にSternらがラビーレーザーの照射で、また、1974年に山本が、YAGレーザーの照射でもエナメル質に耐酸性を生ずるという報告をして以来、この面での研究が多く見られるようになった。

しかし、これらは臨床応用という面での研究の試みで、その耐酸性メカニズムの解明の研究は少ない。今回私たちは、レーザー照射によるエナメル質の溶解抑制効果を組織学的に解明するため、サイドウィッチ法を用いて、偏光顕微鏡下で連続的に観察することによって、非常に興味ある結果を得たので報告する。

実験材料として、ヒト新鮮抜歯上頸中切歯を使用した。実体顕微鏡下で、破折、亀裂発点、石灰化不全などの見られない歯を選別した。この歯の表面に、CO₂レーザーを15〜20J/cm²で照射し、controlとしては同様に選別された無照射歯を使用した。

薄切平行切片はスライドグラスおよびカバーグラス間に接着固定し、作用液流出しパルプシエルを用いて、作用液0.1N酵酸緩衝液pH5.0を注入しながら同時に16mm自動撮影装置を用いて記録観察を行った。

実験結果：今回私たちは、エナメル質表層にCO₂レーザー照射を行い、未照射との間で、偏光顕微鏡を用いて連続的に観察を行った結果、以下の所見を得た。

1) CO₂レーザー照射歯ではcontrolに比べて、5〜6倍程度の脱灰時間を要し、明らかにエナメル質の耐酸性を認めた。

2) Controlでは表層エナメル質の脱灰時における過程では、黄色酸化変性を示す皮膚なる黄色を認めた。

CO₂レーザー照射を用いて、その黄色酸化変性を示す皮膚なる黄色を認めた。この入射を表層に照射した効果が見られないことから、表層酸化変性の脱灰のみが進行するようである。

3) 作用液の浸透の結果と思われる暗赤色部下の赤色層を示す、実験ではその本質を明らかにすることができなかった。

(J. Jpn. Soc. Laser Dent. 4: 85〜62, 1993 Reprint requests to Dr. Kumazaki)

Key words=Caries : Laser : Enamel’s resistance to acid

キーワード＝歯蝕：レーザー：エナメル質の耐酸性

* 5-31, Otemae 1-chome, Chuo-ku, Osaka, 540 JAPAN TEL 06-943-6521 FAX 06-943-8051
* 〒540 大阪市中央区大手前 1-5-31 TEL 06-943-6521 FAX 06-943-8051
The first application of laser technology to dentistry was for the removal of caries infected material and the preparation of cavities. However, ever since reports by Sognnaes (1965) and Stern (1972) using ruby lasers and Yamamoto (1974) using a YAG laser, much attention has been focused on the laser’s potential to enhance the enamel’s resistance to acid.

These previous reports concentrated on research for clinical applications, and very little research has pursued the exact mechanism of this phenomenon. In order to better understand histologically how laser irradiation can result in inhibiting caries in enamel, the authors continuously observed laser irradiated enamel with a polarizing microscope using the sandwich method and obtained some very significant results.

In our research, we used human upper, central incisors which had been freshly extracted. These were first cleaned with a scaler and engine brush and then carefully observed with an ordinary microscope to eliminate teeth which were broken, cracked, had white spots, or were deficient in calcium.

The surface of the teeth finally chosen were irradiated with a CO2 laser at a power of 5 J/cm2. Teeth used as controls were chosen in the same way but never irradiated.

Thin parallel samples of the teeth were secured between a specimen slide and a cover glass by coating the slide with a drop of 5% toluene solution which was then heated at 50~55°C by a constant temperature apparatus for 2 days to polymerize the solution. The decalcifying solution had a pH of 5.0 and was introduced with a micro-tube pump. The decalcification time was 30 hours, and the solution was administered at a rate of 6 ml per hour. The decalcifying solution, 0.1 N acetic acid buffer with pH 5.0, was introduced by a micro-tube pump made by Tokyo Rikakikai and the decalcification process was simultaneously observed and recorded with a polarizing microscope, automatic exposure cinemicrographic equipment model 1 CPMA, and a 16 mm Cine Bolex Camera (Bolex), all made by Nippon Kogaku.

A 16 mm automatic slow motion camera was used to record changes over long periods of time; the camera was set to take 1 frame every 30 seconds and all developing was performed with an automatic developer.

**Experimental Materials and Methods**

1. **Sample Preparation**

The teeth used were freshly extracted upper, central incisors of people in their twenties. After extraction, the teeth were immersed in a 70% alcohol solution and refrigerated.

After cleaning and removal of impurities from the tooth surfaces, the teeth were observed with an ordinary microscope to eliminate those that were cracked or damaged.

The teeth were observed with the sandwich method followed by Ishii and Yoshida (1971). First 0.5 mm samples were cut in a saline solution with the Sugimoto model SI cutting device for the hard tissue of teeth. Then these pieces were made into parallel 20~25 μm slices with the HT hard tissue polishing apparatus N-100. (Each slice was 25±5 μm.) Finally, a microradiogram was made of each slice to eliminate those which were imperfectly formed or showed other abnormalities.

2. **Adhesive Selection**

In order to fix thin slices for a long time, Ishii et al. (1971) investigated the possibilities of commonly available bonds, araldite (ciba), Cemedine, epoxy and balsam resins. The authors determined that polyvinyl methyl ether toluene would give the best results for fixing thin slices, especially for use over a long period of time. It was also determined that a 5% solution of toluene would give better results than the 3% one used by Ishii et al.

3. **Preparing the Samples for Observation**

As shown in Figure 1., the samples made by the method just described were fixed between a slide and cover slide in the following way:

a. The center of the slide was coated with a drop of 5% toluene and then heated in a constant temperature apparatus at 50~55°C for 2 days to polymerize it (Fig. 1a). In order to
provide a route for easy delivery of the active solution, a groove was cut into the center of the slide with a glass cutter.

b. A critical point when fixing a thinly sliced sample to the slide is the exact amount of pressure necessary to make a good sample. This procedure is carefully carried out using a low power microscope, and it is especially important to make sure the slide is not contaminated during the procedure (Fig. 1b).

c. Next the cover glass is pressed down on the sample. The cover glass must be pressed down very carefully to avoid the formation of minute bubbles. This completes the preparation of a sample of exposed enamel surface for observation (Fig. 1c).

d. The active solution is introduced from one direction by a micro pump and drawn off the other way by another micro pump. If the in and out flow of the solution is not balanced, it could leak and damage the instrument. To prevent this, a 3 cm hole is made in a 15 cm laboratory dish and then a cover glass is fixed over the hole with vinyl adhesive. This prevents damage to the instruments due to leakage of the decalcifying solution (Fig. 1d).

4. Decalcifying Solution

The decalcifying solution was 0.1N acetic acid buffer with pH 5.0 and the decalcifying time was determined to be 24 hours.

A micropump made by Tokyo Rikakikai was used to deliver and draw off the solution, and in order to make the procedure as simple as possible, all the parts including the slide, intake tube, and drain tube were made into a single unit which could be attached to the microscope. A laboratory dish was used to prevent the leakage of the solution from damaging the microscope.

Figure 2 is a cross sectional diagram of the experimental apparatus. The apparatus consists of a solution container, a solution intake device and a solution drain device. The container is 3.5 mm in diameter, and the solution is delivered to the sample under the microscope by a micro pump. After the solution has decalcified the enamel, it is drawn out by another micro pump through a 0.5 mm silicon rubber tube (the same as the tube used for delivery).

The micro pumps must be carefully adjusted so that the delivery and draining of the solution are balanced. If not tiny bubbles forming in the area being observed or an over flow of the solution could make it impossible to carry out the experiment (Fig. 3).

As mentioned above, a groove in the center of the slide is provided for the tubes attached to
the delivery and drain pumps so that the solution passes over the sample.

5. Solution Flow Rate

The volume of the solution used and the speed with which it flows over the sample are critical factors in the effect the solution has on the enamel. In order to accurately regulate this flow, alumina particles with a diameter of 0.5 µm were mixed in distilled water, and the movement of these particles was observed with a microscope. In this way the micro pumps could be adjusted to maintain an optimum, constant flow. This observation of the rate of the flow of the solution was performed at a distance of 10 µm from the sample.

In this way we found it quite easy to adjust the pumps for a constant flow of 3 sec/mm past the tooth surface. The experiment was performed with the part of the solution stream 10 µm from the sample flowing at a rate of 3 sec/cm. The tooth surface exposed to the flow was the surface with the smoothest curve such as the buccal surface rather than surfaces with more complicated shapes such as the incisal edge or the lingual surface.

However, a natural tooth’s shape is rarely perfectly regular, and differences in the rate of decalcification when observing different areas of the same sample can probably be considered the result of slight differences in the flow rate.

The decalcifying solution was kept at a constant temperature of 20°C in order to standardize results as much as possible.

6. Experimental and Observational Methods

Figure 4 shows the experimental set up. The 2 micro tube pumps (Tokyo Rikakikai Co.), one each for delivery and drain, were used to maintain the flow of the decalcifying solution, 0.1N acetic acid buffer with a pH of 5.0. The results were observed and recorded simultaneously with an automatic exposure cinemicrographic device (model ICFMA Nikon) and 16 mm Cine Bolex Camera (Bolex) mounted on a polarizing microscope (model POH 3 Nikon).

The 16 mm Cine camera was usually used for observation and recording, but in some cases the 35 mm polarizing microscope camera was used (Fig. 4).

The 16 mm automatic slow motion camera was used to clearly record the subtle changes which occurred over a long period of time. This camera was set to take 1 frame every 15 seconds, and all film development was performed with automatic developing equipment.

The 16 mm camera is superior to the 35 mm microscopic camera for clearly analyzing and timing early changes.

A polarizing microscope was used rather than an ordinary microscope, and when necessary, a Softex CMR-2 was used to determine the degree of decalcification of a sample. Exposure conditions were 10 kV, 3 mA and 5 minutes and observations were made at powers of 100×, 200×, and 400×.

Experimental Results

After the upper, central incisors extracted from patients in their twenties were prepared as described above, they were treated with a decalcifying solution, 0.1N acetic acid buffer with a pH of 5. The time allowed for decalcification was 24 hours and the process was continuously observed and recorded with automatic exposure cinemicrographic equipment and a 16 mm cine
Bolex camera. The results are shown in figures 5~14.

No appreciable changes were observed for about the first 3 hours. Decalcification of the surface enamel apparently requires more time than other areas (Fig. 5).

After about 3 and a half hours, decalcification of the surface enamel was clearly apparent. The width of the dark, decalcified area grew to about 1 μm. As time went on, the dark area remained about 1 μm in width and penetrated deeper into the enamel (Fig. 6).

The beginning of the penetration of the decalcifying solution can be identified by the red, refracted layer below the surface. After approximately 9 hours, the dark area widens and a red refraction band appears directly below the decalcified area. We observed the beginning of the appearance of a red refraction band, which was probably a remineralized layer, directly below the dark area, i.e., the area where decalcification was most advanced (Fig. 7).

It was also observed that the penetrating solution advanced along the Retzius lines. After approximately 15 hours, the dark area had penetrated far below the surface, and the process of decalcification began to take on a rather complex nature. The double refracted area in the center spread through out the enamel below the surface, and a yellow refraction image, which was probably the image of remineralized material, began to appear. The dark refracted area ended at the termination of the Retzius lines, and it was clear that the penetration of the solution was spreading out along this area (Fig. 8).

Figure 9 was taken after 20 hours. There was no longer any unaffected enamel, the dark area had spread out over a large area, and there was a clear image of yellow refraction. A completely decalcified area can be observed outside the red refraction area on the surface. Red refraction can be observed along the Retzius lines, which shows their great resistance to decalcification.

Figure 10 was taken after 24 hours. The subsurface enamel is almost completely decalcified.

Figure 11 shows the enamel which has been irradiated with a CO₂ laser immediately after the initial application of the decalcifying solution.

Figure 12 was taken after 1 hour, hardly any change can be observed on the surface.

Figure 13 was taken after 2 hours, the width of the dark area has grown somewhat.

Figure 14 was taken after 5 hours, the width of the dark area is about 1 mm, which is about the same as a control tooth after 2 hours.

Figure 15 shows the beginning of the appearance of a red refracted layer and is about the same as a control tooth after 3 hours.

Figure 16: The red refracted layer has begun to appear, but the red refracted image of what is probably a remineralized layer is not yet clear.

Figure 17: This is after 8 hours of exposure to the decalcifying solution. The black lines are about 2~3 μm wide and indicate great resistance to decalcification.

Figure 18: Observation of decalcification of surface enamel and a 2~3 μm line of great resistance to decalcification just below the surface.

Figure 19: The red refraction can be observed everywhere beneath the surface, but there is no yellow refraction image. Apparently decalcification is proceeding only in the surface of the enamel.

Figure 20: This was taken after 30 hours. Decalcification is progressing in the surface of the enamel, but the dark area has not spread more than about 5 μm below the surface, indicating great resistance to decalcification.

Discussion

The authors investigated the fundamental pattern of enamel destruction using a 0.1N acetic acid buffer (pH 5.0) and observed the process with a 16 mm camera attached to a polarizing microscope and set for time Lapse photography. The ability of CO₂ laser irradiation (5 J/cm²) to retard caries on the tooth surface was also evaluated.

Although the first signs of demineralization of control specimens caused by an organic acid
Fig. 5  3 hours (×150)
Fig. 6  3.5 hours (×150)
Fig. 7  9 hours (×150)
Fig. 8  15 hours (×150)
Fig. 9  20 hours (×150)
Fig. 10  24 hours (×150)
Fig. 11  0 hour (×150) with Laser Irradiation
Fig. 12  1 hour (×150) with Laser Irradiation
buffer solution often appear within 1 or 2 hours, laser irradiated specimens showed no change at this stage and the expansion of dark areas, considered to be the earliest stage of demineralization, was just barely observable after 5 hours.

In the control specimens, a red refraction zone appeared after 5 hours. There was a yellow compound refraction zone inside this with a dark spot (probably a sign of remineralization) at the deepest level. The red refraction zone did not appear clearly in the irradiated teeth and was just barely visible after 8 hours. This was a large time difference compared to the control specimens, and the dark area assumed to be a zone of remineralization did not change to any great extent.

The irradiated specimens seemed to have a strong resistance to demineralization at a level about 2 or 3 μm below the surface of the enamel, and demineralization occurred only at the surface of the enamel. Although demineralization of the irradiated teeth spread below the surface, it stopped, at least temporarily, once it reached the Retzius striae, and after that spread out sideways following the line of the Retzius striae. The progress of this demineralization was considerably slower than that in the control specimens. Although demineralization occurred in the perikymata, it did not reach the Retzius striae.

It is particularly significant that the yellow compound refraction zone did not appear in the irradiated teeth and that demineralization occurred only at the surface of the enamel. Even after 30 hours, the demineralization at the surface and the dark area about 5 μm deep did not expand and showed great resistance to acid. This is probably due to the laser irradiation of the tooth surface.

Conclusion

The following results were obtained by continuously observing the decalcification of the surface enamel of teeth irradiated with a CO₂ laser and those not irradiated:

1) Decalcification time for the enamel of irradiated teeth was 5~6 times greater than that for teeth not irradiated. This clearly indicates the greater acid resistance of irradiated enamel.

2) During the decalcification process of control teeth, we observed a highly advanced stage of decalcification, which appeared as a yellow double refracted image, and a seemingly mineralized layer deeper than the yellow double refracted image.

In irradiated enamel, no yellow double refracted image appeared, and we observed black lines of great resistance to decalcification about 5~6 μm below the enamel surface. Decalcification progressed only in the surface of the enamel.

3) We were not able to clarify the nature of the red refracted layer which occurred directly below the dark area and seemed to be the result of the penetration of the decalcifying solution.

Reference


