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医療技術評価総合研究事業

新たな歯科治療技法等による治療技術開発に関する総合研究

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1　研究組織

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2　研究概要

近年・開発されてきた新たな歯科治療技法並びに材料等による治療技術法等を検討再評価することにより、新しい治療体系の構築及び歯科治療技術の質の向上を図るとともに、これらの効果的な臨床応用に際して電子情報の普及・活用を計るためのシステムを確立し、国民に時代の進歩にそった良質な医療を提供し、現代の多様化するニーズに答えることを目的として本研究を行うものである。

上記の目的を遂行するために下記のような研究を行う。

・【研究代表者：新潟大学歯学部岩久正明教授・研究協力者：新潟大学歯学部岡本明講師】：初年度のレーザーを用いたウ蝕病巣の削除法の基礎的研究、硬組織及び軟組織への影響、殺菌効果（２年目)、疼痛や不快感の発生などについて明らかにしてその臨床応用（３年目）を計る。また、研究全体の総括を計る。

・【研究協力者：東京医科歯科大学歯学部黒崎紀正教授】：初年度のウ蝕患部溶解・削除法の効果、患者への影響などを検討し（２年目)、臨床応用のためのシステムの確立（３年目）を計る。

・【研究分担者・日本歯科大学新潟歯学部小倉英夫教授、研究協力者・昭和大学歯学部宮崎隆教授】：初年度にCAD/CAMによる歯科修復物製作方法ならびに精度について基礎的研究を行うと共に、その使用材料についての物性を調べ、さらにその性能を検討，評価する（２年目）。その結果に基づき、実用化のために臨床システムの確立を計る〔３年目〕。

・【研究分担者・北海道大学歯学部小口春久教授】：環境ホルモンの一つとして挙げられているビスフエノールＡを含有しない歯科材料の性質向上と汎用性のある新しい歯科材料の開発を目的として、初年度は現在の基礎研究を継続し、二年目は生体親和性などの生物学的性能を調べ、最終年に臨床試験および臨床システムの確立を計る。

・【研究分担者・大阪歯科大学中村正明教授】：初年度、咬合咀嚼機能の維持回復について各種修復材料の生体適合性を *in vitro* の環境で評価する技術を研究開発し、さらに生体適合性に関するデータベースを構築し（２年目)、その臨床応用のシステムを確立する（３年目)。

・【研究協力者・東京歯科大学井上孝助教授】：各種処置法の生体への安全性を

調べるための病理組織学的研究を行う。

・【研究協力者・愛知学院大学歯学部栗田賢一教授】：診断、治療における電子情報の臨床応用，普及の研究を行う。

3　研究目的

近年・開発されてきた新たな歯科治療技法並びに材料等による治療技術法等を検討再評価することにより、新しい治療体系の構築及び歯科治療技術の質の向上を図ることを目的として本研究を行うものである。

本研究では、これらの点ですでにかなりの基礎的研究を進めてきた材料、技法について、その臨床応用に取り組むもので、ほぼこの研究申請期間中にかなりのものが実現可能と考えられ、これらの確立により、現在おこなわれている治療内容を一層進歩させ、優れた歯科医療を国民へ提供することが可能となるものと考えられる。

・研究分担（岩久）：ウ蝕の感染組織の削除法については従来の回転切削法での振動や騒音による患者の不快感がおおきく、その解決のために新しいアプローチが求められる。感染部溶解削除法とレーザーによる除去法について検討を行い、患者にとって快適な治療法の確立を目指す。なお、これらは既にかなりの成果を得ており申請期間内で十分な成果が期待できる。

・研究分担（小倉）： 実用に適する CAD/CAM 装置の機構ならびに材料について考究する。鋳造，重合あるいは焼成などの従来の歯科修復物作製方法において見られる気泡や収縮などの問題点を改善するために，また，歯科材料の選択肢を多くして現在の修復用材料に替わる材料を適用可能とするために本研究が必要である。歯科修復物が CAD/CAM によって良好な再現性をもって容易に作製されるようになれば，従来の修復物作製法において見られた問題点が解決され，より良質な歯科修復物による治療が可能となる。また，材料の選択肢が増えるので，原料の高騰などによって起こる問題を回避しやすくなる。

・研究分担（小口）：環境様ホルモン物質を含まない歯科材料を開発することが本研究の目的である。歯質接着性、フッ素徐放性、抗蝸蝕性の長所を有するグラスアイオノマーセメントの機械的性質を向上させるため、高強度で生

体親和性に優れるガラス繊維粒子を利用した新材料を開発する。これによって・汎用性があり、簡便な操作で長期にわたる生物学的安定性と機械的強度を有する材料が開発され、ウ蝕治療の充填材、シーラント材、合着材などの応用に期待される。

・研究分担（中村）：現在の動物福祉に対する世界的な趨勢や臨床試験における患者への負担の軽減などの立場から *in vitro* 環境でより詳細に生体適合性を評価する必要性は高い．臨床で使用されている材料を含めて生体適合性のデータベースを構築し，新しく開発された材料の相対的評価を行うことが可能となれば，動物実験を大幅に削減できるばかりか，臨床試験の実施に対しても有力な情報を提供できると考えられる．

4　倫理面への配慮

・新たな歯科治療材料等による治療・診断技術開発等

倫理面への配慮については、本研究に用いているレーザー装置は、歯の切削用としてすでに厚生省の正式認可を受けたものであるが、患者への使用に際しては従来の方法との相違について詳細に説明し本人の選択により使用を決め、なお使用の途中でも患者の意思によりいつでも中断し、他の方法に変更し、また、術後の経過観察も十分に行うことにしている。

・咬合咀嚼機能の維持回復に関する新材料の開発、検討

倫理面への配慮：市販のCAD/CAM装置については粉塵発生の問題はほとんどないが，研究開発の途上にある装置においては粉塵の発生が危惧されることもありうる。このような場合は，研究者の安全が確保されるよう必ず粉塵防護策を講じた上で研究を行う。

・医科関連疾患における歯科医療技術の臨床応用

倫理面への配慮・汎用性かつ耐久性がある生体に安全なガラス繊維強化型グラスアイオノマーセメントを開発することは、疫学的見地から、環境ホルモンのビスフエノールＡを含む危険性のあるレジン材料を使用しなくても良くなり、実用後の社会的貢献は大きく、本研究は国民の健康と安全な生活に寄与できる。

・診断、治療における電子情報の臨床応用、普及

口腔内環境をシミュレートした動的環境下で抽出して種々な細胞への影響を調べる際には、確立された動物細胞の使用によっているため，倫理面については特段の配慮を要しないと思われる.

5　研究内容

1）　う蝕病巣除去法に関する研究

＊ Carisolv によるう蝕病巣溶解除去法に関する研究

黒崎紀正、礪波健一

・これまでの研究論文（別刷印刷）

・現在の研究

Carisolvゲル中のアミノ酸が齲蝕象牙質に及ぼす影響

Carisolv ゲルには次亜塩素酸ナトリウムと３種類のアミノ酸が含まれる．本教室では Carisolv ゲル中の次亜塩素酸ナトリウムが齲蝕象牙質に与える影響を検討し，報告した．しかしながら，同じく Carisolv ゲルの構成成分である３種類のアミノ酸が，Carisolv ゲルの齲蝕象牙質除去能に与える影響は明らかではない．そこで，本教室では齲窩を通る面でスライスカットされた象牙質薄片を，次亜塩素酸ナトリウム水溶液，次亜塩素酸ナトリウム水溶液とアミノ酸の混合物，および純水のそれぞれを用いて処理した．そして，その処理面を SEM 観察し，比較することで３種類のアミノ酸が，Carisolv ゲルの齲蝕象牙質除去能に与える影響を検討中である．

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これ以降は雑誌/図書等に掲載された論文となりますので下記の
「研究成果の刊行に関する一覧表」をご参照ください。

「研究成果の刊行に関する一覧表」

Carisolv Gel によるう蝕象牙質の軟化について
五十嵐 公、橋本佳奈、礪波健一、清水チエ、他
日本歯科保存学雑誌 41(4) 1998 P.704-707

Carisolv によって齲蝕を除去した後の窩壁象牙質面
橋本佳奈、礪波健一、清水チエ、他
日本歯科保存学雑誌 42(3) 1999 P.619-622

Carisolv Gelがう蝕象牙質コラーゲンに与える影響
礪波健一、高木 亨、荒木孝二 他
日本歯科保存学雑誌 42(5) 1999 P.1040-1044

＊ レーザーを用いたウ蝕病巣の削除法の基礎的研究

岩久正明、岡本　明

・これまでの研究論文（投稿中論文）

・現在の研究

ウ蝕の感染組織の削除法については従来の回転切削法での振動や騒音による患者の不快感が大きく、その解決のために新しいアプローチとしてレーザー装置に着目した。患者にとって快適な治療法の確立を目指すため、まずはじめにレーザーを用いた際の硬組織及び軟組織への影響を検討し、続いて、ウ蝕病巣の削除法の基礎的研究や、殺菌効果、疼痛や不快感の発生などについて検討を行っている。

Immunohistochemical study on pulpal response in rat molars after cavity preparation by Er:YAG laser

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Er:YAGレーザーによる窩洞形成後の歯髄反応に関する免疫組織化学的研究

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Introduction

Dentinal cavities for caries treatment have traditionally relied on mechanical hard tissue surgery with low-speed and high-speed handpieces. These devices are used to for caries removing and cavity preparation. However, these mechanical devices are associated with an irritating noise level and, frequently, with uncomfortable vibrations transmitted throughout patients' jaws and craniofacial tissues. Therefore, development of alternative methods is desired for more comfortable treatment in dental clinic instead of the rotary cutting device.

Recently, lasers have been tried in dental fields. It was first used in dentistry in 1964 by Goldman et al. They used a ruby laser on caries in teeth and demonstrated the possibility of laser therapy in dental practice. And now, dental lasers have been investigated for use in caries removal (Myers and Myers, 1985), desensitization (Gelskey et al., 1993), resin bonding (Cooper et al., 1988), as an adjunct in root canal therapy (Dederich et al. 1984), and in prevention/control of dental caries (Yamamoto et al., 1980). Clinical trials of the Er:YAG laser for caries treatment have gradually increased, and promising results have been reported (Keller et al., 1995; Kumazaki., 1995; Gimble et al., 1995; Ishikawa et al., 1996; Keller et al., 1998). The Er:YAG laser systems thus have been a prime potential alternative.

The nature of laser's light is determined by its wavelength. Close attention has been paid to the clinical applicability of the Er:YAG laser whose light qualities are different form other laser beams. The Er:YAG laser is near infrared laser with a wavelength of 2.94 μm. This wavelength is identical to peak wavelength of the light most readily absorbed by water and tooth. When used on living tissue with a sufficient water content, this laser causes a rapid evaporation of the water in the most superficial layer of tissue, without greatly affecting the tissue in the neighboring area. When compared with conventional dental devices, this apparatus has the following advantages: (1) there is no metallic sound or sounds of rotation, and (2) there is no vibration during its use.

Many pathological and histological researches have been reported regarding an influence of the Er:YAG laser to the dental pulp. Wigdor et al. (1993) prepared cavities 1.0 mm in depth into the dentin of dogs' teeth with the Er:YAG, Nd:YAG, and CO2 lasers. Their results showed that the Er:YAG laser produced the least damage to the pulp tissues as compared with the other lasers and a high speed handpiece after 4 days, and that the Er:YAG laser-treated pulps were similar in appearance to the untreated control teeth. Another study demonstrated that the pulp response to Er:YAG laser application would appear to be similar to the response from high-speed handpiece application in dogs (Sonntag et al., 1996). However, these approaches have not sufficiently addressed the effects of lasing on pulpul function.

Alkaline phosphatase (ALPase) activity has been suggested as a suitable marker for the differentiative function of dental pulp dentinogenesis (Yoshiki et al., 1971). It has been reported that the ALPase activity was stimulated in affected subodontoblastic layer after cavity preparation with a bur (Aiba., 1983).

DNA fragmentation and programed cell death are known to be associated with biological process, which take place in a variety of cells involved in tooth formation. Apoptotic cells in the dental pulp have been reported in human and rodent teeth (Bronckers et al., 1996; Vermelin et al,. 1996; Franquin et al., 1998; Nishikawa and Sasaki, 1999). This phenomenon has shown to occur in pulp tissue after cavity preparation with a bur in the rat molar (Bronckers et al., 1996).

Various types of immunocompetent cells are known to be present in normal dental pulp (Jontell et al., 1987, 1988; Jontell and Bergenholtz, 1992; Okiji et al., 1992; Ohshima et al., 1994; Yoshiba et al., 1996). Class II MHC antigen expressing cells that can absorb and process complex antigens and present them to lymphocytes, which is a function essential to the initiation of immune responses (Steinman., 1991). Changes in the distribution and the responses of class II-expressing cells to experimentally induced pulpitis (Bergenholtz et al., 1991), cavity preparation (Ohshima et al., 1995), and caries (Yoshiba et al., 1996, 1998) have been reported. Recently, intimate associations of MHC class II-expressing cells and the nerve fibers

have been demonstrated in human carious teeth, suggesting their functional interactions in the dental pulp (Yoshiba et al., 1998).

In the present study, to investigate the effect of Er:YAG laser on pulp tissue used for cavity preparation, we analyzed the immunolocalization for ALPase using an antibody to tissue non-specific alkaline phosphatase (TNAP), and detected DNA fragments using the transferase-mediated, biotin-dUTP nick end-labeling (TUNEL) procedure (Gavrieli et al., 1992). Furthermore, double immunofluorescence staining was performed to analyze distributional relationship between MHC class II antigen-expressing cells and nerve fibers using OX6-polyclonal antibody and PGP 9.5-monoclonal antibody, respectively. And we compared the findings to those of cavity preparation by a traditional bur.

Materials and methods

Operative procedures

Sixty upper first molars in thirty male Wistar rats, 8 weeks old, were used in this study. They were divided into two groups; one was for a bur and the other was for an Er:YAG laser procedure. The animals were anesthetized with sodium pentobarbital (30mg/kg, i.p.). A cervical cavity was prepared on the mesial surface of the tooth by use of a micromotor with a tungsten carbide bur (diameter 0.8 mm) or a ML22 pulsed Er:YAG laser (Erwin; HOYA Corp., Japan, and J. MORITA Mfg Corp., Japan) under water cooling. Laser output energy setting was 70mJ/pulse. Cavity depth was about 0.4mm and each cavity was left open without any further treatment. Some untreated teeth were used as a control.

Tissue preparation

At 5 min, 6 h, 12 h, 1, 3, and 7 days after cavity preparation, the animals were anesthetized as described above and perfused with Ringer solution followed by 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer (pH7.4). The maxillae were removed and immersed in the same fixative for additional 6 h. Following decalcification in 5% EDTA (pH7.4) for 2 weeks at 4 °C, the specimens were dehydrated with an increasing concentration of ethanol and embedded in paraffin, and sectioned at a thickness of 5 μm for TNAP immunohistochemistry and TUNEL methods. For double immunofluorescence staining of OX6 and PGP 9.5, some specimens were immersed in phosphatase-buffer saline (PBS) which contained sucrose in gradually strengthening contractions of 10, 20, and 25%. They were then rapidly frozen in liquid nitrogen and sliced into 25 μm sections with a Cryotome (AS620M, SHANDON, England).

Immunolocalization of TNAP

Immediately after deparaffination, the sections were rehydrated in PBS and treated with in 0.03% H_2O_2 in PBS for 30 min at room temperature to inhibit endogenous peroxidase. The sections were then pre-incubated for 1h with PBS containing 1% bovine serum albumin (BSA-PBS), and thereafter incubated with antiserum against rat TNAP diluted 1:100 in BSA-PBS for 24h at 4 °C. After being rinsed with PBS, the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulins (Dakopatts, Glustrup, Denmark) diluted 1:100 in BSA-PBS for 1h at room temperature. Following a PBS rinse, they were immersed in a mixture of diaminobenzidine (0.05%) and H_2O_2 (0.008%) in 0.05M TRIS-HCl buffer (ph7.6) for 4 min at room temperature for visualization of immunoreactivity. These sections were counter stained by hematoxylin, and observed under a light microscopy. As a

negative control, non-immune rabbit serum at the same dilution was used instead of anti-TNAP antiserum.

TUNEL staining

Nuclear DNA fragmentation was detected on paraffin embedded sections, using an Apop Tag® detection kit (Oncor, Gaithersburg, MD, USA), according to a digoxigenin-based modification of the original TUNEL method introduced by Gavrieli et al. (1992). These sections were counter stained by hematoxylin, and observed under a light microscopy.

Double immunofluorescence for OX6 and PGP 9.5

The double immunofluorescence staining was performed as described by Yoshiba et al. (1998). Free-floating sections were pre-incubated for 60 min with BSA-PBS, and thereafter incubated with OX6-monoclonal antibody recognizing Ia antigens (Serotec, Oxford, England) diluted 1:5000 in PBS for 24h at 4 °C. After rinsing with PBS for 12h, they were incubated with Cy3-conjugated goat anti mouse IgG (Jackson Immunoresearch Laboratories, Inc.,West Grove, PA) diluted 1:400 in PBS for 12h at 4 °C, and then rinsed with PBS for 12h. The sections were then incubated with a rabbit anti human PGP 9.5 polyclonal antibody (Ultraclone Co. Ltd, Cambridge, U.K) diluted 1:2800 in PBS for 24h at 4 °C, rinsed with PBS for 12h, and finally incubated with fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit immunoglobulins (DAKO) diluted 1:40 in PBS for 12h at 4 °C. The sections were examined under a fluorescence microscope. Control sections were stained using normal mouse serum, normal rabbit serum, or PBS as the primary antibody.

Results

Immunolocalization of TNAP

In a control (untreated) group, no or sparse immunoreactivity for TNAP was observed in the subodontoblastic layer (data not shown).

Both of the laser and bur groups showed the similar histological findings in most of the experimental teeth. However, the distribution pattern for TNAP was different between the two experimental groups.

After 5 min postoperatively, the odontoblasts under cavity preparation suffered from severe damages. They were separated from predentin, and lost their elongated profiles. The immunolocalization of TNAP was observed just beneath the odontoblast layer under the cavity lesion in both the laser and bur groups. However, in the laser group, a strong immunoreactivity was observed in a wider range, as compared with the bur group (Fig. 1).

At 6 h after cavity preparation, immunoreactivity for TNAP in the subodontoblastic layer appeared to extend in both groups, where it was more widely and intensely seen in the laser group (Fig. 2).

The TNAP immunoreactivity extended in the pulp tissue in both the laser and bur groups with progress of time, and they showed similar findings at 7 days postoperatively, when reparative dentin formation had started. Intense TNAP was observed entirely in the pulp tissue under a cavity (Fig. 3).

Non-immune rabbit serum did not show any immunoreactivity when used instead of the antibody to TNAP.

TUNEL staining

In a control group, any TUNEL-positive cells were not found in pulp tissues (data not shown).

At 5 min and 6 h after cavity preparation, TUNEL-positive cells were not recognized under the cavity lesion in both experimental groups (data not shown).

From 12 h to 1 day postoperatively, some TUNEL-positive cells were observed under the odontoblast layer in the bur group. On the other hand, a few positive-cells were seen mainly within the odontoblastic layer under cavity lesion in the laser group (Fig. 4).

From 3 to 7 days after procedure, in the laser group, there were no TUNEL-positive cells in and around the odontoblast layer at the cavity lesion. In the bur group, however, a few TUNEL-positive cells were still observed under the odontoblast layer (Fig. 5).

Double immunofluorescence for OX6 and PGP 9.5

In a control group, immunostaining with an OX6 antibody revealed distinct reactions in many cells located predominantly beneath the odontoblastic layer in the dental pulp. And a small number of PGP 9.5-immunopositive nerve fibers were observed inside the pulp.

Throughout the examined stages, no difference was observed between the laser and bur groups for the immunostainings of OX6 and PGP 9.5 antibodies. At 5 min after cavity preparation, OX6-positive dendritic cells and PGP 9.5-immunoreactive nerve fibers with a dotted appearance were observed just beneath the cavity lesion. PGP 9.5-immunoreaction was also recognized on the odontoblasts (Fig. 6).

From 12 h to 1 day after preparation, as compared with the previous stage, an increasing number of OX6-immunopositive cells accumulated along the pulp-dentin border and extended their process deep into the dentinal tubules (Fig. 7). PGP 9.5-immunopositive nerve fibers were not clearly observed around the odontblastic layer.

After 3 days postoperatively, many OX6-positive dendritic cells were appeared inside the pulp around the cavity lesion (Fig. 8). However, no cytoplasmic processes

of the immunopositive cells were found in the dentinal tubules. PGP 9.5-immunopositive nerve fibers began to gather under the cavity lesion.

At 7 days after procedure, the localization of OX6-immunopositive cells was similar to that recognized at 3 days after cavity preparation (Fig. 9). PGP 9.5-immunopositive nerve fibers beaded in appearance gathered around the cavity lesion, and some were extended into odontoblastic layer (Fig. 10).

In a negative control section, no specific labeling was observed (data not shown).

Discussion

This study demonstrated that both the Er:YAG laser and bur groups exhibited the similar features at the histological level, although the differences in distribution patterns of the TNAP immunoreactivity and TUNEL-positive cells are evident between the two experimental groups.

In the pulp tissue, ALPase activity in the subodontoblastic layer is highest and closely related to dentin formation, and it has been suggested as a suitable marker of the differentiative function during dentinogenesis (Yoshiki and Kurahashi, 1971). ALPase has been reported to be stimulated in affected subodontoblastic layer after cavity preparation by an enzyme histochemical technique (Aiba, 1983). In the present study, ALPase detection was performed with an antibody to TNAP. TNAP has shown to be detected at high levels in hard tissues including bone, cartilage, and tooth (Hoshi et al., 1997). In the mouse developing incisor, localization of TNAP was detected in cells of the subodontoblastic layer and the basolateral portion of odontoblasts. The present study demonstrated that the TNAP immunoreactivity in the Er:YAG laser irradiated group was significantly higher at the early stage after cavity preparation, as compared with the bur group.

In the human dental pulp cells in vitro, the laser irradiation has shown to enhance the ALPase activity and the formation of calcified nodules (Ohbayashi et al., 1999). This result would support our observations that the laser induced the increasement of TNAP immunoreactivity, especially in the subodontoblastic layer. However, the mechanisms by which the ALPase activity in the pulp cells was enhanced by the laser irradiation are unknown.

It is known that the pressure waves are generated when the pulsed hard laser is irradiated to the tissues. Irradiation of pulsed Nd:YAG laser to the thigh bone of the rat under the non-overload conditions induced a large amount of bone formation on the contralateral surface of the bone, which may be due to the pressure waves (Ninomiya, 1998, personal communication). The pressure waves are thought to be transmitted to the contralateral surface of the bone, and activated the osteoblasts.

Concerning the changes in temperature by the laser irradiation, the lesser increase in intrapulpal temperature during the Er:YAG laser preparation in comparison to the conventional bur has been reported and the Er:YAG laser used with a water cooling dose not produce significant pulpal temperature changes (Burkes et al. 1992; Glockner et al., 1998). Therefore, intense and wide range immunoreactivity for TNAP observed in the Er:YAG laser group may be due to the pressure waves occurring in the pulp chamber rather than increase in intrapulpal temperature.

TUNEL-positive cells in the pulp tissue have been reported in human and rodent teeth (Bronckers et al., 1996; Vermelin et al., 1996; Franquin et al., 1998; Nishikawa and Sasaki, 1999). DNA fragmentation has demonstrated to be elicited in odontoblasts and underlying pulpal tissue of mature erupted rat molars after cavity preparation using a bur (Bronckers et al., 1996). In the present study, at 12 h postoperatively, TUNEL-positive cells were recognized in the pulp tissue corresponding to a cavity prepared either by a bur or an Er:YAG laser. This result demonstrated that DNA fragmentation was also induced during cavity preparation by using Er:YAG laser and suggested that the Er:YAG laser injured pulpal cells including

odontoblasts under the conditions of this experiment. During cell or tissue necrosis, DNA degradation is a secondary event initiated by acute mechanical, physical or chemical lesion. It has been suggested that the TUNEL procedure makes no distinction between DNA fragmentation either generated during program cell death or by necrosis. DNA fragments generated in both ways will be stained by TUNEL staining method (Kressel and Groscurth 1994).

In the present study, the number of TUNEL-positive cells was a very few contrary to our expectation. The apoptotic cells are phagocytized within a few hours (Wyllie et al., 1980; Bursch et al., 1990) by neighboring cells (Savill et al., 1993) or macrophages (Kim., 1995). This rapid phagocytosis of apoptotic cells could explain the low number of TUNEL-labeled cells observed in our study. Since 3 days after cavity preparation by the Er:YAG laser, TUNEL-positive cells were not observed in the pulp in contrast to the bur group, where they remained after 7 days postoperatively. The reason for this difference is unknown, but the laser irradiation might enhance phagocytotic activity in the pulp tissue. Apoptotic fragments have shown to be eliminated mainly by MHC Class II-expressing cells, including OX6-positive dendritic cells, and by MHC Class II-negative macrophages in the rat incisor (Nishikawa and Sasaki, 1999).

Class II MHC antigen-expressing cells, especially, dendritic cells and macrophages, play an important role in the immune system as antigen presenting cells (Steinman et al., 1986). The pulp tissue contains many class II MHC antigen-expressing cells under physiological conditions, locating predominantly around the odontoblasts (Okiji et al., 1992; Ohshima et al., 1994; Yoshiba et al., 1996). The localization of class II MHC antigen-expressing cells has been shown to change after cavity preparation using a bur (Ohshima et al., 1995). The OX6-immunopositive cells accumulated and the cytoplasmic process extended into the dentinal tubules at 12h to 1d after cavity preparation. The present findings also demonstrated the same phenomenon after cavity preparation by an Er:YAG laser as well as by a bur. In human teeth, the similar localization of class II MHC antigen expressing cells was