©Operative Dentistry, 2019, **44-4,** 336-347

Clinical Research

Clinical and Histological Study on Direct Pulp Capping With CO_2 Laser Irradiation in Human Teeth

M Suzuki • C Kato • S Kawashima • K Shinkai

Clinical Relevance

CO_2 laser irradiation was effective in controlling bleeding and exudate from mechanically exposed pulp. Dentin bridge formation in teeth capped with CO_2 laser was equivalent to that in teeth capped with calcium hydroxide cement at 12 months posttreatment.

SUMMARY

The study aimed to histologically evaluate wound healing of exposed human pulp on direct pulp capping using super-pulsed CO_2 laser preirradiation. In this single-blind clinical trial, 28 third molar teeth of 17 volunteers were randomly capped with either CO_2 laser irradiation (n=14) or Dycal (calcium hydroxide cement; n=14) and restored using resin composite. The laser was operated in super-pulsed mode (pulse duration, 0.2 ms; interval, 5.8 ms; 0.003 J/pulse). The irradiation conditions were a power output of 0.5 W, an irradiation time of 15 seconds, repeat mode (10-ms irradiation and 10-ms intervals, for a total beam exposure time of 7.5 seconds), total applied energy of 3.75 J, and an activated air-cooling system. Each tooth was extracted at six or 12 months posttreatment and prepared for histological evaluation. We evaluated the parameters of pulp tissue disorganization, inflammatory cell infiltration, reparative dentin formation (RDF), and bacterial penetration. There were no significant differences between groups for all parameters at each postoperative period (Mann-Whitney U-test, $p>0.05$). CO_2 laser irradiation completely controlled bleeding and exudate from the exposed pulp. The CO_2 laser group had a tendency to delay RDF compared with the Dycal group, but 4 of 7 teeth from the CO_2 laser group showed a complete dentin bridge at 12 months posttreatment.

*Masaya Suzuki, DDS, PhD, Department of Operative Dentistry, The Nippon Dental University School of Life Dentistry at Niigata, Niigata, Japan

Chikage Kato, DDS, PhD, Comprehensive Dental Care Unit, The Nippon Dental University Niigata Hospital, Niigata, Japan

Satoki Kawashima, DDS, PhD, Department of Operative Dentistry, The Nippon Dental University School of Life Dentistry at Niigata, Niigata, Japan

Koichi Shinkai, DDS, PhD, Department of Operative Dentistry, The Nippon Dental University School of Life Dentistry at Niigata, Niigata, Japan

*Corresponding author: 1-8 Hamaura-cho, Chuo-ku, Niigata, 951-8580, Japan; e-mail: collagen@ngt.ndu.ac.jp

DOI: http://doi.org/10.2341/18-030-C

INTRODUCTION

Preventing pulp devitalization helps extend the longevity of the tooth, especially with an immature tooth. Therefore, dentists try to avoid removing dental pulp. Direct pulp capping (DPC) is an effective method for treating accidentally exposed pulp that may occur during cavity preparation,

traumatic injury, or removal of deep carious lesions in clinical practice. Numerous materials for DPC, including calcium hydroxide (CH) and mineral trioxide aggregate (MTA), have been developed, and their effectiveness and biocompatibility have been reported.[1-3]

On the other hand, managing exposed pulp surfaces is a common issue and one of the most important factors in the clinical success of DPC or pulpotomy notwithstanding the type of pulp-capping materials used.[2,3] Accordingly, some studies have focused on the method of hemorrhage control and disinfection at the site of exposed pulp before a capping material is dressed.[4-7] Sodium hypochlorite (NaClO) at different concentrations is an effective and well-known surface-acting solvent for vital pulp tissue.[4,8-11] However, dentists sometimes encounter rebleeding and exudate from exposed pulp tissue while placing capping material or applying restorative materials, even after achieving hemostasis with NaClO. Hafez and others[10] reported that application of 3% NaClO was effective for hemorrhage control, but renewed bleeding sometimes occurred at the margin between the exposed pulp and dentin after etching or priming. They suggested that the renewed bleeding may be responsible for the initial inflammation. Kitasako and others[12] histologically observed pulp tissue protrusion into the cavity at the periphery of the exposed area via exudate from pulp tissue after chemical lavage despite a well-controlled procedure, small mechanical exposure, healthy pulp, and local anesthesia. If bleeding reoccurs at the exposed area of the pulp after applying the DPC material, it is clinically complicated to remove the capping material and then stem the bleeding again.

A number of studies in dentistry have been conducted using a laser in pulp-capping experiments.[13-17] Wavelength is a highly influential factor for obtaining adequate results from laser treatment because of the different characteristics of laser beams (ie, a laser beam is selected according to its intended purpose). A CO_2 laser emits infrared light with a wavelength of 10.6 μm, which is efficiently absorbed by water in vital tissue. CO_2 laser energy causes the water to vaporize and certain morphological changes to occur, including carbonization, hemocoagulation, and protein denaturation in the target tissue.[18] CO_2 laser is often used as a substitute for a steel scalpel when cutting soft tissue, as it achieves excellent hemostasis as a result of its thermal action.[19] Several studies showed increased success of DPC and pulpotomy assisted by CO_2 laser irradiation compared with the conventional method.[20-23] Our laboratory examined the rat pulp response to irradiation with a super-pulsed CO_2 laser to achieve hemostasis and disinfection of the exposed pulp prior to applying a capping material.[24,25] CO_2 laser irradiation was effective for field control and controlling hemorrhage from the exposed pulp without severe inflammation. But the specimen from the CO_2 laser group demonstrated an irregular fibrous dentin matrix near the denatured and carbonized tissue and a tendency to delay dentin bridge formation compared with applying CH cement or an adhesive resin system without laser irradiation.

The present study aimed to histologically evaluate wound healing of exposed human pulp after DPC using a self-etch adhesive system and CO_2 laser preirradiation compared with a commercially available CH cement (Dycal, Dentsply Caulk, Milford, DE, USA). The null hypothesis of this study was that CO_2 laser irradiation would not affect pulpal healing and reparative dentin formation (RDF) in exposed human pulp.

METHODS AND MATERIALS

This single-blind clinical trial was evaluated and approved by the Ethics Committee of the Nippon Dental University School of Life Dentistry at Niigata (receipt and permission number: ECNG-H-102). Prior to enrollment in the trial, all volunteers were informed of the benefits and risks and clearly instructed on reporting their pain history as well as the influence of the treatment that they would receive. Thereafter, informed consent forms were signed by all volunteers. One operator was responsible for all of the procedures in the dental clinic of the Nippon Dental University Niigata Hospital. This study was conducted in full accordance with the World Medical Association Declaration of Helsinki.

One or two teeth per volunteer were included in the study. Teeth were randomly assigned to the CO_2 laser group or the Dycal group. A simple randomization method was used for selection of volunteers who provided two teeth. Namely, the first tooth was assigned to be treated with CO_2 laser irradiation and the second tooth was to be treated with Dycal. A total of 28 mature permanent third molar teeth scheduled to be extracted from 17 volunteers were randomly capped with either CO_2 laser irradiation (n=14) or Dycal (n=14) and restored using resin composite (Figure 1). None of the tested teeth had any caries or history of pulpitis. The volunteers included 15 women and two men ranging in age from 18 to 33 years. Mean age ± standard deviation at the time of treatment was 21.8 ± 3.45 years.

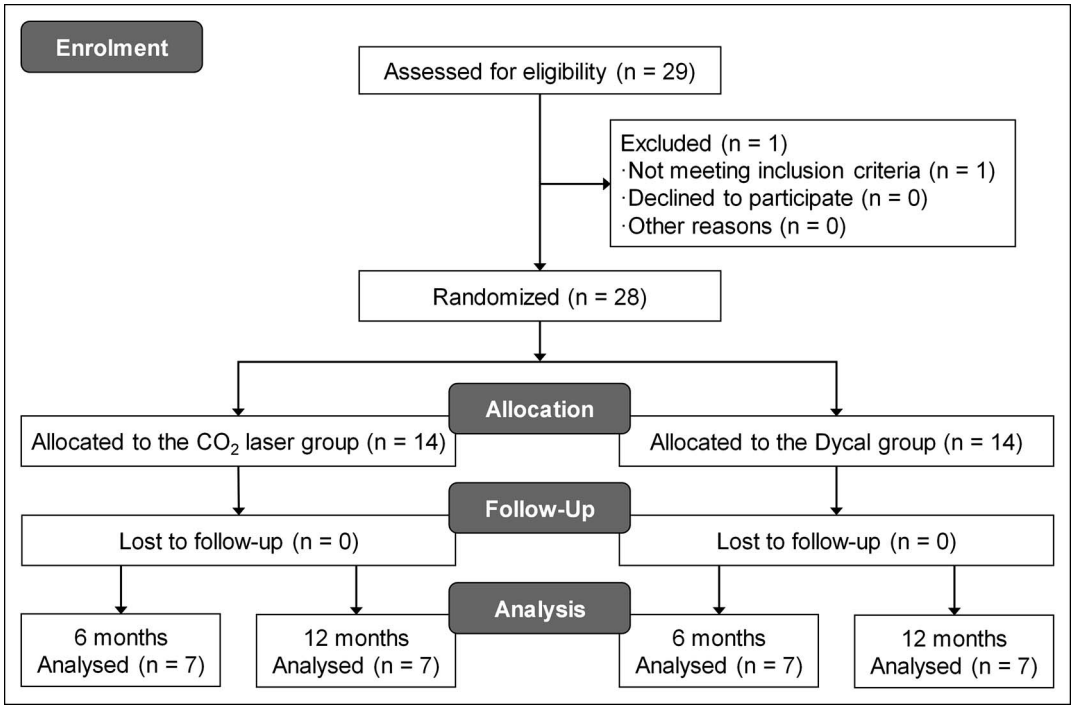

Figure 1. *Diagram showing the flow of volunteers.*

CO_2 Laser

An Opelaser PRO (Lot No. PG-049, Yoshida Dental Mfg Co, Tokyo, Japan) was used for CO_2 laser irradiation. The specifications of this laser were wavelength of 10.6 μm, power output of 0.5 to 5 W (changeable at 0.1-W increments), focus beam diameter of 0.15 mm (at a distance of 10 mm from the tip of the handpiece), and green guide beam with a wavelength of 532 nm. The laser was operated in super-pulsed mode (pulse duration: 0.2 ms; interval: 5.8 ms; 0.003 J/pulse). Irradiation conditions included a power output of 0.5 W, an irradiation time of 15 seconds, repeat mode (10-ms irradiation and 10-ms intervals, for a total beam exposure time of 7.5 seconds), a beam diameter of 0.15 to 1.09 mm (at approximate distances of 10-20 mm from the exposed pulp surface), energy density of 0.32 to 16.99 J/cm^2 per pulse, total applied energy of 3.75 J, and an activated air-cooling system. The laser handpiece was kept moving in a small circular motion during laser irradiation to avoid heat concentration in one area.

Clinical Procedure

All teeth were examined by preoperative clinical tests using a pulp vitality tester (Vitality scanner model 2006, Lot No. 26-10027, Kerr Co, Danbury, CT, USA) and radiographs to confirm the normal condition of pulp and periodontal and periapical tissues. The teeth were polished with a cleaning brush and isolated with a rubber dam or cotton rolls after local anesthesia with 2% lidocaine containing epinephrine bitartrate (ORA Injection Dental Cartridge, Lot No. 24730, Showa Yakuhin Kako Co, Tokyo, Japan). The operating field was cleaned with 0.025% benzalkonium chloride, rinsed with water spray, and disinfected with diluted iodine tincture. Class 1 cavities were prepared on the occlusal surface using an FG No. 440 regular cut diamond point (ISO 015, Shofu Inc, Kyoto, Japan) in a high-speed handpiece under copious amounts of water spray. The pulp was then exposed with a CA No. 3 steel round bur (ISO 012, Shofu Inc) in a low-speed handpiece under distilled water. A new bur was used for each tooth. Alternate irrigation with 6% NaClO (Purelox, Lot No. 4053, Oyalox Co, Tokyo, Japan) and 3% hydrogen peroxide (H_2O_2, Oxydol, Lot No. FUA0033, Daiichi Sankyo Co, Tokyo, Japan) were performed several times to remove operative debris, including dentin chips, and achieve hemostasis. The cavity was then rinsed with normal saline. The excess water was removed using sterilized, small cotton pellets, and the cavity was gently air dried. In the CO_2 laser group (n=14), the exposed pulp was irradiated with the CO_2 laser under the specified conditions and directly capped with a two-step self-

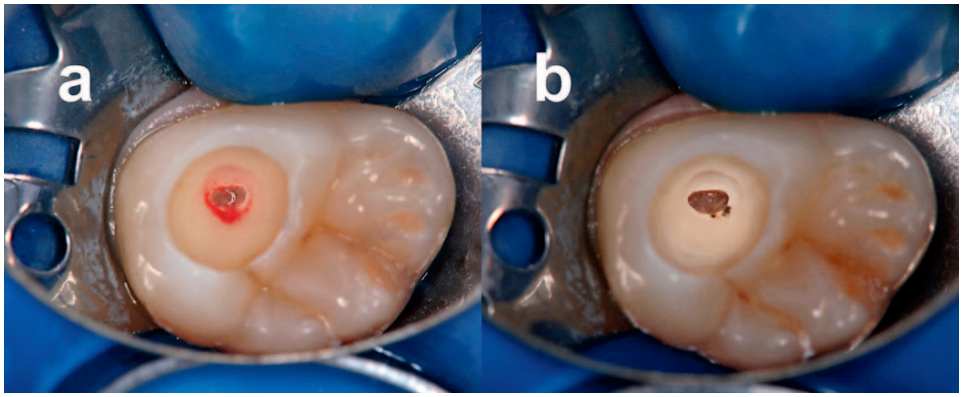

Figure 2. *Clinical photographs of the CO_2 laser group (specimen 6). (a): Bleeding from exposed pulp. (b): Pulp surface irradiated with CO_2 laser.*

etching adhesive system, Clearfil SE Bond (SE, Kuraray Noritake Dental Inc, Tokyo, Japan; Figure 2). In the Dycal group (n=14), the exposed pulp was directly capped with Dycal, and SE was applied to the cavity. SE comprised two components, whereby the primer was applied, left for 20 seconds, and gently air dried, and the bonding agent was applied and then photopolymerized for 10 seconds at 1000 mW/cm^2 with a light-emitting diode light-curing unit (Pencure 2000, Lot No. AF0051, J. Morita Mfg Co, Kyoto, Japan). Light intensity was measured using a curing radiometer (Optilux radiometer model 100, Lot No. 144060, KaVo Dental Systems Japan Co, Tokyo, Japan). Clearfil Majesty LV, a flowable resin composite (Kuraray Noritake Dental Inc), was filled into the cavity floor and photopolymerized for 40 seconds following direct capping and bonding procedures. Clearfil Majesty, a hybrid restorative resin composite (Kuraray Noritake Dental Inc), was added to the cavity using a three- or four-increment filling technique and photopolymerized for 40 seconds for each filling. The composition of the materials used in this study is shown in Table 1. All materials were applied according to the manufacturers' instructions. Excess material and occlusal contact were removed using a superfine diamond point in a high-speed handpiece under copious water spray. Restorations were polished using a silicon point for resin composite one week postoperatively, and all teeth were clinically examined at one week and one-, three-, six-, and 12-month intervals. The volunteers were asked about their experiences regarding symp-

Table 1: *Composition of the Different Materials Used in This Study*

Material	Lot No.	Composition	Manufacturer
Clearfil SE Bond			
Primer	01158A	HEMA, MDP, hydrophilic aliphatic dimethacrylate, *dl*-camphorquinone, accelerators, water, dyes	Kuraray Noritake Dental Inc
Bond	01741A	Bis-GMA, HEMA, MDP, hydrophobic aliphatic methacrylate, colloidal silica, *dl*-camphorquinone, initiators, accelerators, others	
Clearfil Majesty LV	0305BA	TEG-DMA, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, *dl*-camphorquinone, accelerators, pigments, others	Kuraray Noritake Dental Inc
Clearfil Majesty	0017CA	Bis-GMA, TEG-DMA, hydrophobic aromatic dimethacrylate, silanated glass ceramics, surface treated alumina micro filler, silanated silica filler, *dl*-camphorquinone, accelerators, pigments, others	Kuraray Noritake Dental Inc
Dycal	120319	Base paste: 1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, calcium tungstate, iron oxide pigments Catalyst paste: calcium hydroxide, n-ethyl-o/p-toluene sulfonamide, zinc oxide, titanium dioxide, zinc stearate, iron oxide pigments	Dentsply Caulk

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEG-DMA, triethylene glycol dimethacrylate.

Table 2: *Duration of Actual Observation (Days)*

	Six-Month Observation Group							12-Month Observation Group						
Specimen No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CO_2 laser	176	178	182	182	183	185	189	360	360	362	364	366	367	372
Dycal	175	181	182	182	182	186	188	359	362	363	364	364	365	366

toms and signs including postoperative sensitivity and pain, and pulp and periapical responses to the electric pulp test, percussion, palpation, and mobility were examined.

Specimen Preparation

The teeth were extracted after either the six or 12 month observation (Table 2) under local anesthesia, and the apical thirds of the roots were removed for penetration of the fixative solution. They were immediately immersed in 4% paraformaldehyde phosphate buffer solution (pH 7.4; Wako Pure Chemical Industries Co, Tokyo, Japan) at 4°C for one week. The excess tissue was trimmed off the teeth, which were decalcified with 10% EDTA decalcifying solution (pH 7.4, Wako Pure Chemical Industries Co) at room temperature for three months. After the resin composites in the decalcified teeth were carefully removed, the teeth were rinsed with running water for six hours and then dehydrated in ascending grades of ethanol, dealcoholized in xylene, and embedded in paraffin. Serial sections of 5-μm thickness were cut using a sliding microtome (Jung HistoSlide 2000R, Leica Microsystems, Wetzlar, Germany) and stained with Mayer hematoxylin and eosin (H&E) and gram bacterial staining. The stained sections were observed under a light microscope (Eclipse E1000, Nikon Co, Tokyo, Japan), and pulp tissue disorganization (PTD), inflammatory cell infiltration (ICI), RDF, and bacterial penetration (BP) were evaluated according to the following criteria,[26] which are modified versions of the ISO 10993 and 7405 standards, to allow detailed evaluation of the changes in pulp tissue following DPC.

PTD

Score 1: Normal or almost normal tissue morphology (none)

Score 2: Odontoblast layer disorganization but the deep part of the pulp appears normal (mild)

Score 3: Loss of general tissue morphology (moderate)

Score 4: Necrosis in the coronal one-third or more of the pulp (severe)

ICI

Score 1: Absence or presence of a few scattered inflammatory cells in the pulp (none)

Score 2: Mild acute/chronic cell lesions (mild)

Score 3: Moderate inflammatory cell lesions seen as abscesses or densely stained infiltrates of polymorphonuclear leukocytes, histiocytes, and lymphocytes in one-third or more of the coronal pulp and/or the mid-pulp (moderate)

Score 4: Pulp necrosis due to severe degree of infection or lack of tissue in one-half or more of the pulp (severe)

RDF

Score 1: No dentin bridge formation (none)

Score 2: Initial dentin bridge formation extending to not more than one-half of the exposure site (initial)

Score 3: Partial/incomplete dentin bridge formation extending to more than one-half of the exposure site but not completely closing the exposure site (partial)

Score 4: Complete dentin bridge formation (complete)

BP

Score 1: Absence of stained bacterial profiles in any part of the section (none)

Score 2: Presence of stained bacterial profiles along the mesial or distal walls of the cavity (mild)

Score 3: Presence of stained bacterial profiles within cut dentinal tubules or pulpal walls of the cavity (moderate)

Score 4: Presence of stained bacterial profiles within the dental pulp (severe)

In addition, the following histologic features were recorded: hemorrhaging, dentin chips (location, size, and number), and irritation (reactionary) dentin formation.

Measurement of the Diameter of Exposed Pulp Area

The diameter of the exposed pulp area was measured using a stereomicroscope (Measuring Microscope

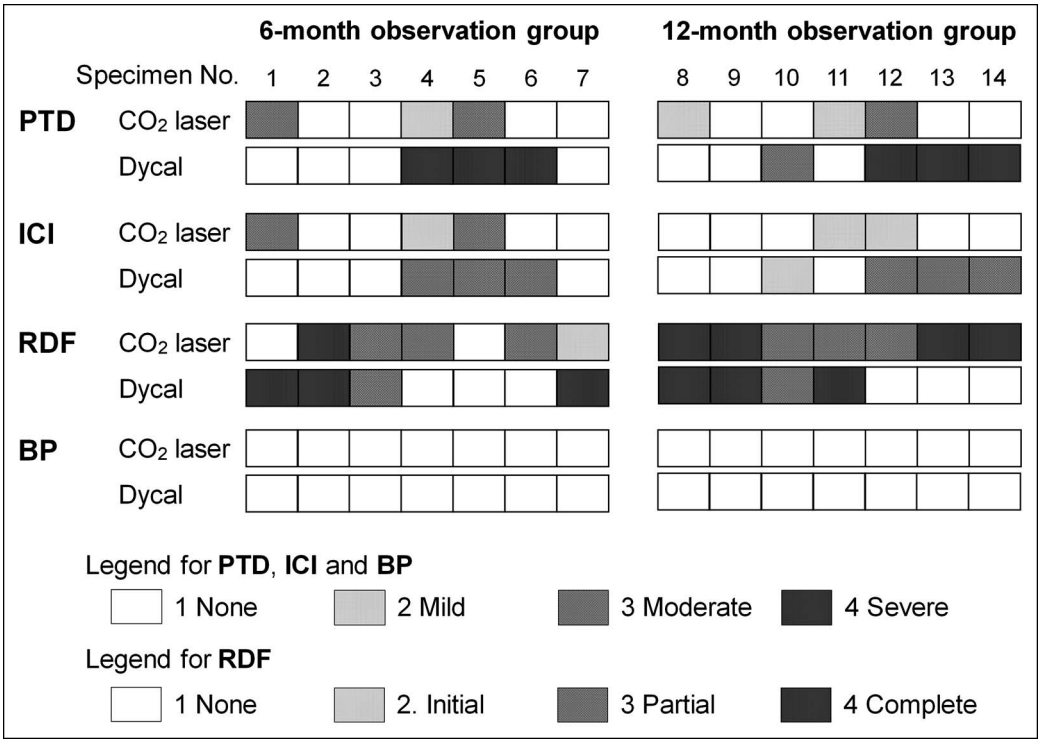

Figure 3. *Overview of the results of histopathological evaluation. PTD, pulp tissue disorganization; ICI, inflammatory cell infiltration; RDR, reparative dentin formation; BP, bacterial penetration.*

MM-40, Nikon Co), and the widest dimension was recorded as the size of the pulp exposure in the H&E-stained section of the specimen.

Statistical Analysis

The results of the histopathological evaluation were statistically analyzed using the Mann-Whitney U test for between-group differences during each observation period. The exposure size data were analyzed using two-way analysis of variance, testing for capping methods (two levels) and observation periods (two levels). Statistical procedures were performed at a significance level of 0.05 using SPSS statistical software (version 14.0J, Base System SC, IBM SPSS Japan, Tokyo, Japan).

RESULTS

Clinical Assessment

From the results of the examination and medical history obtained, slight and temporary pain induced by cold water was present in specimen 1 treated with the CO_2 laser at five months posttreatment, specimen 1 treated with Dycal at three months posttreatment, specimen 4 treated with Dycal at five months posttreatment, and specimen 11 treated with Dycal at two months posttreatment; this condition disappeared spontaneously within a few weeks. The other teeth showed no symptoms, and all the tested teeth had a vital response to the electric pulp test performed immediately prior to tooth extraction.

Diameter of Exposed Pulp Area

The diameter (mm, mean ± SD) of exposed pulp area in each group and observation period was 1.18 ± 0.25 for the CO_2 laser group at six months, 1.28 ± 0.34 for the CO_2 laser group at 12 months, 1.36 ± 0.14 for the Dycal group at six months, and 1.12 ± 0.14 for the Dycal group at 12 months. There were no significant differences between the groups in pulp exposure size ($p>0.05$).

Histopathological Evaluation

The results of the histopathological evaluation are summarized in Figure 3. Representative histopathological images of each group are shown in Figures 4 to 9. The results of the Mann-Whitney U test for histopathological evaluation showed no significant differences between the CO_2 laser group and the Dycal group for all parameters at each observation period ($p>0.05$).

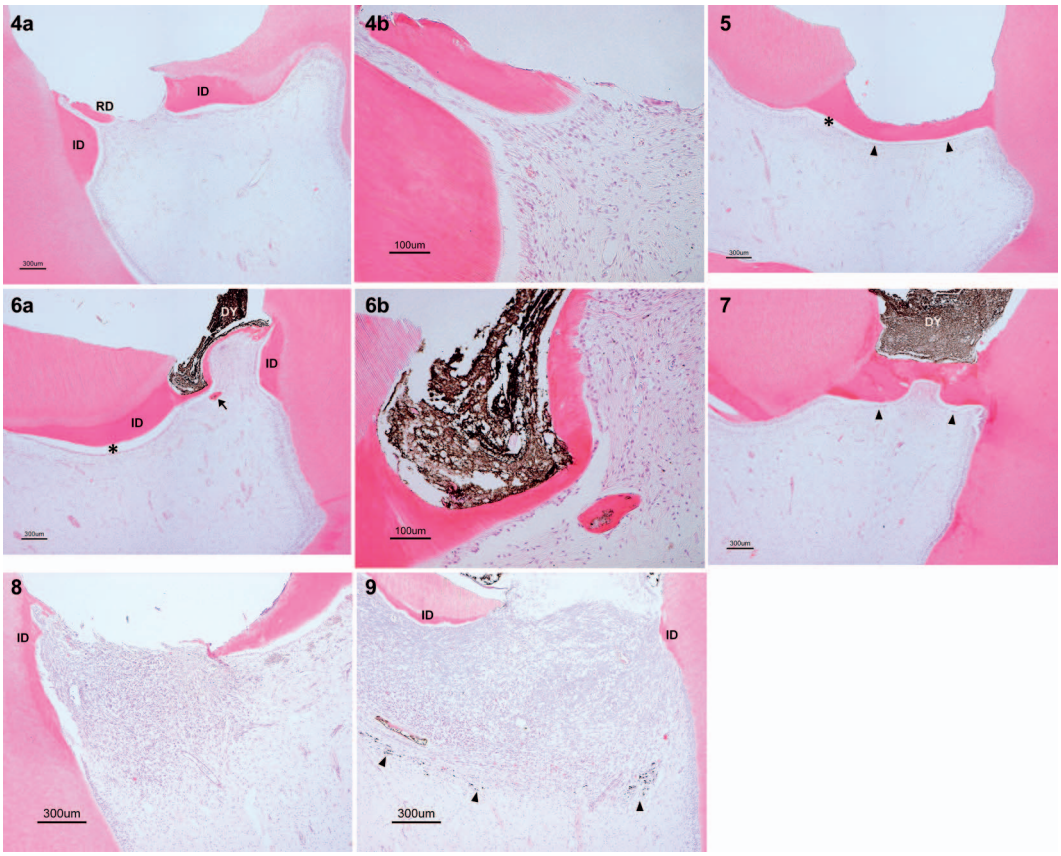

Figure 4. *Representative histologic image of the CO_2 laser group (six months, specimen 7). Irritation (reactionary) dentin (ID) formation is observed at the pulpal dentin walls connecting the cavity floor with the dentinal tubules. Newly formed and initial reparative dentin (RD) can be seen at the exposed pulp surface. Pulpal morphology is normal. (H&E staining, original magnification: a = 40× and b =100×)*

Figure 5. *Representative histologic image of the CO_2 laser group (12 months, specimen 9). A complete dentin bridge has been formed in this specimen. Newly reorganized odontoblast-like cells (arrowheads) are seen beneath the dentin bridge and are connected to the primary odontoblasts at both ends of the exposure. The space (*) between those layers and the dentin bridge is a sectioning artifact. Pulpal morphology is normal. (H&E staining, original magnification = 40×)*

Figure 6. *Representative histologic image of the Dycal group (six months, specimen 2). A thin and complete dentin bridge has formed adjacent to the black material (Dycal, DY). The dentin bridge is continuous with irritation dentins (ID) at both ends of the exposure. Denticle-like reparative dentin (arrow) has formed around the $Ca(OH)_2$ agent particles beneath the dentin bridge. The space (*) is a sectioning artifact. Pulpal morphology is normal. (H&E staining, original magnification: a = 40× and b = 100×)*

Figure 7. *Representative histologic image of the Dycal group (12 months, specimen 8). The pulp exposure site was completely closed with a relatively thick dentin bridge adjacent to Dycal (DY). Newly reorganized odontoblast-like cells (arrowheads) can be seen beneath the dentin bridge. This is an excellent example of pulpal healing and repair. (H&E staining, original magnification = 40×)*

Figure 8. *Representative histologic image of the CO_2 laser group (six months, specimen 5). A collection of inflammatory cell infiltrations including mononuclear leukocytes and macrophages near the pulp exposure site. The mid-pulp has a normal morphology. A small amount of irritation (reactionary) dentin (ID) formation is observed at the pulpal dentin walls, but reparative dentin formation and reorganization of the odontoblastic layer have not occurred. (H&E staining, original magnification = 40×)*

Figure 9. *Representative histologic image of the Dycal group (12 months, specimen 13). Severe tissue disorganization (necrosis) extends to the coronal one-third or more of the pulp. A small amount of irritation (reactionary) dentin (ID) formation is observed at the pulpal dentin walls, but no reparative dentin is evident. Some particles of $Ca(OH)_2$ agent (arrowheads) are scattered among the fibroblasts at the mid-pulp. (H&E staining, original magnification = 40×)*

After Six Months—One tooth from the CO_2 laser group and three from the Dycal group showed complete RDF and exhibited non-PTD and non-ICI. In teeth showing dentin bridge formation, the reorganized layers of newly formed odontoblast-like cells were seen just beneath the reparative dentin. Two teeth from the CO_2 laser group and three from the Dycal group showed no RDF but exhibited PTD and ICI. None of the specimens stained positively for bacteria.

After 12 Months—All of the teeth from the CO_2 laser group showed RDF, including three teeth with

partial RDF and four with complete RDF. A small amount of heat denaturation and carbonized dentin remained at the pulp-exposed area in a few teeth. In contrast, the Dycal group showed one tooth with partial RDF, three with complete RDF, and three with no RDF. Severe PTD and moderate ICI were observed in the teeth with no RDF. The reparative dentin formed by the Dycal group in the early stage tended to include the osteodentin type, while that formed by the CO_2 laser group exhibited the tubular type. Irritation (reactionary) dentin was observed at the pulpal dentin wall around the pulp-exposed area in both groups. These dentins were induced by stimulation of cavity preparation that came through the dentinal tubules. The reparative dentin and irritation dentin had varied thicknesses, which did not depend on the capping method. None of the specimens stained positively for bacteria.

DISCUSSION

Bacterial microleakage was identified as an important factor in pulpal inflammation. Several studies have described that the response of the dentin pulp complex does not depend on the dental materials used for pulp capping but on the materials' ability to prevent bacterial microleakage.[12,26-31] In this study, there was no bacterial invasion to the cavity or the pulp chamber, and none of the volunteers, except for four cases, complained of any pain throughout the observation periods. Nevertheless, some teeth from both groups showed an inflammatory response when evaluated using light microscopy. In particular, severe inflammation was observed in six teeth from the Dycal group (three each at six and 12 months posttreatment). These findings indicate that bacterial leakage is not the only cause of failure in DPC. We identified two major reasons for the presence of severe inflammation.

The first reason was trauma to the pulp associated with the deep cavity preparation using a diamond point in a high-speed handpiece and the mechanical pulp exposure using a steel round bar in a low-speed handpiece. Generally, dentin caries in vital teeth indicate certain protective responses. Acids produced by bacteria in carious lesions dissolve the hydroxyapatite of the tooth substance into calcium and phosphate ions. These ions and saliva form carious crystals in dentinal tubules that are formed by precipitating calcium phosphate, such as brushite (dicalcium phosphate dihydrate), whitrokite (beta-tricalcium phosphate), and octacalcium phosphate, which is termed *sclerotic dentin*.[32,33] In addition, carious dentin gradually produces tertiary (reactionary or reparative) dentin along the pulpal wall in response to irritation due to BP. The barrier protection of tertiary dentin is superior because there is no continuity between the affected permeable tubules of the regular primary dentin and those within the tertiary dentin.[34,35] Sclerotic and tertiary dentins also occur with aging, and it is believed that they reduce exogenous stimuli and tooth pain due to the existence of impermeable dentin.[36,37] We selected only intact teeth, and the volunteers were relatively young and were not expected to have the above-mentioned protective barrier. Therefore, fast and furious treatment stimuli, such as deep cavity preparation, might highly affect pulp tissue and cause inflammation.

The second reason was biocompatibility and stimulation of the pulp tissue with the capping materials. CH and similar cement, such as Dycal, have been used as the gold standard material for DPC for many decades because of their antibacterial effect and ability to stimulate repair and induce RDF.[1,26,38-40] In addition, Dycal has the disadvantage of forming a necrotic layer at the pulp exposure site because of its high alkaline pH (11-13), which should play a protective role in deep pulp but reduces the volume of pulp tissue simultaneously, thereby increasing the risk of pulp morbidity.[1,40] The current Dycal formula used in this study has been improved to a lower pH (9-11)[41]; however, some studies have indicated the cytotoxicity of Dycal.[42,43] Moreover, Dycal specimens with inflammation of the pulp tissue in this study showed black particles of CH preparation diffused into the deep part of the pulp caused by the dissolution of Dycal (Figure 9). Recently, many clinicians have used MTA as their first choice of capping material in substitution for CH because of its higher biocompatibility with pulp cells.[42-44] Several *in vivo* studies have reported that MTA forms dentin bridges earlier, more frequently, and of a greater thickness than those observed with CH.[45,46] However, we used Dycal as the control in this study because we wanted to compare the results of this experiment with those of our previous studies, which also used Dycal as the control.

On the other hand, we selected the adhesive system as the capping material for the CO_2 laser group to evaluate the individual efficiency of CO_2 laser irradiation without any agents to facilitate pulpal repair and RDF. There are arguments for and against using adhesive resins as the DPC agent. Some studies on DPC with adhesive resins have

shown favorable results due to good adhesive bonding that eliminates microleakage in DPC treatment.[10,12,29,30,47,48] In contrast, some studies concluded that adhesive resins should not be applied to exposed pulp because they do not allow histopathological repair of pulp tissue with moderate or severe inflammation due to a poor biocompatibility and cytotoxic effect regardless of bacterial invasion.[49-54] Pameijer and Stanley reported that adhesive systems using the "total etch" technique have been shown to have disastrous effects during DPC in primates.[49] Common adhesive components including bisphenol A-glycidyl methacrylate, urethane dimethacrylate, 2-hydroxyethyl methacrylate, triethylene glycol dimethacrylate, and others display toxic effects in pulp cells such as fibroblasts and odontoblasts and might cause an inflammatory response to the dental pulp and hinder pulpal healing.[55-58] Several studies, including previous studies of our laboratory, revealed that teeth capped with adhesive resins showed delayed RDF compared with those capped with Dycal or MTA but almost normal tissue morphology without severe reactions was seen throughout the observation period.[25,26,59-62] Some studies reported that SE was a reliable adhesive agent to both enamel and dentin,[63] which provides sufficient marginal sealing to prevent BP,[64,65] and that the cytotoxicity of SE was relatively lower than that of other adhesive systems.[57,58] Lu and others[59,60] indicated that SE had good biocompatibility to pulp tissue when used for DPC. The results of this study showed that SE did not allow BP. Fewer teeth showed pulp inflammation in the CO_2 laser group compared with the Dycal group, although the difference was not statistically significant. We suppose that an adhesive resin may not be the most suitable material for DPC, but SE may be available for DPC. In addition, the denaturation tissue at the exposed surface coagulated by the CO_2 laser might prevent resin monomer from diffusing the pulp tissue.

The results of statistical analysis for the histopathological evaluation revealed no significant differences between the groups for RDF, but three of seven teeth in the Dycal group showed complete RDF at six months posttreatment, whereas the CO_2 laser group had only one tooth that exhibited complete RDF. These findings indicated a tendency similar to our previous study using rat pulp in that initiation of dentin bridge formation in teeth capped with Dycal appears earlier than in teeth capped with CO_2 laser irradiation.[25] It was suggested that the total laser energy was too strong for the rat pulp to form a dentin bridge, as the volume of rat pulp is very small. As a result, CO_2 laser irradiation caused thermal damage and created relatively thick denaturation tissue around the pulp-exposed area, which might inhibit pulp tissue from forming a dentin bridge. In another study, we reported that delayed pulp tissue healing following CO_2 laser irradiation might be related to the thickness of the heat-denatured layer, which increases in proportion to the intensity of laser irradiation.[66] Thus, the reaction of the pulp is largely affected by the irradiation settings and the device as well as the wavelength.[13-17,20-25,67,68] In this study, the heat-denatured tissue formed by CO_2 laser irradiation was scarcely observed around the exposed pulp area histopathologically. The exposed pulp irradiated with the CO_2 laser was closed by the complete dentin bridge in four cases at 12 months posttreatment despite the absence of any pharmacological agents. The exposed pulp surface showed a chalky white or light brown discoloration after irradiation with the CO_2 laser, which might have been due to coagulation and protein denaturation by the photothermal effect. It is known that CO_2 laser irradiation with high energy creates dark brown or black carbonized tissue. Therefore, it is speculated that the total energy of CO_2 laser irradiation performed in this study might be relatively low in human pulp and might not completely interfere with dentin bridge formation. Lasers also yield a photoactive effect (low reactive-level laser/light therapy [LLLT]) that demonstrates a reversible reaction, which contributes to providing the biostimulation effect followed by an accelerated wound-healing process.[69,70] It is not clear whether the effect of LLLT contributed to the results of this study. Generally, lower energy and longer irradiation time compared with those of this study are needed to obtain the effect of LLLT in the clinic. Therefore, it was suggested that there was little effect of LLLT under the irradiation conditions in this study.

Alternate irrigation with 6% NaClO and 3% H_2O_2 was performed to control bleeding and remove dentin chips, debris, and coagulum in both groups. Hemostasis was achieved with alternate irrigation in all the teeth; however, teeth without laser irradiation gradually accumulated exudate around the exposed site following chemical lavage, while teeth irradiated with the CO_2 laser exhibited neither secondary bleeding nor exudate until the filling of the resin composite into the cavity was completed because of artificial scab formation at the irradiated

area because of the photothermal effect. It should provide smooth and accurate capping and restoration regardless of the type of capping materials used. One of the great advantages of CO_2 laser is its ability to work without making contact with the pulp tissue, which helps avoid bacterial infection. Moritz and others reported that the most important effects of CO_2 laser irradiation are sterilization and scar formation as well as minimal hematoma formation in the exposed pulp area due to the thermal effects of laser irradiation.[22] Laser irradiation should minimize hematoma formation between the pulp tissue and the capping material and allow close contact between them.

Based on the findings of this study, we accepted the null hypothesis that CO_2 laser irradiation would not affect pulpal healing and RDF in the exposed human pulp. However, further studies using various irradiation conditions and combining the use of capping materials and CO_2 laser irradiation are required to determine the most suitable irradiation conditions and procedure to increase the success rate in DPC with CO_2 laser.

CONCLUSIONS

Under the experimental conditions of this study, CO_2 laser irradiation completely controlled exudate as well as bleeding of the mechanically injured human pulp. DPC with the self-etching adhesive system following CO_2 laser irradiation without carbonization of the exposed pulp demonstrated favorable pulpal healing and dentin bridge formation that was comparable to that with Dycal.

Acknowledgements

This study was supported by a Grant-in-Aid for Young Scientists (B) (grants JP18791412 and JP24792040) from the Japan Society for the Promotion of Science (JSPS). The authors would like to thank Enago (www.enago.jp) for the English language review.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Research Ethics Committees Northern Ireland. The approval code for this study is ORECNI, 16/NI/0101.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 30 May 2018)

REFERENCES

1. Stanley HR (1989) Pulp capping: conserving the dental pulp—can it be done? Is it worth it? *Oral Surgery, Oral Medicine, and Oral Pathology* **68(5)** 628-639.
2. Cox CF, Tarim B, Kopel H, Gürel G, & Hafez A (2001) Technique sensitivity: biological factors contributing to clinical success with various restorative materials *Advances in Dental Research* **15(1)** 85-90.
3. Hilton TJ (2009) Keys to clinical success with pulp capping: a review of the literature *Operative Dentistry* **34(5)** 615-625.
4. Silva AF, Tarquinio SB, Demarco FF, Piva E, & Rivero ER (2006) The influence of haemostatic agents on healing of healthy human dental pulp tissue capped with calcium hydroxide *International Endodontic Journal* **39(4)** 309-316.
5. Accorinte Mde L, Loguercio AD, Reis A, & Holland R (2007) Effects of hemostatic agents on the histomorphologic response of human dental pulp capped with calcium hydroxide *Quintessence International* **38(10)** 843-852.
6. Cannon M, Cernigliaro J, Vieira A, Percinoto C, & Jurado R (2008) Effects of antibacterial agents on dental pulps of monkeys mechanically exposed and contaminated *Journal of Clinical Pediatric Dentistry* **33(1)** 21-28.
7. Bal C, Alacam A, Tuzuner T, Tirali RE, & Baris E (2011) Effects of antiseptics on pulpal healing under calcium hydroxide pulp capping: a pilot study *European Journal of Dentistry* **5(3)** 265-272.
8. Senia ES, Marshall FJ, & Rosen S (1971) The solvent action of sodium hypochlorite on pulp tissue of extracted teeth *Oral Surgery, Oral Medicine, and Oral Pathology* **31(1)** 96-103.
9. Rosenfeld EF, James GA, & Burch BS (1978) Vital pulp tissue response to sodium hypochlorite *Journal of Endodontics* **4(5)** 140-146.
10. Hafez AA, Cox CF, Tarim B, Otsuki M, & Akimoto N (2002) An *in vivo* evaluation of hemorrhage control using sodium hypochlorite and direct capping with a one- or two-component adhesive system in exposed nonhuman primate pulps *Quintessence International* **33(4)** 261-272.
11. Elias RV, Demarco FF, Tarquinio SB, & Piva E (2007) Pulp responses to the application of a self-etching adhesive in human pulps after controlling bleeding with sodium hypochlorite *Quintessence International* **38(2)** e67-e77.
12. Kitasako Y, Inokoshi S, & Tagami J (1999) Effects of direct resin pulp capping techniques on short-term response of mechanically exposed pulps *Journal of Dentistry* **27(4)** 257-263.
13. Louw NP, Pameijer CH, Ackermann WD, Ertl T, Cappius HJ, & Norval G (2002) Pulp histology after Er:YAG laser cavity preparation in subhuman primates—a pilot study *South African Dental Journal* **57(8)** 313-317.
14. Todea C, Kerezsi C, Balabuc C, Calniceanu M, & Filip L (2008) Pulp capping—from conventional to laser-assisted therapy (I) *Journal of Oral Laser Applications* **8(2)** 71-82.

15. Todea C, Kerezsi C, Balabuc C, Calniceanu M, & Filip L (2008) Pulp capping—from conventional to laser-assisted therapy (II) *Journal of Oral Laser Applications* **8(3)** 147-155.

16. Komabayashi T, Ebihara A, & Aoki A (2015) The use of lasers for direct pulp capping *Journal of Oral Science* **57(4)** 277-286.

17. Javed F, Kellesarian SV, Abduljabbar T, Gholamiazizi E, Feng C, Aldosary K, Vohra F, & Romanos GE (2017) Role of laser irradiation in direct pulp capping procedures: a systematic review and meta-analysis *Lasers in Medical Science* **32(2)** 439-448.

18. Hendrick DA & Meyers A (1995) Wound healing after laser surgery *Otolaryngologic Clinics of North America* **28(5)** 969-986.

19. Fitzpatrick RE & Goldman MP (1995) Advances in carbon dioxide laser surgery *Clinics in Dermatology* **13(1)** 35-47.

20. Dang J, Wilder-Smith P, & Peavy GM (1998) Clinical preconditions and treatment modality: effects on pulp surgery outcome *Lasers in Surgery and Medicine* **22(1)** 25-29.

21. Moritz A, Schoop U, Goharkhay K, & Sperr W (1998) The CO_2 laser as an aid in direct pulp capping *Journal of Endodontics* **24(4)** 248-251.

22. Moritz A, Schoop U, Goharkhay K, & Sperr W (1998) Advantages of a pulsed CO_2 laser in direct pulp capping: a long-term *in vivo* study *Lasers in Surgery and Medicine* **22(5)** 288-293.

23. Elliott RD, Roberts MW, Burkes J, & Phillips C (1999) Evaluation of the carbon dioxide laser on vital human primary pulp tissue *Pediatric Dentistry* **21(6)** 327-331.

24. Suzuki M, Katsumi A, Watanabe R, Shirono M, & Katoh Y (2005) Effects of an experimentally developed adhesive resin system and CO_2 laser irradiation on direct pulp capping *Operative Dentistry* **30(6)** 702-718.

25. Suzuki M, Ogisu T, Kato C, Shinkai K, & Katoh Y (2011) Effect of CO_2 laser irradiation on wound healing of exposed rat pulp *Odontology* **99(1)** 34-44.

26. Medina VO III, Shinkai K, Shirono M, Tanaka N, & Katoh Y (2002) Histopathologic study on pulp response to single-bottle and self-etching adhesive systems *Operative Dentistry* **27(4)** 330-342.

27. Brännström M & Nyborg H (1972) Pulpal reaction to composite resin restorations *Journal of Prosthetic Dentistry* **27(2)** 181-189.

28. Cox CF, Keall CL, Keall HJ, Ostro E, & Bergenholtz G (1987) Biocompatibility of surface-sealed dental materials against exposed pulps *Journal of Prosthetic Dentistry* **57(1)** 1-8.

29. Tsuneda Y, Hayakawa T, Yamamoto H, Ikemi T, & Nemoto K (1995) A histopathological study of direct pulp capping with adhesive resins *Operative Dentistry* **20(6)** 223-229.

30. Akimoto N, Momoi Y, Kohno A, Suzuki S, Otsuki M, Suzuki S, & Cox CF (1998) Biocompatibility of Clearfil Liner Bond 2 and Clearfil AP-X system on nonexposed and exposed primate teeth *Quintessence International* **29(3)** 177-188.

31. Murray PE, Hafez AA, Smith AJ, Windsor LJ, & Cox CF (2003) Histomorphometric analysis of odontoblast-like cell numbers and dentine bridge secretory activity following pulp exposure *International Endodontic Journal* **36(2)** 106-116.

32. Rowles SL, & Levine RS (1973) The inorganic composition of arrested carious dentine *Caries Research* **7(4)** 360-367.

33. Shimizu C, Yamashita Y, Ichijo T, & Fusayama T (1981) Carious change of dentin observed on longspan ultrathin sections *Journal of Dental Research* **60(11)** 1826-1831.

34. Smith AJ, Cassidy N, Perry H, Bègue-Kirn C, Ruch JV, & Lesot H (1995) Reactionary dentinogenesis *International Journal of Developmental Biology* **39(1)** 273-280.

35. Pashley DH, Pashley EL, Carvalho RM, & Tay FR (2002) The effects of dentin permeability on restorative dentistry *Dental Clinics of North America* **46(2)** 211-245.

36. Stanley HR, Pereira JC, Spiegel E, Broom C, & Schultz M (1983) The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age *Journal of Oral Pathology* **12(4)** 257-289.

37. Pashley EL, Talman R, Horner JA, & Pashley DH (1991) Permeability of normal versus carious dentin *Endodontics & Dental Traumatology* **7(5)** 207-211.

38. Zander HA (1939) Reaction of the pulp calcium hydroxide *Journal of Dental Research* **18** 373-379.

39. Stanley HR & Lundy T (1972) Dycal therapy for pulp exposures *Oral Surgery, Oral Medicine, and Oral Pathology* **34(5)** 818-827.

40. Tronstad L (1974) Reaction of the exposed pulp to Dycal treatment *Oral Surgery, Oral Medicine, and Oral Pathology* **38(6)** 945-953.

41. Gandolfi MG, Siboni F, Primus CM, & Prati C (2014) Ion release, porosity, solubility, and bioactivity of MTA Plus tricalcium silicate. *Journal of Endodontics* **40(10)** 1632-1637.

42. Poggio C, Ceci M, Dagna A, Beltrami R, Colombo M, & Chiesa M (2015) *In vitro* cytotoxicity evaluation of different pulp capping materials: a comparative study *Arhiv za Higijenu Rada i Toksikologiju* **66(3)** 181-188.

43. Ranjkesh B, Isidor F, Kraft DCE, & Løvschall H (2018) *In vitro* cytotoxic evaluation of novel fast-setting calcium silicate cement compositions and dental materials using colorimetric methyl-thiazolyl-tetrazolium assay *Journal of Oral Science* **60(1)** 82-88.

44. Takita T, Hayashi M, Takeichi O, Ogiso B, Suzuki N, Otsuka K, & Ito K (2006) Effect of mineral trioxide aggregate on proliferation of cultured human dental pulp cells *International Endodontic Journal* **39(5)** 415-422.

45. Faraco IM Jr & Holland R (2001) Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement *Dental Traumatology* **17(4)** 163-166.

46. Accorinte Mde L, Holland R, Reis A, Bortoluzzi MC, Murata SS, Dezan E Jr, Souza V, & Alessandro LD (2008) Evaluation of mineral trioxide aggregate and calcium

hydroxide cement as pulp-capping agents in human teeth *Journal of Endodontics* **34(1)** 1-6.

47. González-López S, & Bolaños-Carmona V (2005) Long-term evolution of a case of direct pulp capping by adhesion to dentin *Quintessence International* **36(10)** 797-803.
48. Kitasako Y, Ikeda M, & Tagami J (2008) Pulpal responses to bacterial contamination following dentin bridging beneath hard-setting calcium hydroxide and self-etching adhesive resin system *Dental Traumatology* **24(2)** 201-206.
49. Pameijer CH & Stanley HR (1998) The disastrous effects of the "total etch" technique in vital pulp capping in primates *American Journal of Dentistry* **11(Special Issue)** S45-S54.
50. Hebling J, Giro EM, & Costa CA (1999) Biocompatibility of an adhesive system applied to exposed human dental pulp *Journal of Endodontics* **25(10)** 676-682.
51. de Souza Costa CA, Lopes do Nascimento AB, Teixeira HM, & Fontana UF (2001) Response of human pulps capped with a self-etching adhesive system *Dental Materials* **17(3)** 230-240.
52. Hörsted-Bindslev P, Vilkinis V, & Sidlauskas A (2003) Direct capping of human pulps with a dentin bonding system or with calcium hydroxide cement *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **96(5)** 591-600.
53. da Silva LA, de Freitas AC, de Carvalho FK, de Queiroz AM, Nelson-Filho P, & Porto-Neto ST (2009) Direct pulp capping with a self-etching adhesive system: histopathologic evaluation in dogs' teeth *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **108(1)** e34-e40.
54. Silva GA, Gava E, Lanza LD, Estrela C, & Alves JB (2013) Subclinical failures of direct pulp capping of human teeth by using a dentin bonding system *Journal of Endodontics* **39(2)** 182-189.
55. Yoshii E (1997) Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity *Journal of Biomedical Materials Research* **37(4)** 517-524.
56. About I, Camps J, Mitsiadis TA, Bottero MJ, Butler W, & Franquin JC (2002) Influence of resinous monomers on the differentiation *in vitro* of human pulp cells into odontoblasts *Journal of Biomedical Materials Research* **63(4)** 418-423.
57. Huang FM & Chang YC (2002) Cytotoxicity of dentine-bonding agents on human pulp cells *in vitro International Endodontic Journal* **35(11)** 905-909.
58. Kusdemir M, Gunal S, Ozer F, Imazato S, Izutani N, Ebisu S, & Blatz MB (2011) Evaluation of cytotoxic effects of six self-etching adhesives with direct and indirect contact tests *Dental Materials Journal* **30(6)** 799-805.
59. Lu Y, Liu T, Li X, Li H, & Pi G (2006) Histologic evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide in beagles *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **102(4)** e78-e84.
60. Lu Y, Liu T, Li H, & Pi G (2008) Histological evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide on human pulp tissue *International Endodontic Journal* **41(8)** 643-650.
61. Nowicka A, Parafiniuk M, Lipski M, Lichota D, & Buczkowska-Radlinska J (2012) Pulpo-dentin complex response after direct capping with self-etch adhesive systems *Folia Histochemica et Cytobiologica* **50(4)** 565-573.
62. Kawashima S, Shinkai K, & Suzuki M (2016) Effect of an experimental adhesive resin containing multi-ion releasing fillers on direct pulp-capping *Dental Materials Journal* **35(3)** 479-489.
63. Kaaden C, Powers JM, Friedl KH, & Schmalz G (2002) Bond strength of self-etching adhesives to dental hard tissues *Clinical Oral Investigations* **6(3)** 155-160.
64. Belli S, Zhang Y, Pereira PN, & Pashley DH (2001) Adhesive sealing of the pulp chamber *Journal of Endodontics* **27(8)** 521-526.
65. Osorio R, Toledano M, de Leonardi G, & Tay F (2003) Microleakage and interfacial morphology of self-etching adhesives in class V resin composite restorations *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **66(1)** 399-409.
66. Ogisu T, Suzuki M, Shinkai K, & Katoh Y (2008) Effect on pulp healing of CO_2 laser irradiation and direct pulp capping with experimentally developed adhesive resin systems containing reparative dentin promoting agents *Journal of Oral Laser Applications* **8(4)** 257-273.
67. Shoji S, Nakamura M, & Horiuchi H (1985) Histopathological changes in dental pulps irradiated by CO_2 laser: a preliminary report on laser pulpotomy *Journal of Endodontics* **11(9)** 379-384.
68. Jukic S, Anic I, Koba K, Najzar-Fleger D ,& Matsumoto K (1997) The effect of pulpotomy using CO_2 and Nd:YAG lasers on dental pulp tissue *International Endodontic Journal* **30(3)** 175-180.
69. Utsunomiya T (1998) A histopathological study of the effects of low-power laser irradiation on wound healing of exposed dental pulp tissues in dogs, with special reference to lectins and collagens *Journal of Endodontics* **24(3)** 187-193.
70. Marques NC, Neto NL, Rodini Cde O, Fernandes AP, Sakai VT, Machado MA, & Oliveira TM (2015) Low-level laser therapy as an alternative for pulpotomy in human primary teeth *Lasers in Medical Science* **30(7)** 1815-1822.