Antibacterial and Odontogenesis Efficacy of Mineral Trioxide Aggregate Combined with CO_2 Laser Treatment

Tuan-Ti Hsu, MS,[*] Chia-Hung Yeh, PhD,[†] Chia-Tze Kao, DDS, PhD,[‡§] Yi-Wen Chen, PhD,[†] Tsui-Hsien Huang, DDS, PhD,[‡§] Jaw-Ji Yang, PhD,[*] and Ming-You Shie, PhD[†]

Abstract

Introduction: Mineral trioxide aggregate (MTA) has been successfully used in clinical applications in endodontics. Studies show that the antibacterial effects of CO_2 laser irradiation are highly efficient when bacteria are embedded in biofilm because of a photothermal mechanism. The aim of this study was to confirm the effects of CO_2 laser irradiation on MTA with regard to both material characterization and cell viability. **Methods:** MTA was irradiated with a dental CO_2 laser using directly mounted fiber optics in the wound healing mode with a spot area of 0.25 cm^2 and then stored in an incubator at 100% relative humidity and 37°C for 1 day to set. The human dental pulp cells cultured on MTA were analyzed along with their proliferation and odontogenic differentiation behaviors. **Results:** The results indicate that the setting time of MTA after irradiation by the CO_2 laser was significantly reduced to 118 minutes rather than the usual 143 minutes. The maximum diametral tensile strength and x-ray diffraction patterns were similar to those obtained without CO_2 laser irradiation. However, the CO_2 laser irradiation increased the amount of Ca and Si ions released from the MTA and regulated cell behavior. CO_2 laser–irradiated MTA promoted odontogenic differentiation of hDPCs, with the increased formation of mineralized nodules on the substrate's surface. It also up-regulated the protein expression of multiple markers of odontogenic and the expression of dentin sialophosphoprotein protein. **Conclusions:** The current study provides new and important data about the effects of CO_2 laser irradiation on MTA with regard to the decreased setting time and increased ion release. Taking cell functions into account, the Si concentration released from MTA with laser irradiation may be lower than a critical value, and this information could lead to the development of new regenerative therapies for dentin and periodontal tissue. *(J Endod 2015;41:1073–1080)*

Key Words

Antibacterial, CO_2 laser, human dental pulp cell, mineral trioxide aggregate, odontogenic

Periradicular surgery may be performed in the presence of infection or when orthograde endodontic treatment is contraindicated or considered unfeasible (1). Despite the high antimicrobial efficacy of traditional disinfectants *in vitro*, clinical research has shown bacterial persistence within the root canal after cleaning and shaping procedures (2–4). Therefore, it is very important to investigate advanced endodontic disinfection strategies that are effective in eliminating biofilm bacteria in the root canals. Several root filling materials play an essential role in the control of reinfection by entombing residual organisms that may remain after instrumentation, irrigation, and intracanal medication. The ideal root-end filling material should have antibacterial activity to prohibit bacterial growth, in addition to possessing good cellular properties to enhance tissue formation (5), even though the antibacterial ability of these materials is well-known (6).

Mineral trioxide aggregate (MTA) has been successfully used in clinical applications in endodontics (7). Not only does MTA have good biocompatibility (8), but it also has been verified to promote hard tissue formation (9, 10). Calcium silicate–based materials have been used in dentin replacement restorative materials in dentistry (11–13). We recently showed that calcium silicate–based materials stimulate the proliferation and differentiation of primary human dental pulp cells (hDPCs) (14–16) and human periodontal ligament cells (17) *in vitro*. The inorganic ions released from calcium silicate–based materials have been found to affect cell behavior and osteogenic and angiogenic marker protein secretion (15–19). In addition, although many studies have been published on the antibacterial properties of MTA, there remains some controversy regarding the underlying mechanisms (5). Although MTA releases Ca ions and leads to an alkaline microenvironment with a higher pH value, which may suppress bacterial growth (5), it has also been shown that the calcium silicate particles themselves may permeate the bacteria (20) with possible antibacterial consequences (21).

Laser light can be applied to stimulate bacteria capable of bioremediation (22), and lasers have been successfully used in many fields, including medicine, industry, and the military. With regard to clinical use, there are several commercially available lasers, including CO_2, diode, and erbium:yttrium aluminum garnet lasers (17). The advantage of laser light over conventional treatments with chemical agents is its ability to partially travel through the biofilm (23). Research shows that CO_2 laser irradiation has highly efficient antibacterial effects when bacteria are embedded in biofilm because of a photothermal mechanism (24). However, another study reported that laser light can increase fibroblast proliferation and collagen synthesis and reduce inflammation

From the *Institute of Oral Science, [‡]School of Dentistry, and [§]Department of Stomatology, Chung Shan Medical University Hospital, Taichung City, Taiwan; and [†]3D Printing Medical Research Center, China Medical University Hospital, Taichung City, Taiwan.

Address requests for reprints to Dr Ming-You Shie, 3D Printing Medical Research Center, China Medical University Hospital, Taichung City, Taiwan or Prof Jaw-Ji Yang, Institute of Oral Science, Chung Shan Medical University Hospital, Taichung City, Taiwan. E-mail address: eviltacasi@gmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2015.02.031

(25, 26). In addition, laser light can promote periodontal cell differentiation and, thus, has the potential to enhance periodontal tissue regeneration (17).

The aim of this study was to assess the effects of CO_2 laser irradiation on MTA with regard to material characterization and cell viability. We hypothesized that CO_2 laser irradiation destroyed the surface structure of MTA and increased the release of ions. After CO_2 laser irradiation, the antibacterial ability of MTA was investigated and compared with that of calcium hydroxide. This study also investigated the odontogenic protein expression for regenerative endodontics.

Materials and Methods

Preparation of MTA Specimens

The MTA used in this study was prepared according to the manufacturer's instructions. MTA powder (0.2 g) was mixed with distilled H_2O in a liquid/powder ratio of 0.3 mL/g to make the cement. The resulting cement was then used to fully cover each well of a 96-well plate (GeneDireX, Las Vegas, NV) to a thickness of 2 mm. The specimens were then irradiated with a dental CO_2 laser with an output of 10,600 nm (Opelaser Pro; Yoshida Co Ltd, Tokyo, Japan) using directly mounted fiber optics in the wound healing mode with a spot area of 0.25 cm^2 and stored in an incubator at 100% relative humidity and 37°C for 1 day to set. Before cell experiments, all specimens were sterilized by immersion in 75% ethanol followed by exposure to ultraviolet light for 1 hour.

Setting Time, pH Variation, and Strength

After the powder was mixed with distilled H_2O, the cements were placed into a cylindric mold and treated with the CO_2 laser before being stored in an incubator at 37°C and 100% relative humidity for hydration. The setting time of the cements was tested according to standards set by ISO 9917-1. To evaluate the setting time, each material was analyzed using Gilmore needles (456.5 g), and setting was completed when the needle failed to create a 1-mm-deep indentation in 3 separate areas. The variation in the pH value of the MTA during the setting process was measured with a pH meter (IQ120 miniLab pH meter; IQ Scientific Instruments, San Diego, CA). The diametral tensile strength (DTS) testing was conducted on an EZ Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 1 mm/min. The maximal compression load at failure was obtained from the recorded load-deflection curves. At least 10 specimens from each group were tested.

Phase Composition and Morphology

The crystalline phases of MTA treated with the CO_2 laser were examined using x-ray diffraction (XRD; Shimadzu XD-D1, Kyoto, Japan) over the 2-theta range from 20°–50°, with a scanning speed of 1°/min. The morphologies of the MTA specimens before and after immersion in simulated body fluid (SBF) solution were examined under a scanning electron microscope (JSM-6700F, JEOL, Tokyo, Japan) operated in the lower secondary electron image mode at 3-kV accelerating voltage.

In Vitro Soaking

It is believed that the prerequisite for materials to bond to natural bone is the formation of a "bonelike" apatite layer, an indicator of bioactivity (the ability to form a chemical bond with living tissue) (21). To evaluate the *in vitro* bioactivity, the cements were immersed in a 10-mL SBF solution at 37°C. The SBF solution, of which the ionic composition is similar to that of human blood plasma, consisted of 7.9949 g NaCl, 0.3528 g $NaHCO_3$, 0.2235 g KCl, 0.147 g K_2HPO_4, 0.305 g $MgCl_2 \bullet 6H_2O$, 0.2775 g $CaCl_2$, and 0.071 g Na_2SO_4 in 1000 mL distilled H_2O and was buffered to a pH of 7.4 with hydrochloric acid and trishydroxymethyl aminomethane ($[CH_2OH]_3CNH_2$) (7, 11, 13, 21, 27). All chemicals used were of reagent grade. The solution in the shaker water bath exhibited no change under static conditions. After soaking for 1 day, the specimens were removed from the tube, and their surface microstructures were examined.

Antibacterial Properties

To investigate the antibacterial effects of the MTA irradiated with CO_2 laser treatment, *Staphylococcus aureus* in Luria Broth (LB) culture media (4.0×10^4 bacteria per mL) was cultured with MTA materials for 24 hours. Aliquots of 0.1 mL from each group were then mixed with 0.9 mL PrestoBlue (Invitrogen, Grand Island, NY) for 10 minutes; after which, the solution in each well was transferred to a new 96-well plate. Plates were then read in a multiwell spectrophotometer (Hitachi, Tokyo, Japan) at 570 nm with a reference wavelength of 600 nm. Cells cultured on the tissue culture plate without the cement were used as the control (Ctl). The results were obtained in triplicate from 3 separate experiments in terms of optical density. The agar diffusion test is considered the standard method to test for antibiotic susceptibility (8, 28, 29); thus, to further confirm the inhibitory effects of MTA treated with the CO_2 laser on the growth of bacterial strains, we performed an agar diffusion assay. Four sterile 6-mm disk-shaped samples from each of the 4 experimental groups were placed on cultured agar plates containing bacterial lawns of *S. aureus*. The inhibition zones (in mm) were determined after 1 day of incubation.

Ion Concentration

The Ca and Si ion concentrations on Dulbecco modified Eagle medium (DMEM) were analyzed using an inductively coupled plasma atomic emission spectrometer (OPT 1 MA 3000DV; Perkin-Elmer, Shelton, CT) after culturing for different time points. Six samples were immersed in 10 mL DMEM and measured for each data point. The results were obtained in triplicate from 3 separate samples for each test.

hDPC Isolation and Culture

hDPCs were freshly derived from caries-free, intact premolars that were extracted for orthodontic treatment purposes as described previously (13, 14). The patient gave informed consent, and approval from the Ethics Committee of the Chung Shan Medicine University Hospital was obtained (CSMUH No. CS14117). The tooth was split sagittally with a chisel. The pulp tissue was then immersed in phosphate-buffered saline (PBS; Caisson, North Logan, UT) solution and digested in 0.1% collagenase type I (Sigma-Aldrich, St Louis, MO) for 30 minutes. After being transferred to a new plate, the cell suspension was cultured in DMEM (Caisson) supplemented with 20% fetal bovine serum (GeneDireX) and 1% penicillin (10,000 U/mL)/streptomycin (10,000 mg/mL) (Caisson) in a humidified atmosphere with 5% CO_2 at 37°C, and the medium was changed every 3 days. The cells were subcultured through successive passaging at a 1:3 ratio until they were used for experiments (passages 3–7). The odontogenic differentiation medium was DMEM supplemented with 10^{-8} mol/L dexamethasone (Sigma-Aldrich), 0.05 g/L L-Ascorbic acid (Sigma-Aldrich), and 2.16 g/L glycerol 2-phosphate disodium salt hydrate (Sigma-Aldrich).

Cell Viability

Before the *in vitro* cell experiments, MTA specimens were sterilized by soaking in 75% ethanol followed by exposure to ultraviolet light for 1 hour. After direct culture for various time periods, cell viability was evaluated using the PrestoBlue assay, which is based on the detection of mitochondrial activity. Thirty microliters of PrestoBlue solution and