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Research Article

Antibacterial and Antifungal Effect of 405 nm Monochromatic Laser on Endodontopathogenic Microorganisms

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The purpose of this study is to evaluate the usefulness of 405 nm monochromatic laser irradiation as an alternative management for prevention and/or treatment of endodontic infections. A monochromatic laser-emitting device equipped with a 405-nm laser diode was developed. Using this device, the effect of 405 nm laser irradiation on the growth of *Porphyromonas gingivalis, Prevotella intermedia, Enterococcus faecalis*, and *Candida albicans*, which are microorganisms associated with persistent endodontic infections, was evaluated by viable colony counting. As a result, the irradiation with a 405 nm laser had a significant bactericidal/fungicidal effect on *P. gingivalis, P. intermedia*, and *C. albicans*, whereas the growth of *E. faecalis* was not affected by the irradiation. The inhibition rate in *P. gingivalis* and *P. intermedia* was ~60% and ~80%, respectively, following irradiation at 0.2 W for 300 sec. The inhibition rate in *C. albicans* was ~90% following irradiation at 0.2 W for 1200 sec. These results indicate that 405 nm monochromatic laser irradiation exerts a bactericidal/fungicidal effect on these microorganisms. The present study clearly demonstrates that 405 nm laser irradiation is a promising alternative management strategy for prevention and/or treatment of endodontic infections.

1. Introduction

When root canal treatment is performed correctly, the success rate is generally high. Several follow-up studies have reported overall success rates of 53–90% [1–4]. Many endodontic failures are believed to be due to persistent intracanal infection by microorganisms that resist antimicrobial chemotherapy and have endured periods of nutrient deprivation in treated root canals [5–7]. Although it is generally agreed that the optimal approach in these cases is to perform conventional retreatment, the outcome of retreatment is not always satisfactory [4, 8, 9], demonstrating the difficulty of completely eliminating the microorganisms from the infected root canals.

The microbiological profiles of persistent endodontic infections have been analyzed by a variety of methods including conventional culture systems [4, 10–12], polymerase chain reaction [13], reverse-capture checkerboard analysis [14], and pyrosequencing [15]. Evidence from these studies has shown that the microbial flora from persistent endodontic infections differs markedly from that found in untreated necrotic dental pulp, quantitatively as well as qualitatively. Bacteria isolated most frequently from root canals with persistent/secondary endodontic infections included *Enterococcus faecalis*, *Streptococcus spp., Pseudoramibacter alactolyticus*, *Propionibacterium propionicum,* and *Filifactor alocis* [16]. Particularly, *E. faecalis* was the species most commonly isolated from persistently infected root canals [12, 17]. Interestingly, *Candida*

albicans, one of the resident fungi in the oral cavity, and *Porphyromonas gingivalis* and *Prevotella intermedia*, two of the major periodontopathogenic microorganisms, were also isolated with relatively high frequency [11–13, 16–19]. These findings suggest that, for an optimal outcome of endodontic treatment, these microorganisms should be eliminated from the infected root canals or at least reduced significantly to levels where periradicular tissue healing is not hampered.

Photodynamic therapy (PDT), which is usually performed using photosensitizers such as 5-aminolevulinic acid and porfimer sodium, was originally developed to target tumor cells [20]. Recently, PDT has been used as an antimicrobial therapy with some photosensitizers to target a variety of microorganisms, such as *P. gingivalis*, *Fusobacterium nucleatum*, *Propionibacterium acnes, S. aureus, S. mutans,* and *E. coli* [21–26]. Guffey and Wilborn reported that 405 nm blue light had a bactericidal effect on two aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [25, 26]. In our previous study, we investigated the most effective wavelength for inhibiting *P. gingivalis* growth and confirmed that 400–410 nm blue light was the most effective for inhibiting this bacterium even without a photosensitizer [27]. *P. gingivalis* is known as a black-pigmented anaerobic pathogen implicated not only in marginal periodontitis but also in apical periodontitis. This bacterium is also known to degrade hemoglobin for its growth to produce protoporphyrin which could act as an endogenous photosensitizer, making this bacterium photosensitive [28]. In fact, some other studies have also reported the bactericidal effects of laser irradiation on periodontopathogenic bacteria including *P. gingivalis* [21, 23, 27–30]. These findings suggest the feasibility of PDT for both apical and marginal periodontitis.

In the present study, we investigated the use of a 405 nm monochromatic laser in the prevention and/or treatment of persistent endodontic infections under more realistic conditions. We developed a monochromatic laser-emitting device equipped with a 405 nm blue laser diode and evaluated the effects of this irradiation on four microorganisms associated with persistent endodontic infections (*P. gingivalis*, *P. intermedia*, *E. faecalis*, and *C. albicans*).

2. Materials and Methods

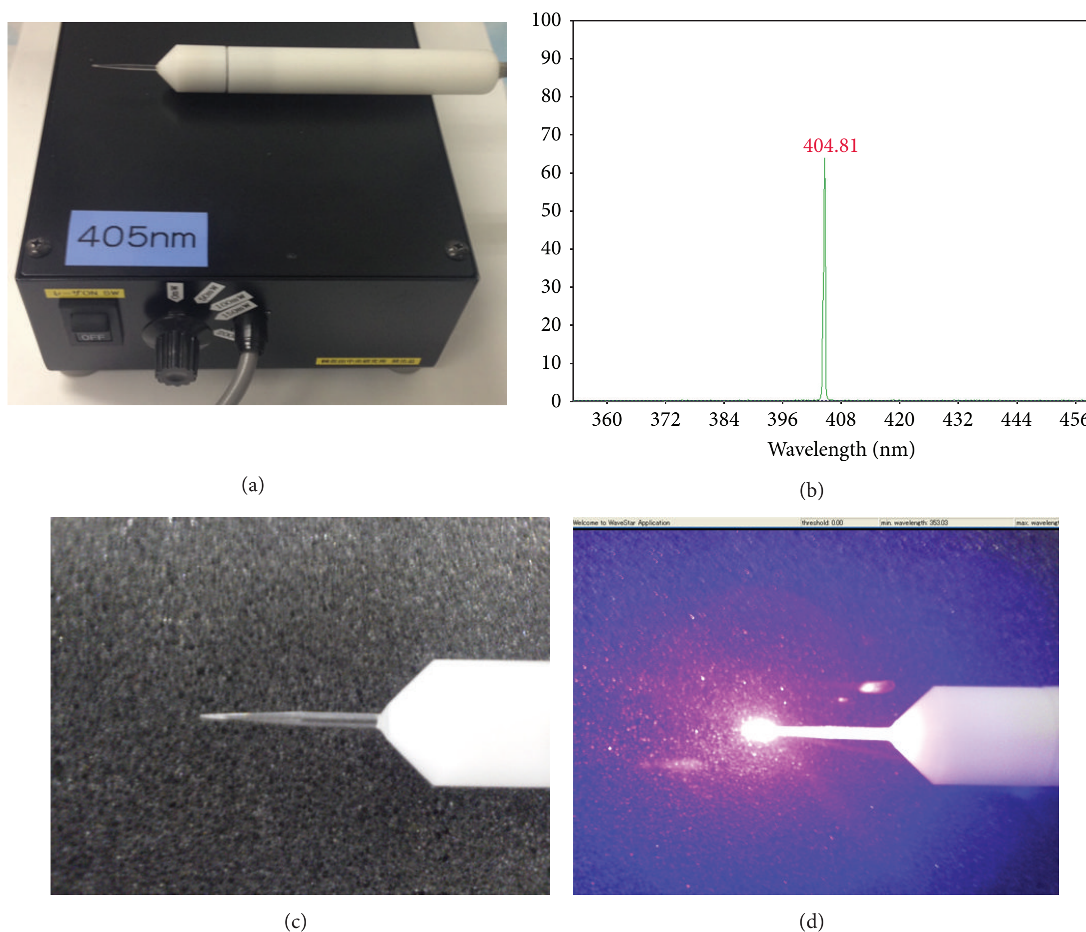
2.1. Development of Laser-Emitting Device. The experiments in the present study were conducted using a laser-emitting device equipped with a monochromatic 405 nm laser diode that was developed by Osada Electric Co. Ltd., Tokyo, Japan. The output was adjustable between 0.05 W and 0.2 W, and the tapered irradiation tip was made from acrylic resin, with a diameter at the apex of 50 μm (Figure 1(a)). The emission of the laser was confirmed by a spectrophotometer (Anritsu Keiki Co. Ltd., Tokyo, Japan) (Figure 1(b)). The acrylic irradiation tip was tapered to 50 μm in diameter at the apex, which is appropriate for endodontic treatment (Figures 1(c) and 1(d)). To eliminate the possibility that heat emitted from the irradiation tip may have affected the viability of the microorganisms, the temperature of the acrylic irradiation tip was analyzed during the irradiation using an infrared thermography (FLIR Systems Inc., Australia). This analysis showed that the irradiation tip did not generate sufficient heat to cause damage to living microorganisms. The temperature of the tip was 23.2°C (room temperature) before irradiation, rose to 29.8°C in the first 300 sec of irradiation, and declined to 28.5°C in the next 300 sec at an output of 0.2 W (Figure 2). The temperature remained stable thereafter.

2.2. Microorganisms and Culture. Four types of oral pathogenic microbes, *P. gingivalis* ATCC33277, *P. intermedia* ATCC25611, *E. faecalis* ATCC19433, and *C. albicans* ATCC18804, were used in this study. *E. faecalis* was aerobically cultured in 3 mL of brain heart infusion broth (BHI) (Becton Dickinson and Company, MD, USA) supplemented with 0.5% yeast extract, 0.05% L-cysteine, and 0.025% resazurin. *P. gingivalis* and *P. intermedia* were anaerobically cultured in 3 mL of brain heart infusion broth (BHI) (Becton Dickinson and Company) supplemented with 0.5% yeast extract, 0.05% L-cysteine, 0.0005% hemin, 0.0001% vitamin K_1, and 0.025% resazurin in an anaerobic cabinet (Sanyo Electric Co. Ltd., Osaka, Japan) with an atmosphere of 80% N_2, 10% CO_2, and 10% H_2 overnight at 37°C. Thereafter, 100 μL of each culture solution was inoculated into 10 mL of BHI and cultured anaerobically for two days at 37°C. Each bacterial solution was adjusted to 4×10^5 colony forming units (CFUs)/mL for *E. faecalis*, 2×10^5 CFU/mL for *P. gingivalis,* and 6×10^3 CFU/mL for *P. intermedia*. These bacterial solutions were used for the 405 nm laser exposure experiments.

The colonies of *C. albicans* cultured aerobically for 2 days on Sabouraud dextrose agar (Nissui, Tokyo, Japan) were suspended in 10 mL of Tryptic soy broth (Becton Dickinson) containing 5% anhydrous dextrose and were cultured aerobically at 37°C under gentle agitation for 2 more days. The fungal solution of *C. albicans* was adjusted to 1×10^6 CFU/mL and used for the 405 nm laser exposure experiments.

2.3. Irradiation with 405 nm Monochromatic Laser. Aliquots (10 μL) of each homogeneous bacterial/fungal solution were aspirated into a 20 μL microtip by a micropipette; then the irradiation device tip (50 μm in diameter at the apex) was inserted into the bacterial solution in the microtip, kept static in position, and irradiated (Figures 3(a) and 3(b)). The irradiation was performed under constant exposure time (300 sec) at various output powers of 0.05 W, 0.1 W, 0.15 W, and 0.2 W for *P. gingivalis*, *P. intermedia,* and *E. faecalis* and under constant output of power (0.2 W) at various irradiation times of 300 sec, 600 sec, and 1200 sec for *C. albicans*. All of the treatment for *P gingivalis and P. intermedia* was carried out in an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, MI).

2.4. Viable Colony Count. Following irradiation, each bacterial/fungal solution was applied to agar plates: Tryptic soy agar plates for *E. faecalis*, blood agar plates for *P. gingivalis* and *P. intermedia*, and Sabouraud dextrose agar plates for *C. albicans*. Survival of the microorganisms was determined by counting CFUs after aerobic incubation (for *E. faecalis* and



(a) (b) (c) (d)

Figure 1: (a) Prototype monochromatic laser-emitting device, with output adjustable between 0.05 W and 0.2 W. (b) Spectrophotometry confirming the monochromatic laser emission at a wavelength of 405 nm. (c) Tapered irradiation tip, made from acrylic resin, with a diameter at the apex of 50 μm. (d) Emission of 405 nm monochromatic laser light from laser tip.

C. albicans) or anaerobic incubation (for *P. gingivalis* and *P. intermedia*) for 48 h at 37°C.

2.5. Statistical Analysis. For multiple group comparisons, the data were analyzed by one-way ANOVA. The significance of individual differences was evaluated using the Mann-Whitney *U* test with Bonferroni correction. $P < 0.05$ was considered significant.

3. Results

The effects of 405 nm laser irradiation on each bacterial strain were analyzed by the colony forming capacity of each strain before and after irradiation for 300 sec at a variety of outputs (0.05, 0.1, 0.15, and 0.2 W). No inhibition of *P. gingivalis* growth was noted following 300 sec irradiation at an output power of 0.05 W, whereas a significant inhibition was observed following irradiation at output power of 0.1 W or higher (Figure 4(a)). The inhibition rate was ~50% at 0.1 and 0.15 W and ~60% at 0.2 W. Interestingly, significant inhibition of *P. intermedia* growth was observed following irradiation even at an output power of 0.05 W. The inhibition rate was ~40% at 0.05 W, ~70% at 0.1 W, and ~80% at 0.15 W and 0.2 W (Figure 4(b)). In contrast, no significant inhibition of *E. faecalis* growth was observed under any of the irradiating conditions examined (Figure 4(c)). These results demonstrate that 405 nm laser irradiation has a significant bactericidal effect on *P. gingivalis* and *P. intermedia* but not on *E. faecalis*. No inhibitory effect on *C. albicans* growth was noted upon 0.2 W irradiation for 300 sec (60 J), whereas irradiation for 600 sec (120 J) and 1200 sec (240 J) resulted in significant growth inhibition rates of ~60% and ~90%, respectively (Figure 4(d)).

4. Discussion

Endodontic infections consist of a complex mix of bacterial/fungal microorganisms, and therapeutic management still depends on conventional chemomechanical root canal preparation, which sometimes fails to yield a satisfactory outcome [1–9]. Consequently, there is a need for an alternative management strategy for infected root canals that is easy to perform, less invasive for patients, and repeatable.

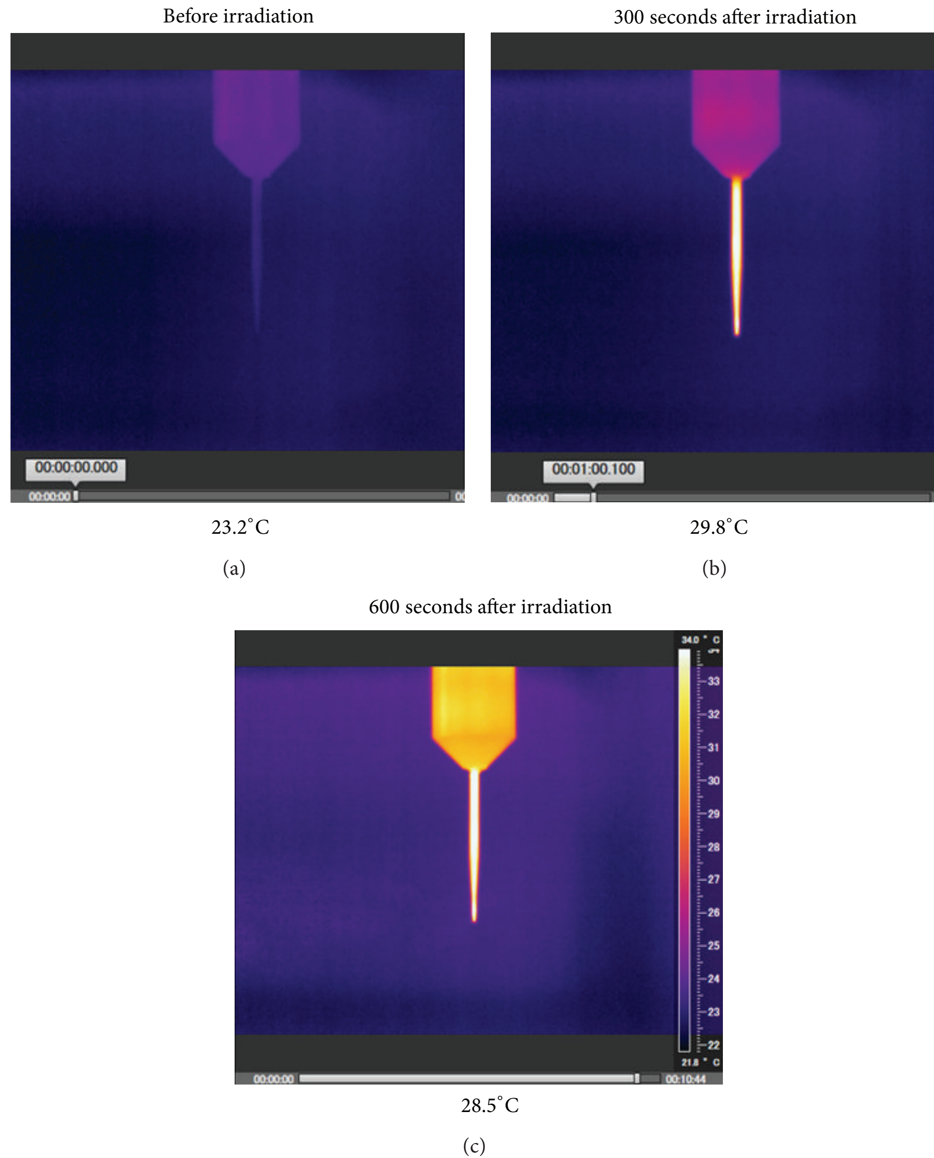
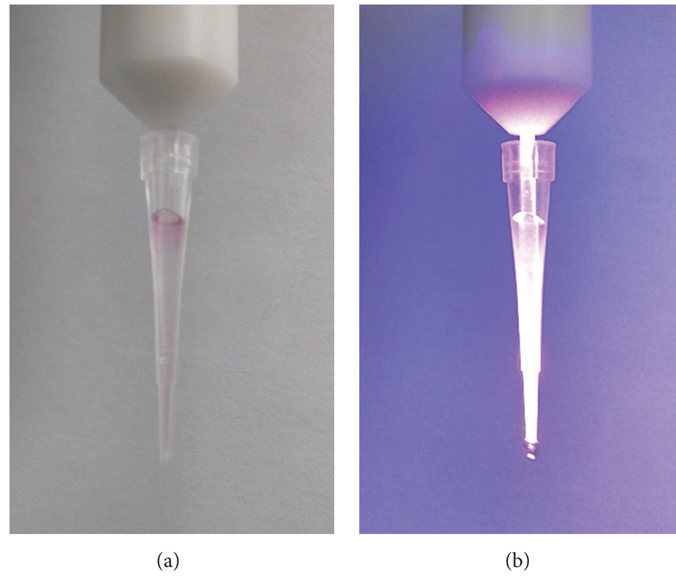


Figure 2: Change in temperature of acrylic irradiation tips. (a) Before laser irradiation; (b) 300 sec after laser irradiation; and (c) 600 sec after laser irradiation.

In our previous study, we demonstrated that *P. gingivalis* growth is specifically suppressed by 405 nm blue laser irradiation, even without an exogenous photosensitizer. These findings suggested that 405 nm laser irradiation is a promising means for eradicating *P. gingivalis* from infected root canals and periodontal lesions [29]. Based on these results, in the present study we developed a prototype 405 nm laser-emitting device designed for endodontic treatment to investigate whether irradiation exerts bactericidal and/or fungicidal effects on microorganisms associated with persistent endodontic infections, such as *P. gingivalis*, *P. intermedia*, *E. faecalis,* and *C. albicans*. We found that 405 nm laser irradiation inhibited *P. gingivalis* growth in an output power-dependent manner. This result is consistent with our previous study. Interestingly, *P. intermedia* growth was inhibited by the irradiation to an even greater extent than *P. gingivalis*. Given that *P. intermedia* is known to be relatively resistant to a variety of antibiotics compared with other pathogenic bacteria [18], this finding confirms the possibility that 405 nm laser irradiation is a promising method for treating and preventing persistent endodontic infections. In contrast, 405 nm laser irradiation had no inhibitory effect on *E. faecalis*, one of the major endodontopathogenic bacteria [17]. Interestingly, the growth of *C. albicans* was significantly inhibited by irradiation at duration of greater than 600 sec at an output of 0.2 W. This result indicates the possibility that 405 nm laser irradiation is effective for eliminating fungi as well as bacteria.

The mechanisms underlying the inhibitory effects of 405 nm laser irradiation and the differences between bacterial strains are still unclear. One possibility is that microorganisms sensitive to 405 nm laser light may have some substance which acts as an endogenous photosensitizer and that the



(a) (b)

Figure 3: (a) Microtip following aspiration of microbial solution. (b) Irradiation of the microbial solution following insertion of the irradiation device tip into the microbial solution.

Porphyromonas gingivalis ATCC33277
(CFU/dish)
Control 0.05 0.1 0.15 0.2
(W)
(a)

Prevotella intermedia ATCC25611
Control 0.05 0.1 0.15 0.2
(W)
(b)

Enterococcus faecalis ATCC19433
(CFU/dish)
Control 0.05 0.1 0.15 0.2
(W)
(c)

Candida albicans ATCC18804
Control 300 s (60 J) 600 s (120 J) 1200 s (240 J)
(d)

Figure 4: (a) Effect of 300 sec laser irradiation on *P. gingivalis* growth. (b) Effect of 300 sec laser irradiation on *P. intermedia* growth. (c) Effect of 300 sec laser irradiation on *E. faecalis* growth. (d) Effect of laser irradiation at 0.2 W output on *C. albicans* growth. $^{*}P < 0.05$.

sensitivity may vary according to the level or type of this substance. Some studies using black-pigmented bacteria such as *P. gingivalis* have suggested that endogenously produced porphyrins might act as photosensitizers under light irradiation, leading to a reduction in bacterial growth and viability [31, 32]. Further investigations will be able to resolve this issue in the near future.

The present study clearly demonstrates that 405 nm laser irradiation is a promising alternative management strategy for persistent endodontic infections.

5. Conclusions

Irradiation with a 405 nm laser had a significant bactericidal/fungicidal effect on *P. gingivalis*, *P. intermedia,* and *C. albicans*. These findings demonstrate that 405 nm laser irradiation is a promising alternative management strategy for prevention and/or treatment of persistent endodontic infections.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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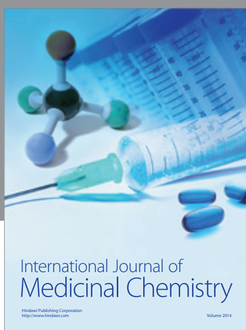
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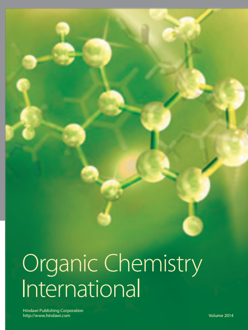
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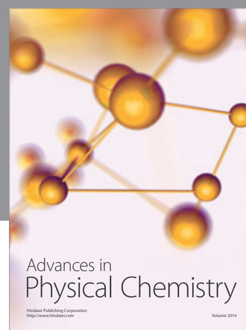
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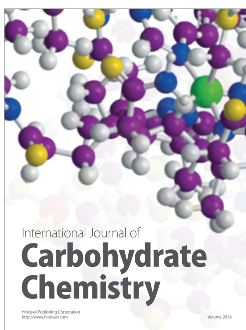
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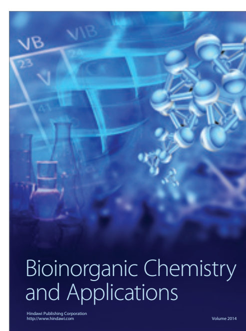
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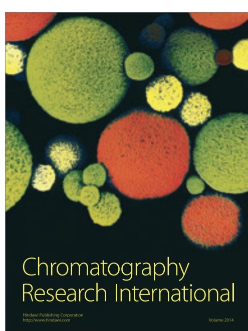
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