Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications
Prague, Czech Republic, August 11-13, 2014
Paper No. 147

Deposition of Fluorescent Magnetic Nanoparticles into Dentinal Tubules

Pavel Polenik, Jan Netolicky
Stomatological Clinic/ Charles University, Medical Faculty
Alej Svobody 80, Pilsen, Czech Republic
polenik@fnplzen.cz; netolicky@fnplzen.cz

Abstract - Current mechanical and chemical possibilities of bacterial suppression within infected root canals are not completely effective and especially dentinal tubules seems be a dominant problem.

From this point of view, the use of nanoparticles with antibacterial effect could be very attractive for better results of endodontic therapy. The aim if this study was to evaluate *in vitro* possibility of magnetic nanoparticles to penetrate into dentinal tubules. Photon-initiated photoacoustic streaming and exposure to magnetic field was tested for improvement of nanoparticles penetration. Root canals of 20 extracted single-rooted human teeth were mechanically spreaded. Subsequent irrigation was performed by trypsin - EDTA solution. As an active medium, the fluorescent nano-screen MAG-DEAE particles were used. Waterlase iPlus laser was used as a source of photon-initiated photoacoustic streaming. After this treatment 15 teeth was placed on neodymium magnet with tearing force 492 N for 20 minutes and 5 teeth served as controls. After 24 hours all teeth were splitted and examined by confocal laser-scanning microscope, fluorescent microscope, SEM and SEM/Energy dispersive X-ray spectrometry.

Confocal laser-scanning microscopy and fluorescent microscopy revealed in experimental as well as control teeth closing of entrances into dentinal tubules by clusters of nanoparticles. Limited penetration around 100 µm was detected in SEM by control teeth, whereas more than 1000 µm long distance of dentinal tubules was occupied by nanoparticles in experimental teeth. The same differences were confirmed by SEM/Energy dispersive X-ray spectrometry. Under the conditions of this *in vitro* study, photon-initiated photoacoustic streaming in combination with magnetic field significantly increased penetration of magnetic nanoparticles into dentinal tubules. Obtained results seems be promising for next phase of research, i.e. antibacterial effect of nanoparticles.

Keywords: Root canal, Nanoparticles, Photoacoustic streaming, Magnetic, Dentinal.

1. Introduction

Infected root canals are an everyday problem in dental practice. Complicated anatomical proportions of root canals and a complex of dentinal tubules provide very good conditions for colonization and spreading of bacterial infection. Consequently microbial decontamination of colonized spaces is essential for healing of clinical manifestations of infected root canals. Although the large volume of the contaminating bacteria is removed during mechanical and chemical debridement, residual bacteria are readily detectable in approximately one-half of teeth at the time of obturation (Byström, Sundqvist, 1983).Current mechanical and chemical possibilities of bacterial suppression are not completely effective and especially dentinal tubules seems be a dominant problem (Byström, Sundqvist, 1983). The depth of chemicals penetration to the lumen of dentinal tubules is limited to 100 µm, whereas location of bacteria can be more than 1000 µm (Haapasalo, Ørstavik, 1987). Presence of a "smear layer" after mechanical instrumentation reduces the effectiveness of irrigations and intracanal antimicrobials in disinfecting dentinal tubules (Berutti et al., 1997). Different agitation techniques have been proposed to improve the efficacy of irrigants, including agitation with endodontic instruments, plastic files, sonic and ultrasonic devices and more recently laser devices (Gu et al., 2009). Laser energy may be used to activate irrigants in different ways. The more recent laser technique is based on a photon-induced photoacoustic streaming and generation of expansions and successive implosion of irrigants causing a movement of fluids because of a secondary cavitation effect (DiVito et al., 2012).

Next idea for improvement of antibacterial strategy is the use of nanoparticles with antibacterial effect for better accessibility of residual bacteria inside the dentinal tubules and narrow parts of root canal ramifications. From this point of view, the aim of this study was to evaluate *in vitro* possibility of magnetic nanoparticles to penetrate into dentinal tubules. Photon-initiated photoacoustic streaming and exposure to magnetic field was tested for improvement of nanoparticles penetration.

2. Material and Methods

20 extracted single-rooted human teeth were stored in 0,12% chlorhexidine for 3 weeks. Teeth were trepanned from occlusion with high-speed drill under water cooling. The next instrumentation sequence consisted from Gates burs 4 and 2 and Hedstroem files ISO 15 to ISO 50. Subsequent irrigation was performed by 2 ml trypsin (0,5%)-EDTA (0,2%) solution for 4 minutes and root canals were dried out by paper points. As an active medium, the fluorescent nano-screen MAG-DEAE particles with diameter 200 nm and density 1,25g/cm^3 were used and mixed (ratio 1:1) with 0,1% Triton X-100 in order to achieve better hydrophilic properties. Active medium was injected into root canals and shortly agitated by root instrument. In all teeth Waterlase iPlus laser was used as a source of photon-initiated photoacoustic streaming. Shock waves were initiated by 320 nm radial firing tip by 0,75 W, 20 Hz, 10% air and 0% water. Vertical spiral movement inside root canal was used 4 times in one minute. In this moment was treatment finished in 5 teeth (control group).After laser treatment 15 teeth was placed on neodymium magnet with tearing force 492 N for 20 minutes and subsequently incubated in humid chamber by 37^{o}C. After 24 hours of incubation, all teeth were splitted along the long axis and examined by confocal laser-scanning microscope, fluorescent microscope, SEM and SEM/Energy dispersive X-ray spectrometry. Moreover, in 3 teeth ground sections along the long axis was prepared for examination of dentinal tubules by optical microscopy. Manual grinding was done in two steps, first with rough carborundum stone till a section of 1 mm was obtained. Grinding was further done using fine carborundum stone till the section of 0,4 mm thickness was left. Finally, the Arkansas stone was used and the section of 0,3 mm was refined and mounted in Canadian balsam. Subsequently, the sections were viewed and evaluated under microscope.

3. Results

After mechanical and chemical treatment of root canals, absence of smear layer and opened dentinal tubules were visible (Fig. 1).

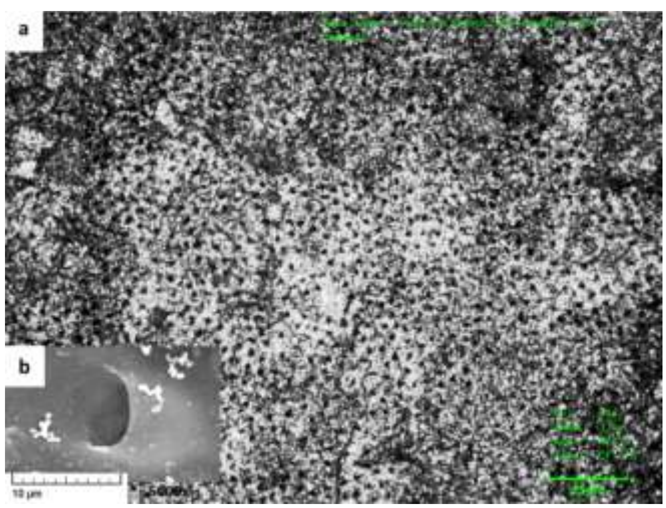

Fig. 1-a: Root canal wall in confocal laser-scanning microscope before application of nanoparticles. Entrances of dentinal tubules are completely visible and free of smear layer. b: Entrance of dentinal tubule in SEM (5000x) and small clusters of nanoparticles located around its margin.

Fluorescent microscope revealed in experimental as well as control teeth closing of entrances into dentinal tubules by clusters of nanoparticles (Fig. 2).

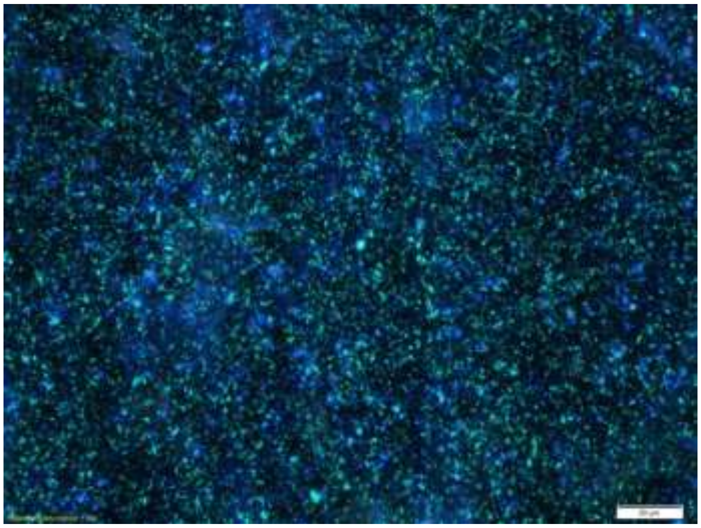

Fig. 2 Surface of root canal wall covered by clusters of fluorescent magnetic nanoparticles after laser and magnetic treatment (fluorescent microscopy).

Penetration of nanoparticles was detected within dentinal tubules on ground sections and by confocal laser-scanning microscopy. Limited penetration of dark nanoparticles around 100-200 µm was detected after laser treatment only (Fig. 3-a). On the border between root canal (R.C.) and root dentine (R.D.) are located clusters of nanoparticles and inside dentinal tubules dark pigmented zone of infiltration. More than 1000 µm long distance of dentinal tubules was occupied by nanoparticles in experimental teeth (Fig. 3-b).

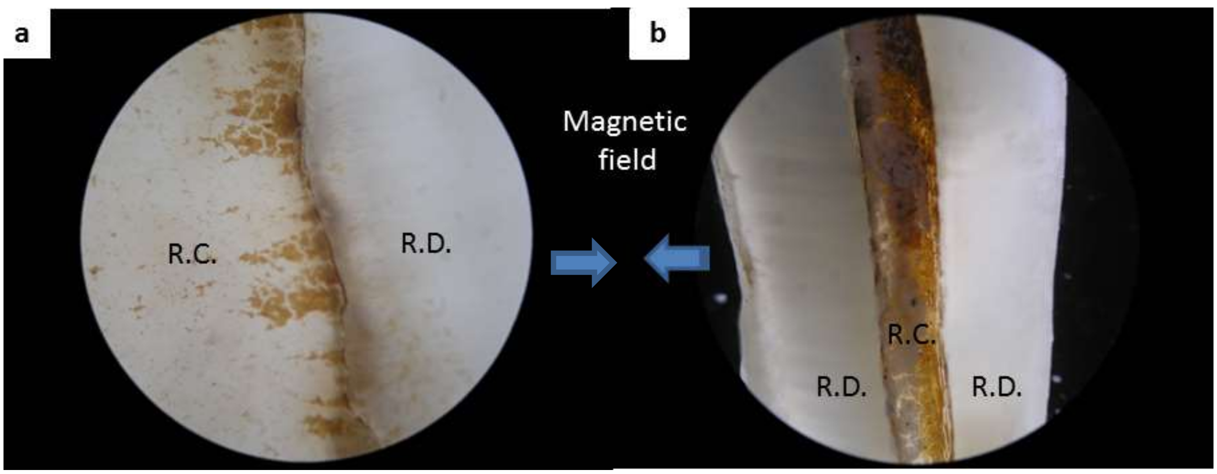

Fig. 3-a: Clusters of nanoparticles on the root canal wall and their limited penetration after laser treatment. b: Nanoparticles inside root canal. Left side after laser and magnet application. Nearly complete infiltration of root dentine by nanoparticles (dark color), right side without influence of magnetic field and without discoloration.

Further, the effect of photon-initiated photoacoustic streaming and magnetic field was examined by confocal laser-scanning microscope. Manifestation of this treatment was visible as clusters of nanoparticles on the surface of dentine wall and the same detection of accumulated nanoparticles within dentinal tubules as in previous examination (Fig. 4).

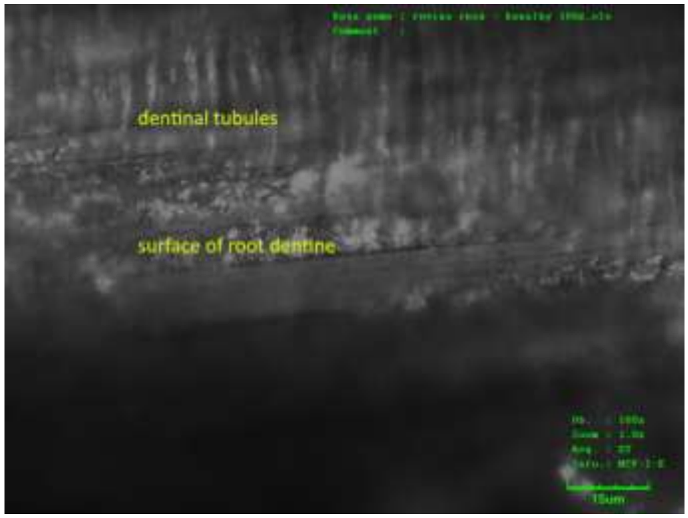

Fig. 4 Root canal dentine wall with adjacent layer of root dentine. Accumulated nanoparticles are apparent as a granular structure on the surface of root dentine as well as inside of dentinal tubules.

Microscopically detected differences of nanoparticles penetration into dentinal tubules between experimental and control group were confirmed by SEM/Energy dispersive X-ray spectrometry. Iron ions – typical constituent of used nanoparticles were detected up to more than 1000 µm of dentinal tubules of experimental teeth (Fig. 5), but complete absence was typical for control teeth (Fig. 6).

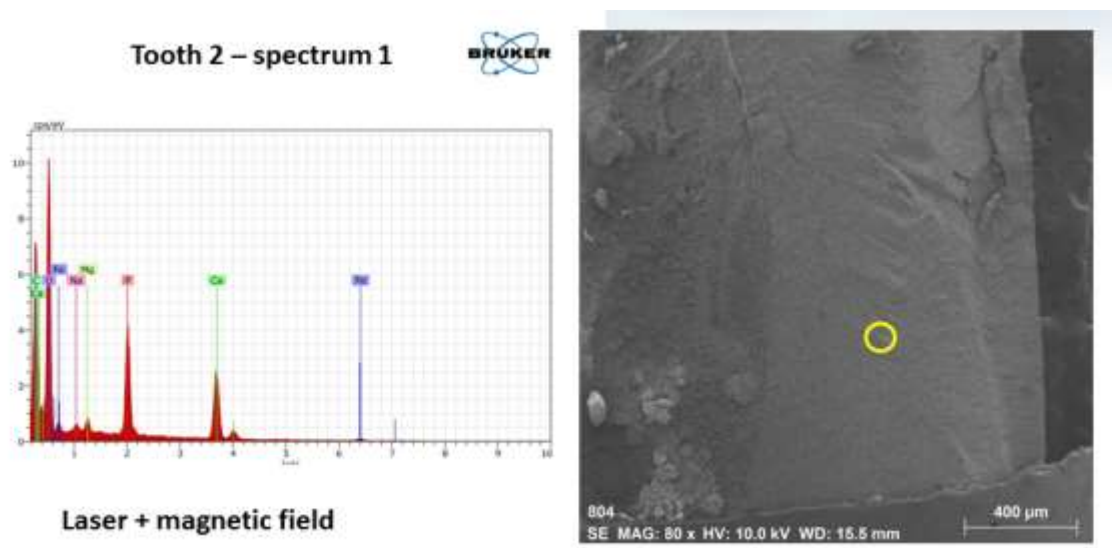

Fig. 5 Positive detection of Fe ions by SEM/Energy dispersive X-ray spectrometry in root dentin of teeth treated by laser and photon – initiated photoacoustic streaming.

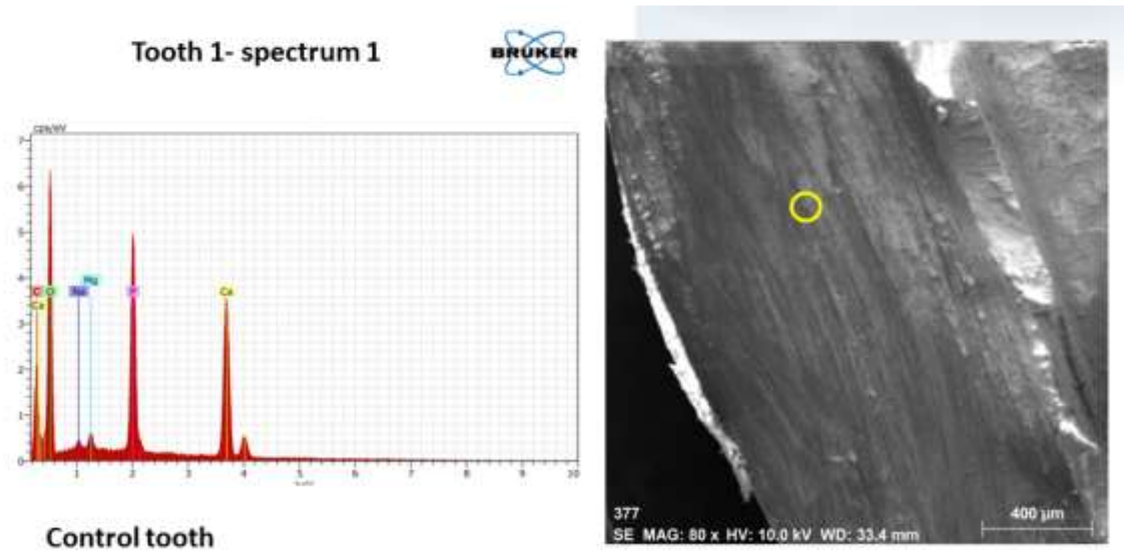

Fig. 6 Negative detection of Fe ions by SEM/Energy dispersive X-ray spectrometry in root dentin of teeth treated by laser and photon – initiated photoacoustic streaming.

4. Conclusions

Under the conditions of this *in vitro* study, significant differences in penetration of fluorescent magnetic nanoparticles were detected in the teeth treated by photon-initiated photoacoustic streaming and by magnetic field in comparison with the teeth without this treatment. Elimination of smear layer and opening of entrances of dentinal tubules are very important conditions for penetration and subsequent transport of nanoparticles into dentinal tubules. Obtained results seems be promising for next phase of research, i.e. antibacterial effect of nanoparticles.

Acknowledgment

This work was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic (grant number NT/13334).

References

Berutti E., Marine R., Angeretti A. (1997). Penetration ability of different irrigants into dentinal tubules. J Endod, 23, 725-727.

Byström A., Sundqvist G. (1983). Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol,55, 307-312.

DiVito E., Peters O.A., Olivi G. (2012). Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation.

Lasers Med Sci, 27, 273-280.

Gu L.S., Kim J.R., Ling J., Choi K.K., Pashley D.H., Tay F.R. (2009). Review of contemporary irrigant agitation techniques and devices. J Endod, 35, 791-804.

Haapasalo M., Ørstavik D. (1987). In vitro infection and disinfection of dentinal tubules. J Dent Res, 66, 1375-1379.