

Assessment of Efficacy of Photoactivated Disinfection Versus Double Antibiotic Paste Disinfection in Regeneration of Immature Infected Teeth. A comparative vivo study

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Abstract:

Aim: To investigate the efficacy of photo activated disinfection (PAD) Disinfection Versus Double Antibiotic paste Disinfection in Regeneration of Immature Infected Teeth. **Material and Methods:** 24 patients with immature, non-vital single rooted teeth presenting with or without signs and/or symptoms of periapical pathology were included in the study. Cases are divided into three groups according to the disinfection protocol, eight patients each group (n=8). **Group I:** Double Antibiotic paste (DAP) was injected in the canal after drying the canal with sterile paper points. **Group II:** Photosensitizers (Azulenocyanine)⁵ is inserted in the canals and agitated in the canal, then activated with diode laser 940 nm used in cycles 4 x 2mm/sec for disinfection of canal. **Group III:** Photosensitizers (Azulenocyanine)⁵ is inserted in the canals and agitated in the canal, then activated with diode laser 940 nm used in cycles 4 x 2mm/sec for disinfection of canal then Double Antibiotic paste (DAP) is injected in the canal after drying the canal with sterile paper points. **Results:** Overall results of our study, there was no a significant difference between the three groups, but group III "Double Antibiotic paste and Photoactivated disinfection" showed higher significant values than the other two groups. **Conclusion:** PAD may be an adjunctive procedure in disinfection of immature necrotic teeth.

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1. Introduction

Regenerative endodontics are biologically based procedures designed to replace damaged structures such as dentin-pulp complex by using stem cells, scaffolds and growth factors.¹ In regeneration, we depend more on irrigants and intra canal medicaments for achieving proper disinfection.² 3Photo-activated disinfection (PAD) is a new approach for elimination of intra-canal micro-organisms. PAD is based on the interaction of a photosensitive antibacterial agent and a light source. It uses a nontoxic dye named photosensitizer (PS) and low-intensity visible light. In presence of oxygen, the PS molecules adhere to the bacterial membrane. Irradiation with a specific wavelength of the light produces singlet oxygen, resulting in rupture of the microbial cell wall.⁴

2. Materials and methods.

2.1 selection of patients.

24 patients with immature, non-vital single rooted teeth presenting with or without signs and/or symptoms of periapical pathology were included in the study from the clinic of Faculty of Dentistry, Ainshams University, Cairo, Egypt.

2.2 cases classification

Cases are divided into three groups according to the disinfection protocol, eight patients each group (n=8).

Group I: 8 patients were treated as follows:

Double Antibiotic paste (DAP) was injected in the canal after drying the canal with sterile paper points

Group II: 8 patients were treated as follows:

Photosensitizers (Azulenocyanine)⁵ is inserted in the canals and agitated in the canal for 60 seconds using an endodontic file to ensure maximum penetration of the dye, then activated with diode laser 940 nm used in cycles 4 x 2mm/sec for disinfection of canal.

Group III: 8 patients were treated as follows:

Photosensitizers (Azulenocyanine)⁵ is

inserted in the canals and agitated in the canal for 60 seconds using an endodontic file to ensure maximum penetration of the dye, then activated with diode laser 940 nm used in cycles 4 x 2mm/sec for disinfection of canal then Double Antibiotic paste (DAP) is injected in the canal after drying the canal with sterile paper points.

Methods of evaluation

Patients were recalled at 3,6,9 and 12 months. Follow up included the clinical assessment of pain and/or swelling and standardized radiographic assessment which included the following:

An increase in root length.

An increase in root thickness.

A decrease in apical diameter.

statistical analysis

Two-way ANOVA followed by post hoc Tukey test was used to compare between more than two groups in non-related samples.

3. Results

3.1 Increase in root length (showed in Table 1)

There was no significant difference between samples treated with different treatments (p=0.544). Samples treated with Laser& DAP showed the highest mean value followed by samples treated with Laser while samples treated with DAP showed the lowest mean value.

3.2 Increase in dentine thickness (showed in table 2)

There was no significant difference between samples treated with different treatments (p=0.555). Samples treated with DAP showed the highest mean value followed by samples treated with Laser & DAP while samples treated with Laser showed the lowest mean value.

3.3 Decrease in apical diameter (showed in table 3)

There was a significant difference between samples treated with different treatments ($p < 0.001$). Samples treated with Laser & DAP showed the highest mean value followed by samples treated with DAP while samples treated with laser showed the lowest mean value.

4. Discussion

In the present study, we tested the efficiency of Double antibiotic paste versus Photoactivated disinfection of immature infected teeth. Apexification is a conventional treatment modality for these cases in which either calcium hydroxide paste is used to induce an apical barrier or mineral trioxide aggregate (MTA) is placed as an apical barrier in order to achieve closure of the apex.⁵

This technique has several disadvantages because it requires multiple visits during long period of time, requiring compliance, and the long-term use of calcium hydroxide might alter the mechanical properties of dentin, making the tooth more susceptible to fractures. Therefore, even if apexification closure is successful, the long-term prognosis is questionable because of eventual root fracture.⁶ Revascularization, regeneration, and revitalization are commonly used terms to describe the regrowth of de novo tissue within the root canal space.

Tagelsir et al 2016⁷ concluded from his study that root canal disinfection in regenerative endodontics offers 2 main challenges in comparison with regular endodontic treatment. The first challenge is that omission of mechanical instrumentation during endodontic regeneration keeps the bacterial biofilm on the root canal walls intact without enough access to chemical irrigants and medication. The second important challenge in disinfection protocols of endodontic regeneration is maintaining stem cell viability and bioactive properties of root dentin throughout the disinfection procedure. Indeed, high concentrations of sodium hypochlorite (NaOCl) and antibiotic intracanal

medicaments such as triple antibiotic paste (TAP) and double antibiotic paste (DAP) were found to be toxic to stem cells from apical papillae, negatively affect stem cell attachment and proliferation on dentin, and disrupt the release of growth factors from dentin.

The concept of photodynamic inactivation requires microbial exposure to either exogenous or endogenous photosensitizer molecules, followed by visible light energy, typically wavelengths in the red/near-infrared region that cause the excitation of the photosensitizers resulting in the production of singlet oxygen and other reactive oxygen species that react with intracellular components and consequently produce cell inactivation and death.⁸ Jenks et al 2016⁹ concluded in his study that a plenty of recent in vitro evidence recommended the use of low concentrations of NaOCl (Martin et al., 2014) and antibiotic pastes (Althumairy, Teixeira, & Diogenes, 2014; Kim et al., 2015) in an attempt to create a balanced disinfection protocol that can eliminate root canal pathogens without damaging stem cells and dentin endogenous proteins within the root canal system. In first session for the three groups involved in this study after access was done no mechanical instrumentation as Diogenes et al 2014¹⁰ published his review stated that canals for immature open apex teeth have compromised fragile underdeveloped dentinal walls represent a contraindication for mechanical instrumentation; thus, chemical debridement remains the main form of disinfection in REPs. Regarding the Sodium hypochlorite it was used for removal of necrotic remnants of pulp tissue Kim et al 2018¹¹ in his review stated that it is the most commonly used antiseptic irrigating solution in root canal therapy (Zehnder 2006, Mohammadi 2008).

For chemical disinfection in first group in this study DAP was used as TAP consisting of metronidazole, ciprofloxacin and minocycline, has been successfully used to disinfect the canals in RET, achieving closure of the apex and thickening of the dentinal walls. A potential disadvantage when using TAP is the possibility of tooth discoloration due to minocycline. The

double antibiotic paste (DAP) that excludes minocycline has also been successfully used for RET. 12 Therefore, disinfection during regenerative endodontics may require antimicrobial agents with considerable levels of substantivity (Fouad, 2011). Indeed, both TAP and DAP were suggested to have an extended residual antibiofilm effect after their removal (Sabrah et al., 2015). Additionally, DAP was proposed to have longer residual antibiofilm properties in comparison to similar concentrations of TAP (Sabrah, et al., 2015).13

Alagzni et al 201514 resulted that methylcellulose-based DAP and MTAP gels significantly reduced biofilm formation by both species of tested bacteria at all dilutions, regardless of the length of gel aging time. Additionally, the DAP and MTAP gels demonstrated significant reduction of biofilm formation by both of the tested bacterial species. The minimum bactericidal concentrations of DAP and TAP against both *E. faecalis* and *P. gingivalis* were 0.14 and 0.3 mg/mL, respectively.

In principles, Chrepa et al 201415 stated that it uses a nontoxic photosensitizer that is selectively absorbed in a target tissue and a low-intensity light source. Upon photo-induced activation of the photosensitizer, in the presence of oxygen, a series of reactions produce free radicals and singlet oxygen molecules leading to bacterial eradication. Singlet oxygen diffuses to a distance of approximately 100 nm with a half-life of <0.04 microseconds. The photodynamic effect or the extent of tissue/cell damage depends on the type, dose, incubation time, and localization of the photosensitizer; the availability of oxygen; the wavelength of light (nm); the light power density measured in mW/cm²; and the light energy fluency.

In the light of this study there was no significant difference between samples treated with different treatments regarding increase in root length and increase in dentine thickness as in reviews by Saoud et al 201616, kim et al 2018 (Chen et al. 2012, Alobaid et al. 2014, Kahler et al. 2014, Tong et al. 2017)11 ,

Thomson et al 201017.

A general agreement between this study and Nagai et al 201718 using same photosensitizer dye Azulenocyanine (Azc) with Diode Laser 940 Nm. The combination of aPDT with MB and either the 650 or 940 nm laser, the combination of Azc with the 940 nm laser, and MB significantly reduced the number of *S. mutans* in the infected dentin plates. Bezgin et al 201519 presented in his review that in the first report of successful revascularization, a double antibiotic paste of metronidazole and ciprofloxacin was used as an intracanal disinfectant, which approves selection of DAP for disinfection in this study.

After disinfection in the three groups in this study induction of bleeding was done as an agreement with Huang et al 200820 review. Finally MTA was for closure and sealing of root canal orifice after induction of bleeding coinciding with Torabinejad et al 201821 study

5. conclusion

Under the circumstances of this study, it can be concluded that:

1. DAP and PAD were equally successful disinfection protocols with regard to regenerative endodontic procedure of immature necrotic teeth.

2. Regenerative approach was a successful treatment option for the permanent immature teeth with necrotic pulp, which was evident by continued root growth and resolution of periapical radiolucency.

Table (1): The mean, standard deviation (SD) values of increase in root length of different groups.

Difference points	Difference in root length (mean±SD)			p-value
	DAP	Laser	Laser& DAP	
Baseline- 3 months	0.86±0.34	0.95±0.77	1.10±0.70	0.840ns
3- 6 months	1.06±0.21	0.73±0.67	1.36±0.61	0.206ns
6-9 months	0.52±0.31 ^B	0.70±0.42 ^{AB}	1.30±0.31 ^A	0.010*
9-12 months	0.72±0.35	1.13±0.93	0.60±0.25	0.354ns
Overall	3.16±0.83	3.52±2.60	4.36±0.75	0.544ns

Table (2): The mean, standard deviation (SD) values of increase in root dentin thickness of different groups.

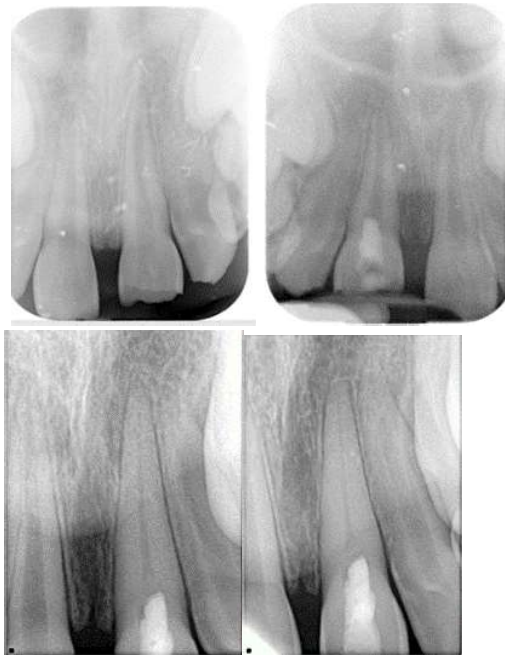
Difference points	Difference in root thickness (mean±SD)			p-value
	DAP	Laser	Laser& DAP	
Baseline- 3 months	0.28±0.16	0.25±0.19	0.40±0.21	0.419ns
3- 6 months	0.38±0.23	0.20±0.13	0.34±0.09	0.171ns
6-9 months	0.28±0.08	0.23±0.08	0.28±0.16	0.736ns
9-12 months	0.38±0.29	0.33±0.22	0.24±0.11	0.604ns
Overall	1.32±0.54	1.02±0.50	1.26±0.40	0.555ns

Table (3): The mean, standard deviation (SD) values of decrease in apical diameter of different groups.

Difference points	Difference in apical diameter (mean±SD)			p-value
	DAP	Laser	Laser& DAP	
Baseline- 3 months	0.38±0.15 ^{AB}	0.15±0.12 ^B	0.42±0.15 ^A	0.013*
3- 6 months	0.14±0.05 ^B	0.13±0.08 ^B	0.36±0.05 ^A	<0.001*
6-9 months	0.22±0.18 ^B	0.17±0.10 ^B	0.48±0.08 ^A	0.003*
9-12 months	0.26±0.21	0.15±0.10	0.34±0.21	0.233ns
Overall	0.94±0.28 ^B	0.60±0.13 ^B	1.60±0.26 ^A	<0.001*

Means with different small letters in the same column indicate significant difference, while means with different capital letters in the same row indicate significant difference *; significant (p<0.05) ns; non-significant (p>0.05)

Representative case:



A: preoperative radiograph - B: 3 months -
C: 6 months - D: 12 months

Figure (1): Radiograph showing representative case of group I (Double Antibiotic paste)

6. References:

1. Jung, C., Kim, S., Sun, T., Cho, Y. B. & Song, M. Pulp-dentin regeneration: current approaches and challenges. *Journal of Tissue Engineering* 10, (2019).
2. Galler, K. M. Clinical procedures for revitalization: current knowledge and considerations. *Int. Endod. J.* 49, 926–936 (2016).
3. Bago Jurić, I. & Anić, I. The Use of Lasers in Disinfection and Cleaning of Root Canals: a Review. *Acta Stomatol. Croat.* 48, 6–15 (2014).
4. Mohan, D. et al. Photoactivated disinfection (PAD) of dental root canal system – An ex-vivo study. *Saudi J. Biol. Sci.* 23, 122–127 (2016).
5. Lin, J., Zeng, Q., Wei, X., Zhao, W., Cui, M., Gu, J., ... & Ling, J. (2017). Regenerative endodontics versus apexification in immature permanent teeth with apical periodontitis: a prospective randomized controlled study. *Journal of endodontics*, 43(11), 1821-1827
6. Estefan, B. S., El Batouty, K. M., Nagy, M. M., & Diogenes, A. (2016). Influence of age and apical diameter on the success of endodontic regeneration procedures. *Journal of endodontics*, 42(11), 1620-1625.
7. Tagelsir, A., Yassen, G. H., Gomez, G. F., & Gregory, R. L. (2016). Effect of antimicrobials used in regenerative endodontic procedures on 3-week-old *Enterococcus faecalis* biofilm. *Journal of endodontics*, 42(2), 258-262.
8. Plotino, G., Grande, N. M., & Mercade, M. (2019). Photodynamic therapy in endodontics. *International endodontic journal*, 52(6), 760-774.
9. Jenks, D. B., Ehrlich, Y., Spolnik, K., Gregory, R. L., & Yassen, G. H. (2016). Residual antibiofilm effects of various concentrations of double antibiotic paste used during regenerative endodontics after different application times. *Archives of oral biology*, 70, 88-93.
10. Diogenes, A. R., Ruparel, N. B., Teixeira, F. B., & Hargreaves, K. M. (2014). Translational science in disinfection for regenerative endodontics. *Journal of endodontics*, 40(4), S52-S57.
11. Kim, S. G., Malek, M., Sigurdsson, A., Lin, L. M., & Kahler, B. (2018). Regenerative endodontics: a comprehensive review. *International endodontic journal*, 51(12), 1367-1388.
12. Valverde, M. E., Baca, P., Ceballos, L., FUENTES, M. V., Ruiz-Linares, M., & FERRER-LUQUE, C. M. (2017). Antibacterial efficacy of several intracanal medicaments for endodontic therapy. *Dental materials journal*, 2016-102.

13. Jenks, D. B., Ehrlich, Y., Spolnik, K., Gregory, R. L., & Yassen, G. H. (2016). Residual antibiofilm effects of various concentrations of double antibiotic paste used during regenerative endodontics after different application times. *Archives of oral biology*, 70, 88-93.
14. Algarni, A. A., Yassen, G. H., & Gregory, R. L. (2015). Inhibitory effect of gels loaded with a low concentration of antibiotics against biofilm formation by *Enterococcus faecalis* and *Porphyromonas gingivalis*. *Journal of oral science*, 57(3), 213-218.
15. Chrepa, V., Kotsakis, G. A., Pagonis, T. C., & Hargreaves, K. M. (2014). The effect of photodynamic therapy in root canal disinfection: a systematic review. *Journal of endodontics*, 40(7), 891-898.
16. Saoud, T. M. A., Ricucci, D., Lin, L. M., & Gaengler, P. (2016). Regeneration and repair in endodontics—a special issue of the regenerative endodontics—a new era in clinical endodontics. *Dentistry journal*, 4(1), 3
17. Thomson, A., & Kahler, B. (2010). Regenerative endodontics—biologically-based treatment for immature permanent teeth: a case report and review of the literature. *Australian dental journal*, 55(4), 446-452.
18. Nagai, Y., Suzuki, A., Katsuragi, H., & Shinkai, K. (2018). Effect of antimicrobial photodynamic therapy (aPDT) on the sterilization of infected dentin in vitro. *Odontology*, 106(2), 154-161.
19. Bezgin, T., & Sönmez, H. (2015). Review of current concepts of revascularization/revitalization. *Dental Traumatology*, 31(4), 267-273.
20. Huang, G. T. J. (2008). A paradigm shift in endodontic management of immature teeth: conservation of stem cells for regeneration. *Journal of dentistry*, 36(6), 379-386.
21. Torabinejad, M., Alexander, A., Vahdati, S. A., Grandhi, A., Baylink, D., & Shabahang, S. (2018). Effect of Residual Dental Pulp Tissue on Regeneration of Dentin-pulp Complex: An In Vivo Investigation. *Journal of endodontics*, 44(12), 1796-1801.

