Evaluation of the Antimicrobial Effect of Pre-Synthesized Novel Antibiotic Electrospun Nanofibers as an Intracanal Delivery Strategy for Regenerative Endodontics: A Randomized Clinical Trial

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Abstract

AIM: The aim of this study is to evaluate the antimicrobial effect of pre-synthesized novel antibiotic loaded electrospun nanofibers and compare it with conventional triple antibiotic paste when used in patients with immature necrotic teeth.

METHODS: Antibiotic loaded nanofibers were fabricated by electrospinning. Thirty-four patients with immature necrotic teeth were included in the study. In the first visit, access cavity preparation was performed to obtain the first bacteriological sample (S1). The canals were thoroughly irrigated using sodium hypochlorite 1.5% and a second sampling was performed (S2). Patients were randomly divided into two groups according to the intracanal medicament used: Modified triple antibiotic paste (MTAP) loaded electrospun nanofibers or MTAP paste. At the second appointment, the third samples (S3) were taken. The intracanal bacterial count was determined using the spread plate culture technique. Scanning electron microscopy (SEM) was used to examine the morphology of the fabricated MTAP loaded electrospun nanofibers.

RESULTS: Both MTAP nanofibers and MTAP paste resulted in significant reduction of bacterial count after the irrigation step. MTAP nanofibers resulted in significantly higher percent reduction of bacterial count (p < 0.05).

CONCLUSIONS: It was concluded that electrospinning technology can be used to fabricate antibiotic containing nanofibers which can result in enhanced disinfection in regenerative endodontic procedures.

Introduction

In the era of regenerative medicine, proper microbial control is mandatory for success of regenerative endodontic procedures (REPs), as regeneration and repair can never occur in presence of a persistent infection [1]. REPs for immature teeth comprise minimal-to-no mechanical instrumentation. That is why they count on the chemical disinfection step and application of intracanal medicaments to achieve proper disinfection. The previous studies showed that the majority of failed regenerative cases were attributed to the presence of a persistent root canal infection [2], [3].

Despite the fact that triple antibiotic paste (TAP) is used in an increasingly number of clinical cases since its introduction by Hoshino [4], still the majority of studies on the antimicrobial effects of the material are in vitro studies carried out under a very controlled environment [5]. Tooth discoloration was mainly attributed to minocycline as a component of the TAP [6]. Minocycline binds to the calcium ions in dentin by chelation, and induces a color change [7]. Minocycline is believed to undesirably affect angiogenesis by reducing vascular endothelial growth factor secretion, which suppresses the neovascularogenesis of endothelial cells [5], [8]. Thus, a number of suggestions were proposed including sealing the coronal dentinal tubules with bonding agent as well as eliminating minocycline using only double antibiotic paste [9]. Replacement of minocycline with cefaclor, amoxicillin, or clindamycin was also proposed. On the other hand, clindamycin is less cytotoxic, exerts a remarkable post-antibiotic activity and even more, can induce a proangiogenic effect similar to vascular endothelial growth factor [10], [11].

Moreover, the toxicity of TAP on stem cells in regenerative endodontics is well established [5], [6]. Regeneration of the pulp dentin complex involves both...
human dental pulp stem cells (HDPSCs) and stem cells of the apical papilla (SCAP) [12], [13], [14], maintaining the viability of both types of cells is crucial for success. Concerns about the deleterious toxic effects on stem cells have led the way for research for more biocompatible means for delivery of the antibiotic [15].

Electrospinning, which refers to “electrostatic spinning,” is an intervention that relies on application of high voltage electric force to draw charged threads from polymer solutions to form nanofiber, where the fiber’s diameter ranges from few to hundreds of nanometers [16]. Electrospinning technology was used to manufacture drug loaded polymer-based nanofibers containing minimal effective minute concentrations for drug delivery strategies. Electrospun nanofibers containing antibiotic have proven to be a promising approach to achieve decontamination of the root canal in vitro [17], [18], [19], [20], [21], [22], [23], [24].

In this study, we fabricated electrospun nanofibers loaded with a modified triple antibiotic paste containing metronidazole, ciprofloxacin, and clindamycin and evaluated its antimicrobial effect. To the best of our knowledge, no previous clinical studies investigated the effect of antibiotic electrospun nanofibers in patients with necrotic immature teeth undergoing regenerative endodontic treatment.

Materials and Methods

Study design

The study was designed as a prospective, parallel, blinded, and randomized clinical trial with 1:1 allocation ratio. The protocol of this study was registered in the national institute of health clinical trials registry [NCT03690960]. The protocol of this study was reviewed and approved by the Institutional review board and ethics committee (IRBs/ECs) in the Faculty of Dentistry - Cairo University. All participants provided signed informed consent. Participants were recruited from the endodontic clinic's out patients at Faculty of dentistry, Cairo University, Cairo governorate. Patients were treated in accordance with the Helsinki Declaration.

Sample size calculation was performed, and a total of 34 patients (divided into two groups of 17 patients each) were included in the study, which set the power of the study at 80%. The Type I error probability related to this test was 0.05. A computer software was used to generate a random sequence. Opaque envelopes containing folded numbered papers were prepared to be dragged by the patients. The patients did not know their treatment group. The laboratory technician and statistician were also blinded to the study groups.

Patient selection

The inclusion criteria included healthy patients between 9 and 25 years old with non-vital, asymptomatic single-rooted tooth with immature root. Exclusion criteria were patients with allergy to any of the used antibiotics, patients taking antibiotics in the past 3 months. Teeth with vital pulp, previously initiated treatment or teeth that cannot be isolated properly were also excluded from the study.

Fabrication of antibiotic containing nanofibers

Polyvinyl alcohol (PVA) (Oxford laboratories, India) was dissolved in sterile saline solution at 7% weight/volume ratio to prepare the polymer solution which was then stirred using a magnetic stirrer (WiseStir, Germany) at 100°C for 2 h [16]. After which 15% citric acid powder (Adwic - El Nasr, Egypt) was added to the solution. Afterward, three antibiotics powder of metronidazole, ciprofloxacin, and clindamycin were added. Equal proportions (335 mg of each drug) were added to the polymer solution. The antibiotic concentration was 30 wt.% relative to the polymer weight [17]. After 1 h of stirring, plastic syringes with a metallic tip needle were loaded with the solution and the solution was electrospun using an electrospinning system (NanoEbers LLC, Egypt). The processing parameters applied were: a flow rate of 0.4 mL/h, the distance between the needle tip and the collecting mandrel was 15-cm, and electrical voltage of 20 kV. The collected nanofibers membrane was heat treated on a glass watch in a dry heat oven for 10 min at 120°C. The nanofibers membrane was then cut into pieces with a final MTAP concentration of 0.1 mg/ml. Finally, the nanofibers were vacuum dried and exposed to UV irradiation for sterilization [11], [23].

Regenerative endodontic procedures

The first appointment

Medical and dental history were obtained from all patients participating in this research. After confirmation of the diagnosis, teeth were anaesthetized and isolated. The crowns and surrounding structures were disinfected with 30% H₂O₂ and 2.5% sodium hypochlorite for 30s after which 5% sodium thiosulfate was used. Sterility was confirmed by swabbing a sample from the crown surface. A sterile round bur was used for preparing the access cavity with sterile saline solution. The first bacteriological samples (S1) were taken by inserting three successive sterile paper points in the canal for 1 min each. Determination of working length was done using apex locator and confirmed using radiograph (Soredex, Digora, USA). Light mechanical preparation was done using stainless
steel manual K-files (MANI, INC., Japan) and the canals were thoroughly irrigated using 20 ml of sodium hypochlorite 1.5% followed by 5 ml of 0.5% sodium thiosulfate. The final wash was done using saline and a second sampling were performed (S2) samples were then immediately placed in sterile ready to fill vials containing the transport media for culturing. All samples were transported to the microbiology laboratory within 1 h [25], [26].

The patients were randomly allocated into two groups according to the type of intracanal medicament:

- **Intervention group**: MTAP nanofibers (prefabricated electrospun nanofibers loaded with metronidazole, ciprofloxacin, and clindamycin) were placed into the root canals using hand pluggers (Sedradent solutions, Egypt).
- **Comparator group**: Equal proportions of antibiotic powders of metronidazole, ciprofloxacin, and clindamycin were combined with saline to make a homogeneous MTAP paste with a creamy consistency and a concentration of 1 g/ml. The mixture was injected into the canal using a syringe that was 2 mm shorter than the working length. Excess antibiotic was removed from the access cavity to a point just below the cementoenamel junction.

**The second appointment**

The second visit was scheduled after 2–3 weeks, the nanofiber or MTAP paste were removed with 5 ml of sterile saline solution and the third samples (S3) were taken in the same previously described manner. Irrigation was done with 17% Ethylenediaminetetraacetic acid (EDTA) and the final wash was done using saline. To induce bleeding into the canal, a manual K-file was inserted beyond the working length. The bleeding was permitted to reach a depth of 3 mm below the cemento-enamel junction. The blood column was left for 3 min to allow for formation of a blood clot followed by a placement of a CollaCote membrane (Zimmer Dental, Carlsbad, CA). Then a 3-mm barrier of mineral trioxide aggregate (Cerkamed, Poland) was placed to reach a level of 2–3 mm beyond the cementoenamel junction. The tooth was restored with glass-ionomer and composite resin [26].

**Evaluation of the MTAP loaded electrospun nanofiber**

SEM (SEM Model Quanta 250 FEG, FEI company, Netherlands) with an Image-J software was used to observe the morphology of the developed nanofibers. An ion sputter (Hitachi Ion Sputter MC1000, Japan) was used to coat the samples with gold particles for imaging [11].

**Statistical analysis**

Statistical analysis was performed with IBM SPSS Statistics (IBM Corporation, Version 25, NY, USA), CFU/ml counts were converted into LOG 10. Parametric data were analyzed using independent t-test for comparisons between two groups and multi-way ANOVA followed by Tukey post hoc test for multiple group comparisons. The significance level was set at p ≤ 0.05 within all tests.

**Results**

A total of 34 patients met the inclusion criteria and were enrolled in the study. Patients were randomized into two groups of 17 patients each. Thirty-four patients were included in the analysis. The mean age in the intervention group (MTAP loaded electrospun nanofibers) was 15.94 ± 5.88 years, while for the comparator group (MTAP paste), the mean age was 17.00 ± 3.61 years with no statistically significant difference between groups (p = 0.532). For the gender, the intervention group (MTAP loaded electrospun nanofibers) consisted of ten males and seven females while the comparator group (MTAP paste) consisted of eight males and nine females with no statistically significant difference (p = 0.49).

**Bacterial count**

Comparison of bacterial count between the study groups (Table 1 and Figure 1) showed that, for aerobic bacteria at S1, there was no statistically
significant difference between bacterial count in both
groups at the start of the treatment, (p = 0.3). At S2,
there was no statistically significant difference between
bacterial count in both groups, (p = 0.6). At S3, there
was a statistically significant higher mean value of bacterial
count recorded in the MTAP paste group compared to
MTAP loaded electrospun nanofibers group, (p = 0.015).
Within each group, there was a statistically significant
difference between bacterial count at S1, S2, and S3
(p < 0.001).

For anaerobic bacteria, at S1, there was no
statistically significant difference between bacterial
count in both groups at the start of the treatment after
access cavity preparation, (p = 0.8). At S2, there was
no statistically significant difference between bacterial
count in both groups, (p = 0.16). At S3, there was a
statistically significant higher mean value of bacterial
count recorded in the MTAP paste group compared to
MTAP loaded electrospun nanofibers group, (p = 0.004).
Within each group, there was a statistically significant
difference between bacterial count at S1, S2, and S3
(p < 0.001).

Percent reduction (%) of the bacterial count

Comparison of the percent reduction of bacterial count between the study groups (Table 2 and
Figure 2) showed that, for aerobic Bacteria from S1
to S2, there was no statistically significant difference
between both groups, (p = 0.8). From S2 to S3, there
was a statistically significant higher mean percent of
reduction in MTAP loaded electrospun nanofibers group
to MTAP paste group, (p = 0.002). From S1
to S3, there was a statistically significant higher percent
of reduction in the MTAP loaded electrospun nanofibers
group to MTAP paste group (p = 0.015).

For anaerobic Bacteria, from S1 to S2, there
was no statistically significant difference between both
groups, (p = 0.1). From S2 to S3, there was a statistically
significant higher percent of bacterial reduction in
MTAP loaded electrospun nanofibers group compared
to MTAP paste group, (p < 0.001). From S1 to S3, there
was a statistically significant higher percent of bacterial
reduction in the MTAP loaded electrospun nanofibers
group compared to MTAP paste group, (p = 0.005).

Scanning electron microscope imaging (SEM) of the fabricated nanofibers

SEM imaging confirmed the ability to fabricate antibiotic containing nanofibrous structures with
relatively uniform diameters in the nano-submicron scale. The fiber diameter of the antibiotic containing
loaded electrospun nanofibers ranged between 400 nm–600 nm. At 500× magnification the SEM images
showed a dense, compact, nonwoven nanofibrous mesh with even architecture. At 2500× magnification, a nanofibrous 3D network consisting of well-defined randomly oriented loaded electrospun nanofibers with interconnected pores was observed. At 6000× magnification, The SEM images showed

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### Table 1: Descriptive statistics and test of significance comparing the bacterial count (log10) between the study groups

| Bacterial count | Aerobic, mean ± SD | Anaerobic, mean ± SD |
|-----------------|-------------------|----------------------|
|                | S1                | S2                  | S3                  | P       | S1                | S2                  | S3                  | p       |
| MTAP nanofiber  | 6.35 ± 0.35       | 5.27 ± 0.8          | 0.72 ± 1.61         | <0.001* | 6.17 ± 0.42       | 5.02 ± 0.71          | 0.24 ± 0.97          | <0.001* |
| MTAP paste      | 6.23 ± 0.33       | 5.05 ± 1.9          | 2.6 ± 2.5           | <0.001* | 6.19 ± 0.37       | 4.04 ± 2.7           | 2.18 ± 2.4           | <0.001* |
| p               | 0.3 (NS)          | 0.6 (NS)            | 0.015               |         | 0.8 (NS)          | 0.16 (NS)            | 0.004               |         |

*Significant, Significance level P ≤ 0.05. NS: Non-significant, SD: Standard deviation, MTAP: Modified triple antibiotic paste, S1: First bacteriological sample, S2: Second sampling, S3: Third sample.

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that the loaded electrospun nanofibers have a smooth, uniform surface morphology and structure without any beads along the fiber. Moreover, at higher magnification 10000 X, noticeable branching of the fibers was noted along with larger interfiber spaces (Figure 3).

### Discussion

Regenerative endodontic procedures for the management of necrotic immature teeth have been widely investigated and applied. Due to the fact that necrotic immature teeth have thin dentinal walls that mandates the use of minimal instrumentation, disinfection relies mainly on chemical methods [3], [28].

In our study, electrospinning technology was used to fabricate the modified triple antibiotic loaded nanofibers. PVA is an exceptional polymer with admirable properties as biocompatibility, inertness and safety. PVA is FDA approved for clinical application in humans and is widely used in a number of pharmaceutical products [23]. Another interesting feature, the polymer is readily soluble in water which eliminated the need to use toxic solvents. The polymer (PVA) was dissolved at a concentration of 7% W/V in the solvent to ensure production of smooth bead free nanofibers in the nanoscale. At a lower concentration the stretching force was weak resulting in fragmentation of the fiber jet into droplets, while increasing the polymer’s concentration results in larger diameter nanofibers [29], [30].

In our study, the parameters used to obtain a continuous steady jet for the PVA/antibiotic solutions were 20 kV voltage, a flow rate of 0.4 ml/h, and a 15 cm needle to collector distance.

Various methods were implemented to incorporate antibiotics into nanofibers via electrospinning including blending, coaxial electrospinning, encapsulation, and attachment. In our study, blending was used before the electrospinning process to actively incorporate the antibiotic within the polymer solution. Blending electrospinning is a widely used form of electrospinning being a simple and versatile method as long as proper solubility of the antibiotic in the polymer solution is achieved [31]. The antibiotic mixture was added to the polymer solution at a 25–35 Wt.%, this concentration was previously applied successfully in numerous studies [11], [17], [18].

In our study, a modified antibiotic combination was used, where minocycline was replaced with clindamycin. Minocycline results in severe adverse effects that counterbalance the regenerative process as a result of inhibition of angiogenesis and toxic effects on stem cells [12], [13]. In addition, minocycline causes tooth discoloration because it binds to calcium ions by chelation and form insoluble complexes [9]. In our study, a two-visit revascularization protocol was followed. A recent systematic review concluded that the success rate of single visit REPs was much lower than the multiple visit success rate for REPs [32]. Root canals were irrigated using 1.5% sodium hypochlorite as it was proved that it has lower toxicity to stem cells in the apical tissues compared to full concentration 5.25% sodium hypochlorite [33].

For the comparator group, antibiotics were mixed with saline to form a slurry thick mix, the same 1 gm/ml concentration was used before and showed successful results [25], [26], [27]. For regenerative endodontic procedures, it is now recommended to use intracanal antibiotics at concentrations of 0.1–1 mg/ml to maintain stem cell viability [33]. However, this low concentration always results in a liquid mixture difficult to apply inside the root canal [26]. The use of MTAP in the form of nanofibers allowed for the use of the minimum yet effective recommended antibiotic concentration in the intervention group.

Although culture technique comes with limitations, numerous studies have shown that success of treatment is directly related to decrease in number of microorganisms [34], [35]. To decrease the odds of false positive results, teeth were isolated with rubber
dam, the tooth, and the rubber dam were disinfected and sterility control samples were collected, after which a sterile bur was used to access the pulp chamber [36].

In our study, SEM was used to examine the fabricated MTAP loaded electrospun nanofibers' morphology as the nanofibers are non-conductive [37]. Sputter coater was used before scanning to deposit a thin nanolayer of gold ions to ensure obtaining images with the highest resolution [38]. Our results showed that successful electrospinning of PVA polymer solution containing antibiotic for local drug delivery is possible. The antibiotic containing nanofibers were continuous and even. No bead formation was detected under scanning electron microscopy. The fiber diameter ranged between 400 nm and 600 nm; smaller diameter nanofibers are known to provide more support cell proliferation compared to larger diameters approaching 1000 nm [39]. Moreover, noticeable branching of the nanofibers was noticed along with large inter-fiber spaces which can be of great value in regenerative procedures [39].

Overall, bacterial levels were significantly decreased with each phase of the treatment. At S1, after access cavity preparation, all teeth sampled were positive for bacteria. No statistically significant difference between groups was found indicating similar participants at the start of the trial. The mean counts were close to other studies in immature teeth [25], [26], [40]. However, it was higher than the previous studies performed on teeth with mature roots. This may be credited to the fact that sampling in a tooth with a very large canal space is much easier and more conductive to higher bacterial growth [25].

At S2, after irrigation with sodium hypochlorite, all cases showed a significant reduction in the aerobic, anaerobic, and total bacterial count. This agrees with other studies results [25], [26], [40]. However, in the present study, the majority of teeth still continued to be positive for bacterial analysis after the 1.5% sodium hypochlorite irrigation this comes in disagreement with Nagata et al. [26]. This disagreement in the antimicrobial efficacy after the irrigation protocol may be attributed to the much higher concentration of sodium hypochlorite (6%) used in their study. However, the toxicity of such high concentration to DPSC and SCAP is now well-established and this concentration is no more recommended [12], [33].

After intracanal medicament placement at S3, there was a further significant reduction in the CFU count for both groups. This further reduction comes in agreement with the previous studies [25], [26], [27]. Our results showed a significant higher bacterial reduction after intracanal medicament placement in both groups compared to the bacterial reduction after the irrigation step. This comes in agreement with the results of the study by de-jesus-soares [25]. The anatomy of the root canals of immature teeth might have enhanced the effectiveness of the used medicaments, along with the minimal instrumentation implemented and lower concentration of irrigants, contributed to the more pronounced effect of the intracanal medicament in regenerative endodontics [6].

Comparing both study groups, regarding the bacterial count no statistically significant difference was found between groups at S1 and S2. However, a statistically significant higher number of bacterial cells was found in the MTAP paste group at S3. A statistically significant higher mean value of percent reduction was recorded in the MTAP loaded electrospun nanofibers group compared to the MTAP paste group from S2 to S3 and from S1 to S3. This significant difference may be accredited to the sustained release nature of the MTAP loaded electrospun nanofibers as a drug delivery method. The previous studies showed that nanofibrous scaffolds containing antibiotic mixtures exhibited a consistent and sustained antimicrobial effect of for a period of 21 day inside the root canals [11], [18].

Conclusions

From the findings of our study, it could be concluded that electrospinning technology is a successful method for fabrication of MTAP loaded electrospun nanofibers. Substitution of minocycline by clindamycin in the formula of triple antibiotic paste resulted in production of smaller diameter nanofibers, a potent antimicrobial effect while avoiding the undesirable tooth discoloration. Along with the ease of placement of nanofibers inside the root canals, sustained drug release and proposed nontoxic effect on stem cells, MTAP loaded electrospun nanofibers had a statistically significant higher antimicrobial effect in our study compared to MTAP paste.

References

1. Lin LM, Kahler B. A review of regenerative endodontics: Current protocols and future directions. J Istanbul Univ Fac Dent. 2017;51(3 Suppl 1):S41-51. https://doi.org/10.17096/jiufd.53911
PMid:29354308
2. Lopes L, Neves JA, Botelho J, Machado V, Mendes JJ. Regenerative endodontics procedure: An umbrella review. Int J Environ Res Public Health. 2021;18(2):754. https://doi.org/10.3390/ijerph18020754
PMid:33561086
3. Almutairi W, Yassen GH, Aminoshariae A, Williams KA, Mickel A. Regenerative endodontics: A systematic analysis of the failed cases. J Endod. 2019;45(5):567-77. https://doi.org/10.1016/j.joen.2019.02.004
PMid:30905573
4. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K,
et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996;29(2):125-30. https://doi.org/10.1111/j.1365-2591.1996.tb01173.x
PMid:9206436

5. Bains RB, Verma P, Pandey PT. Systematic review of the antimicrobial efficacy of triple antibiotic paste used as intra-canal medicament in teeth with primary endodontic infection. Asian J Oral Heal Allied Sci. 2021;11(2):1-7. https://doi.org/10.25299/AJOHAS_3_2021

6. Do Couto AM, Espaladori MC, Leite AP, Martins CC, de Aguilar MC, Abreu LG. A systematic review of pulp revascularization using a triple antibiotic paste. Pediatr Dent. 2019;41(5):341-53.
PMid:31648664

7. Montero-Miralles P, Martín-González J, Alonso-Espeleota O, Jiménez-Sánchez MC, Velasco-Ortega E, Segura-Egea JJ. Effectiveness and clinical implications of the use of topical antibiotics in regenerative endodontic procedures: A review. Int Endod J. 2018;51(9):918-88. https://doi.org/10.1111/iej.12913
PMid:29480932

8. Jung HJ, Seo I, Jha BK, Suh SI, Suh MH, Baek WK. Minocycline inhibits angiogenesis in vitro through the translational suppression of HIF-1α. Arch Biochem Biophys. 2014;545:74-82. http://dx.doi.org/10.1016/j.abb.2013.12.023
PMid:24412777

9. Dos Santos LG, Chisina LA, Springmann CG, de Souza BD, Pappen FG, Demarco FF, et al. Alternative to avoid tooth discoloration after regenerative endodontic procedure: A systematic review. Braz Dent J. 2018;29(5):409-18. https://pmid:30517438

10. Dubey N, Xu J, Zhang Z, Nör JE, Bottino MC. Comparative evaluation of the cytotoxic and angiogenic effects of minocycline and clindamycin. An in vitro study. J Endod. 2019;45(7):882-9. https://doi.org/10.1016/j.joen.2019.04.007
PMid:31133343

11. Karczewski A, Feitosa SA, Hamer EI, Pankajakshan D, Gregory RL, Spolnik KJ, et al. Clindamycin-modified triple antibiotic nanofibers: A stain-free antibacterial intracanal drug delivery system. J Endod. 2018;44(1):155-62. https://doi.org/10.1016/j.joen.2017.08.024
PMid:29061356

12. Sabrah HA, Yassen GH, Liu W, Goebel W, Gregory RP. The effect of diluted triple and double antibiotic pastes on dental pulp stem cells and established Enterococcus faecalis biofilm. Clin Oral Investig. 2015;19(8):2059-66. https://doi.org/10.1007/s00784-015-1423-6
PMid:25689981

13. Alghilan MA, Windsor LJ, Palasuk J, Yassen GH. Attachment and proliferation of dental pulp stem cells on dentine treated with different regenerative endodontic protocols. Int Endod J. 2017;50(7):667-75. https://doi.org/10.1111/iej.12669
PMid:27272393

14. Negm MI, El-Shafei JM, Roshdy NK, Eid GE. Determination of mesenchymal stem cell origin during bleeding-induced regenerative endodontic procedure using 2-step real-time reverse-transcription polymerase chain reaction (qRT-PCR). Acta Sci Dent Sci. 2018;2(6):5-10.

15. Mallishery S, Shah T. Regenerative endodontics looking inward. J Adv Med Med Res. 2020;32(7):83-98. https://doi.org/10.9734/jammr/2020/v32i730454

16. Seo SJ, Kim HW, Lee JH. Electrospun nanofibers applications in dentistry. J Nanomater. 2016;2016:5931946. https://doi.org/10.1155/2016/5931946

17. Albuquerque MT, Ryan SJ, Münchow EA, Kamocka MM, Gregory RL, Valera MC, et al. Antimicrobial effects of novel triple antibiotic paste-mimic scaffolds on actinomyces naeslundii biofilm. J Endod. 2015;41(8):1337-43. https://doi.org/10.1016/j.joen.2015.03.005
PMid:25917945

18. Albuquerque MT, Evans JD, Gregory RL, Valera MC, Bottino MC. Antibacterial TAP-mimic electrop spun polymer scaffold: Effects on P. gingivalis-infected dentin biofilm. Clin Oral Investig. 2016;20(2):387-93. https://doi.org/10.1007/s00784-015-1577-2
PMid:26319981

19. Porter ML, Münchow EA, Albuquerque MT, Spolnik J, Hara AT, Bottino MC. Effects of novel 3D antibiotic-containing electrop spun scaffoldson dentin discoloration. J Endod. 2017;42(1):106-12. https://doi.org/10.1016/j.joen.2016.09.013
PMid:26602451

20. Pankajakshan D, Albuquerque MT, Evans JD, Kamocka MM, Gregory RL, Bottino MC. Triple antibiotic polymer nanofibers for intracanal drug delivery: Effects on dual species biofilm and cell function. J Endod. 2016;42(10):1490-5. https://doi.org/10.1016/j.joen.2016.07.019
PMid:27663615

21. Azabi A. The Antimicrobial Efficacy of Innovative 3D Triple Antibiotic Paste-mimic Tubular Scaffold against Actinomyces Naeslundii. Doctoral Dissertation, Indiana University; 2015. http://dx.doi.org/10.17912/C2/1607

22. Kumar S. Nanofiber incorporated intracanal medicaments and its antibacterial effect against Enterococcus faecalis biofilm in an in vitro study. Sifonoforos. 2019;1(12):1-4. Available from: http://repository-tmmgru.ac.in/epirnten/10530 [Last accessed on 2021 Sep 10].

23. Vaishali A, Varma KM, Bhupathi PA, Bharath TS, Ramesh MV, Varma PV. In vitro evaluation of antimicrobial efficacy of 2% chlorhexidine loaded electrop spun nanofibers. J Pierre Fauchard Acad (India Sect). 2017;31(2-4):105-8. https://doi.org/10.1016/j.jpfa.2017.01.006

24. Verma P, Chaturvedi T, Gupta V, Srivastava R, Srivastava A. Evaluation of metronidazole nanofibers in patients with chronic periodontitis: A clinical study. Int J Pharm Investig. 2012;2(2):213. Available from: http://www.ijpionline.org/text.asp?2012/2/2/107/100707

25. de-Jesus-Soares A, Prado MC, Nardello LC, Pereira AC, Cerqueira-Neto AC, Nagata JY, et al. Clinical and molecular microbiological evaluation of regenerative endodontic procedures in immature permanent teeth. J Endod. 2020;46(10):1445-54. https://doi.org/10.1016/j.joen.2020.07.005
PMid:32681848

26. Nagata JY, Soares AJ, Souza-Filho FJ, Zaia AA, Ferraz CC, Almeida JF, et al. Microbiological evaluation of traumatized teeth treated with triple antibiotic paste or calcium hydroxide for 2% chlorhexidine gel in pulp revascularization. J Endod. 2014;40(6):778-83. https://doi.org/10.1016/j.joen.2014.01.038
PMid:24862703

27. Arruda ME, Neves MA, Diogenes A, Mdalá I, Guilherme BP, Siqueira JF, et al. Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: A randomized clinical trial. J Endod. 2018;44(10):1474-9. https://doi.org/10.1016/j.joen.2018.07.005
PMid:30144986

28. Pereira AC, de Oliveira ML, Cerqueira-Neto AC, Gomes BP, Ferraz CC, de Almeida JF, et al. Treatment outcomes of pulp revascularization in traumatized immature teeth using calcium hydroxide and 2% chlorhexidine gel as intracanal medication. J Appl Oral Sci. 2020;28(55):e20200217. https://doi.org/10.1590/1678-7757-2020-0217

29. Teixeira MA, Amorim MT, Felgueiras HP. Poly(vinyl alcohol)-based nanofibrous electrop spun scaffolds for tissue engineering
30. Pillay V, Dott C, Choonara YE, Tyagi C, Tomar L, Kumar P, et al. A review of the effect of processing variables on the fabrication of electrospun nanofibers for drug delivery applications. J Nanomater. 2013;2013:789289. https://doi.org/10.1155/2013/789289

31. Vlachou M, Siamidi A, Kyriakou S. Electrospinning and drug delivery. In: Electrospinning and Electrospraying Techniques and Applications. London: Intech Open Books; 2019. p. 1-22. https://doi.org/10.5772/intechopen.86181

32. Rossi-Fedele G, Kahler B, Venkateshbabu N. Limited evidence suggests benefits of single visit revascularization endodontic procedures a systematic review. Braz Dent J. 2019;30(6):527-35. https://doi.org/10.1590/0103-6440201902670

33. American Association of Endodontics. Clinical Considerations for a Regenerative Procedure (New, Revised 6-18-16). Chicago, Illinois: American Association of Endodontics; 2016. p. 1-6. Available from: http://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderations.pdf [Last accessed on 2016 Oct 21].

34. Sathorn C, Parashos P, Messer HH. How useful is root canal culturing in predicting treatment outcome? J Endod. 2007;33(3):220-5. https://doi.org/10.1016/j.joen.2006.11.006

35. Cameron R, Claudia E, Ping W, Erin S, Ruparel NB. Effect of a residual biofilm on release of transforming growth factor β1 from dentin. J Endod. 2019;45(9):1119-25. https://doi.org/10.1016/j.joen.2019.05.004

36. El-Tayeb MM, Abu-Seida AM, El Ashry SH, El-Hady SA. Evaluation of antibacterial activity of propolis on regenerative potential of necrotic immature permanent teeth in dogs. BMC Oral Health. 2019;19(1):174. https://doi.org/10.1186/s12903-019-0835-0

37. Sarhan WA, Azzazy HM, El-Sherbiny IM. Honey/chitosan nanofiber wound dressing enriched with allium sativum and cleome droserifolia: Enhanced antimicrobial and wound healing activity. ACS Appl Mater Interfaces. 2016;8(10):6379-90. https://doi.org/10.1021/acsami.6b00739

38. He P, Li Y, Huang Z, Guo ZZ, Luo B, Zhou CR, et al. A multifunctional coaxial fiber membrane loaded with dual drugs for guided tissue regeneration. J Biomater Appl. 2020;34(8):1041-51. https://doi.org/10.1177%2F0885328219894001

39. Sarhan WA, Azzazy HM. Apitherapeutics and phage-loaded nanofibers as wound dressings with enhanced wound healing and antibacterial activity. Nanomedicine. 2017;12(17):2055-67. https://doi.org/10.2217/nmm-2017-0151

40. Sabharwal S, Bhagat SK, Gami KS, Siddhartha A, Rai K, Ahluwalia Y. An in vivo study to compare anti microbial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on microorganisms in the root canal of immature teeth. J Int Soc Prev Community Dent. 2019;9:263-8. https://doi.org/10.4103/jispcd.JISPCD_400_18

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