Systemactic Review

Cytocompatibility and Antimicrobial Efficacy of Antibiotic Eluting Nanofibrous Scaffolds in Regenerative Endodontics: A Systematic Review

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Abstract

The integration of tissue engineering advancements in regenerative endodontic procedures has opened the doors to many exciting possibilities in the domain of scaffolds. Nanofibrous scaffolds – a tissue engineering marvel – have been used successfully for local drug delivery in wound dressing that has found its place in regenerative endodontic procedures. Its special properties due to its porous 3D structure allow it to become a perfect vehicle for triple antibiotic pastes. This not only eliminates the cytotoxicity, but also maintains the antibacterial properties of the latter. The aim of this systematic review is to evaluate the antimicrobial efficacy of antibiotic eluting nanofibers and its effect on human dental pulp stem cell (hDPSCs) based on published *in vitro* studies. This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. An electronic literature search on all publications was performed by two independent researchers across Google Scholar, PubMed, and Science Direct. These articles were selected to answer the following research question – are antibiotic eluting nanofibers effective against multispecies biofilms? The data were extracted by two independent researchers. The search resulted in 316 titles from all databases, out of which 12 studies met the inclusion criteria. The results showed that antibiotic eluting nanofibers containing a combination of metronidazole, ciprofloxacin, and minocycline show antibacterial efficacy against multispecies biofilms while maintaining the viability of the hDPSCs. Only metronidazole-containing scaffolds were shown to have no cytoxicity against hDPSCs, but its antibacterial was limited only to *Porphyromonas gingivalis*. The result showed the potential of antibiotic-eluting nanofibrous scaffolds in regenerative endodontic procedures as antibacterial agents against offending organisms commonly found in root canals.

Keywords: Antibiotic-eluting nanofibrous scaffolds, Electrospinning, Polydiaxone suture, Triple antibiotic pastes.

Introduction

The treatment of immature permanent teeth whose pulps have been necrosed as a result of traumatic injury or dental caries has always posed a challenge. Earlier, they were subjected to apexification using calcium hydroxide or MTA which led to the formation of a hard tissue barrier, which did not allow for the continued development of root. In recent years, the focus has shifted to regenerative endodontic procedures to regain pulp vitality and the continued development of the root in length, as well as thickness in immature permanent teeth with necrosed pulps.[1]

Regenerative endodontics – a subset of tissue engineering – is a triad of biomimetic scaffolds, bioactive growth factors, and stem cells which can be defined as a "biologically based procedure designed to replace damaged tooth structures, including dentine and root structures, as well as cells of the pulp-dentine complex."[2]

Access this article online

Website: www.jrad.co.in

DOI: https://doi.org/10.53064/jrad.2022.14.6.429

It can be roughly divided into two categories cell-free and cell-based regenerative endodontic therapy, the day-to-day clinical practice usually adopts the cell-free type whereas the cell-based approach is still in the clinical trial stage.[3]

These procedures will not be successful without a thorough disinfection of the root canal space, which can be achieved through mechanical instrumentation, as well as the judicious use of antibiotic pastes. Antibiotic pastes are effective in disinfecting necrotic infected pulps as well as creating

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Received: Aug. 07, 2023; **Accepted:** Sep. 10, 2023; **Published:** Sep. 18, 2023

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How to cite this article: Kumar NK, Brigit B, Bhumralkar S, Naik SB, Merwade S, Bhargavi H. Cytocompatibility and Antimicrobial Efficacy of Antibiotic Eluting Nanofibrous Scaffolds in Regenerative Endodontics: A Systematic Review. J Res Adv Dent 2023;14(6):1-10.

an environment conducive to regenerative processes, but one of their main demerits is a detrimental effect on the viability of stem cells of apical papilla when used in higher concentrations.[4]

Nanofibrous scaffolds, a product of tissue engineering, are not a novel concept in the field of medicine and it has been previously used as a local drug delivery system in wound dressings for its bioactivity.[5]

In REP, it acts as a bioactive scaffold due to its unique 3D structure that imitates the extracellular matrix allowing a sustained drug delivery. The method of manufacturing nanofibrous scaffolds is through electrospinning or phase separation, which permits the operator to control the fiber morphology.[6]

The structure of the resulting nanofibrous scaffold allows for a substantive release (range of 70–1000 mg) of the antibiotics similar to that seen in chlorhexidine. The controlled release will not only allow for a prolonged action of the antibiotic, but also reduce the cytotoxic effect of the antibiotic on human dental pulp stem cells (hDPSCs), permitting the apical closure of root in immature permanent tooth.[7]

Extensive research is being conducted to manufacture and customize patient-specific 3-D bioactive scaffolds loaded with antibiotic-eluting fibers. This review aimed to provide a systematic evaluation of published *in vitro* and animal studies on antibiotic-eluted nanofibrous scaffolds, their effect on biofilms commonly found in the root canal system, and the viability of hDPSCs.

Materials and Methods

The literature review was constructed on the basis of Preferred Reporting Items for Systematic reviews and Meta-analyses statement as shown in Table 1 PRISMA 2020 CHECKLIST. The articles were selected to address the following research questions:

Table 1: PRISMA 2020 checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Headine
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	-
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Lines 30-33 introduction
Objectives	4	Provide an explicit statement of the objective (s) or question (s) the review addresses.	materials and methods
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	subheading search strategy and study selection
Information sources	6	Specify all databases, registers, websites, organizations, reference lists, and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Under the subheading search strategy and study selection
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used.	Under the subheading search strategy and study selection
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Under the subheading data extraction
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	-
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	-
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Under Table 1

(*Contd*...)

Table 1: (*Continued*)

Section and Topic	Item #	Checklist item	Location where item is reported
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool (s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Under quality assessment
Effect measures	12	Specify for each outcome the effect measure (s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	Under Table 2
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	-
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice (s). If meta-analysis was performed, describe the model (s), method (s) to identify the presence and extent of statistical heterogeneity, and software package (s) used.	No statistical analysis performed
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	-
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	-
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Flow diagram under the subheading "search strategy and study selection"
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Data extraction
Study characteristics	17	Cite each included study and present its characteristics.	Table 2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table 1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 2
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	-
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	-
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	-
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	Under the subheading discussion
	23c	Discuss any limitations of the review processes used.	Under the subheading "discussion"
	23d	Discuss implications of the results for practice, policy, and future research.	Under the subheading discussion

(*Contd*...)

Table 1: (*Continued*)

Section and Topic	Item #	Checklist item	Location where item is reported
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	-
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	-
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	-
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Not applicable
Competing interests	26	Declare any competing interests of review authors.	NOT APPLICABLE
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

1. Are antibiotic-eluting nanofibers effective against multispecies biofilms?
2. Do they have an effect on the viability of hDPSCs for regenerative endodontic procedures?

Search strategy and study selection

A literature search on all publications was performed by two independent researchers without a time limit across three independent databases – PUBMED/MEDLINE, Science Direct, and Google Scholar. The language was limited to English. The following keywords were used for search strategies: (((NANOFIBROUS SCAFFOLDS) AND ANTIBIOTIC ELUTING FIBERS) OR BIOACTIVE SCAFFOLDS OR ELECTROSPINNING OR PHASE SEPARATION) AND ((DENTIN PULP REVASCULARISATION OR REGENERATIVE ENDODONTICS).

The inclusion criteria for the *in vitro* studies included the following points:

1. Nanofibrous scaffolds should be incorporated with antibiotic-eluted fibers
2. The studies should be relevant toward regenerative endodontic procedures only
3. The studies should be in the English language
4. The studies included were taken from year 2010 to 2018.

Data extraction

The search resulted in 316 articles across all databases. After removing 12 duplicates, 275 out of 304 records were rejected on the basis of title and abstract screening. The remaining 29 records were scrutinized, out of which two were eliminated as the full texts were unavailable. On further evaluation, 15 of the 27 remaining records were dismissed on the basis of unspecified methodology. The remaining 12 studies were subjected to quality assessment. All qualified publications were written in English as shown in Figure 1.

Table 2: Risk of bias

Author	Score	Risk of bias	Reason for scoring
Feng *et al*. 2010	8	Low	All criteria met
Bottino *et al*. 2013	8	Low	All criteria met
Palasuk *et al*. 2014	8	Low	All criteria met
Kamocki *et al*. 2015	6	Moderate	Scaffold characterization not mentioned
Albuquerque *et al*. 2015	6	Moderate	Scaffold characterization not mentioned
Albuquerque *et al*. 2015	8	Low	All criteria met
Pankajakshan *et al*. 2016	6	Moderate	Scaffold characterization not mentioned
Kamocki *et al*. 2016	6	Moderate	Inoculation of dentin slices not mentioned
Albuquerque *et al*. 2016	6	Moderate	Scaffold characterization not mentioned
Albuquerque *et al*. 2017	6	Moderate	Scaffold characterization not mentioned
Karczewski *et al*. 2018	7	Low	All criteria met
Bottino *et al*. 2018	7	Low	All criteria met

Quality assessment

A customized risk of bias questionnaire consisting of eight parameters was devised on the basis of OHAT Risk of Bias tool. All 12 articles met the quality assessment criteria and were selected for the systematic review. Two investigators independently reviewed and scored the articles for quality assessment. One animal study is included in this systematic review and it follows the ARRIVE guidelines for reporting animal research. A scoring of (0–3) presented with high risk, (4–6) moderate risk, and[7,8] low risk of bias as shown in Table 2.

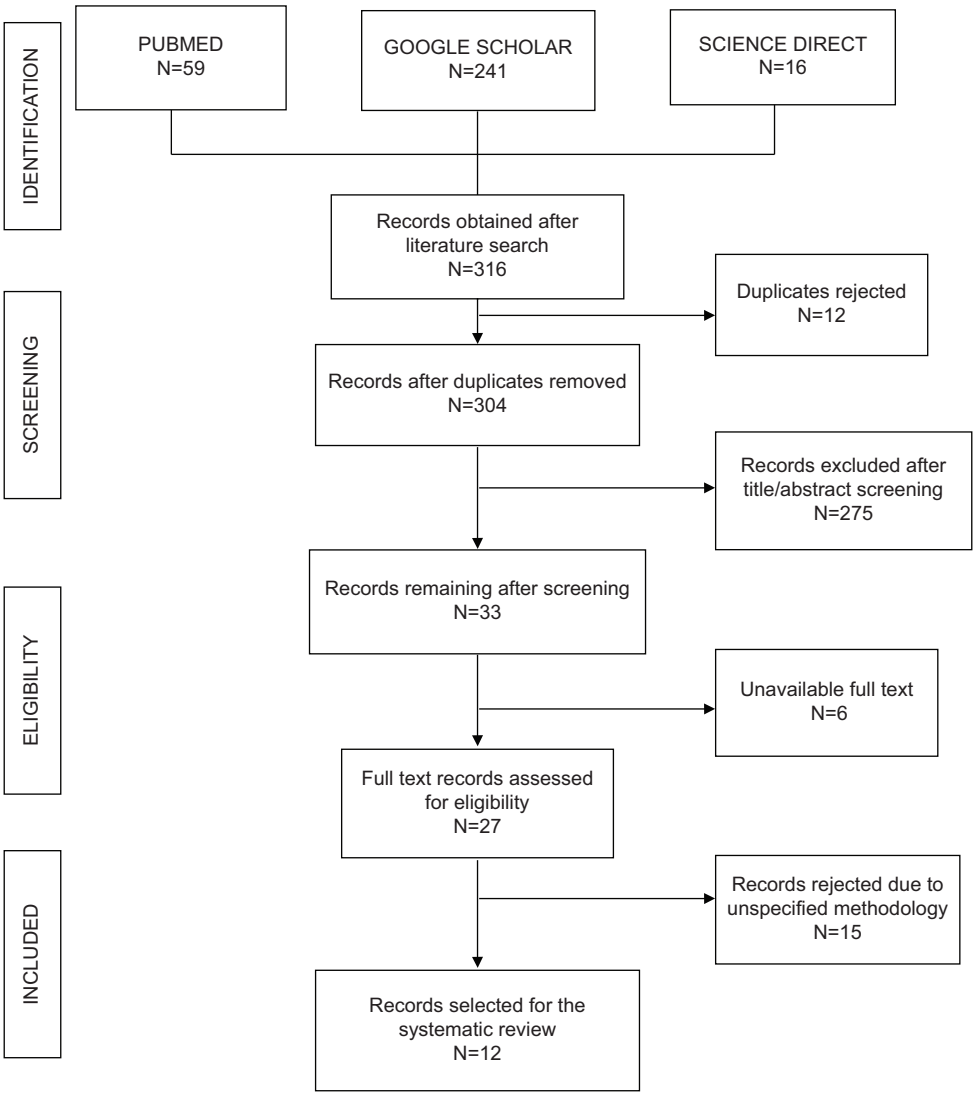


Figure 1: Flowchart for search strategy and study selection

Quality assessment criteria

1. Use of dentin slices with similar dimensions
2. Description of sample size collection
3. Did selection of study participants result in appropriate comparison groups?
4. Did the study account for any confounding variables?
5. Were experimental conditions identical along all study groups?
6. Was there evidence of incomplete data? If so, was it accounted for?
7. Is the method of nanofiber production specified?
8. Was the scaffold characterization elaborated?

Data regarding the fabrication of nanofibrous scaffolds, the type of material used, research methodology, and antibacterial efficacy against established biofilms as well as the cytotoxic effect of antibacterial-eluted nanofibrous scaffolds were subjected to evaluation as given in Table 3.

Discussion

This review investigated the action of antibiotic eluting nanofibers on multispecies biofilms commonly found in the root canal system as well as its effect on the viability of hDPSCs. All but one study included has been performed *in vitro*. Results were consistent across all studies.

Nanofibrous scaffolds are manufactured either by electrospinning or phase separation, which are processes by which polymers can be drawn into fibers imitating the complex porosities found in an extracellular matrix. The complex structures become excellent vehicles for drug delivery of antibiotics, allowing a sustained drug release, as well as counteracting the cytoxicity of antibiotics on hDPSCs, which are the backbone of regenerative endodontic procedures.

They are commonly manufactured using polydioxanone (PDS) suture material, which resulted in an average suture

Table 3: Results

Author/year	Scaffold used and cytocompatibitlity	Scaffold parameters	Antibiotics used	Micro-organisms tested against	Study design	Results
Feng *et al*. 2010[8]	PLGA nanospheres post seeded into PLLA scaffolds (Phase separation, sugar leaching technique)	Pore diameter: 250–425 µm Sphere diameter: 280 nm–410 nm Based on molecular weight and LA/GA ratio: PLGA50–6.5K PLGA50–64K PLGA75–113K PLGA85–142K	Doxycycline	*S. aureus* *E. coli* Bacterial inhibition calculated Bacterial inhibition (%)= abs *(Ic-Is)*/*(Ic)* × 100	In-*vitro*	Diameter of scaffold: PLGA50-6.5K NS smallest diameter-300 nm PLGA85-142K NS had the largest diameter Release of doxycycline: PLGA50-6.5K NS-scaffold PLGA50-64K NS-scaffold 1st day- 70% DOXY 2 weeks-95% DOXY PLGA75-113K NS-scaffold 1st day-50% DOXY 2 weeks-85% DOXY 6 weeks-95% DOXY PLGA85-142K NS 1st day-25% DOXY 6 weeks-80% DOXY Antibacterial efficacy: 70% inhibition for *S.aureus* 40% inhibition for *E. coli* at day 42
Bottino *et al*. 2013[9]	Polydioxanone Electrospinning	Fiber diameter: MET-1000–1100 nm approx. CIP–0.800–900 nm approx. Tensile strength: MET-4.5–6.5 Mpa CIP-2.5–5.5 Mpa	Ciprofloxacin Metronidazole	*P. gingivalis* *E. faecalis* Spiral plating	*In vitro*	Drug release % — 48 h 5 wt% CIP-22.4% — 8.6±0.78 µg 25 wt% CIP–27.52% — 57.5±6.3 µg 5 wt% MET-51.4% — 28.68±2.3 µg 25 wt% MET-44.6% — 88.6 µg Zone of inhibition • All CIP-containing mats significantly inhibited growth of both *Ef* and *Pg* • MET-containing mats significantly inhibited the growth of *Pg* at 25 wt% MET
Palasuk *et al*. 2014[10]	Polydioxanone Electrospinning Cytocompatibility test: hDPSCs grown in Low glucose *Dulbecco's Modified Eagle's Medium*	Fiber diameter: 541.43±246.73 nm - 770.09±295.71 nm Tensile strength (Mpa): 3.45±0.51-1:3MET/CIP 3.53±0.52-3:1MET/CIP 4.32±0.53-PURE PDS 4.38±0.46-MET only 5.13±0.67-CIP only 6.05±0.63 1:1MET/CIP	Metronidazole Ciprofloxacin	*E. faecalis*-Tryptic soy broth *P. gingivalis*-Brain Heart Infusion *F. nucleatum*-Brain Heart Infusion	*In-vitro*	• Tensile strength of G3 (1:1 MET/CIP) significantly greater than pure PDS • A significant reduction in cell viability as compared to Pure PDS Zones of inhibition Antibiotic-containing scaffolds inhibited growth of *Ef, Pg* and *Fn*, Pure MET did not inhibit the growth of bacteria G2 – CIP only G3 – 1:1 MET / CIP } greatest zones of inhibition G4 – 1:3MET / CIP

(*Contd...*)

Table 3: (*Continued*)

Author/year	Scaffold used and cytocompatibitlity	Scaffold parameters	Antibiotics used	Micro-organisms tested against	Study design	Results
Kamocki *et al.* 2015[7]	Polydioxanone Electrospinning Cytocompatibility test: hDPSCs grown in Low glucose Dulbecco's Modified Eagle's Medium Harvested by: Trryptinization WST 1 assay: Effect of *CIP* on hDPSCs	-	Ciprofloxacin	*E. faecalis* grown in-Brain Heart Infusion	*In vitro*	• Cytotoxicity assays revealed decrease in cell viability between 5% and 25% CIPwt • No inhibitory effects seen on hDPSCs between 1 and 2.5%CIPwt • Significant inhibition of *E. faecalis* at a concentration between 1% and 2.5%
Albuquerque *et al.* 2015[13]	Polydioxanone Electrospinning	-	Ciprofloxacin	Infected dentine slices inoculated with *E. faecalis*	*In-vitro*	Dentine specimens infected with *E. coli*- PDS+25% CIP was able to completely eliminate the bacterial surface From SEM analysis.
Albuquerque *et al.* 2015[14]	Polydioxanone-electrospinning	Fiber diameter (SEM) TAP mimic-718–125 nm Tensile strength TAP Mimic – 2–3 Mpa PDS-3–4 Mpa	Metronidazole Ciprofloxacin Minocycline	Dentine slices were inoculated with *A. naeslundii*	*In-vitro*	Dentin specimens infected with *A. neaslundii* -most of the colonies were eliminated (dentine discolouration seen due to formation of agglomerates) Drug release in microgram Day 1 / Day 3 / Day 7 / Day 14 / Day 28 MET 1000 / 931.8 / 811.2 / 742.4 / 687.6 CIP 850.3 / 853.9 / 667.2 / 619.6 / 528.2 MINO 712.2 / 410.2 / 69.8 / 0 / 0
Pankajakshan *et al.* 2016 [15]	Polydioxanone Electrospinning Cytocompatibility test: hDPSCs cultured in alpha-minimum essential medium		Metronidazole Ciprofloxacin Minocycline	Dentin slices inoculated with *A. naeslundii* and *E. faecalis*	*In-vitro*	CLSM analysis showed Triple antibiotic containing nanopolymer showed a significant reduction in *A. naeslundii* and *E. faecalis* Qualitative representative CLSM images showed enhanced DPSC adhesion and spreading on triple antibiotic-containing treated dentin when compared with TAP
Kamocki *et al.* 2016[7]	Polydioxanone Electrospinning Cytocompatibility test: hDPSCs grown in Low glucose Dulbecco's Modified Eagle's Medium Harvested by: Tryptinization WST 1 assay: Effect of antimicrobial agent on hDSPCs LIVE/DEAD® assay: The effects of antibiotic-containing scaffolds on hDPSC viability	Fiber diameter-(SEM): Sub-micron fibre diameter, smaller than pure PDS	Metronidazole Ciprofloxacin	-	*In-vitro*	Drug release after 14 days The mean MET release pure MET-1870±663.1 µg/69% 3:1 MET/CIP-1260±229 µg/63% 1:1 MET/CIP-1068±305.9 µg/93% 1:3 MET/CIP-536±71.6 µg The mean CIP release pure CIP-966±166.6 µg/32% 3:1 MET/CIP-418±96.8 µg/63% 1:1 MET/CIP-674±105 µg 1:3 MET/CIP-900±96.8 µg CELL VIABILITY Higher % of dead cells, was observed after exposure to CIP-containing scaffold Groups exposed to MET and pure PDS, showed no change in proliferation

(*Contd...*)

Table 3: (*Continued*)

Author/year	Scaffold used and cytocompatibitlity	Scaffold parameters	Antibiotics used	Micro-organisms tested against	Study design	Results
Albuquerque *et al*. 2016[17]	Polydioxanone-Electrospinning	-	Metronidaxole Ciprofloxacin Minocycline	Dentine slices Inoculated with *P. gingivalis* and cultured onto blood agar plates to measure Bacterial counts log10 CFU/mL	*In-vitro*	Infected dentine slices SEM images indicate that Dentine specimens infected with *P. gingivalis* - 25% TAP mimic scaffold were able to completely eliminate bacteria bacteria were not considered viable due to the observation of irregularities and porosities in their membrane surfaces Culture plates 25 wt.% TAP-mimic polymer scaffold and the positive control (i.e., TAP solution) led to complete bacterial elimination
Albuquerque *et al*. 2017[18]	Polydioxanone-Electrospinning	-	Metronidazole Ciprofloxacin Minocycline	Dentine slices Inoculated with *E. coli*, *A. naeslundii* and *S. sanguinis*	*In-vitro*	CLSM analysis showed that Dentin slices infected with *E. coli*, *A. naeslundii* and *S. sanguinis* were completely eliminated in the group with antibiotic eluted nanofibrous scaffolds
Karczewski *et al*. 2018[11]	Polydioxanone Electrospinning Cytocompatibility test: hDPSCs grown in Low glucose Dulbecco's Modified Eagle's medium	Fiber diameter-(SEM): CLIN- 352±128 nm CLIN-m- 349±128 nm Tensile strength: CLIN- 7.2±1.0 MPa CLIN-m fibers 2.60±1.6 MPa	Clindamycin Metronidazole Ciprofloxacin	Cultured in tryptic soy broth *A. naeslundii* *E. faecalis* Cultured in BHI *A. actinomycetemcomitans* *F. nucleatum*	*in-vitro*	Antimicrobial activity A fairly homogeneous and consistent antimicrobial activity with a slight decrease in inhibition over 21 days was seen Dentin discolouration Dentin discolouration not noted in CLIN/CLINm group Cytotoxicity Cytotoxicity was noted both Clin and CLIN-m groups but the latter showed cell viability above 50 and 70%
Bottino *et al*. 2018[12]	Polydioxanone electrospinning	Fiber diameter-(SEM): Antibiotic free (1026.1 [320.6–2295.2 nm]) Triple antibiotic-eluting fibers (898.5 [625.7–1799 nm])	Clindiamycin Metronidazole Ciprofloxacin	*In-vitro* Dentin slices inoculated with *A. naeslundii* *In-vivo* Sterile sponges soaked in 0.9% saline and mixed with supragingival plaque from dog's teeth placed into ten immature permanent double-rooted Upper and lower premolars, Teeth were left open (non-restored) to the oral cavity	*In-vitro* *In-vivo*	Antimicrobial activity (CSLM and SEM) The scaffold is patient specific and modifiable *In-vitro*- showed 99.1–99.4% reduction of bacterial cell viability of *A. naeslundii*. TAP showed 100% reduction in bacterial viability *In vivo* (Canine model-periapical disease induced) - TAP treated group – complete apical closure (osteodentin like tissue noted) TA-3D$_c$- Complete apical closure (osteodentin like tissue noted)

E. faecalis: *Enterococcus faecalis*, *A. naeslundii*: *Actinomyces naeslundii*, *P. gingivalis*: *Porphyromonas gingivalis*, *F. nucleatum*: *Fusobacterium nucleatum*, *S. sanguinis*: *Streptococcus sanguinis*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

diameter of 350–1100 nm, and an average tensile strength of 3.43 MPa–7.6 MPa.[9-12]

Six studies included in this review employed triple antibiotic paste or its modification in which clindamycin replaced minocycline, three studies employed double antibiotic paste that contained a combination of ciprofloxacin and metronidazole, and three studies included only doxycycline or ciprofloxacin. There was no difference in antibiotic efficacy in any of combinations of the above-mentioned drugs. The antibiotic-eluting nanofibers were able to inhibit the growth of either one or all of the following bacterial films, that is, *Enterococcus faecalis*, *Actinomyces naeslundii*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Streptococcus sanguinis*.[7-15]

According to the study by Feng *et al*., doxycycline, when incorporated into PLLA scaffolds seeded with PLGA nanospheres, inhibited 40% and 70% growth of *E. faecalis* and *S. sangunis* respectively, as compared to plain NS scaffolds. The DOXY-loaded NS scaffold, apart from its antibacterial efficacy, showed a two-pronged result, that is, an osteoconductive effect as well as a sustained drug delivery of doxycycline over 6 weeks after an initial burst, showing substantivity similar to 0.12% chlorhexidine.[8] Ciprofloxacin and metronidazole, when incorporated into nanofibrous scaffolds, showed a controlled release of the drug from 48 h to 28 days.[7,9,14] In addition, all ciprofloxacin-containing scaffolds were able to inhibit the growth of *E. faecalis*, *P. gingivalis*, and *F. nucleatum*.[9,10]

Pure PDS-containing scaffolds did not inhibit the growth of bacteria. Concomitantly, it did not seem to have an adverse effect on hDPSCs. Pure metronidazole, when incorporated into scaffolds, had the potential to behave as a "potential stem cell proliferation inducer" as it can modulate proliferation through the release of inflammatory cytokines."[7]

Metronidazole is effective against *P. gingivalis* but not against *E. faecalis*, as the former is an obligate anaerobe and the latter, a facultative anaerobe.[16]

Triple antibiotic paste, when used on its own at a concentration of 1 mg/dL, is highly cytotoxic toward hDPSCs. Subsequently, when it is incorporated into nanofibrous scaffolds at a concentration of 1–2.5% CIP by weight, it showed no cytotoxic effects, while its antibacterial efficacy against *E. faecalis* remained constant.[7] Similarly, bimix antimicrobial-eluting nanofibers used showed no cytotoxicity (>90% cell viability) or slight toxicity (60–90% cell viability) without affecting the antibacterial activity.[10]

One of the drawbacks of using triple antibiotic pastes is the potential dentinal staining caused by minocycline. This was counteracted in a study in which minocycline was replaced by clindamycin-modified triple antibiotic pastes. The antibacterial efficacy as well as the strength of the modified triple antibiotic eluting nanofibers remained unaffected.[11]

An *in vitro* as well as *in vivo* study conducted by Bottino *et al*. in 2018 proved the efficacy of TAP in combination with PDS nanofibrous scaffolds against *A. naeslundii* – an offending microorganism found in open apex cases – with a 99.4% reduction in bacterial viability. In addition, when periapical disease was induced in a canine model, both TAP and TAP-3D scaffold created an appropriate environment for the apical closure of the immature apex by deposition of osteodentin. It paves the way for future studies to follow similar disinfection strategies for the closure of immature permanent teeth using nanofibrous scaffolds.

Conclusion

The literature review shows the potential of antibiotic-eluting nanofibrous scaffolds in regenerative endodontic procedures as antibacterial agents against offending organisms commonly found in root canals. A controlled release of antibiotics from the scaffolds at a specific concentration shows antibacterial efficacy while maintaining the viability of hDPSCs. Metronidazole, a commonly used antibiotic, seems to be effective against *P. gingivalis*, but not against *E. faecalis*. Hence, a combination drug therapy with nanofibrous scaffolds is recommended for a better antibacterial effect. To date, studies have been performed in the laboratory on extracted teeth. There is a need to perform further studies, particularly *in vivo* studies.

Acknowledgment

The authors deny any conflicts of interest with respect to this study.

References

1. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: A way forward. J Am Dent Assoc 2016;147:372-80.
2. Lin LM, Kahler B. A review of regenerative endodontics: Current protocols and future directions. J Istanb Univ Fac Dent 2017;51 3 Suppl 1:S41-51.
3. Lin LM, Huang GT, Sigurdsson A, Kahler B. Clinical cell-based versus cell-free regenerative endodontics: Clarification of concept and term. Int Endod J 2021;54:887-901.
4. Gathani KM, Raghavendra SS. Scaffolds in regenerative endodontics: A review. Dent Res J (Isfahan) 2016;13:379-86.
5. Pilehvar-Soltanahmadi Y, Dadashpour M, Mohajeri A, Fattahi A, Sheervalilou R, Zarghami N. An overview on application of natural substances incorporated with electrospun nanofibrous scaffolds to development of innovative wound dressings. Mini Rev Med Chem 2018;18:414-27.
6. Smith LA, Ma PX. Nano-fibrous scaffolds for tissue engineering. Colloids Surf B Biointerfaces 2004;39:125-31.
7. Kamocki K, Nör JE, Bottino MC. Dental pulp stem cell responses to novel antibiotic-containing scaffolds for regenerative endodontics. Int Endod J 2015;48:1147-56.
8. Feng K, Sun H, Bradley MA, Dupler EJ, Giannobile WV, Ma PX. Novel antibacterial nanofibrous PLLA scaffolds. J Control Release 2010;146:363-9.
9. Bottino MC, Kamocki K, Yassen GH, Platt JA, Vail MM, Ehrlich Y, *et al.* Bioactive nanofibrous scaffolds for regenerative endodontics. J Dent Res 2013;92:963-9.
10. Palasuk J, Kamocki K, Hippenmeyer L, Platt JA, Spolnik KJ, Gregory RL, *et al.* Bimix antimicrobial scaffolds for regenerative endodontics. J Endod 2014;40:1879-84.

11. Karczewski A, Feitosa SA, Hamer EI, Pankajakshan D, Gregory RL, Spolnik KJ, *et al.* Clindamycin-modified triple antibiotic nanofibers: A stain-free antimicrobial intracanal drug delivery system. J Endod 2018;44:155-62.
12. Bottino MC, Albuquerque MT, Azabi A, Münchow EA, Spolnik KJ, Nör JE, *et al.* A novel patient-specific three-dimensional drug delivery construct for regenerative endodontics. J Biomed Mater Res B Appl Biomater 2019;107:1576-86.
13. Albuquerque MT, Valera MC, Moreira CS, Bresciani E, de Melo RM, Bottino MC. Effects of ciprofloxacin-containing scaffolds on enterococcus faecalis biofilms. J Endod 2015;41:710-4.
14. 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;41:1337-43.
15. 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;42:1490-5.
16. Gaetti-Jardim Júnior E, Landucci LF, Lins SA, Vieira EM, de Oliveira SR. Susceptibility of strict and facultative anaerobes Isolated from endodontic infections to metronidazole and beta-lactams. J Appl Oral Sci 2007;15:539-45.
17. Albuquerque MT, Evans JD, Gregory RL, Valera MC, Bottino MC. Antibacterial TAP-mimic electrospun polymer scaffold: Effects on *P. gingivalis*-infected dentin biofilm. Clin Oral Investig 2016;20: 387-93.
18. Albuquerque MT, Nagata J, Bottino MC. Antimicrobial efficacy of triple antibiotic-eluting polymer nanofibers against multispecies biofilm. J Endod 2017;43:S51-6.