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Electrospun Poly-Lactic Acid Nanofibers for Dual Drug Delivery in Regenerative Endodontics: Ciprofloxacin and Metronidazole Loading and Characterization

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Aim: This study presents the synthesis, material characterization, and evaluation of the antimicrobial activity of antibiotic-loaded scaffolds.
Materials and methods: Metronidazole (MID) or Ciprofloxacin (CIP) was incorporated into a polylactic acid (PLA) polymer solution at a concentration of 25 wt.% and then processed into Fibers. Neat PLA nanofibers were used as controls. Characterization techniques, including scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), degradation tests, and drug release assessments, were employed. The antimicrobial properties were tested against *Porphyromonasgingivalis* (*Pg*) and *Enterococcus faecalis* (*Ef*).
Results: SEM imaging showed Fibers with submicron diameters, and FTIR confirmed the successful incorporation of the antibiotics. Data analysis demonstrated a gradual and sustained release of the drugs from the scaffolds over 48 hours. Antimicrobial activity was confirmed via agar diffusion assays, showing efficacy against the tested bacteria.
Conclusion: Antibiotic-loaded electrospun PLA scaffolds could serve as biologically safe antimicrobial drug delivery systems for use in regenerative endodontics.

Keywords: Electrospinning; PLA Nanofibers-scaffold; Drug delivery; Regenerative Endodontic Procedure.

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Introduction

Regenerative endodontics aims to restore the functionality of damaged dental pulp, particularly in cases of necrotic pulps and apical periodontitis.[1] A critical challenge in this field is the effective disinfection of the root canal system while preserving the viability and differentiation potential of stem cells. [2] Traditional methods, such as the use of highly concentrated antibiotic pastes (e.g., triple antibiotic paste, TAP), have shown limitations, including cytotoxicity, difficulty in removal, and interference with growth factor release. These drawbacks necessitate the development of alternative strategies that can provide controlled and sustained antimicrobial activity without compromising cell viability.[3]

Electrospun nanofibers are highly versatile materials characterized by their nanostructured morphology, high surface area, and customizable porosity, which closely mimic the extracellular matrix (ECM) of natural tissues. These properties make them particularly valuable in dental tissue engineering, where they support cell adhesion, growth, and differentiation, ultimately promoting tissue regeneration. 4

Synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), poly-l-lactic acid (PLLA), polycaprolactone (PCL) and polylactic-glycolic acid (PLGA) have been effectively used as scaffold materials in pulp tissue engineering.These materials are non-toxic, biodegradable, and possess flexibility and regenerative capabilities, enabling them to replicate extracellular matrix (ECM) structures. They exhibit enhanced cell adhesion and growth, while allowing precise control over their physicochemical properties, such as mechanical stiffness, degradation rate, porosity, and microstructure. [5]

Electrospun nanofibers provide a biomimetic environment that facilitates cell attachment, proliferation, and migration. Their high porosity and controlled fiber alignment support tissue-specific cellular behaviors.[6]Techniques for fabricating nanofibrous scaffolds include self-assembly, phase separation, and electrospinning, with the latter offering advantages in precision and scalability. Factors like voltage, flow rate, and collector distance critically influence fiber morphology and scaffold properties.[7]

Electrospun nanofibers have shown promise in regenerating the pulp-dentin complex. For instance, PLA scaffolds enhance odontogenic differentiation of dental pulp stem cells (DPSCs), while bioactive nanoparticle-polymer composites improve mechanical and biological properties.[8,9]Nanofibrous scaffolds loaded with antibiotics have also demonstrated effective drug delivery, suggesting their utility in managing infections.[10]

Over the past years, a myriad of studies has been performed thatinvestigated the synthesis of antibiotic-incorporated scaffolds, hypothesizing that regulating the release rate of significantly lower level, yet effective, antibiotic doses—compared to double and triple antibiotic pastes—could enhance the viability of human dental pulp stem cells (hDPSCs) while maintaining antimicrobial efficacy. [11, 12, 13]

Electrospunnanofibrous scaffolds, particularly those made from biocompatible and biodegradable polymers like polylactic acid (PLA), offer a promising solution. These scaffolds can mimic the extracellular matrix, providing a supportive environment for cell proliferation and tissue regeneration. Additionally, they can serve as effective drug delivery systems, allowing for the controlled release of antibiotics at lower, yet effective, concentrations.[5]

This study focuses on the development and characterization of PLA nanofibrous scaffolds loaded with ciprofloxacin (CIP) and metronidazole (MID) for dual drug delivery in regenerative

endodontics. Electrospun scaffolds can provide a sustained release of antibiotics, reducing the risk of cytotoxicity associated with high concentrations of drugs. The combination of CIP and MID targets a broad spectrum of endodontic pathogens, including both gram-positive and gram-negative bacteria. PLA is a well-established biomaterial that is non-toxic, biodegradable, and capable of supporting cell attachment and proliferation.The nanofibrous structure of the scaffolds closely resembles the native extracellular matrix, promoting cell guidance and tissue regeneration.[5,13]

The null hypothesis was adopted in this study which was thatthe incorporation of ciprofloxacin (CIP) and metronidazole (MID) into electrospunpolylactic acid (PLA) nanofibrous scaffolds does not significantly enhance the antimicrobial efficacy, drug release profile, or biocompatibility compared to neat PLA scaffolds.

This hypothesis will be tested through a series of experiments, including material characterization, drug release assessments, and antimicrobial activity tests, to determine whether the antibiotic-loaded scaffolds offer significant advantages over non-loaded scaffolds in the context of regenerative endodontics.

Materials and methods

This study was conducted after the approval of the Research Ethics Committee of the Faculty of Dentistry at Ain Shams University in Egypt. "FDASU-RECID0618016".

Preparation of different nanofibrous PLA scaffolds.

Preparation of polymer solution for freeloaded nanofibers

PLA (10% w/v) was dissolved in a mixture of Chloroform and Acetone (70:30 v/v) with magnetic stirring for 30min at 700rpm at room temperature, in a closed vessel till a homogenous system was obtained. The solution obtained was then subjected to electrospinning.[5]

Preparation of polymer solution for (Ciprofloxacin&Metronidzole) CIP-MID loaded nanofibers

Metronidazole (25% w/w of polymer ratio (10% w/v), i.e. 35 mg ofMetronidazole per ml of the polymer solution).[11]Metronidazole and PLA were dissolved in a mixture of Chloroform:Acetone (70: 30 v/v) on a magnetic stirrerfor 24h to obtain a homogenous system.

While Ciprofloxacin (25 % w/w of polymer ratio(10% w/v), i.e. 35 mg of CIP per ml of the polymer solution) [11] was dissolved in Chloroform in a separate closed vessel with continuous stirring at 700rpm for 24h at room temperature.

Then, both resulting solutions were mixed at 700rpm for 6h till reaching a homogenous system. Afterwards, the obtained polymeric solution impregnated with the double antibiotic DAP was subjected to electrospinning.[14,15]

Both the prepared solutions were dispensed into a 4mL syringe with stainless steel 20G needles separately.

The electrospinning was performed using NANON-01A Electrospinner (MECC Co., LTD., Japan). The used potential voltage differential was 20kV, and the needle-collector was held at an 80 cm constant distance. A syringe pump supplied rates of 0.5 mL/h. Every experiment was carried out in natural light (25°C ± 0.2 temperature and 36 - 42% relative humidity). The fibers were collected at room temperature (RT) on an aluminum-foil-covered rotating mandrel.

The electrospun scaffoldswere dried in a desiccator at RT for 48h under vacuum to remove any remaining solvent and stored at 4°C.[16]

Characterization for the nano-scaffolds

Scanning electron microscopy "SEM"

The average fiber diameter and fiber distribution were determined by SEM imaging.

The morphology of the prepared electrospun-free and loaded nanofibers was detected using SEM.[17] Samples preparation for SEM imaging included: samples fixation on aluminum foil, followed by Gold coating for 45s under Argon atmosphere using a coating system (MED020, Bal-Tech, USA). The measurements were conducted using an acceleration voltage of 20 kV and a working distance of 10mm. The diameters of 50 single fibers were measured using ImageJ software (National Institutes of Health, USA). Fiber diameter was calculated and reported based on (50 randomly chosen measurements per image) at the same magnification (5000×).[18,19]

Fourier Transform Infrared Spectroscopy

"FTIR" with Attenuated Total Reflection (ATR) was performed to characterize the chemical structure and investigate the possibility of interaction of chemical bonds between drug and polymer[16] and to analyze pure PLA, CIP, MID and the prepared electrospun CIP-MID loaded nanofibers.[19,20]

FTIR spectrophotometer (Thermo Nicolet AVATAR 330, Madison, WI, USA) was used to analyze pure PLA, CIP, MID and the prepared electrospun CIP-MID loaded nanofibers.[19,20]

The instrument was outfitted with a single reflection detector (Jasco ATR PRO470-H). The measurements were recorded in absorbance mode, and a background was obtained before each measurement.

At room temperature, 100 scans were acquired for each spectrum between 400 and 4000 cm^{-1}, at a resolution of 4 cm^{-1}.

For data acquisition, the Spectra Manager-II software (Jasco, Easton, MD, USA) was utilized.[20]

X-ray Diffraction Calorimetry (XRD)

XRD (Shimadzu XRD-6000 diffractometer, Japan) was performed to examine the crystallinity of the pure PLA, CIP, MID and the prepared electrospun CIP-MID loaded nanofibers.

The XRD was run at 40 kV and 40 mA utilizing Cu-K radiation in the range of (2Θ) 5°- 60° with a scanning rate of 0.05°/s at ambient temperature. The area integration approach was used to determine the crystallinity level.[21]

Entrapment Efficiency EE (%) of CIP-MID loaded nanofibers was estimated as follows; specific weight of around 10 mg of loaded nanofibers were chopped and dissolved in 10 mL PBS (pH=7.4) for 60 minutes using a magnetic stirrer, then filtrated through Millipore (0.45 µm).

A UV/VIS Spectrophotometer (Shimadzu UV-1601 PC, Japan) measured the quantity of CIP and MID at 271nm and 320 nm, respectively.The test was performed in triplicate, and the findings were computed as mean ±SD. The process's drug EE (%) was calculated as follows:[21]

$$EE(\% = \frac{Amountofdruginnanofibers\ (mg)}{Initalamountofdrug\ (mg)} X100$$

In-vitro drug release

was tested to determine the accumulative amount of released drugs at different time intervals, using the Dialysis Tube Technique.[22]

A specific weight of CIP-MID loaded nanofibers mat was immersed in 1mL of PBS (pH =7.4) and deposited in a dialysis (12,000-14,000 M.wt cut-off) that was well blocked at both ends. The latter was then immersed in 50 mL of PBS (pH = 7.4) and swirled on a magnetic stirrer at 37°C for 8h at a continuous rate of 100 rpm. At each time interval (0.5, 1, 2, 3, 4, 5, 6, 8 h,24hand 48hr), an aliquot of the sample (3mL) was removed and replaced with fresh PBS (pH = 7.4). The

amount of CIP and MID emitted was UV-VIS spectrophotometrically quantified against PBS (pH =7.4) at predetermined wavelength 271nm and 32 nm, respectively.[21]

Degradation test

In an attempt to expect the degradation time of the prepared nanofibers in-vivo, this test was carried out. Strips of the free and prepared CIP-MID nanofibers (4 × 4 mm) were placed in closed containers filled with 20 mL of PBS (pH=7.4). Samples were then transferred to the mini-incubator (Mini ICT 5.4, Falc Instruments, Italy) and kept at 37°C ± 0.5∘ C (pH = 7.4), for 4 weeks. Samples were then removed from the incubator and left for drying in a desiccator at room temperature, for one week. At the end of the experiment, dried materials were analysed by SEM.[23]

Agar Diffusion Test

Phosphate buffered saline (PBS, pH=7.4; GIBCO BRL, Grand Island, New York, USA) aliquots containing antibiotics released from the scaffolds were prepared. Rectangular-shaped scaffolds (n=3/group, 4.0±0.2 mg) were cut and disinfected by UV light (1 min each side) and rinsed twice with sterile PBS.

E. faecalis (ATCC 29212) was cultured aerobically in Tryptic Soy Broth for 24h in 5%CO2 at 37°C. Meanwhile, *P. gingivalis* (ATCC 33277) was cultured for 24h anaerobically in Brain Heart Infusion Broth (BHI) containing 5g/L Yeast Extract UF (Ultra-Filtered) in an anaerobic GasPak jar.[24] Then, 100µL of each bacterial suspension was swabbed onto Brain Heart Infusion agar plates (Oxoid, USA) to create a lawn of bacteria.

Each Agar plate was divided into 2 zones (1 containing a plain scaffold and the other an antibiotic-containing scaffold. Plates were incubated following the bacterial strain they carry (aerobic or anaerobic). After 24h of incubation, the diameters (in mm) of the clear zones of growth inhibition were recorded.[11]

Results

Fabricated scaffolds' morphology (SEM)

Scanning electron microscope imaging of the fabricated scaffolds (both neat Polylactic nanofibrous scaffold (Fig. 1a) and double antibiotic-loaded Polylactic acid nanofibrous scaffold) (Fig. 1b) showed the presence of nanofibrous fibers with relatively homogeneous sizes in the nano-submicron range.

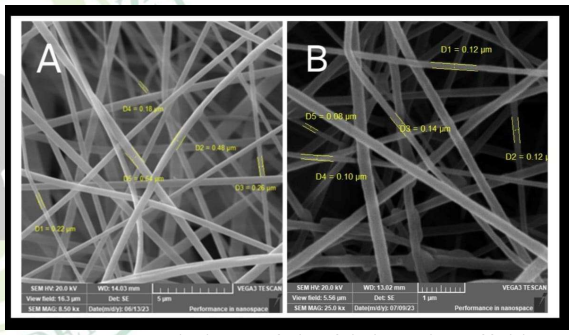

Figure 1: Morphology of the fabricated scaffolds as **shown by Scanning electron microscope imaging: (A) neat polylactic acid nanofiber, (B) Ciprofloxacin/Metronidazole loaded polylactic acid nanofiber .**

The resulting nanofibers were well-defined and oriented randomly as shown in (Fig. 1a&1b). The surface morphology of neat polylactic acid nanofibrous scaffold was smooth and uniform with a fiber diameter larger than the loaded mats.

while Double antibiotic-loaded nano-fibrous sacffoldswere also smooth and uniform but with beads & signs of drug deposition were observed on the surface of the nanofibers with a fiber diameter thinner than the neat polylactic acid fiber scaffold.

Recorded readings for the diameter of free and loaded nanofibers were in the nano-diameter ranges: 336±162.7 nm & 112±22 nm –respectively.

FTIR (Figure 2)
According to the Olefin stretching vibrations, the following were recorded:

FTIR spectra of pure CIP, MID, PLA, and CIP-MID loaded nanofibers were presented,to evaluate potential molecular interactions and verify the chemical composition of the formulated nanofibers.(Figure 2e)

At 2922.95 cm^{-1}, the most prevalent PLA bands were observed due to asymmetric CH_2 stretching, while those observed at 2855.36 cm^{-1} appeared to be caused by symmetric CH_2 stretching. (Figure 2a)

Furthermore, the peaks at 1750 cm^{-1} and 1458.38 cm^{-1} were attributed to carbonyl stretching, while those at 1182.66 cm^{-1} and 1090.55 cm^{-1} were attributed to C-O, C-C, and asymmetric C-O-C stretching.
Peaks at 3528.49 cm^{-1} and 1623.41 cm^{-1} in the FTIR spectra of CIP are due to O-H stretching and quinolone structure, respectively. (Figure 2b)

The peaks between 1385.67 cm^{-1} and 1451 cm^{-1} are related to C-O, and the peaks between 1311.86 and 1269.21 cm^{-1} belong to the carboxylic acid's O-H group.
In addition, the presence of amine stretching with a peak at 3383.5 cm^{-1} and 1750 cm^{-1}, ketone stretching with a peak at 1707.45 cm^{-1}, and alkyl halide stretching with a peak at 1017 cm^{-1}.

MID peaks appeared at 3416.90 cm^{-1}, 3100.26 cm-1 and 1625.92 cm^{-1} related to O-H, C-H and C=O, respectively. Peaks around 1466.56 cm^{-1} and 1373.58 cm^{-1} related to symmetric and asymmetric stretching of the N=O group, respectively. Stretching of C-C, C-O, and C-N groups appeared at 1433.18 cm^{-1}, 1156.73 cm^{-1} and 1074.86 cm^{-1}, respectively.(Figure 2C)

Finally, FTIR spectra of CIP-MID-loaded nanofibers showed no interaction between CIP, MID, and PLA, as evidenced by the presence of characteristic peaks for each drug. (Figure 2D)

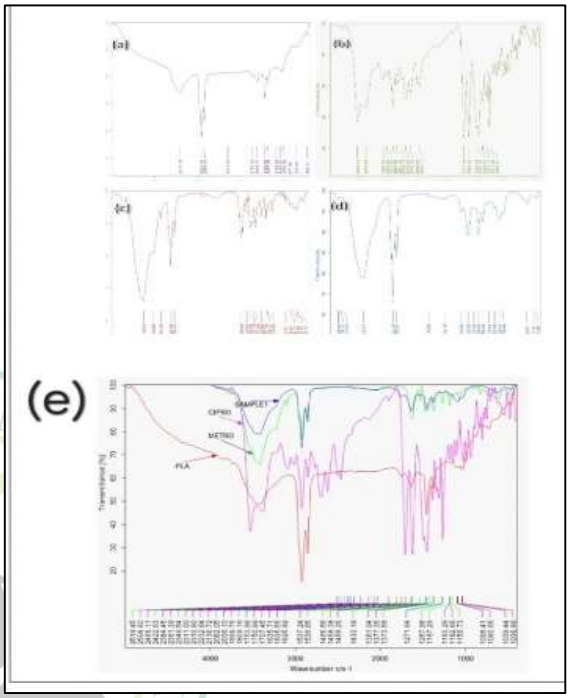

Figure 2: Fourier Transform Infrared Spectroscopy spectra of (a)PolyLacticAcid, (b)Ciprofloxacin(c)Metronidazole and (d)Ciprofloxacin-Metronidazole loaded nanofibers. (e) Fourier Transform Infrared Spectroscopy spectra of PolyLacticAcid, Ciprofloxacin , Metronidazole and Ciprofloxacin-Metronidazole loaded nanofibers Collectively.

FTIR results confirmed

a) The presence of chemical compatibility between drugs and polymer.
b) The stability of CIP and MID which were successfully entrapped within the prepared nanofibers.

XRD results

the patterns of pure PLA, CIP, MID, and the prepared CIP-MID-loaded nanofibers.
PLA showed intensity with a broad maximum appearing at 2Θ angle of 16°, emphasizing that the PLA lacked polymorphic crystalline transition.
Characteristic sharp peaks of crystallinity of CIP appeared at 2Θ angles of 8.5°, 10° and 20°.

MID showed numerous sharp peaks at 2Θ angles of 12.4°, 15.2°, 18.4°, 25.2°, 26.4°, 28.5°, 30° and 35.2°. The diffraction of CIP-MID-loaded nanofibers showed a decrease in the intensity of characteristic peaks for CIP and MID. This indicated the embedding of each drug within the formed nanofibers in an amorphous form.**(Figure 3).**

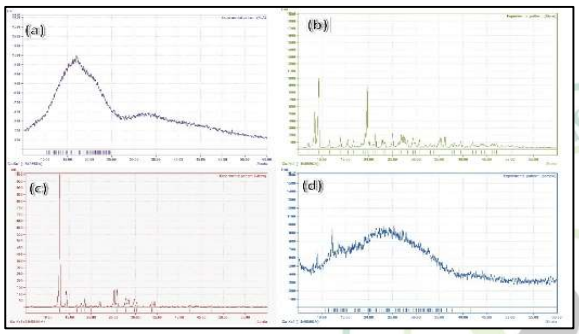

Figure 3 :X-ray Diffraction Calorimetry patterns of (a) PolyLactic Acid, (b) Ciprofloxacin (c) Metronidazole and (d) Ciprofloxacin-Metronidazole loaded nanofibers.

Degradation test

During the experimental period (6weeks); it was optically observed by scanning electron microscope for any change in the shape of both nanofibrous scaffolds.

At the end of the 15 days, it was observed that the nanofiber mats (nest PolyLacticAcidnanofibrous scaffold & double antibiotic-impregnated polylactic acid nanofibrous scaffold) structure began to undergo deformation and partial deterioration by visual inspection in vitro.

At the near end of the experiment (6th week) it is evident that double antibiotic loaded nanofibrous scaffold degraded faster and was nearly fully degraded at the end of 1 month by 90%. . (Fig,4C,D)

The neat PolyLacticAcidnano-fibrous scaffold deteriorates with longer incubation time. The thin, well-formed PolyLacticAcid fibers that were put together into a thick framework showed a nearly complete degradation by 85% from the total sample. (Fig,4A,B)

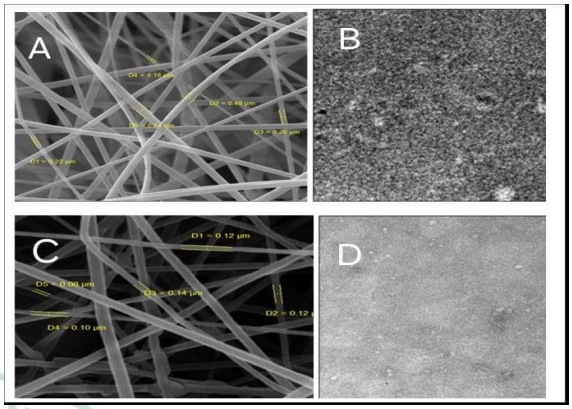

Figure 4: (A&B) showing the degradation pattern of nanofibers mats during the degradation process over the experimental period for the neat PolyLacticAcidnano-fibrous scaffold . (C& D) showing the degradation pattern of nanofibers mats during the degradation process over the experimental period for the antibiotic loaded polylactic acid nano-fibrous scaffold.

Entrapment Efficiency "EE" (%)

EE (%) recorded for CIP-MID loaded nanofibers were 93.8 ± 2.5 % for CIP and 95.1 ± 3.2 % for MID.

Drug release test

Depicts the cumulative drug released (%) of CIP and MID from the prepared loaded electrospun nanofiber against corresponding CIP and MID dispersion. (Figure 5)

It showed, first, a burst release effect till 8hrs as shown in (Figure5 a& b) followed by a sustained drug released till 48h as shown in (Figure 5E&F)from the prepared nanofiber-impregnated scaffold compared to the corresponding dispersion. Incomplete dissolution was observed from CIP dispersion compared to MID dispersion.

Agar Diffusion Test

Antibiotic-loaded nano fibrous polylactic acid scaffolds inhibited the growth of *Enterococcus faecalis* and *Porphyromonasgingivalis.*antibiotic-loaded polylactic acid nanofibrous scaffold had larger inhibition zones than the neat polylactic acid nanofibrous scaffolds.

The inhibition zones on *Porphyromonasgingivalis* were smaller than those of *Enterococcus faecalis*.(Figure 6 A,B)

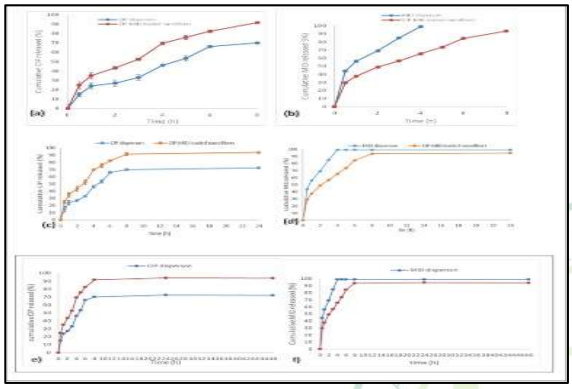

Figure 5: In-vitro release study of (CIP/MID) Ciprofloxacin- Metronidazole loaded nanofibers against corresponding Ciprofloxacin (CIP) and Metronidazole(MID) dispersion, respectively to 8hrs(a&b) then till 24hrs(c&d) then till 48hrs (e&f). Error bars represent standard deviation.

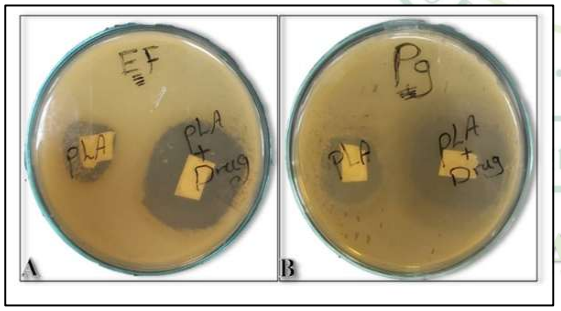

Figure 6: Agar plates showing inhibitions zones produced by different test scaffolds on both A:E. faecalisand,B:P. gingivalis.

Discussion

The selection of the most effective method for eliminating root canal infections before regenerative endodontic procedures should be based on two key outcomes: maximizing antibacterial effectiveness while preserving the proliferation and differentiation potential of pulp stem cells.[25]

Over the past years, numerous studies have been focusing on the synthesis of antibiotic-containing scaffolds based on the central hypothesis, that controlling the release rate of significantly lower (compared to DAP and TAP) -yet effective doses- might enhance human dental pulp stem cell viability (hDPSC), while preserving antimicrobial activity.[11,12,26]This, in turn,could improve the outcomes of regenerative endodontics,especially if there is also, an additional supportive skeleton (scaffold).[27]

Regarding scaffold material selection; In the present study PLA was selected as the supporting material& was used as a carrier for antibiotics (CIP and MID) because it is biodegradable, biocompatible, and can support the proliferation and attachment of various cells.The high porosity and large surface area of the nano-fiber mat are expected to increase the interaction between the (CIP and MID) and tissue and also modulate the cellular function to promote regeneration. [30,31,32]

Regarding antibiotic selection; 35 mg of Metronidazole & Ciprofloxacin added per ml of the polymer solution) based on previously reported data demonstrating minimal cell toxicity and significant antimicrobial efficacy against different endodontic pathogens P. gingivalis (Pg) and E. faecalis (Ef) .[11] One of the major goals of endodontic therapy is to eliminate microbial infection, typically a multi-species infection.[33] The majority of intracanal bacteria are strict anaerobes, thus MID is the first choice of antibiotic.[33] CIP, a DNA gyrase inhibitor, is very effective against gram-negative pathogens but has very limited effect against gram-positives. Consequently, it is often combined with MID in the treatment of mixed infections.[34]

A previous study demonstrated that, at clinically used concentrations, the antibiotics found in both Triple & double antibiotic Paste have deleterious effects on the survival of stem cells from the apical papillae and that the survival rates increased as the antibiotic concentrations decreased to 1 mg/mL or less.

The use of a biocompatible antibiotic-containing scaffold should enhance antimicrobial activity without the side effects of staining and cell toxicity.[35]

Regarding method of fabrication in this study,Electrospinning is a versatile technique widely used to fabricate fine fibers from both synthetic and natural polymers due to its simplicity and compatibility with almost any soluble polymer. This method also enables the incorporation of various additives, including medications, to impart specific properties to the final product. It has been extensively employed in the creation of nanofibrous scaffolds, offering control over fiber diameter, alignment, and material composition. The ability to adjust fiber diameter and pore size optimizes conditions for cell differentiation and proliferation. Additionally, electrospun materials enhance cellular interactions, improve protein absorption by providing binding sites for cell receptors, and feature a high surface area-to-volume ratio.[36]

Regarding fabrication protocol ; The selection of an appropriate solvent is crucial in nanofiber fabrication through electrospinning. Key considerations include the solvent's solubility and boiling point. Volatile solvents are preferred due to their ability to evaporate and dehydrate quickly, which is essential for producing uniform nanofibers. However, solvents with excessively low boiling points should be avoided, as they can evaporate too rapidly, leading to needle clogging. Conversely, high boiling point solvents may not fully evaporate before the fibers reach the target, causing the formation of flat, ribbon-shaped fibers rather than round ones. Therefore, cautious solvent selection is necessary to ensure proper nanofiber morphology during electrospinning.[37]

Regarding SEM results; The diameter of CIP-MID-loaded nanofibers was less than the diameter of the free nanofibers. This is thought to be connected to the increase in conductivity of electrospinning solutions caused by the addition of the CIP and MID as, the difference in diameter could be related to the ratio of drug to polymer solution in aspects of viscosity, conductivity and surface tension of the formed systems. [20]

The results from this study were similar to a study by Zeng etal andSuner etal where the diameters of drug-loaded nanofibers are smaller than the diameters of neat PLA. The use of dilute polymer solutions in the synthesis of nanofibers causes a decrease in their viscosity.[38,39]

Several researchers have reported on the effects of various parameters on the morphology of PLA fibers. Gu and Ren found that the diameter of PLA fibers increases with higher polymer concentration, while it decreases with an increase in the applied voltage.[40]

Regarding Fourier Transform Infrared Spectroscopy in our study; Fourier Transform Infrared Spectroscopy spectra of ciprofloxacin andmetronidazole-loaded nanofibers showed no interaction between Ciprofloxacin, Metronidazole and Polyactic Acid.This is evidenced by the presence of the characteristic peaks for each drug, suggesting a chemical compatibility between drugs and polymer. In addition to that it also confirmed the stability of ciprofloxacin andmetronidazole which were successfully entrapped within the prepared nanofibers. Which coincides with other studies in literature. [41,42]

Regarding degradation, thetime to complete degradation of PLA-based biomaterials in aqueous conditions depend on numerous factors including the form of the biomaterial itself (bulk, porous, fibrous, and powder), its molecular weight, polydispersity, crystallinity, surface energy, additives, and microstructure. Typical PLA biomaterials with low specific surface area (bulk materials) undergo degradation in

volume and their molecular mass decreases due to the contact with water. Acidic degradation products of the material, which are released in aqueous and biological environments, accelerate the degradation process. [43]

Regarding drug release pattern; This burstdrug release, followed by sustained drug release from the prepared nanofibers, aligns with findings previously reported by Immich et al. Additionally, the incomplete dissolution observed in the CIP dispersion further supports these findings. Immich et al. noted that the drug release pattern from PLA nanofibers exhibited a sudden burst followed by a sustained release, which is consistent with the release kinetics observed in our study. [44]

Researchers stated that there were 3 major mechanisms of release from electrospun PLA nanofibers: desorption, diffusion and degradation. Their results showed that the initial burst was due to the desorption of the loaded drug from the outermost surface fibers. This was usually followed by the so-called diffusion-mediated release. Furthermore, membranes with lower fiber density exhibit high porosity, which facilitates the free movement of water and enhances drug release.[43]Conversely, membranes with higher fiber densities have reduced porosity, causing drug molecules to be packed more tightly and thus limiting drug delivery. Generally, drug-loaded electrospun PLA nanofiber mats display solution-diffusion kinetics, and the release timeline can be adjusted through various processing factors such as fabrication techniques, choice of drug and its ionization state, and whether uniaxial or coaxial electrospinning is used. [43]Additionally, coaxial electrospinning minimizes desorption from the PLA surface as a primary release mechanism by encapsulating the drug within the core. When the drug is contained in a core surrounded by a porous PLA sheath, diffusion-driven release kinetics are observed. On the other hand, encapsulation within a solid PLA sheath promotes degradation as the main release mechanism.[45]

In a one-day in vitro study, a significant burst release of tetracycline hydrochloride from PLA nanofibers was observed. This rapid drug release was explained by weak physical interactions between the model drug and PLA.[45]

Moreover, our drug release findings align with those from several other studies investigating antibiotic release from nanofibrous structures and further support the effectiveness of electrospun nanofibers for controlled drug delivery applications. [2,10,11,12,13.15]Overall, this comprehensive analysis emphasizes the efficacy of the electrospun nanofibers in drug release, highlighting both the initial burst and sustained release phases while considering the variations in performance between the different formulations.

Regarding Antimicrobial properties,the inhibition zones on *Porphyromonasgingivalis* were smaller than those of Enterococcus faecalis indicating higher antibacterial efficacy against *Enterococcus faecalis*.For neat PLA mats, this could be due to two factors: the production of lactic acid during biodegradation and the possible presence of chloroform traces. The acidic by-product from PLA tends to have an antibacterial effect. Additionally, chloroform has been reported to exhibit some degree of antibacterial activity.[46] In the case of PLA+DoubleAntibiotic mats, the inhibition zones were larger and could be mainly due to the diffusion of the antibiotics released. [13,15] Analysis of data from the agar diffusion test further confirmed the efficacy of CIP-MID-containing mats against both bacteria. Herein, the incorporation of CIP, a gyrase inhibitor into the electrospun mats led to a

significant inhibition of growth of both *Ef* and *Pg*. [12]

Upon closer observation of our antibacterial data clearly showed that the electrospinning process did not negatively affect antimicrobial properties. This finding aligns with similar studies that examined the incorporation of a wide range of drugs. These studies also demonstrated that electrospinning does not compromise therapeutic properties. [2,10,11,12,13.15]

Despite the promising results, this study has several limitations: In Vitro Limitations; The experiments were conducted in vitro, which may not fully replicate the complex biological environment of the root canal system. Further, in vivo studies are needed to validate the efficacy and biocompatibility of the scaffolds in a clinical setting. Limited Antibiotic Combinations: Only CIP and MET were tested in this study. Future research could explore other antibiotic combinations or concentrations to optimize antimicrobial efficacy and minimize potential cytotoxicity. Cell Viability and Differentiation; The study focused on antimicrobial properties and drug release but did not evaluate the effects of the scaffolds on stem cell viability, proliferation, or differentiation. Future work should incorporate cell-based assays to assess these critical parameters.

Conclusion

This study successfully demonstrated the potential of electrospunpolylactic acid (PLA) nanofibrous scaffolds loaded with ciprofloxacin (CIP) and metronidazole (MID) as a dual drug delivery system for regenerative endodontics. Furthermore, the biodegradability of PLA makes it an ideal material for supporting cell proliferation and tissue regeneration in endodontic applications. These findings suggest that antibiotic-loaded PLA nanofibrous scaffolds could serve as a promising alternative to traditional antibiotic pastes, offering controlled drug delivery, reduced cytotoxicity, and enhanced regenerative outcomes.

Declarations

Ethics approval and consent to participate

This study was conducted after the approval of the Research Ethics Committee of the Faculty of Dentistry at Ain Shams University in Egypt (FD ASU- REC/ ID 09171).

Competing interests

The authors declare that they have no competing interests.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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