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Original Article

Antimicrobial Activity of PCL Nano Scaffold Incorporated with Zinc Oxide Nanoparticles of Varied Concentrations with and without Injectable Platelet-rich Fibrin: An *in vitro* Study

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Periodontitis, a chronic inflammatory disease induced by microbiome imbalance, progressively leads to the destruction of periodontal tissues. The development of scaffolds for periodontal tissue regeneration necessitates enhanced antibacterial treatments to mitigate infection risks and address the escalating issue of antibiotic resistance. This study investigates the antibacterial properties of electrospun polycaprolactone (PCL) scaffolds incorporated with varying concentrations of zinc oxide nanoparticles (ZnO-NPs). Additionally, the influence of injectable platelet-rich fibrin (i-PRF) on the antibacterial efficacy of the scaffolds was examined. PCL scaffolds were fabricated through electrospinning. ZnO-NPs via wet chemical synthesis. The antimicrobial efficacy of PCL, both with and without injectable PRF, was evaluated against S. *mutans* and P. *gingivalis* using PCL:ZnO NPs at varying doses. The study was conducted using SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA). The findings were displayed as the mean percentage of inhibition. S. *mutans* and P. *gingivalis* were significantly inhibited by ZnO concentrations (0.1% and 0.2%) compared to PCL alone in antimicrobial activity analysis without i-PRF. Incorporating i-PRF further enhanced the antimicrobial activity of PCL-ZnO scaffolds against both bacterial strains, with higher ZnO concentrations showing greater effectiveness. Higher concentrations of ZnO-NPs enhance inhibitory effects, indicating a dose-dependent relationship. Combining i-PRF with PCL-ZnO scaffolds further improves clinical outcomes in periodontal tissue regeneration.

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Introduction

Periodontitis, an inflammatory illness, commonly affects the tissues that provide support to the teeth [1]. Behavioral, environmental, systemic, and epigenetic factors all contribute to the outcome. Untreated periodontitis can lead to tooth loss as it progressively damages the tissues [2]. To reduce the number of microorganisms, it is essential to disturb or eliminate biofilms located below the gum line in individuals who are susceptible to periodontitis [3]. Thus, the objective of pocket/root instrumentation is to stimulate a robust immune response against infection by decreasing the bacterial load in the patient below the threshold level that causes sickness [4].

The conventional approaches for managing periodontitis involve surgical intervention to address severe pockets or abnormalities, as well as mechanical cleaning techniques such as scaling and root planning [5]. The most highly regarded method for treating periodontal disease is still the mechanical removal of plaque biofilm through supra- and subgingival debridement [6]. Six regrettably,

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pathogenic germs may persist in gingival tissues or other inaccessible areas that cannot be reached by periodontal instruments, rendering mechanical cleaning ineffective in certain cases [7]. Systemic or local administration of antibiotics can be employed to address the re-establishment of periodontopathogens. Regenerating periodontal tissues completely can be challenging, depending on the size of the pocket or the inflamed area [8]. Bioresorbable or non-resorbable barrier membranes are regenerative techniques employed to direct tissue or bone regeneration. Researchers are currently studying the application of cell-based and gene therapies, bone anabolics, laser treatments, and other emerging technologies to regenerate periodontal tissue [9,10]. Regardless of the level of success achieved by membrane-based guided tissue regeneration (GTR) or guided bone regeneration (GBR) procedures in clinical settings, it is crucial to maintain a sterile environment to ensure effective tissue regeneration [11].

In recent times, GTR or GBR membranes have been enhanced by incorporating antimicrobial drugs into them, so increasing their efficacy in combating local infections [12]. The adverse consequences associated with antibiotics contribute to the emergence of antibiotic resistance, a major global concern that hampers the effectiveness of these medications [13]. Given the availability of alternative antibacterial agents for the development of biomaterials in GTR or GBR applications, it is crucial to utilize them in order to produce regenerative technologies that are more secure for clinical implementation [14]. Nanoparticles (NPs) are attracting attention as a possible alternative to antibiotics [15]. Inorganic ions and metallic oxide (MO) nanoparticles have shown antibacterial properties [16].

The MO-NPs that have been extensively explored and are widely recognized are zinc oxide (ZnO), copper oxide (CuO), iron oxide (Fe_2O_3), magnesium oxide (MgO), titanium oxide (Ti_2O), and silver oxide (Ag_2O). The presence of Cu-NPs and Ag-NPs can lead to the eradication of bacteria by the emission of Cu^{2+} and Ag^{+} ions, respectively [17]. Ongoing research is being conducted on the antibacterial characteristics of ZnO NPs, which are a form of MO-NP, at both the micro- and nano-scales. Decreasing the particle size of ZnO to the nanoscale reveals its notable antibacterial properties [18]. The bactericidal processes of nanoscale ZnO are distinct because it can interact with bacterial surfaces and/or infiltrate the bacterial core. Possible processes include the generation of reactive oxygen species (ROS), the emission of antimicrobial ions (mostly Zn^{2+} ions), and the direct interaction between ZnO nanoparticles and bacterial cell walls, leading to the disruption of bacterial cell integrity. As a result of this characteristic, their utilization in enhanced biological applications such as medication delivery, wound healing, cancer therapy, and bioimaging became essential [19,20].

Polycaprolactone (PCL) scaffolds are widely used in tissue engineering due to their ability to break down naturally and their flexibility. They are utilized for various purposes in tissue engineering, such as for bone, cartilage, skin, teeth, and nerves. Regrettably, PCL scaffolds lack inherent antibacterial properties, which is a significant disadvantage. Implantation can lead to subsequent infections [21]. Consequently, a variety of supplementary substances and drugs were examined as prospective combinations with PCL scaffolds in order to address this issue. Recently, there have been findings that electrospun polymer-based scaffolds or membranes with ZnO possess antibacterial properties, promote cell growth, and aid in wound healing [22]. Further research is needed to determine the effectiveness of these membranes against periodontopathogens, such as GTR or GBR membranes. Previous studies only focused on the ZnO-scaffolds' impact on Staphylococcus aureus (G+) and Escherichia coli (G-)[23].

A growing number of people are choosing to use platelet concentrates to alleviate the symptoms of periodontal disease. There is a wealth of research on the regenerative and anti-inflammatory properties of platelet concentrates. Platelet concentrates' ability to repair tissue is due to the several growth factors that they produce [24]. An innovative leukocyte-enriched platelet concentrate called injectable platelet-rich fibrin (i-PRF) promotes tissue regeneration and wound healing. i-PRF produces a dynamic fibrin gel while it is still liquid, which it uses to release growth factors such platelets, leukocytes, type 1 collagen (COL1), osteocalcin (OC), and others gradually [25]. Platelet concentrates are effective against a range of infections, such as S. *aureus*, E. *coli*, *Klebsiella pneumoniae*, and S*treptococcus oralis*, due to their antibacterial and tissue-regenerative properties [26]. The antibacterial qualities of platelet concentrates are still unclear. Leukocytes are known to have antibacterial properties; these cells are found in platelet concentrates at much higher concentrations than in whole blood [27]. Kour et al. (2018) investigated the antibacterial activity of three distinct platelet concentrates against P. gingivalis and Aa: platelet-rich plasma (PRP), platelet-rich fraction (PRF) and iso-PRF. All three platelet concentrations exhibited antibacterial activity; however, PRF was less effective than PRP and I-PRF [28]. To completely understand all of the numerous features of i-PRF, including its antibacterial capacity, more research is necessary given its ease of manufacturing and excellent compatibility with a variety of biomaterials.

Standard strains of *P. gingivalis* and *S. mutans*, two common oral bacteria, were tested using electrospun poly(ε-caprolactone) (PCL) with and without i-PRF to assess the antibacterial efficacy of various ZnO-NP concentrations.

Materials and Methods

Zinc acetate dihydrate $(Zn\ CH_3COO)_2.2H_2O$ (as a zinc source) and sodium hydroxide (NaOH) (Sigma Aldrich, Germany) were used as precursors to fabricate the ZnO nanopowder. Deionized water was used as the solvent. The research methodology employed was an *in vitro* approach. This study adheres to the 2008 amendments of the 1975 declaration of Helsinki and received approval from the institutional ethical committee for human subjects at Saveetha University in Chennai, India (IHC registration number: SDC/PhD/01). A power analysis was conducted using the G-Power 3.1.3 software to determine the minimal sample size needed to detect statistically significant differences with a 90% power and a 5% acceptable margin of error (α).

Synthesis of zinc oxide nanoparticles (ZnO-NPs)

NaOH solution was slowly added (drop-wise) to the $(Zn\ CH_3COO)_2.2H_2O$ solution at room temperature to obtain ZnO particles, along with vigorous stirring. The ensuing reaction led to the formation of a white precipitate in the solution, which was then centrifuged at 3000 rpm for 30 minutes to remove the supernatant and obtain the precipitate containing nano ZnO. The precipitate was washed and dried in an electric oven at 90°C for 2 hours and crushed using a mortar to obtain the nano ZnO powder [29-31]. The average particle size) (>100 ZnO NPs) was determined using X-ray diffraction (XRD) and energy-dispersive X-ray spectroscopy (EDX) and microtopography was analysed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) imaging (Supplementary data figures 1 to 4).

Fabrication of electrospun PCL nano scaffold

A bespoke solvent blend consisting of 60% acetone and 40% dimethyl carbon in a 3:2 proportion was employed to dissolve 7.5 grams of PCL polymer (Sigma Aldrich, Stuttgart, Germany) with

an estimated molecular weight of 80,000 g/mol [32,33]. To prepare the solution, the polymer was dissolved in a mixture of solvents and agitated at a temperature of 45°C for a duration of 2 hours. In order to standardize this PCL solution, we incorporated 7.5 mg of ZnO-NPs to achieve a concentration of 0.1% and 15 mg to achieve a concentration of 0.2% by weight [32]. In order to ensure uniformity, the mixture was continually agitated while being kept at room temperature overnight. In order to distribute the NPs more widely and create a more uniform solution, an ultrasonicator was used. The concoction was meticulously inserted into a 10 ml syringe equipped with a 20 gauge needle. The material was subjected to electrospinning at a voltage of 12 kV and a flow rate of 3 mL/h, while maintaining a distance of 12 cm from the collector. The electrospinning technique was conducted by Holmarc, Opto-mechatronics Pvt. Ltd., an indian company. A rotating collector was surrounded by aluminum foil to catch fibers. Following the removal of the scaffolds from the collector, they underwent vacuum treatment at ambient temperature for a duration of 24 hours to eliminate any residual solvent. The thickness of the PCL scaffold was approximately one hundred micrometers. Finally, the scaffolds were preserved at room temperature by placing them in a tight plastic bag. Zinc oxide (ZnO) nanoparticles were added to the electrospun PCL scaffold at quantities ranging from 0.1% to 0.2%.

Preparation of injectable platelet-rich fibrin

Only individuals who do not smoke and are in good overall health were eligible for consideration. Individuals were disqualified from participation if they had any systemic illnesses, had been prescribed anticoagulant or antiplatelet medication within the past three months, or had recently used systemic antibiotics. A group of five young adults, aged between 25 and 35, who were in good health, donated blood as volunteers. The samples were put in uncoated plastic tubes and promptly centrifuged at 700 revolutions per minute (with a maximum relative centrifugal force of 60 g) for three minutes without an anticoagulant. The microcentrifuge (KW 80, Almicro Instruments, Haryana, India) was used for the centrifugation [34]. Using the synthesized nano scaffolds, thirty-six separate experimental groups were built: The following are the categories: Six groupings distinguished the subjects: PCL alone was used for Group I; Group II received PCL combined with 0.1% ZnO; Group III received PCL combined with 0.2% ZnO; Group IV received PCL combined with I-PRF; Group V received PCL combined with 0.1% ZnO and I-PRF; Group VI received PCL combined with 0.2% ZnO and I-PRF. Six groups in all, with groups IV-VI getting I-PRF and groups I-III not, Every sample in the I-PRF groups got 0.5 milliliters of the chemical.We compared the performance of every group against common microorganisms Streptococcus mutans and *P. gingivalis.*

Determination of antibacterial activity

The antibacterial action was evaluated using microdilution in 96-well microplates. Two strains of *Staphylococcus mutans* and *Pseudomonas gingivalis*, respectively, purchased from the ATCC, were the study's bacteria: 33277.2 and 25175 respectively. The trial called for *P. gingivalis* to be incubated five days oxygen-free in human anticoagulated whole blood. The Brucella agar was then topped with a vitamin K1-hemin solution before the bacteria were grown. *S. mutans* was cultivated on TYS20B agar under an aerobic conditions at 37°C for 48 hours. From 500μg/mL to 0.06μg/mL, the levels of the ethanolic and oily phases were methodically dropped on 96-well plates with bacterial suspensions ($5x10^5$ CFU/mL). To preserve sterility, a broth solution was utilized; gentamicin (16μg/mL) was the negative control used to limit bacterial growth. Following 13 hours of an initial incubation time set at 37°C for *S. mutans*, the plates were then placed under aerobic conditions for 22 hours for *P. gingivalis*. Using an ELISA plate reader to measure the variations in optical density (OD) at 630 nm, one might assess the evolution of microorganisms. Following visual inspection of the region, the optical density (OD) was measured to track bacterial growth. Formula let one find the percentage of inhibition.

Percentage of inhibition = [(OD of control - OD of test)/(OD of control)] × 100

Statistical analysis

In order to conduct the analysis, SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA) was utilized. Following the use of the Bonferroni test to account for multiple comparisons, post-hoc analysis was conducted using the results of the Analysis of Variance (ANOVA). Significant results were defined as P-values less than 0.05.

Results

Significant variations were noted across the groups in terms of the antibacterial effectiveness of PCL scaffolds containing varying quantities of zinc oxide nanoparticles, with or without injectable PRF, against Streptococcus mutans ($p < 0.001$). The inhibition rates varied from 50.82% in Group V (PCL + 0.1% ZnO + I-PRF) to 54.82% in Group III (PCL + 0.2% ZnO), and 59.82% in Group VI (PCL + 0.2% ZnO + I-PRF). Among the several classes, Class II (PCL + 0.1% ZnO) had the highest level of inhibition, with a percentage of 31.46%. Class IV (PCL + I-PRF) showed a lower level of inhibition at 15.03%, while Class I (Plain PCL) had the lowest level of inhibition at 10.99%. Based on the results of the pairwise comparisons, the addition of ZnO significantly enhanced

Table 1: Overall and individual pair-wise inter-group comparison of antimicrobial activity between the groups against *Streptococcus mutans*

Overall Inter-group Comparison			
Groups	Percentage of Inhibition		p-value
	Mean	SD	
Group I	10.990	0.060	< 0.001*
Group II	31.460	0.057	
Group III	54.820	0.057	
Group IV	15.030	0.057	
Group V	50.820	0.057	
Group VI	59.820	0.057	

Individual pair-wise Inter-group Comparison			
Groups		MD	p-value
Group I	Group II	20.470	< 0.001*
	Group III	43.830	< 0.001*
	Group IV	4.040	1.000
	Group V	39.830	< 0.001*
	Group VI	48.830	< 0.001*
Group II	Group III	23.360	< 0.001*
	Group IV	16.430	< 0.001*
	Group V	19.360	< 0.001*
	Group VI	28.360	< 0.001*
Group III	Group IV	39.790	< 0.001*
	Group V	4.000	1.000
	Group VI	5.000	1.000
Group IV	Group V	35.790	< 0.001*
	Group VI	44.790	< 0.001*
Group V	Group VI	9.000	1.000

Group I = Plain PCL; Group II = PCL 0.1% ZnO; Group III = PCL 0.2% ZnO; Group IV = PCL + I-PRF Scaffold; Group V = PCL 0.1% ZnO + I-PRF; Group VI = PCL 0.2% ZnO + I-PRF
p-value based on Analysis of Variance (ANOVA) followed by post-hoc analysis using Bonferroni test after adjusting for multiple comparisons
* = Statistically Significant ($p < 0.05$)

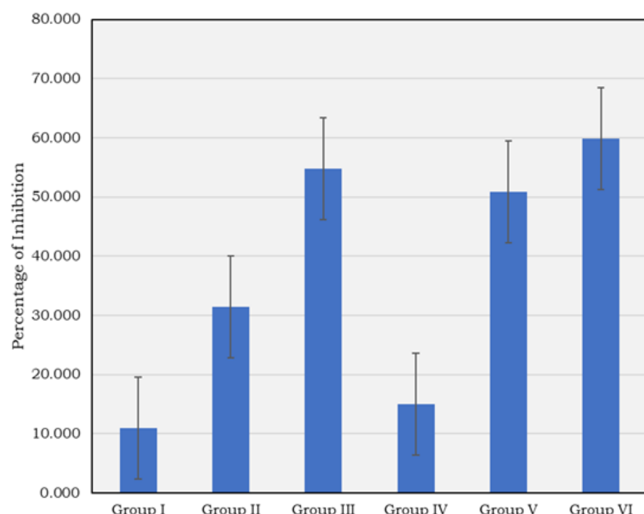

Figure 1 : Comparison of antimicrobial activity between the groups against *Streptococcus mutans*

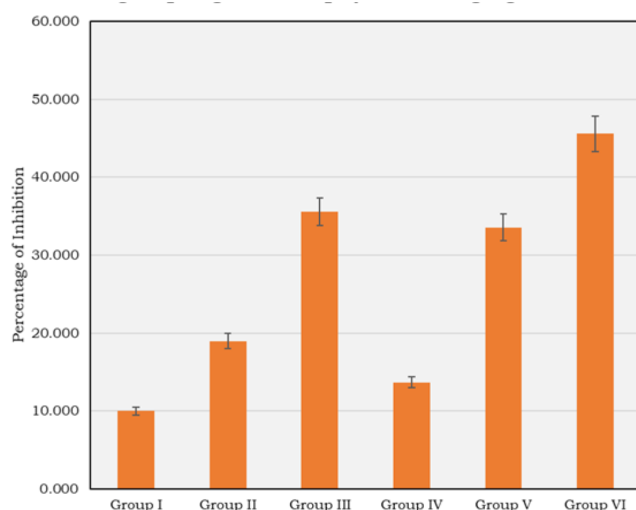

Figure 2 : Comparison of antimicrobial activity between the groups against *Porphyromonas gingivalis*

the antibacterial activity, particularly at higher doses. The inclusion of i-PRF significantly increased the effectiveness against microorganisms, particularly in Group VI (refer to table 1 and figure 1).

Table 2: Overall and individual pair-wise inter-group comparison of antimicrobial activity between the groups against *Porphyromonas gingivalis*

Overall Inter-group Comparison			
Groups	Percentage of Inhibition Mean	SD	p-value
Group I	10.006	0.008	< 0.001*
Group II	18.990	0.057	
Group III	35.570	0.057	
Group IV	13.670	0.057	
Group V	33.570	0.057	
Group VI	45.570	0.057	

Individual pair-wise Inter-group Comparison			
Groups		MD	p-value
Group I	Group II	8.984	< 0.001*
	Group III	25.564	< 0.001*
	Group IV	3.664	1.000
	Group V	23.564	< 0.001*
	Group VI	35.564	< 0.001*
Group II	Group III	16.580	< 0.001*
	Group IV	5.320	1.000
	Group V	14.580	< 0.001*
	Group VI	26.580	< 0.001*
Group III	Group IV	22.200	< 0.001*
	Group V	2.000	1.000
	Group VI	10.000	< 0.001*
Group IV	Group V	19.900	< 0.001*
	Group VI	31.900	< 0.001*
Group V	Group VI	12.000	< 0.001*

Group I = Plain PCL; Group II = PCL 0.1% ZnO; Group III = PCL 0.2% ZnO; Group IV = PCL + I-PRF Scaffold; Group V = PCL 0.1% ZnO + I-PRF; Group VI = PCL 0.2% ZnO + I-PRF
p-value based on Analysis of Variance (ANOVA) followed by post-hoc analysis using Bonferroni test after adjusting for multiple comparisons
* = Statistically Significant ($p < 0.05$)

Similarly, there were significant differences in the antibacterial effectiveness of PCL scaffold groups that were exposed to different amounts of zinc oxide nanoparticles, with or without injectable PRF, against Porphyromonas gingivalis ($p < 0.001$). Group VI (PCL + 0.2% ZnO + I-PRF) showed the highest inhibition rate of 45.57 percent, followed by Group III (PCL + 0.2% ZnO) with an inhibition rate of 35.57 percent, and Group V (PCL + 0.1% ZnO + i-PRF) with an inhibition rate of 33.57 percent. Group II, consisting of PCL with 0.1% ZnO, exhibited an inhibition rate of 18.99%. Group IV, comprising of PCL with i-PRF, showed an inhibition rate of 13.67%. The lowest inhibition rate of 10.006% was observed in Group I, which used plain PCL. The pairwise comparisons revealed that the antibacterial activity of Groups III, V, and VI was significantly higher than that of Group I ($p < 0.001$). Aside from that, there were big differences ($p < 0.001$) between Group II and Groups III, V, and VI, as well as between Group IV and Groups V and VI (refer to table 2 and figure 2).

Discussion

The higher mean values show that the inhibition was stronger when there were higher amounts of ZnO. When compared to PCL alone, both strains showed a lot of inhibition at amounts of 0.1% and 0.2%. Intergroup studies showed that the antimicrobial activity was very different between the groups that did not receive i-PRF. When the mean values of Group I (PCL alone) were compared to those of Groups II and III (PCL ZnO, 0.1% and 0.2%, respectively), they were much higher. This means that there was more prevention against *P. gingivalis,* which means things got better. Furthermore, the analysis of ZnO concentrations demonstrated a direct correlation with the dosage; specifically, Group III exhibited significantly higher average values compared to Group II, indicating the potential efficacy of employing higher ZnO concentrations in combating *P. gingivalis* infections.

Research suggests that the addition of zinc is a viable method for modifying the membrane to possess antibacterial properties [35]. The proportion of inhibition exhibited varied bacterial functions across all tested dosages. The samples that were analyzed showed antibacterial effectiveness against the pathogens tested, thanks to the specific quantities of ZnO-NPs.

The dose-dependent antibacterial activity of ZnO-loaded membranes, with a minimum concentration of 5 wt.%, was confirmed in our experiment. An increase in ZnO concentration could lead to a decrease in both the bacterial load and the number of assessed species. However, once the biofilms reached the 72-hour phase, the antibacterial efficacy of the Zn-NM started to decrease [36]. This experiment also observed a dose-dependent relationship (≥0.2%) between the antibacterial activity of ZnO-NPs coupled with PCL and electrospun.

Upon the structural failure of the scaffold, ZnO-NPs or Zn^{2+} ions are discharged into the broth culture media, resulting in the suppression of bacterial proliferation. When bacteria come into touch with solid ZnO-NPs, they may undergo mechanical cell membrane disintegration [37].

Zinc oxide nanoparticles (ZnO-NPs) have a positive charge in aqueous solutions, which is opposite to the negative charge usually found on the surface of bacterial cell walls. The electrostatic attraction between bacteria and ZnO nanoparticles leads to the accumulation of the nanoparticles on the bacterium's surface, causing a change in their zeta potential. Cell death is a result of further damage to the potassium channels in cell membranes [39,40]. ZnO-NPs are insoluble in water. Due to its hydrophobic nature, PCL membranes with ZnO would exhibit reduced wettability. Given that GTR or GBR membranes are in close contact with moisture, such as fluids at the site of periodontal surgery, it is essential for these membranes to have strong wettability. Prior studies have indicated that the use of hydrophilic scaffolds can enhance cell proliferation and wound healing by increasing cell affinity [41,43]. ZnO-NP chains exhibit malleability, compatibility with diverse materials, and solubility in a broad spectrum of organic solvents at ambient temperature. The objective of this work is to demonstrate the antibacterial properties of electrospun PCL by combining it with i-PRF, a platelet concentrate derived from the patient's own blood, and introducing ZnO-NPs into the mixture. Scaffolds frequently incorporate i-PRF to enhance their hydrophilicity and hence promote greater regeneration capacity [27,29].

In general, when comparing the two strains, there were noticeable differences in antibacterial activity when i-PRF was present. The addition of ZnO to PCL matrices had a significant impact, with higher concentrations of ZnO proving more successful than using PCL alone. The incorporation of ZnO into PCL composites, particularly when combined with PRF, demonstrates potential as a therapy for oral bacterial infections. This is supported by pair-wise comparisons that clearly demonstrate the significant impact of ZnO concentrations on inhibiting bacterial development. Group VI demonstrated enhanced efficacy against both strains, with further enhancements observed upon the incorporation of i-PRF into PCL scaffolds [44]. The findings of this work demonstrate that the inclusion of ZnO, particularly at elevated concentrations, significantly enhances the antibacterial effectiveness of PCL scaffolds.

Platelet concentrates have regenerative and antibacterial action against a variety of bacteria including *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Streptococcus oralis*. Our results support earlier research demonstrating that i-PRF kills *P. gingivalis* more effectively than *Aggregatibacter actinomycetemcomitans* [43].

i-PRF's improved antibacterial properties could be a result of its absence of synthetic compounds. i-PRF is more potent than standard platelet concentrates since it contains more platelets and other cells like leukocytes [45]. Ghanaati et al.'s "low-speed concept" offers validity for this notion by stressing the need of blood centrifugation techniques [46]. Lower speed centrifugation results in a fibrin clot formation that preserves more leukocytes among other cell types. The higher platelet and cell concentration in i-PRF is thought to enhance its already remarkable antibacterial properties [45]. Platelet concentrates' possible mechanisms for antimicrobial activities are bacterial binding, clustering, and internalization; release of antimicrobial peptides; and synthesis of oxygen metabolites including superoxide, hydrogen peroxide, and hydroxyl-free radicals [27]. Platelets directly destroy pathogens by means of antibody-dependent cell cytotoxicity (ADCC), a mechanism triggered upon contact with them. Platelet concentrates destroy bacteria because they include platelet microbicidal proteins (PMPs). Several PMPs might be able to eradicate germs. Included among the substances here are fibrinopeptide B, platelet basic protein, platelet factor 4, connective tissue activating peptide 3, thymosin beta 4, and platelet factor 4. These PMPs enter bacterial cells and change their permeability, therefore stopping the synthesis of essential components.

By means of localized antibacterial capabilities, combining electrospun PCL scaffolds with ZnO-NPs and I-PRF into a composite membrane should improve therapeutic treatments. These results add to the amount of knowledge already in publication that supports this assertion. Combining their antibacterial features could reveal insights into bacterial resistance processes, strategies to increase inhibitory activity, and means to extend the interaction between ZnO-NPs and i-PRF.

Conclusion

Our results revealed substantial differences in antibacterial activity among the groups, indicating that the dosages of ZnO significantly suppressed bacterial growth, particularly against *S. mutans* and *P. gingivalis*. There was a clear relationship between the dose and the mean percentage of inhibition. This indicates that the level of ZnO added to PCL changed as the concentrations increased, resulting in a more powerful inhibition. Furthermore, the suppressive impact of ZnO was enhanced by the inclusion of i-PRF; higher quantities of ZnO resulted in more efficient eradication of both bacteria.

Clinical Significance

The combination of injectable platelet-rich fibrin (i-PRF) and electrospun polycaprolactone (PCL) scaffolds augmented with zinc oxide nanoparticles (ZnO-NPs) shows significant potential for periodontal tissue regeneration. ZnO-NPs enhance the antimicrobial properties of PCL scaffolds, effectively inhibiting the growth of key oral pathogens, Streptococcus mutans and Porphyromonas gingivalis, in a dose-dependent manner. The addition of i-PRF further enhances the antibacterial efficacy of the scaffold. This innovative approach could lead to the development of advanced, infection-resistant biomaterials for periodontal regeneration, potentially mitigating the issue of antibiotic resistance and reducing the reliance on systemic antibiotic therapy. This novel strategy represents a promising advancement in periodontal tissue engineering, offering a robust solution for maintaining oral health and addressing periodontal disease.

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Supplementary data available online

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