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Comparative study of bacterial growth between PTFE membrane and zirconium membrane. *In vitro* study

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Abstract
Introduction: Bacterial adhesion to biomaterials used in guided bone regeneration procedures represents a critical factor for the success of treatments in implantology. Microorganisms associated with persistent infections with *Enterococcus faecalis* have the ability to survive, form biofilms, and resist adverse conditions.
Objective: Evaluate which surface of the PTFE and Zirconia membranes (advanced ceramic membrane based on zirconium dioxide ZrO_2) has greater growth and bacterial contamination of the *Enterococcus faecalis* strain, *in vitro* study.
Materials and methods: The researchwas quantitative, observational, longitudinal, experimental, and *in vitro*, with PTFE and zirconia membranes inoculated with the bacteria under controlled conditions and evaluated at 2 and 7 days. Bacterial growth was identified using the imprint technique and with the count of CFU/ml, which were transformed to log10 for statistical analysis (non-parametric Mann-Whitney and Wilcoxon tests) with a 95% confidence level.
Results: The PTFE membranes exhibited greater bacterial growth compared to the zirconia membranes at both evaluated time points. Likewise, bacterial proliferation was evidenced during the incubation period, with a statistical variation having a p-value <0.05.
Conclusion: Zirconia membranes exhibit lower bacterial colonization by *Enterococcus faecalis* compared to PTFE membranes, making them a priority alternative in guided bone regeneration procedures and implantology.

Keywords: PTFE Membrane, Zirconia, *Enterococcus faecalis*, Oral Implantology, Dental Biomaterials

Introduction

Guided bone regeneration (GBR) is a surgical procedure that employs a barrier membrane to prevent soft tissue growth while allowing new bone formation (Abtahi *et al*., 2023) [1]. Various factors, such as dental extraction, trauma, periodontal disease, and previous failed treatments, can lead to significant alveolar bone loss; in these situations, guided regeneration techniques are a fundamental tool for restoring the patient's function and esthetics (Marian *et al*., 2024) [2]. Therefore, one of the available surgical techniques is the guided bone regeneration procedure (Marian *et al*, 2024) [2].
Barrier membranes are used in bone regeneration second surgical intervention for their removal, while resorbable membranes are biodegradable and have resorption rates. Regardless of the types of membranes, they differ in their physical and biomaterial characteristics (Milillo *et al*, 2021) [4]. Tissue compatibility, space maintenance, blood clot stabilization, cellular occlusion, mechanical strength, and predictable resorption rates are also considered (Pellegrini *et al*., 2024) [5].
Experimental and clinical studies using bioresorbable and non-resorbable membranes, such as polytetrafluoroethylene (PTFE), titanium meshes, collagen with polylactic acid, and copolymers, are associated with infections and bacterial exposure in bone regeneration (Abtahi *et al*., 2023) [1]. In bone regeneration, risks of infection, pain, graft rejection, and even limited regenerated tissue are considered; another common problem in implantology is partial or total loss of the alveolar structure due to dental extractions and dental treatments, which pose risks in terms of bone resorption (Liu *et al*., 2025) [6]. The exposure of membranes allows for bacterial contamination during surgery or in the regeneration stage (Ortiz *et al*., 2006) [7].
Enterococcus faecalis is a facultative anaerobic gram-positive microorganism and is the main pathogen associated with endodontic treatment failure (Coronel *et al*., 2026) [8]. Its presence is in the root canals and surrounding bone. It is considered a key factor in bone loss (Flanagan, 2017) [9]. The growth and contamination occur on the inner or outer surface of the membrane in contact with the epithelium (Blancas *et al*., 2021) [10]. Similarly, they colonize alone, in pairs, or in chains, and appear as coccobacilli when Gram-stained from agar plate samples. It is a microorganism that grows optimally at 35°C; however, growth has been observed between 10 and 45°C (Gutiérrez *et al*., 2023) [11].
The influence of membranes on bone regeneration is the ability to resist bacterial colonization, and the study of risk factors such as surface texture, biomaterial characteristics, and porosity changes adherence (Shi *et al*., 2025) [12]. The evaluation of the presence of *Enterococcus faecalis* in non-resorbable membranes that are removed in a second surgery, except in some reinforcement treatments that are left in place for a longer time (Pupo Marrugo *et al*., 2014) [13]. Non-resorbable membranes are materials approved for clinical use. They maintain structural integrity and are left on tissues for a long time. Their function is temporary, and once completed, they are removed, but they are susceptible to post-surgical bacterial contamination (Burgos, 2005) [14].
Among the risks is the presence of the bacterium *Enterococcus faecalis*, which is considered resistant, survives in hostile environments, and colonizes surgical surfaces, including membranes (Romero *et al*., 2025) [15]. The evaluation of dental membranes in the presence of

bacteria that possess the ability to colonize and infect and are capable of synthesizing a wide variety of proteins under adverse environmental conditions, such as high pH (Rodríguez-Niklitschek *et al*., 2015) [16]. The verification of the bacteria's ability to form biofilms on smooth surfaces such as PTFE and Zirconia membranes, which can compromise bone regeneration in the presence of soft-tissue infection (Vargas, 2016) [17].

In view of the above, the objective is to compare which surface of the PTFE and Zirconia membranes shows greater growth and bacterial contamination by the *Enterococcus faecalis* strain in an *in vitro* study. To achieve the proposed objective, a quantitative, observational, longitudinal, experimental, and *in vitro* study was conducted.

Methodology

The present study was quantitative, observational, longitudinal, experimental, and *in vitro*, allowing for the evaluation and comparison of the surfaces of 40 non-resorbable barrier membranes (non-resorbable polytetrafluoroethylene (PTFE) and Zirconium) with the bacterial growth of *Enterococcus faecalis*. The sample size was calculated using the formula for comparing means, with a 95% confidence level, 80% statistical power, an estimated deviation of 0.5, and a minimum detectable difference of 0.7 logarithmic units of CFU/ml. Simple random sampling was used for the following subdivision of the sample: Group 1A (n=10): PTFE membrane with an incubation time of 2 days, Group 1B (n=10): PTFE membrane with an incubation time of 7 days, Group 2A (n=10): Zirconia membrane with an incubation time of 2 days, and Group 2B (n=10): Zirconia membrane with an incubation time of 7 days. The dimensions of the dental membranes were 1 x 1 cm.

In the research, non-resorbable polytetrafluoroethylene (PTFE) and Zirconium membranes measuring 1 x 1 cm, new and sterile, were included; non-resorbable manipulated membranes, without aseptic conditions and defective, were excluded.

The preparation, handling, and standardization of the dental membranes were carried out by the researcher. Initially, the membranes were placed on the sterile surface using tweezers and gloves. They were cut to standard dimensions of 1 x 1 cm to ensure uniformity in the experiment. The preliminary cleaning was performed with sterile saline solution and distilled water. Prior to contamination with the microorganism, the dental membranes were sterilized. For groups 1A and 1B with PTFE membranes, sterilization was performed in an autoclave at 121 °C and 15 psi for 15 minutes. For groups 2A and 2B, the zirconia membranes were sterilized by UV radiation for 15 minutes.

To ensure the absence of microbiological contamination, each group was stored in sterile Petri dishes. The Enterococcus faecalis strains were independently cultured on supplemented blood agar plates. The culture plate was inoculated to achieve a higher bacterial load on the surface using 3% trypticase broth supplemented with 0.5% yeast extract, 5 µg/ml hemin, and 1 µg/ml menadione.

The bacterial culture was maintained at 37 °C under anaerobic conditions (a chamber with an atmosphere composed of: 10% CO_2, 5% H_2, and 85% N). The application of the standard inoculum volume on each membrane. Regarding the evaluation of bacterial growth, the preparation was carried out in 1 mL tubes with supplemented agar, covered with a 6 mm disk of the membrane type, sealed with a sterile plastic device, and inoculated with a semi-solid culture of the *Enterococcus faecalis* strain.

The bacteriological procedure was based on the preparation of the culture vials, which were carried out under sterile conditions in a type II laminar flow hood. After incubation for 2 and 7 days, the colony-forming units on the membrane disks were verified using the imprint technique. A Bunsen burner was used to sterilize the area around the culture, and then Gram staining with crystal violet (dye) was performed to detect microorganisms on the study membranes for one minute.

With a binocular optical microscope, the colony count (CFU) was visualized, then quantified using microbiological software, and the bacterial concentration was determined and expressed in CFU/ml. The results were presented in log10, as it facilitates the handling of very large numbers and allows for the simplification of these values for comparison between groups.

In the *in vitro* study, there was no intervention in humans or animals, and microbiological biosafety standards were applied, including the use of personal protective equipment, hygiene and disinfection, handling of microorganisms, and laboratory conduct rules.

The data were statistically analyzed using SPSS version 24. The Shapiro-Wilk normality test was applied at the 95% confidence level because the data had fewer than 50 samples. The non-parametric Mann-Whitney U test and the Wilcoxon test were used, with a p-value < 0.05 considered statistically significant for all comparisons of the presence of microorganisms on the surface of the membranes at different incubation times.

Findings

As observed in Table 1, four groups are identified by the type of non-resorbable barrier membranes (PTFE and zirconia) and the culture time (2 and 7 days). No missing data in the statistical analysis. The Shapiro-Wilk normality test indicated that the data do not follow a normal distribution (p-value < 0.05). The comparison of bacterial growth by membrane type was conducted using the Mann-Whitney U test, and incubation time was analyzed using the Wilcoxon test, at a 95% confidence level.

Table 1: Data distribution

Variable	Results
Total, non-resorbable barrier membranes	40
Number of groups	4
Size of each group	10
Total, non-resorbable barrier membranes	PTFE y Zirconio
Cultivation period	2 y 7 días
Lost data	0

Source: Own creation

In Table 2, the colony-forming unit (CFU) counts of *Enterococcus faecalis* strains (CFU/mL; Log 10) are reported for the 20 non-resorbable barrier membranes of PTFE and Zirconium, divided into two subgroups according to incubation times of 2 and 7 days. The mean with standard deviation is reported for the PTFE sample at 2 days, showing bacterial growth of 4.99 ± 0.02 CFU/ml (Log 10),

and at 7 days, 5.09 ± 0.03 CFU/ml (Log 10), indicating an increase in bacterial colonization from 2 to 7 days. Similarly, the mean and standard deviation of the zirconia sample at 2 days of growth was 4.38 ± 0.02 CFU/ml (Log 10), and at 7 days it was 4.48 ± 0.03 CFU/ml (Log 10), indicating an increase in the number of bacterial colonies as the cultivation time increased.

Table 2: Data of Colony Forming Units UFC/ml- log10

Variable	Nº	Mean	Standard deviation
PTFE (2 days) UFC/ml (Log 10)	10	4,99	0,02
PTFE (7 days) UFC/ml (Log 10)	10	5,09	0,02
Zirconia (2 days) UFC/ml (Log 10)	10	4,38	0,03
Zirconia (7 days) UFC/ml (Log 10)	10	4,48	0,03

Source: Own creation

In Table 3, it presents the statistical comparison of bacterial growth of the PTFE and zirconia membranes at 2 days and 7 days, showing a significant difference (U=0.000; p=0.000).

Table 3: Comparison of CFU/ml (log 10) based on the type of membrane

Cultivation period	Comparison	Nº total of samples	U values from the Mann-Whitney test	p-valor	Interpretation
(2 days) UFC/ml (Log 10)	Membrane PTFE- Zirconia	20	0,000	0,000	Significant
(7 days) UFC/ml (Log 10)	Membrane PTFE- Zirconia	20	0,000	0,000	Significant

Source: Own creation

According to the data in Table 4, regarding the quantification of CFU/mL (log 10) of *Enterococcus faecalis* strains on the PTFE and zirconia membranes based on the cultivation time (2 and 7 days), with a p-value <0.05 indicating a significant difference between the bacterial growth on the PTFE membrane (p= 0.004) and zirconia (p= 0.005) with respect to 2 days and 7 days.

Table 4: Comparison of CFU/ml (log 10) based on the cultivation period

Membrane	Comparison	Total, Nº of samples	p-value from Wilcoxon test	Interpretation
PTFE UFC/ml (Log 10)	2 – 7 days	20	0,004	Significant
Zirconia UFC/ml (Log 10)	2- 7 days	20	0,005	Significant

Source: Own creation

Discussion

In the present study, it was determined that the bacterial growth of *Enterococcus faecalis* strains quantified in CFU/mL was higher in PTFE membranes exposed for 2 and 7 days, with 4.99± 0.02 and 5.09± 0.02. The Zirconia membranes reached values of 4.38±0.03 and 4.49±0.03. The quantitative variation allowed us to identify significant differences according to the Mann-Whitney test, with a p-value < 0.05.

It was shown that the Zirconium membranes in the study exhibited lower microbial adherence of *Enterococcus faecalis* strains, according to Hofferber *et al*. (2018) [18], who consider it an innovative and ideal material for guided bone regeneration due to its high biocompatibility, reduced inflammatory response, and biofilm adhesion compared to titanium. These membranes are used to guide the placement and align the intended position of the dental implant.

Likewise, Ziad *et al*. (2024) [19] determined that guided bone regeneration (GBR) is a predictable approach and that the techniques involve the use of absorbable and non-absorbable membranes. In this context, biocompatibility and adequate mechanical strength maintain space for new bone formation, providing flexibility and adjustability to adapt to the shape of the bone defect.

The suggested conditions for the membranes are a thickness of 0.5 to 1 mm; the membrane rests only on three bony margins: mesial and lateral to the vestibular bone defect area and over the lingual plate to achieve proper support and fit (Ziad *et al*., 2024) [19].

In fact, zirconia membranes, unlike titanium, are brittle ceramic materials with little flexibility, making in situ adaptation difficult when design and manufacturing inaccuracies occur. Extreme caution is required when adapting the fixation screw.

Similarly, Abtahi *et al*. (2023) [1] described that patients undergoing alveolar ridge augmentation with customized zirconium membranes achieve better results in terms of bone gain.

The results of our study demonstrated greater bacterial colonization on PTFE membranes; this difference is associated with physicochemical characteristics such as roughness, surface energy, and hydrophobic properties that directly influence microbial adhesion. Consequently, the polymeric surface of PTFE favors the initial formation of the bacterial biofilm. But ceramic materials like zirconia with smoother surfaces exhibit lower bacterial adhesion capacity, which explains the lower UFC/ml values observed in the results.

Ortiz *et al*. (2006) [7] explained that some membranes used as barriers were designed and developed for guided tissue regeneration, among them PTFE-e, which has a carbon-carbon bond with four fluorine atoms in its polymer chain. Moreover, it is inert and persists after regeneration and is removed in a second operation.

The possibility of membrane contamination arises from exposure during the surgical process and the regeneration stage, implying that bacterial accumulation on the membranes depends on the texture and surface characteristics.

Meanwhile, Sadanandan and Yogendraiah (2025) [20] explained that the main cause of failure in dental treatment is the persistence of various bacteria in bone regeneration, with *Enterococcus faecalis* being the most relevant, as it is a gram-positive anaerobic coccus with high resistance to disinfectant agents, capable of surviving in extreme conditions, environments with scarce nutrients, and highly alkaline pH levels of up to 11.5.

The presence of this bacterium in bone regeneration membranes negatively affects adhesion and leads to reduced development of cortical bone due to post-treatment infection (Abdo *et al*., 2023) [21].

To all this, Sowmiya *et al*. (2025) [22] studied the antibacterial efficiency of bone regeneration barrier membranes on *Enterococcus faecalis* over a 24-hour incubation period, establishing that PTFE showed the least bacterial efficacy, with the least inhibition, indicating that there is a direct relationship between the structural properties, variation in pore size, and composition of the biomaterial with the adhesion of the microorganism and the success rate of the GTR/GBR technique.

The bacterial count CFU/ml in dental membranes shows minimal variation in the growth of *Enterococcus faecalis*, with the PTFE membrane for 2 days being 4.99± 0.02 and the zirconia membrane 4.38± 0.03.

Now, Torres-Solis *et al*. (2025) [23] reported that the prevalence of failures in bone regeneration treatments with non-resorbable membranes is approximately 11-23%, most of which are associated with the presence of bacteria (such as Enterococcus faecalis) that colonize the surface of the biomaterial.

Conclusions

The microbiological analysis conducted in the study showed that the surface of the PTFE membranes exhibited a higher number of Colony Forming Units (CFU/mL) of *Enterococcus faecalis* than that of the zirconia membranes, indicating a greater bacterial adhesion capacity of the polymeric material.

Zirconia membranes exhibit lower levels of bacterial colonization; this depends on their physicochemical properties, with lower surface roughness and greater structural stability limiting the formation of bacterial biofilms. In the clinical setting, it is a promising alternative for guided bone regeneration procedures and implantology therapies.

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