Original Article
http://dx.doi.org/10.1590/1678-7757-2023-0135

JAOS
JOURNAL OF APPLIED ORAL SCIENCE
ISSN 1678-7765

Adjuvant effects of *Saccharomyces cerevisiae* in the treatment of experimental periodontitis in rats undergoing chemotherapy

Valdir Gouveia GARCIA[1,2]
Tiago Esgalha da ROCHA[2]
Natália Amanda GOMES[2]
Daniela Maria Janjácomo MIESSI[2]
Marta Aparecida Alberton NUERNBERG[2]
João Victor Soares RODRIGUES[2]
Jânderson de Medeiros CARDOSO[1]
Edilson ERVOLINO[3]
Letícia Helena THEODORO[2]

Abstract

Surgical procedures, radiotherapy, and chemotherapy, individually or in association, are current oncological treatments. Among the most used chemotherapy drugs, 5-fluorouracil (5FU) is an antimetabolite with a broad spectrum of action. This study evaluated the effects of probiotics (PRO) as an adjuvant to the treatment of experimental periodontitis (EP) in rats immunosuppressed with 5FU. Methodology: 108 rats were randomly allocated to six different groups: EP; SS – systemic treatment with saline solution (SS); 5FU – systemic treatment with 5FU; 5FU+PRO – systemic treatment with 5FU, followed by the local administration of *Saccharomyces cerevisiae*; 5FU+SRP – systemic treatment with 5-FU, followed by scaling and root planing (SRP); and 5FU+SRP+PRO – systemic treatment with 5FU followed by local treatments with SRP and PRO. Immunosuppression was obtained at two points: at the time of ligature installation and after 48 h. Six animals from each group were euthanized at seven, 15, and 30 d and hemimandibles were collected and processed for histopathological, histometric, and immunohistochemical analysis. Data were subjected to statistical analysis (α=5%). Results: At 7 d, the 5FU+PRO group showed less bone resorption and better structured connective tissue compared with the EP, SS, 5FU+SRP, and 5FU+SRP+PRO groups. At 15 d, the 5FU+SRP group showed a greater intensity of the inflammatory response ($p<0.05$). At 30 d, the 5FU+SRP+PRO group showed better structured bone tissue and a higher percentage of bone tissue (PBT) than the EP, SS, 5FU, and 5FU+PRO groups ($p<0.05$). Conclusion: The use of *Saccharomyces cerevisiae* as monotherapy or as an adjuvant to periodontal therapy may have a positive effect on bone repair in immunosuppressed conditions.

Keywords: Cancer. Periodontitis. Periodontology. Probiotics. Chemotherapy. Fluorouracil. Animal model.

Corresponding address:
Leticia Helena Theodoro
Faculdade de Odontologia de Araçatuba - UNESP
- Rua José Bonifácio, 1193 - Centro -
Zip Code: 16015-050 - Araçatuba - SP - Brazil.
Phone: (+55) 18 36362860
e-mail: leticia.theodoro@unesp.br

Received: April 15, 2023
Revised: September 17, 2023
Accepted: October 11, 2023

Editor: Linda Wang
Associate Editor: Daniela Rios Honório

[1]Instituto Latino Americano de Pesquisa e Ensino Odontológico (ILAPEO), Curitiba, PR, Brasil.
[2]Universidade Estadual Paulista (UNESP), Faculdade de Odontologia de Araçatuba, Departamento de Diagnóstico e Cirurgia, Divisão Periodontia, Araçatuba, SP, Brasil.
[3]Universidade Estadual Paulista (UNESP), Faculdade de Odontologia de Araçatuba, Departamento de Ciências Básicas, Araçatuba, SP, Brasil.

Introduction

In 2020, the Global Cancer Observatory (GLOBOCAN) reported the occurrence of 19.3 million new cancer cases and 10 million cancer deaths worldwide.[1] On the other hand, an epidemiological study with North American adults showed that 46% of the population (i.e., 67 million individuals) had periodontitis.[2] Therefore, many of these individuals diagnosed with periodontitis also had some type of malignant neoplasm, which requires the attention of researchers and clinicians.[3]

Surgical procedures, radiotherapy, and chemotherapy, individually or in association, are current oncological treatments.[1,4] Among the most used chemotherapy drugs, 5-fluorouracil (5FU) is an antimetabolite with a broad spectrum of action. It directly affects DNA synthesis and causes side effects in the oral cavity, such as mucositis and xerostomia.[5,6] The influence of 5FU on periodontal tissues was evaluated in recent animal studies, which showed that this drug exacerbates periodontal inflammation, with a greater inflammatory infiltrate and higher RANKL immunostaining scores.[7-12] This not only aggravates the progression of experimental periodontitis (EP) in rats, but also interferes with the response to periodontal treatment.[7-12]

The consensus regarding therapeutic modalities for stage I–III periodontitis is the control and reduction of pathogenic subgingival microbiota by supra- and subgingival mechanical therapy, with scaling and root planing (SRP).[13] However, some sites and/or patients may have a limited response to treatment as a result of periodontal disease manifestations, systemic conditions, or the use of medications that can result in a loss of supporting periodontal tissue, independently of periodontitis.[14-16] It is suggested that these limitations are related to the pathogenic microbiota that prevent the proposed therapy from converting the dysbiotic infectious process into an equilibrium condition, either due to the presence of a residual subgingival biofilm after SRP or due to a chronic inflammatory response despite mechanic debridement.[13] For this reason, animal and clinical studies are underway to evaluate adjuvant therapies that may improve the outcomes of subgingival SRP.[17-20]

Previous animals studies and human clinical trials evaluated the effect of probiotics as an adjuvant therapy for periodontal diseases.[17,20,21] The World Health Organization (WHO) describes probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[22] Compounds such as paraprobiotics (inactivated probiotic microorganisms) and postbiotics (concentrated active bacterial metabolites) have shown promising results in *in vitro* and *in vivo* studies.[18,23] A recent systematic review with meta-analysis evaluated the effects of probiotics on periodontitis, under the hypothesis that, when applied as an adjuvant therapy, they can cause a long-term effect, promoting changes in the subgingival microbiota, with good healing of periodontal pockets and periodontal tissue repair nine to 12 months after SRP.[20] Data from the analyzed studies showed beneficial effects of probiotics in reducing probing depth and bleeding on probing, modulating the host response with a reduction in inflammatory cytokines (TNF-α, IL-1β, and IL17) and an improvement in microbiological patterns similar to the findings of studies using systemic antibiotics.[24,25] Animal experiments have been performed to evaluate the effect of probiotics in healthy systemic[21,26-28] or immunosuppressed conditions.[11,29] A recent study by our group evaluated the effect of the systemic use of *Lactobacillus reuteri* in the treatment of EP in immunosuppressed animals, and showed promising results in controlling periodontal tissue inflammation in rats.[11] However, only one study evaluated the effect of *Saccharomyces cerevisiae* as a local adjuvant in the treatment of EP in healthy animals.[17] In view of the above, there are few studies in the literature that evaluate the effect of probiotics on the control of periodontal disease in immunosuppressed conditions.[11,30] This study considered the hypothesis that the use of *Saccharomyces cerevisiae* as monotherapy or its association with nonsurgical periodontal therapy could minimize the deleterious effects of immunosuppression on the progression of periodontitis. Thus, this study aimed to evaluate the effects of *Saccharomyces cerevisiae* as an adjuvant to the treatment of EP in rats immunosuppressed with 5FU.

Methodology

Animals

This study was conducted on 108 three-month-old male rats (*Rattus norvegicus albinus, Wistar)* weighing

180 to 250 g. They were healthy and able to undergo the procedures. They were kept in a temperature-controlled environment (21±1°C), with 12/12 light cycles, and received food and water *ad libitum*. The research protocol (#00191-2013) was approved by the Animal Ethics Committee in accordance with the ARRIVE guidelines.[30]

Experimental protocol

For the surgical procedures, rats received intraperitoneal injections of ketamine hydrochloride (70 mg/kg; Vetaset, Fort Dodge, Iowa, USA) and xylazine hydrochloride (6 mg/kg; Coopazine, Coopers, São Paulo, Brazil). EP was induced by installing a cotton thread around the lower left first molar. The ligature was maintained for 7 d in the subgingival position.[11]

The ligature was removed and rats were divided into six groups (n=18/group): EP; SS – systemic treatment with saline solution (SS); 5FU – systemic treatment with 5FU and local irrigation with SS; 5FU+SRP – systemic treatment with 5FU followed by local SRP; 5FU+PRO – systemic treatment with 5FU followed by local irrigation with 0.6 mL of the probiotic (PRO); and 5FU+SRP+PRO – systemic treatment with 5FU, local SRP, and irrigation with 0.6 mL of PRO. A schematic representation of all groups can be found in Figure 1.

Systemic treatments

Systemic treatment with 5FU (50 mg/mL; Laboratório Eurofarma, São Paulo, SP, Brazil) consisted of an intraperitoneal injection of 80 mg/kg at the time of ligature installation and 40 mg/kg 48 h later in the 5FU, 5FU+SRP, 5FU+PRO, and 5FU+SRP+PRO groups.[11] Rats in the SS group received a systemic administration of 0.5 mL of SS on the day of ligature installation and 48 h later.[11]

Local treatments

SRP

The SRP procedures were performed by the same experienced and trained operator, blinded to the experimental groups (T.E.R.),[11] with one or two Mini Five manual curettes (Hu-Friedy, Chicago, IL, USA).[11]

Probiotic treatment

PRO (*Saccharomyces cerevisiae*, FR 1972, Florax®; Hebron Farmacêutica, Caruaru, PE, Brazil) was applied locally at three times: immediately after and 48 and 96 h after ligature removal (5FU+PRO) or SRP (5FU+SRP+PRO). Each PRO application consisted of 0.6 mL (1x10^8 CFU of *S. cerevisiae)*, administered directly into the gingival sulcus using a 1 mL syringe and a non-beveled needle (13x4.5 mm).[17]

Histological processing

Six rats from each group were euthanized by

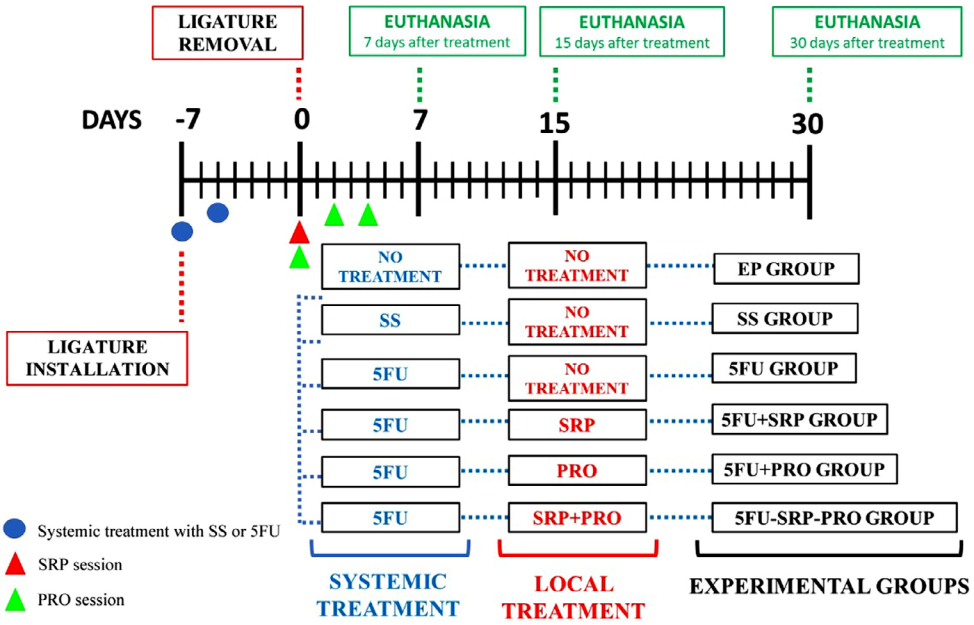

Figure 1- Schematic illustration of the study design. Abbreviations and symbol: EP: no local treatment; SS: systemic treatment with saline solution (SS); 5FU: systemic treatment with 5FU and local irrigation with SS; 5FU+SRP: systemic treatment with 5FU followed by local scaling and root planing (SRP); 5FU+PRO: systemic treatment with 5FU followed by local irrigation with 0.6 mL of the probiotic (PRO); 5FU+SRP+PRO: systemic treatment with 5FU, local SRP treatment and irrigation with 0.6 mL of PRO; blue circle: systemic treatment with 5FU or SS; red triangle: SRP session; green triangle: PRO session

injection of a lethal dose of thiopental (150 mg/kg; Cristália, Produtos Químicos Farmacêuticos Ltda., Itapira, SP, Brazil) at 7, 15, and 30 d after treatment. After fixation, the left hemimandibles were carefully handled and subjected to demineralization in 10% ethylenediaminetetraacetic acid for eight weeks.[21] Subsequently, they were dehydrated by immersion in serial dilutions of alcohol. After paraffin embedding, the samples were sectioned in the sagittal plane, always following the long axis of the tooth, on 4 µm thick slides.[21] Some serial sections of the lower left first molar were collected, mounted on glass slides, and stained with hematoxylin and eosin (HE), while other sections were subjected to immunohistochemical processing.

Histomorphometric and histological analysis

A histomorphometric analysis of alveolar bone loss (ABL),[11] percentage of bone tissue (PBT),[7] percentage of vital bone (PVB),[31] and percentage of non-vital bone (PNVB)[31] in the furcation area (FA) was conducted. A certified and blinded histologist (E.E.) performed the histological analyses.[7] Images were captured using a digital camera (AxioCam®, ZEISS, Gottingen, Germany) coupled to a light microscope (AxioLab®, ZEISS, Gottingen, Germany) and connected to a microcomputer. Histological analyses were performed in the FA, using a scoring system (Table 1),[7] by a calibrated examiner, blinded to the treatments (E.E.). The ABL area was delimited by contouring the entire cementum surface between the bone crest and the roof of the furcation.[7] For these analyses, the FA, the bone tissue area (BTA), the vital bone tissue area (VBT), and the non-vital bone tissue area (NVBT) in the FA were measured in mm^2. The BTA had the same apical limit as the FA and, from this limit, followed the entire external surface of the alveolar bone between the roots. The VBT and NVBT defined the area of vital or non-vital bone tissue between the roots. The PBT, PVB, and PNVB in the furcation region were calculated by multiplying the BTA, VBT, or NVBT consecutively by 100 and dividing by the FA. The ABL, PBT, PVB, and PNVB of each specimen were measured three times by the same examiner (T.E.R.) on different days.[7]

Immunohistochemical analysis

The histological sections were deparaffinized in xylene, hydrated in a decreasing series of ethanol, and subjected to indirect immunoperoxidase. Antigen retrieval was performed by immersing the histological slides in 0.1 M citrate buffer (pH 7.4; Diva Decloaker®, Biocare Medical, Concord, CA, USA) in a pressurized chamber (Decloaking Chamber®, Biocare Medical, Concord, CA, USA) at 95°C for 20 min.[31] The slides with samples from each experimental group were divided into five batches. Each batch was incubated with one of the following primary antibodies: osteoprotegerin (OPG; goat anti-OPG, Santa Cruz Biotechnology, Santa Cruz, CA, USA; SC 8468); tartrate-resistant acid phosphatase (TRAP; goat anti-TRAP, Santa Cruz Biotechnology, Santa Cruz, CA, USA; SC 30833); and RANKL ligand (RANKL; goat anti-RANKL, Santa Cruz Biotechnology, Santa Cruz, CA, USA; SC 7628). The histological slides were counterstained with Harris hematoxylin. Images were captured using a digital camera (AxioCam®, ZEISS, Gottingen, Germany) coupled to a light microscope (AxioLab®, ZEISS, Gottingen, Germany) and connected to a microcomputer. A histologist (E.E.) performed the immunohistochemical analyses. The cells immunolabeled for TRAP were located in the center of the interradicular septum.[11] The coronal limit of this area was the alveolar bone crest, from which it extended apically for 1,000 µm. For RANKL and OPG immunolabeling, a semiquantitative analysis of immunoreaction was performed throughout the FA: score 0, no immunolabeling [total absence of immunoreactive (IR) cells]; score 1, low immunolabeling pattern (1/4 IR cells); score 2, moderate immunolabeling pattern (1/2 IR cells); and score 3, high immunolabeling pattern (3/4 IR cells).[11] Quantitative analysis of TRAP immunostaining was performed by a blinded and calibrated examiner (D.M.J.M.), by counting TRAP-positive cells located in the center of the interradicular septum of the lower first molar in an area of 1,000x1,000 µm, with 200x magnification in three equidistant histological sections[11]. The coronal limit of this area was the alveolar bone crest, from which it extended apically for 1,000 µm.[11] Semiquantitative analyses for RANKL and OPG were performed in the FA at 400x magnification by a certified and blinded histologist (E.E.). RANKL and OPG scores were individually analyzed in each experimental group.[21]

Examiner calibration

Before the histometric and immunohistochemical analyses began, two examiners were trained and calibrated. To this end, they performed two

measurements of ABL, PBT, PVB, and PNVB (T.E.R.), and TRAP (D.M.J.M.) of 24 species, with an interval of one week. Intraexaminer calibration was assessed by the Kappa test (95%).[32]

Statistical analysis

The sample size was calculated considering the PBT in the furcation region as the primary outcome.[33] The secondary outcome was described by the immunostaining pattern and histological features in the FA. Considering the minimum difference of 4% between the treatment means and a standard deviation of 1.3% of the PBT, the results showed a sample size of four animals ($\alpha=0.05$)[7] with a study power of 95% (BioEstat, version 5.3, Instituto Mamirauá, Manaus, Brazil).

Statistical analysis of all data was performed using BioEstat (version 5.3, Instituto Mamirauá, Manaus, Brazil) at a significance level of 5%. The normality of the histometric (ABL, PBT, PVB and PNVB) and immunohistochemical (TRAP) data was assessed using the Shapiro-Wilk test. Intra- and intergroup analyses were subjected to analysis of variance (ANOVA) and Tukey's post-hoc test ($p<0.05$).

Semiquantitative data from the histological analysis of periodontal tissues were subjected to the Kruskal-Wallis test, followed by the Student-Newman-Keuls post-test when a statistically significant difference was detected ($p<0.05$).

Results

Histological analysis

The results of histological analysis are shown in Table 1. The 5FU group showed a greater inflammatory response in the FA compared with the 5FU+PRO group at 15 d ($p<0.05$); the EP, SS, and 5FU+SRP+PRO groups at 15 and 30 d; and the 5FU+SRP group at 30 d ($p<0.05$). The 5FU+SRP group showed a greater inflammatory response than the EP, SS, 5FU+PRO, and 5FU+SRP+PRO groups at 15 d ($p<0.05$). At 30 d, the 5FU+SRP and 5FU+SRP+PRO groups showed a lower inflammatory response than the 5FU+PRO group ($p<0.05$). In the intragroup analysis, the 5FU+SRP group had a lower inflammatory response at 30 d than at days 7 and 15 ($p<0.05$). The 5FU+SRP+PRO group had a greater inflammatory response at 7 d than at 15 and 30 d after treatment ($p<0.05$), while the 5FU+PRO group showed a lower response at 15 d compared with 7 and 30 d ($p<0.05$; Figure 2). The 5FU group showed a greater extent of the inflammatory process than all the other groups at 15 and 30 d ($p<0.05$). In the intragroup analysis, 5FU+SRP, 5FU+PRO, and 5FU+SRP+PRO had a greater extent at 7 d than at 15 and 30 d ($p<0.05$; Figure 2). The 5FU group showed greater external root resorption compared with all the experimental groups at 7 d. In the intragroup analysis, 5FU showed greater root resorption at 7 d than at 30 d ($p<0.05$; Figure 2). In the intragroup analysis for the 5FU+SRP and 5FU+SRP+PRO groups, resorption was greater at 7 d compared with 30 d ($p<0.05$; Figure 2). The pattern of connective tissue structuring in the furcation region was worse in EP and SS than in 5FU+PRO at 7 d ($p<0.05$) and 5FU+SRP and 5FU+SRP+PRO at 30 d ($p<0.05$). The 5FU group showed a worse pattern of connective tissue structuring than 5FU+PRO at 7 and 15 d ($p<0.05$), 5FU+SRP at 15 and 30 d ($p<0.05$), and 5FU+SRP+PRO at 30 d ($p<0.05$). The 5FU+PRO group showed a better pattern of connective tissue structuring compared with the 5FU+SRP and 5FU+SRP+PRO groups at 7 d ($p<0.05$). The 5FU+PRO group had a worse pattern of connective tissue structuring than the 5FU+SRP+PRO group at 30 d ($p<0.05$).

The 5FU+SRP group showed worse connective tissue structuring in the FA at 7 d compared with 15 and 30 d ($p<0.05$). The 5FU+SRP+PRO group, on the other hand, showed better structuring at 30 d than at days 7 and 15 ($p<0.05$; Figure 2). Regarding the pattern of bone tissue structuring in the FA, the 5FU group had worse structuring compared with the 5FU+SRP+PRO group at 15 and 30 d ($p<0.05$) and the EP, 5FU+SRP, and 5FU+PRO groups at 30 d ($p<0.05$). In the intragroup analysis, this structuring pattern was better at 30 d than at 7 d in the 5FU+SRP+PRO group ($p<0.05$; Figure 2).

Histometric analysis (ABL, PBT, PVB and PNVB)

The 5FU+PRO group had high ABL compared with the SS group at 30 d ($p<0.05$; Figure 3). There were no statistically significant intragroup differences ($p<0.05$). The EP group showed higher PBT compared with the 5FU+SRP+PRO group at 7 d ($p<0.05$) and the 5FU, 5FU+SRP, and 5FU+PRO groups at 7 and 15 d ($p<0.05$). Even at 7 d, the 5FU+SRP+PRO group had higher PBT than the 5FU+SRP group ($p<0.05$). At 30 d, the 5FU+SRP+PRO group showed higher PBT

Table 1 Parameters, scores, and distribution of samples in percentage (%) according to the histological analysis of the mandibular first molar in different experimental groups and periods.

PARAMETERS AND SCORES	PERCENTAGE OF SAMPLES (%)																	
	EXPERIMENTAL GROUPS																	
	EP			SS			5FU			5FU+SRP			5FU+PRO			5FU+SRP+PRO		
	7 d	15 d	30 d	7 d	15 d	30 d	7 d	15 d	30 d	7 d	15 d	30 d	7 d	15 d	30 d	7 d	15 d	30 d
INTENSITY OF LOCAL INFLAMMATORY RESPONSE																		
(1) no inflammation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) mild inflammation (up to 1/3 of the cells are inflammatory)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(3) moderate inflammation (from 1/3 to 2/3 of the cells are inflammatory)	33	67	67	33	67	67	-	-	-	-	-	100	33	100	33	-	67	100
(4) severe inflammation (more than 2/3 of the cells are inflammatory)	67	33	33	67	33	33	100	100	100	100	100	-	67	-	67	100	33	-
MEDIAN	3	2	2	3	2	2	3	3*†	3*†	3	3*†	2‡βμ	3	2‡¶β	3¶μ	3	2‡¶	2‡α
EXTENT OF INFLAMMATORY PROCESS																		
(1) no inflammation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) extending only to part of the connective tissue in the furcation region	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(3) extending throughout the connective tissue in the furcation region	33	67	67	33	67	67	-	-	-	-	67	100	-	100	100	-	67	100
(4) extending throughout the connective tissue and bone in the furcation region	67	33	33	67	33	33	100	100	100	100	33	-	100	-	-	100	33	-
MEDIAN	3	2	2	3	2	2	3	3*†	3*†	3	2‡β	2‡β	3	2‡β	2‡β	3	2‡β	2‡β
EXTERNAL ROOT RESORPTION (CEMENTUM AND DENTIN)																		
(1) absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) only inactive resorption areas	-	-	-	-	-	-	-	-	-	-	-	33	-	-	33	-	-	33
(3) few active resorption areas	100	100	100	100	100	100	-	33	100	100	100	67	100	100	67	100	100	67
(4) many active resorption areas	-	-	-	-	-	-	100	67	-	-	-	-	-	-	-	-	-	-
MEDIAN	2	2	2	2	2	2	3*†	3	2β	2‡	2	2	2‡	2	2	2‡	2	2
ALVEOLAR BONE RESORPTION																		
(1) within normal patterns	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) small amount of bone resorption areas	-	-	-	-	-	-	-	-	-	-	-	33	-	-	-	-	-	33
(3) moderate amount of bone resorption areas	100	100	100	67	67	67	-	-	-	-	-	67	100	100	100	-	67	67
(4) large amount of bone resorption areas	-	-	-	33	33	33	100	100	100	100	100	-	-	-	-	100	33	-
MEDIAN	2	2	2	2	2	2	3	3	3	3	3	2‡β	2‡¶	2‡¶	2‡	3α	2	2‡β
CONNECTIVE TISSUE STRUCTURING PATTERN																		
(1) moderate number of fibroblasts and large amount of collagen fibers (dense connective tissue)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) moderate amount of both fibroblasts and collagen fibers	-	-	-	-	-	-	-	-	-	-	-	33	-	-	-	-	-	100
(3) small amount of both fibroblasts and collagen fibers	-	33	33	-	33	33	-	-	-	-	100	67	100	100	67	-	33	-
(4) severe tissue breakdown with necrotic areas	100	67	67	100	67	67	100	100	100	100	-	-	-	-	33	100	67	-
MEDIAN	3	3	3	3	3	3	3	3	3	3	2‡β	2*†‡β	2*‡¶	2‡	2	3α	3	2*†‡ αβμ
BONE ALVEOLAR STRUCTURING PATTERN																		
(1) regularly contoured bone trabeculae lined with active osteoblasts, including areas of new bone formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) irregularly contoured bone trabeculae lined with active osteoblasts and osteoclasts	-	-	-	-	-	-	-	-	-	-	-	33	-	-	-	-	-	33
(3) irregularly contoured bone trabeculae lined with active osteoclasts	67	67	100	67	67	100	-	-	-	67	67	67	67	67	100	33	100	67
(4) areas of necrotic bone and irregularly contoured bone trabeculae lined with active osteoclasts	33	33	-	33	33	-	100	100	100	33	33	-	33	33	-	67	-	-
MEDIAN	2	2	2	2	2	2	3	3	3*	2	2	2‡	2	2	2	3	2‡	2‡β

Symbols: *: significant difference compared with the EP group at the same experimental period; †: significant difference compared with SS at the same experimental period; ‡: significant difference compared with 5FU at the same experimental period; ¶: significant difference compared with SRP at the same experimental period; α: significant difference compared with PRO at the same experimental period; β: significant difference compared with 7 d in the same experimental group; μ: significant difference compared with 15 d in the same experimental group.

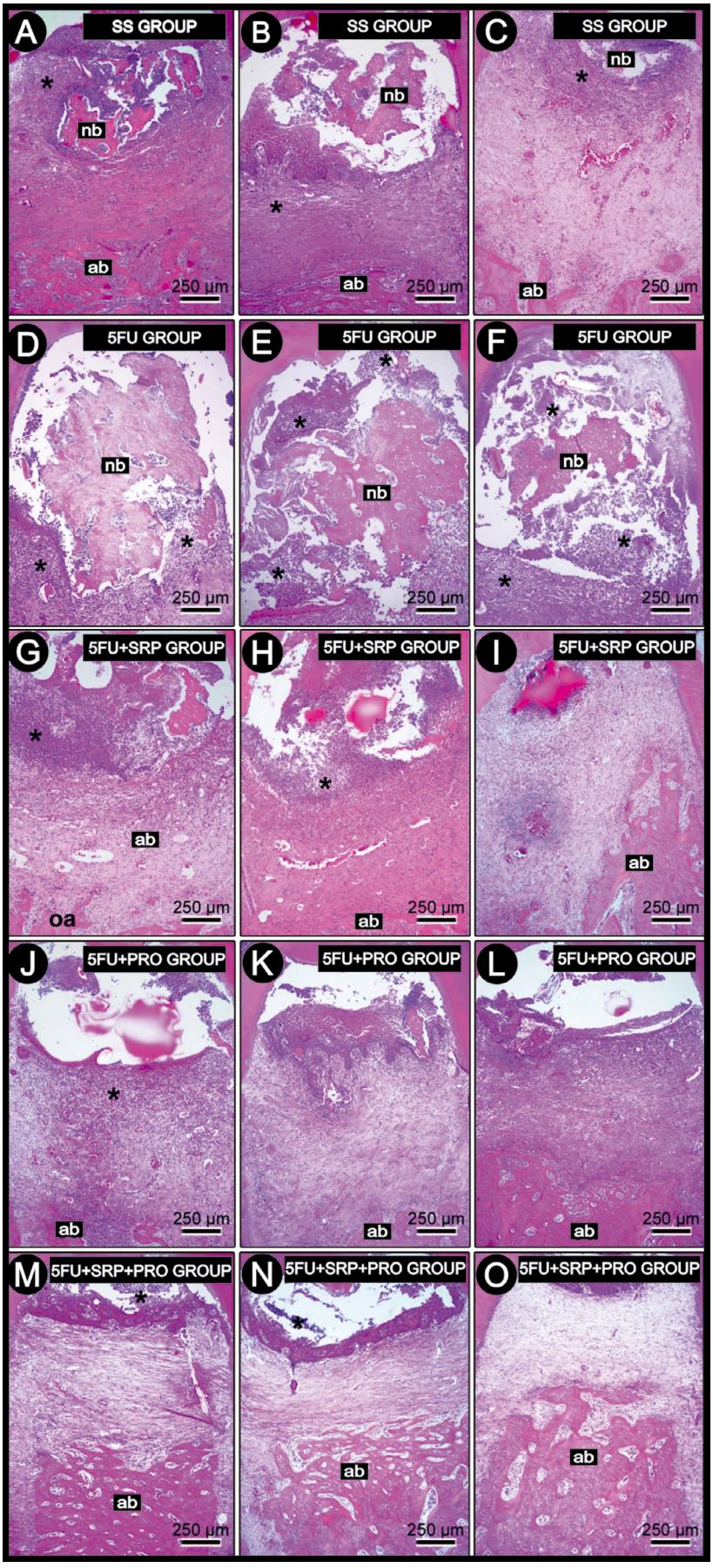

Figure 2- Photomicrographs of the lower left first molar with experimental periodontitis, showing the magnitude of the local inflammatory response, the level of alveolar bone loss, and the periodontal repair process in SS, 5FU, 5FU+SRP, 5FU+PRO e 5FU+SRP+PRO at 7 d (A, D, G, J, M), 15 d (B, E, H, K, N), and 30 d (C, F, I, L, O). Abbreviations and symbols: ab: alveolar bone; nb: necrotic bone; *: inflammatory infiltrate. Staining method: hematoxylin and eosin (H&E). Original magnification: 100x. Scale bars: 250 µm

compared with EP, SS, 5FU, and 5FU+PRO ($p<0.05$). In the intragroup analysis, the EP group had lower PBT at 30 d than at 7 or 15 d ($p<0.05$). The SS and 5FU+SRP groups showed lower PBT at 30 d than at 7 d ($p<0.05$; Figure 3). Regarding PVB, the EP and SS groups had higher PVB than the 5FU+PRO group at 7 d ($p<0.05$) and 5FU and 5FU+SRP at 7 and 15 d ($p<0.05$). The 5FU+SRP+PRO group showed higher PVB than the 5FU group at 7 d ($p<0.05$), the 5FU+SRP group at 7 and 30 d ($p<0.05$), and the EP, SS, 5FU, and 5FU+PRO groups at 30 d ($p<0.05$). 5FU+SRP and 5FU+PRO had higher PVB than 5FU group at 30 d ($p<0.05$). In the intragroup analysis, the EP, SS, and 5FU+SRP groups had lower PVB at 30 d than at 7 d ($p<0.05$), and 5FU+SRP+PRO had higher PVB at 30 d compared with 7 and 15 d ($p<0.05$; Figure 3). The PNVB was lower in the 5FU+SRP group compared with the SS group at 7 d ($p<0.05$). Intragroup analysis showed that the PNVB was lower at 30 d than at 15 d in the EP group ($p<0.05$) and lower at 30 d compared with 7 d in the SS group ($p<0.05$; Figure 3).

Immunohistochemical analysis

There was no statistically significant difference between the groups in the immunostaining of TRAP-positive cells in the different periods ($p>0.05$; Figure 4). For RANKL, a high pattern of immunostaining prevailed at 7, 15, and 30 d in the EP, SS, and 5FU groups. In the 5FU+PRO and 5FU+SRP groups, the immunostaining pattern was predominantly high at 7 d and moderate at 15 and 30 d. The 5FU+SRP+PRO group showed a high immunostaining pattern at 7 d, moderate at 15 d, and moderate to low at 30 d (Figure 5). For OPG, a low staining pattern prevailed at 7, 15, and 30 d in all experimental groups, except at 30 d in 5FU+SRP+PRO, where most samples showed a moderate pattern of immunostaining (Figure 5).

Discussion

There is evidence that cancer patients undergoing chemotherapy develop a more pathogenic oral microbiota during immunosuppression, causing a worsening of periodontal disease during their treatment.[4,6] With the aim of preventing infections and intervening in the immunosuppressive action, this is

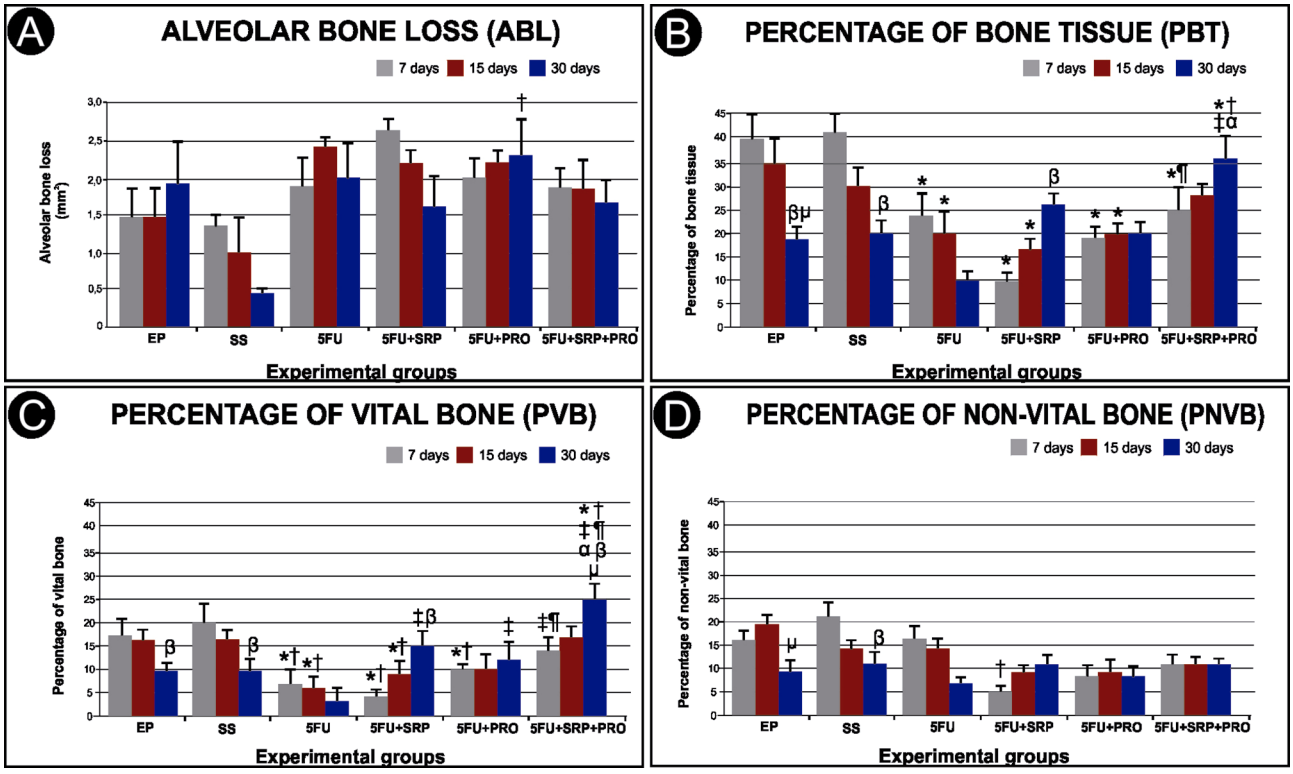

Figure 3- Graphs showing the mean and standard deviation of alveolar bone loss (A), percentage of bone tissue (B), percentage of vital bone (C), and percentage of non-vital bone (D) in the furcation area, according to the groups and periods (mm2). Abbreviations and symbols: *: significant difference compared with the EP group at the same experimental period; †: significant difference compared with SS at the same experimental period; ‡: significant difference compared with 5FU at the same experimental period; ¶: significant difference compared with 5FU+SRP at the same experimental period; α: significant difference compared with 5FU+PRO at the same experimental period; β: significant difference compared with 7 d in the same experimental group; μ: significant difference compared with 15 d in the same experimental group

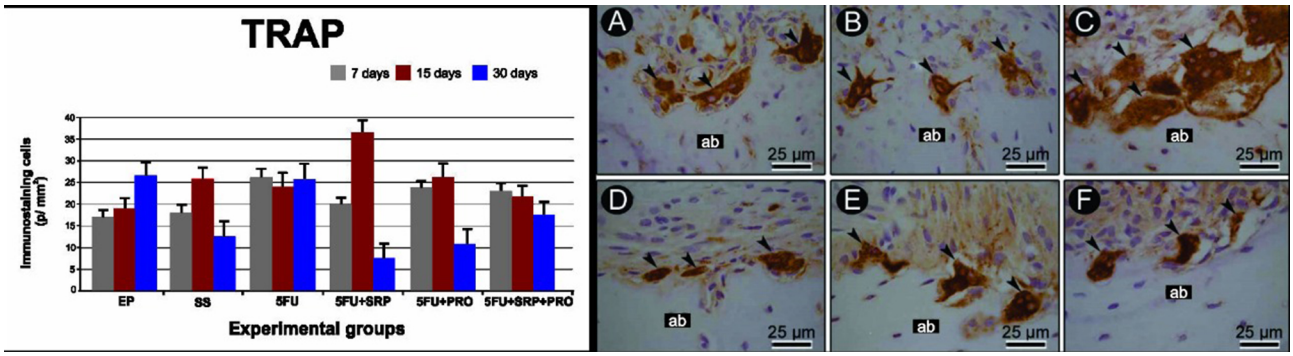

Figure 4- Graph showing the mean and standard deviation of TRAP-positive cells in the furcation area, according to the groups and periods. Photomicrographs showing the immunolabeling pattern for TRAP in the periodontium of the lower left first molar with experimental periodontitis at 7 d in EP (A), SS (B), 5FU (C), 5FU+PRO (D), 5FU+SRP (E), and 5FU+SRP+PRO (F). Abbreviations and symbols: ab: alveolar bone; arrows: osteoclasts cells. Counterstain: Harris hematoxylin. Original magnification: 1000x. Scale bars: 25 µm

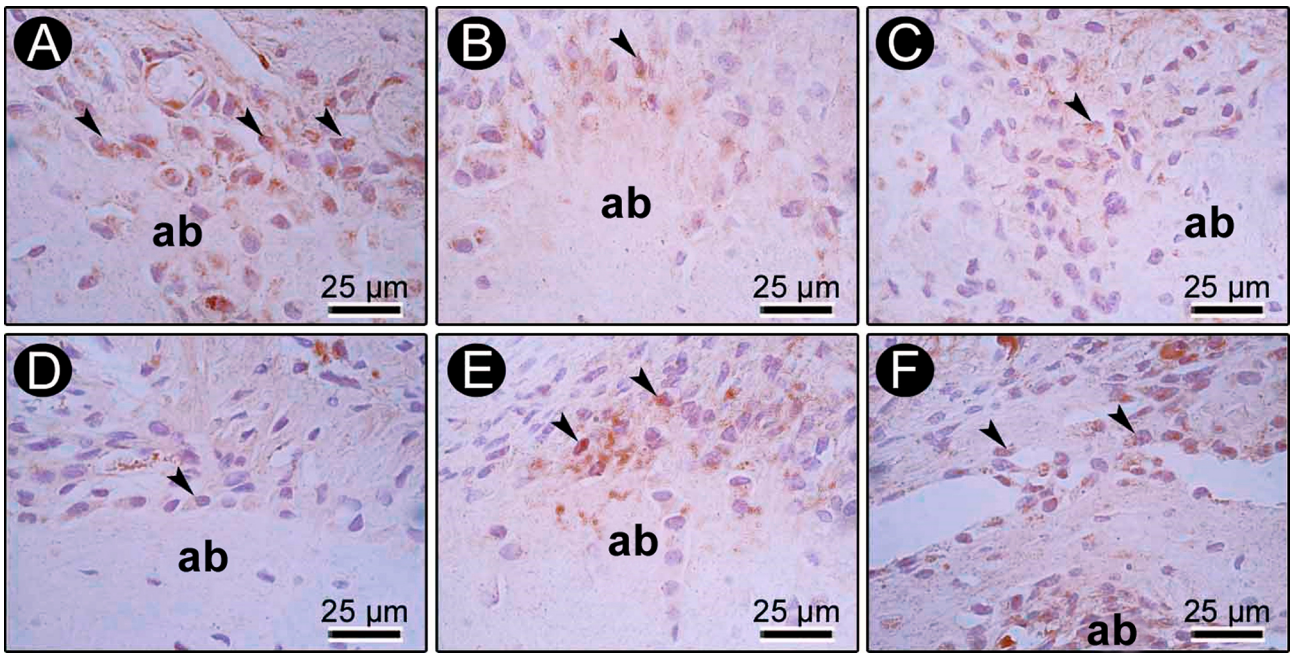

Figure 5- Photomicrographs showing the immunolabeling pattern for RANKL in 5FU (A), 5FU+PRO (B), and 5FU+SRP+PRO (C) at 7 d; and for OPG in 5FU (D), 5FU+PRO (E), and 5FU+SRP+PRO (F) at 30 d. Abbreviations and symbols: ab: alveolar bone; arrows: immunostained cells. Counterstain: Harris hematoxylin. Original magnification: 1000x. Scale bars: 25 µm

the first *in vivo* study to evaluate the hypothesis that local monotherapy with *Saccharomyces cerevisiae* or its association with SRP in the treatment of EP could minimize the effects of immunosuppression, as observed in previous studies by our research group.[7,8] Our findings show that the antineoplastic agent negatively affects bone repair at significant rates, the modulation of the local inflammatory response, and, consequently, the degree of severity of periodontitis.

The ligature-induced EP model has been widely used and is a highly reproducible model to assess the progression and treatment of periodontitis.[34,35] Rats are commonly used in EP studies due to their easy handling, low cost, and biological response, which is very similar to that of humans.[35] The animal ligature model, although limited, is critical to the establishment of cause and effect relationships in advanced therapeutic tests.[36] The changes in the periodontal tissue are similar to those observed in human periodontitis, involving rupture and apical migration of the junctional epithelium.[35,36]

Based on the results of this study, the methods of disease induction should be debated. Immunosuppression was induced by the systemic administration of 5FU in rats with EP. Models of immunosuppression induced by the use of chemotherapeutics have a considerable capacity to mimic the events observed in humans, including the gradual increase in leukopenia.[7] This fact increases the vulnerability of the host and aggravates periodontal disease.[7-12] Rats treated with 5FU showed severe changes in the alveolar bone, toxicity in the oral

mucosa, damage to the epithelial tissue and cellular apoptosis, which facilitates the penetration of bacteria and their by-products into the periodontal tissues. Similar results to this study were presented by other studies that used 5FU as an immunosuppression modality in rats. These showed increased levels of bone resorption in rats with EP and increased bone loss.[7,8] These data are justified by the fact that 5FU is cytotoxic to cells that have a high proliferation and regeneration rate, such as those present in the junctional epithelium.[7,8] As the first line of defense of periodontal tissues against microbial aggression, the rupture of the junctional epithelium facilitates the penetration of bacteria and their products into the periodontal tissues, especially those that constitute the insertion periodontium. This contributes to the progression of periodontal disease,[7,9] a fact also observed in this study.

The beneficial effects of the systemic use of probiotics on periodontal disease have also been reported by other studies that induced EP in animals.[17,21,28] Some randomized controlled clinical trials have also shown favorable results from probiotics in various conditions, such as reducing gingival inflammation and periodontitis.[37-39] Regarding probiotic monotherapy (5FU+PRO), it was observed that even in the absence of mechanical instrumentation, the use of *Saccharomyces cerevisiae* was able to reduce the inflammatory infiltrate. This is due to the possible effects of probiotics on the local immune response, by the release of products that inhibits the growth of oral bacteria. Probiotics also can affect bacterial plaque formation by competing and intervening with bacteria and may even be involved in substrate metabolism.[40] *Saccharomyces cerevisiae* is a yeast biotherapeutic agent that may possess probiotic properties.[41] Furthermore, the cell wall of *Saccharomyces cerevisiae* contains β-glucans, polysaccharides, which have an antimicrobial effect, and activate leukocytes, and stimulate phagocytic activity and the production of inflammatory cytokines.[40]

In the histological analysis, it was observed that rats in the groups in which no modality of local treatment was performed (EP, SS, and 5FU) showed severe signs of periodontal destruction, especially in the initial phase (7 to 15 d) of the repair process. However, the group subjected to SRP showed a more intense inflammatory response at 15 d ($p<0.05$). These results show that the antineoplastic agent exacerbates and sustains the inflammatory response caused by pathogens in periodontal tissues throughout the experimental periods. On the other hand, the pattern of connective tissue structuring showed a greater tendency to repair in the 5FU+PRO group at 7 d compared with the EP and SS groups ($p<0.05$). This result corroborates a recent study that evaluated the effects of antineoplastic agents on healthy periodontal tissues and on the progression of periodontitis in the furcation region.[12] The results also show that the significant ABL in the immunosuppressed groups is a result of exacerbated inflammation.[12] In the 5FU+PRO group, there was less ABL in the FA at 7 d than in all the other experimental groups, and at 30 d compared with the groups subjected to local treatment (5FU+SRP and 5FU+SRP+PRO; $p<0.05$). These data were also confirmed by the analysis of bone resorption, which showed that the 5FU+PRO group had lower parameters, as well as a lower porosity extension than the 7-day treatment groups ($p<0.05$). These findings suggest that the use of *S. cerevisiae* as monotherapy can benefit the tissue repair process and help prevent periodontal disease in immunosuppressed conditions.

Data from the histomorphometric analyses in this study and one of the main parameters for assessing the progression of EP highlighted that monotherapy with *Saccharomyces cerevisiae* was able to modulate the quality of bone tissue to a level compatible with the SS group, i.e., a state of systemic health ($p<0.05$). At 7 d, rats in the 5FU+SRP+PRO group showed a higher PBT compared with the 5FU+SRP group ($p<0.05$), and at 30 d compared with the EP, SS, 5FU, and 5FU+PRO groups ($p<0.05$). At 30 d, an increase in PVB was also observed in 5FU+SRP+PRO compared with all the experimental groups ($p<0.05$). The reduction of these measurements in rats treated with *Saccharomyces cerevisiae* may have influenced the reduction in the process of bone tissue destruction, which is greatly worsened by chemotherapy. Other authors have shown that photonic therapies can reduce ABL in the FA of animals undergoing chemotherapy with 5FU, thus reinforcing the positive effects of adjuvant therapies in mimicking immunosuppression.[8,9]

Further studies are still needed on the protocol of probiotics as a local monotherapy or in association with SRP in the treatment of periodontitis, especially in immunosuppressed conditions. Miessi et al.[11] (2020) showed that probiotic therapy with *Lactobaccillus reuteri* was unable to significantly reduce inflammation

and improve periodontal tissue repair. However, the systemic use of *Lactobaccillus reuteri* promoted greater control of the inflammatory response.[11] Factors related to pathogenic microbiota still generate gaps in the proposed therapies, which affect their ability to convert a dysbiotic infectious process into a homeostatic/commensal balance and/or contain tissue invasion by pathogens, especially in individuals undergoing chemotherapy.[42,43] Therefore, there is a continuous search for therapies that can optimize the results of periodontal treatment, especially in immunosuppressed conditions.

Regarding the immunomodulatory effects of *Saccharomyces cerevisiae,* it was observed that rats in the groups treated with the probiotic (5FU+PRO and 5FU+SRP+PRO) showed different immunostaining profiles directly involved in bone metabolism (TRAP, OPG and RANKL) than the other groups (EP, SS, and 5FU). However, it is worth mentioning that according to the protocol proposed for the 7-, 15-, and 30-day periods, some results deserve to be highlighted, since they describe an immunoinflammatory profile inherent to the simulation of immunosuppression associated with early and late stages of periodontitis. Clinical studies report that after 15 d of chemotherapy, there is an increase in the conditions associated with periodontal disease, as well as a strong presence of periodontal abscess resulting from a significant imbalance in the oral ecosystem.[4,6] The findings of this study point to a high pattern of RANKL immunostaining at 7 d in rats subjected to local treatments (5FU+PRO, 5FU+SRP, and 5FU+SRP+PRO) compared with the other groups (EP, SS, and 5FU). At 15 d, rats that received local treatments showed a moderate pattern of immunostaining for OPG and RANKL, and at 30 d only the 5FU+SRP+PRO group had a low pattern of RANKL immunostaining. This result suggests a modulating effect of probiotic monotherapy on bone formation and resorption processes. Some studies consider the low levels of the RANKL/OPG ratio as a result of SRP and/or probiotic therapy, in addition to mimicking the effects of chemotherapy on periodontal tissues.[11] Moreover, other variables can have a significant influence on the oral environment. The use of photobiomodulation,[10,11] antimicrobial photodynamic therapy,[9,10,44] and regenerative materials[45] may modify clinical and microbiological parameters in periodontal patients, and may also have an effect in association with probiotics. All these variables should be considered in future trials.

Considering the limitations of this study, we highlight the method of inducing immunosuppression using the chemotherapy drug 5FU in two applications[9] and the protocol for using local PRO. Only one study published in the literature used a treatment protocol with the same PRO used in this study.[17] The local effects of *Saccharomyces cerevisiae* are strain-specific and depend on the dose and frequency in which they are administered. Therefore, the results presented in this study cannot be generalized to other probiotic strains. Further studies involving different dosages, therapeutic protocols, and routes of administration of *Saccharomyces cerevisiae*, as well as evaluating the systemic effects of this therapy under immunosuppressed conditions or systemic modifications, are needed so that clinical trials can be performed to evaluate the effects of these beneficial microorganisms and their derivatives in controlling bone loss under immunosuppressed conditions.

Conclusion

Despite the limitations of this study, it can be concluded that *Saccharomyces cerevisiae* used as monotherapy or as adjuvant therapy to SRP can promote a protective effect against alveolar bone loss caused by 5FU in cases of periodontitis in rats. As a modulator of the local inflammatory response and, consequently, of the severity of periodontal disease, *Saccharomyces cerevisiae* has proved to be a promising therapy during and after cancer treatment.

Acknowledgments

This study was funded by the São Paulo Research Foundation (FAPESP; Process No. 2011/20969-0) and the Coordination of Superior Level Staff Improvement – Brazil (CAPES #001).

Conflict of interest

The authors declare no conflict of interest related to this study.

Data availability statement

The datasets generated and analyzed during this study are available from the corresponding author on reasonable request.

Authors' contributions

Garcia, Valdir Gouveia: Conceptualization (Equal); Funding acquisition (Equal); Resources (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal). **Rocha, Tiago Esgalha da:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Software (Equal); Writing – original draft (Equal). **Gomes, Natália Amanda:** Formal analysis (Equal); Investigation (Equal); Software (Equal); Writing – original draft (Equal). **Miessi, Daniela Maria Janjácomo:** Data curation (Equal); Methodology (Equal); Project administration (Equal). **Nuernberg, Marta Aparecida Alberton:** Data curation (Equal); Methodology (Equal); Project administration (Equal). **Rodrigues, João Victor Soares:** Formal analysis (Equal); Investigation (Equal); Software (Equal); Writing – original draft (Equal). **Cardoso, Jânderson de Medeiros:** Data curation (Equal); Methodology (Equal); Project administration (Equal). **Ervolino, Edilson:** Formal analysis (Equal); Investigation (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal). **Theodoro, Letícia Helena:** Conceptualization (Equal); Funding acquisition (Equal); Resources (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal).

References

1- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. doi: 10.3322/caac.21660

2- Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on prevalence of periodontitis in adults in the United States: NHANES 2009 - 2012. J Clin Periodontol. 2015;86(5):611-22. doi: 10.1902/jop.2015.140520

3- Michaud DS, Fu Z, Shi J, Chung M. Periodontal disease, tooth loss, and cancer risk. Epidemiol Rev. 2017;39(1):49-58. doi: 10.1093/epirev/mxx006

4- Villafuerte KR, Martinez CJ, Dantas FT, Carrara HH, Dos Reis FJ, Palioto DB. The impact of chemotherapeutic treatment on the oral microbiota of patients with cancer: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(6):552-66. doi: 10.1016/j.oooo.2018.02.008

5- Hong CH, Hu S, Haverman T, Stokman M, Napeñas JJ, Braber JB, et al. A systematic review of dental disease management in cancer patients. Support Care Cancer. 2018;26(1):155-74. doi: 10.1007/s00520-017-3829-y

6- Villafuerte KR, Dantas FT, Taba M Jr, Messora M, Reis FJ, Carrara HH, et al. Effects of non-surgical periodontal therapy on the cytokine profile in gingival crevicular fluid of breast cancer patients with periodontitis undergoing chemotherapy. Support Care Cancer. 2021;29(12):7505-13. doi: 10.1007/s00520-021-06194-w

7- Garcia VG, Novaes VC, Almeida JM, Longo M, Ervolino E, Bomfim SR, et al. Evaluation of the progression and treatment of experimental periodontitis in rats subjected to chemotherapy with 5-fluorouracil. Support Care Cancer. 2015;23(7):2007-17. doi: 10.1007/s00520-014-2563-y

8- Theodoro LH, Longo M, Ervolino E, Duque C, Ferro-Alves ML, Assem NZ, et al. Effect of low-level laser therapy as an adjuvant in the treatment of periodontitis induced in rats subjected to 5-fluorouracil chemotherapy. J Periodontal Res. 2016;51(5):669-80. doi: 10.1111/jre.12347

9- Theodoro LH, Longo M, Novaes VC, Miessi DM, Ferro-Alves ML, Ervolino E, et al. Low-level laser and antimicrobial photodynamic therapy on experimental periodontitis in rats submitted to chemotherapy by 5-fluorouracil. Support Care Cancer. 2017;25(10):3261-71. doi: 10.1007/s00520-017-3738-0

10- Longo M, Garcia VG, Ervolino E, Ferro-Alves ML, Duque C, Wainwright M, et al. Multiple aPDT sessions on periodontitis in rats treated with chemotherapy: histomorphometrical, immunohistochemical, immunological and microbiological analyses. Photodiagnosis Photodyn Ther. 2019;25:92-102. doi: 10.1016/j.pdpdt.2018.11.014

11- Miessi DM, Garcia VG, Ervolino E, Scalet V, Nuernberg MA, Santos OM Neto, et al. Lactobacillus reuteri associated with scaling and root planing in the treatment of periodontitis in rats submitted to chemotherapy. Arch Oral Biol. 2020;117:1048-25. doi: 10.1016/j.archoralbio.2020.104825

12- Novaes VC, Ervolino E, Fernandes GL, Cunha CP, Theodoro LH, Garcia VG, et al. Influence of the treatment with the antineoplastic agents 5-fluorouracil and cisplatin on the severity of experimental periodontitis in rats. Support Care Cancer. 2022;30(3):1967-80. doi: 10.1007/s00520-021-06586-y

13- Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, et al. EFP Workshop Participants and Methodological Consultants. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47(Suppl 22):4-60. doi: 10.1111/jcpe.13290

14- Albandar JM, Susin C, Hughes FJ. Manifestations of systemic diseases and conditions that affect the periodontal attachment apparatus: case definitions and diagnostic considerations. J Clin Periodontol. 2018;45(Suppl 20):S171-89. doi: 10.1111/jcpe.12947

15- Jepsen S, Caton JG, Albandar JM, Bissada NF, Bouchard P, Cortellini P, et al. Periodontal manifestations of systemic diseases and developmental and acquired conditions: consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and PeriImplant Diseases and Conditions. J Clin Periodontol. 2018;89(Suppl 1):S237-48. doi: 10.1002/JPER.17-0733

16- Donos N, Calciolari E, Brusselaers N, Goldoni M, Bostanci N, Belibasakis GN. The adjunctive use of host modulators in non-surgical periodontal therapy. A systematic review of randomized, placebo-controlled clinical studies. J Clin Periodontol. 2020;47(Suppl 22):199-238. doi: 10.1111/jcpe.13232

17- Garcia VG, Knoll LR, Longo M, Novaes VC, Assem NZ, Ervolino E, et al. Effect of the probiotic *Saccharomyces cerevisiae* on ligature-induced periodontitis in rats. J Periodontal Res. 2016;51(1):26-37. doi: 10.1111/jre.12274

18- Varela-López A, Navarro-Hortal MD, Giampieri F, Bullón P, Battino M, Quiles JL. Nutraceuticals in periodontal health: a systematic review on the role of vitamins in periodontal health maintenance. Molecules. 2018;23(5):1226. doi: 10.3390/molecules23051226

19- Nuernberg MA, Wainwright M, Miessi DM, Scalet V, Olivo MB, Ervolino E, et al. Effects of butyl toluidine blue photosensitizer on antimicrobial photodynamic therapy for experimental periodontitis treatment in rats. Photodiagnosis Photodyn Ther. 2020;31:1018-68. doi: 10.1016/j.pdpdt.2020.101868

20- Ho SN, Acharya A, Sidharthan S, Li KY, Leung WK, McGrath C, et al. A systematic review and meta-analysis of clinical, immunological, and microbiological shift in periodontitis after nonsurgical periodontal therapy with adjunctive use of probiotics. J Evid Based Dent Pract. 2020;20(1):101397. doi: 10.1016/j.jebdp.2020.101397

21- Garcia VG, Miessi DJ, Esgalha T, Gomes NA, Nuernberg MA, Cardoso JM, et al. The effects of Lactobacillus reuteri on the inflammation and periodontal tissue repair in rats: a pilot study. Saudi Dent J. 2022;34(6):516-26. doi: 10.1016/j.sdentj.2022.05.004

22- World Health Organization. Food and Agriculture Organization of the United Nations. Guidelines for the evaluation of probiotics in food [internet]. London, Ontario: WHO; 2002 [cited 2023 Oct 16]. Available from: https://pdf4pro.com/amp/view/guidelines-for-the-evaluation-of-probiotics-in-food-1d59e1.html

23- Vale GC, Mayer MP. Effect of probiotic Lactobacillus rhamnosus by-products on gingival epithelial cells challenged with Porphyromonas gingivalis. Arch Oral Biol. 2021;128:105174. doi: 10.1016/j.archoralbio.2021.105174

24- Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: systematic review and meta-analysis. J Dent. 2016;48:16-25. doi: 10.1016/j.jdent.2016.03.002

25- Hagannathan N, Acharya A, Farn OY, Li KY, Nibali L, Pelekos G. Disease severity, debridement approach and timing of drug modify outcomes of adjunctive azithromycin in non-surgical management of chronic periodontitis: a multivariate meta-analysis. BMC Oral Health. 2019;19(1):65. doi: 10.1186/s12903-019-0754-0

26- Messora MR, Pereira LJ, Foureaux R, Oliveira LF, Sordi CG, Alves AJ, et al. Favourable effects of Bacillus subtilis and Bacillus licheniformis on experimental periodontitis in rats. Arch Oral Biol. 2016;66:108-19. doi: 10.1016/j.archoralbio.2016.02.014

27- Ricoldi MS, Furlaneto FA, Oliveira LF, Teixeira GC, Pischiotini JP, Moreira AL, et al. Effects of the probiotic Bifidobacterium animalis subsp. lactis on the non-surgical treatment of periodontitis. A histomorphometric, microtomographic and immunohistochemical study in rats. PLoS One. 2017;12(6):01799-46. doi: 10.1371/journal.pone.0179946

28- Oliveira LF, Silva PH, Salvador SL, Ervolino E, Casarin R, Figueiredo L, et al. Probiotic consumption can modify the resilience of periodontal tissues in rats under experimental periodontitis. J Periodontol. 2023;94(2):217-29. doi: 10.1002/JPER.21-0555

29- Maia LP, Levi YL, Silva PH, Wons LC, Pitelli LP, Castro JG, et al. Impact of Bifidobacterium animalis subsp. lactis HN019 probiotic in the prevention of periodontitis associated with immunosuppression. J Periodontol. 2023;94(3):389-404. doi: 10.1002/JPER.22-0146.

30- du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. Br J Pharmacol. 2020;177(16):3617-24. doi: 10.1111/bph.15193

31- Ervolino E, Statkievicz C, Toro LF, Mello-Neto JM, Cavazana TP, Issa JP, et al. Antimicrobial photodynamic therapy improves the alveolar repair process and prevents the occurrence of osteonecrosis of the jaws after tooth extraction in senile rats treated with zoledronate. Bone. 2019;120:101-13. doi: 10.1016/j.bone.2018.10.014

32- Zuza EP, Garcia VG, Theodoro LH, Ervolino E, Favero LF, Longo M, et al. Influence of obesity on experimental periodontitis in rats: histopathological, histometric and immmunoistochemical study. Clin Oral Investig. 2018;22(3):1197-208. doi: 10.1007/s00784-017-2207-y

33- Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother. 2013;4(4):303-6. doi: 10.4103/0976-500X.119726

34- Garcia VG, Gualberto EC Junior, Fernandes LA, Bosco AF, Nagata MJ, Casatti CA, et al. Adjunctive antimicrobial photodynamic treatment ofexperimentally induced periodontitis in rats with ovariectomy. J Periodontol. 2013;84:556-65. doi: 10.1902/jop.2012.120163

35- Molon RS, Avila ED, Cirelli JA. Host responses induced by different animal models of periodontal disease: a literature review. J Investig Clin Dent. 2013;4(4): 211-8. doi: 10.1111/jicd.12018

36- Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G. The use of rodent models to investigate host-bacteriainteractions related to periodontal diseases. J Clin Periodontol. 2008;35:89-105. doi: 10.1111/j.1600-051X.2007.01172.x

37- Invernici MM, Salvador SL, Silva PH, Soares MS, Casarin R, Palioto DB, et al. Effects of Bifidobacterium probiotic on the treatment of chronic periodontitis: a randomized clinical trial. J Clin Periodontol. 2018;45(10):1198-210. doi: 10.1111/jcpe.12995

38- Ikram S, Hassan N, Baig S, Borges KJ, Raffat MA, Akram Z. Effect of local probiotic (Lactobacillus reuteri) vs systemic antibiotic therapy as an adjunct to non-surgical periodontal treatment in chronic periodontitis. J Investig Clin Dent. 2019;10(2):123-93. doi: 10.1111/jicd.12393

39- Pelekos G, Ho SN, Acharya A, Leung WK, McGrath C. A double-blind, paralleled-arm, placebo-controlled and randomized clinical trial of the effectiveness of probiotics as an adjunct in periodontal care. J Clin Periodontol. 2019;46(12):1217-27. doi: 10.1111/jcpe.13191

40- Alok A, Singh ID, Singh S, Kishore M, Jha PC, Iqubal MA. Probiotics: a new era of biotherapy. Adv Biomed Res. 2017;7:6-31. doi.org/10.4103/2277-9175.192625

41- Van der Aa Kuhle A, Skovgaard K, Jespersen L. *In vitro* screening of probiotic properties of *Saccharomyces cerevisiae* var. boulardii and food-borne *Saccharomyces cerevisiae* strains. Int J Food Microbiol. 2005;101:29-39. doi: 10.1016/j.ijfoodmicro.2004.10.039

42- Deng ZL, Szafranski SP, Jarek M, Bhuju S, Wagner-Dobler I. Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci Rep. 2017;7(1):3703. doi: 10.1038/s41598-017-03804-8

43- Willis JR, Gabaldón T. The human oral microbiome in health and disease: from sequences to ecosystems. Microorganisms. 2020;8(2):E308. doi: 10.3390/microorganisms8020308

44- Cláudio MM, Nuernberg MA, Rodrigues JV, Belizário LC, Batista JA, Duque C, et al. Effects of multiple sessions of antimicrobial photodynamic therapy (aPDT) in the treatment of periodontitis in patients with uncompensated type 2 diabetes: a randomized controlled clinical study. Photodiagnosis Photodyn Ther. 2021;35:102451. doi: 10.1016/j.pdpdt.2021.102451

45- Gallo S, Pascadopoli M, Pellegrini M, Pulicari F, Manfredini M, Zampetti P, et al. Latest findings of the regenerative materials application in periodontal and peri-implant surgery: a scoping review. Bioengineering (Basel). 2022;9(10):594. doi: 10.3390/bioengineering9100594