Original Research Paper

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Periodontology

FORMULATION AND EVALUATION OF PERIODONTAL IN SITU PROBIOTIC GEL (BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS 2 * 10^9 CFU/G) – AN IN VITRO STUDY

Kavya Gandrakota*	Post Graduate (MDS) Vydehi Institute of Dental Sciences and research Centre, Bangalore, India *Corresponding Author
Shruthi Sethuraman	Reader (MDS) Vydehi Institute of Dental Sciences and research Centre, Bangalore, India
Nisha K J	Professor and Head (MDS, PhD) Vydehi Institute of Dental Sciences and research Centre, Bangalore, India
Vidyadhar K V N	Senior lecturer (MDS) CKS Theja Institute of Dental Sciences, Tirupathi, India

ABSTRACT **Purpose:** The shift from symbiotic environment to dysbiotic environment results in degradation of supporting structures of tooth. Recolonization by the pathogenic bacteria and antibiotic resistance remains the main constraints of various conventional therapies available against periodontal disease. One agent/drug which can ensure a symbiotic environment thereby delaying the recolonization by periodontal pathogens are Probiotics. Locally delivered drug to the target site ensures more positive effect on the pathogens than the other available routes of delivery. In situ gel forming formulations are a novel idea of delivering drugs to patients in a liquid dosage form, yet achieve sustained release of drug for the desired period. Hence the aim of the in vitro study was to formulate and evaluate the properties of probiotic gel containing Bifidobacterium animalis subsp lactis (2 * 10^9 CFU/g) by assessing the in vitro viability and degradation of probiotic gel at 1, 15, 30, 90 days, to assess the in vitro antimicrobial efficacy on periodontal pathogens. **Methods:** The antimicrobial activity of probiotic gel on periodontal pathogens bacterial strains was evaluated by agar diffusion assay by MIC, MBC and zone of inhibition. In vitro degradation of gel was done using UV spectrophotometer at 600nm **Results:** The probiotic gel has shown a strong inhibitory effect against P.gingivalis at 100mg/ml concentration, and against Fusobacterium nucleatum at 200mg/ml concentration and the invitro degradation of gel showed that the gel was not completely degraded by the end of 90 days and has shown bacterium release. **Conclusion:** The present in vitro study has shown that Bifidobacterium animalis subsp lactis gel has exhibited great antibacterial efficacy against both P. gingivalis and F. nucleatum and it can prove to be a potent local drug delivery agent for the treatment of periodontal disease.

KEYWORDS : Probiotics; Porphyromonas gingivalis; Fusobacterium nucleatum; Periodontitis; Bifidobacterium animalis; Antibacterial agents

INTRODUCTION

Elimination of subgingival microflora thereby attaining health of supporting structures of tooth remains the goal of periodontal therapy. [1] This goal cannot be attained by mechanical therapy alone hence paving way for many adjunctive treatment strategies. The different adjunctive therapies that have been used in the treatment of periodontal diseases include use of antibiotics locally or systemically or daily use of mouth washes with antimicrobial components.[1] However these therapies have their own share of side effects which includes altered taste sensation, increase in antibiotic resistance of the periodontal pathogens. [2, 3] Moreover, current ecological theory applied to oral microbiology support the view that antimicrobial agents may not be effective in preventing oral diseases. [4] As it states that these diseases are the outcome of a microbial dysbiosis, the periodontal treatment should involve strategies which are aimed in restoring the ecological balance and microbial- host homeostasis. [5] Probiotics have shown promising results in achieving this.[6]

Probiotics have been widely used in the field of periodontics in the form of lozenges, mouth washes, yoghurts, milk, drinks, capsules, sachets, chewable tablets. [7] Probiotics recently have also been used as LDD in the form of fibers, powder mixed with tragacanth powder and saline as the vehicle. [8] The major drawback of above forms is that they cannot cover up the entire pocket hence providing an opportunity for pathogens to re-inhabit the pit.

This shortcoming could be overcome by in situ gel-forming drug delivery systems prepared using stimuli sensitive polymers that exhibit solution-to-gel (sol-to-gel) phase transitions. As the target of periodontal therapy are the subgingival pathogens present in the periodontal pocket, formulation of the probiotic into a theromreversible gel could give us the advantage of increasing the stay of viable bacteria at the disease site for longer period thereby achieving and maintaining a symbiotic environment resulting in periodontal health.

Poloxamer 188 has been frequently used in the field of periodontics to formulate periodontal gels, tooth pastes and local drug delivery agents. This could prove to be a better vehicle enhancing the residence time of the active substance. [9,10]

Hence the aim of this in vitro study was to formulate and evaluate the properties of probiotic gel containing *Bifidobacterium animalis subsp lactis* (2 x 10^9 CFU/g).

MATERIALS AND METHODS

Formulation of Probiotic Gel:

Weighed quantity of Poloxamer 188 was dissolved in part quantity of double distilled water, the mixture was set aside for 2 hours to allow complete swelling of the polymer. Weighed quantity of sodium benzoate was dissolved in part quantity of double distilled water. Sodium benzoate solution was added to polymer mixture slowly with continuous stirring. Weighed quantities of probiotic was dissolved in another part of double distilled water, the drug solution was added with continuous stirring, the mixture was transferred to the vial and sealed and it was stored in refrigerator until further use. The probiotic gel was prepared in aseptic conditions and subjected to UV light overnight for sterilization.

Bifidobacterium animalis subsp lactis - UBBla-70CFU/g probiotic powder was generously sponsored by Unique Biotech Pvt Ltd, Hyderabad

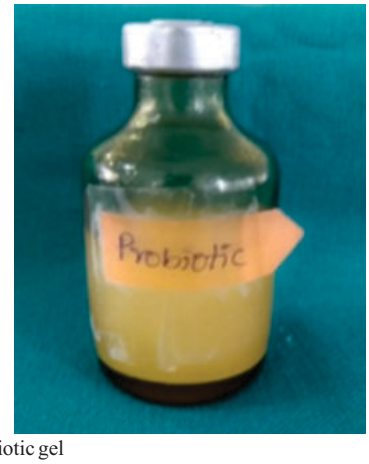


Fig 1: Probiotic gel

Antibacterial test of Probiotic gel against *Porphyromonas Gingivalis* (ATCC33277) and *Fusobacterium nucleatum* (ATCC 25586)

The antimicrobial activity test of Probiotic gel and Chlorhexidine against *P.gingivalis* and F nucleatum were tested by agar well-diffusion method, Wells of 6 mm diameter were punched on specific agar media. About 100µl of pre-cultured test organisms, were spread onto the agar plates. Various concentrations of the sample were loaded into the wells. Duplicated Bacterial plates were incubated at 37ºC for 24 hours, under anaerobic conditions with 5% CO2 and 95% Nitrogen for *P.gingivalis,* and Zone of inhibition were measured and tabulated. [11]

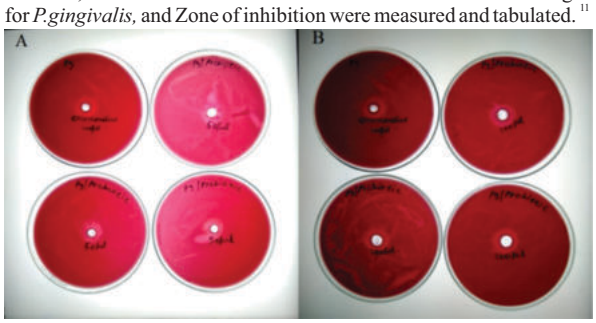


Fig 2: Zone of inhibition of probiotic gel on Porphyromonas gingivalis

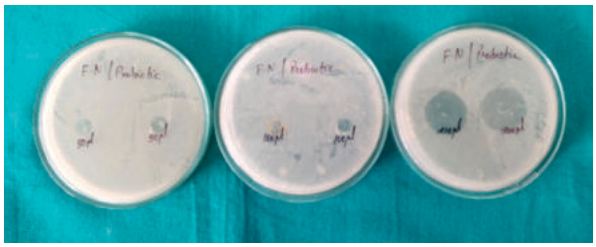


Fig 3: Zone of inhibition of probiotic gel on Fusobacterium Nucleatum

Minimum Inhibitory Concentration (MIC)

The MIC of Probiotic gel against *P.gingivalis and Fusobacterium nucleatum* were determined by micro dilution assay. A separate microplate was used for each bacterial species assessed. The micro plates was incubated for 24 hr at 37°C. The optical density of each well was evaluated after incubation using a spectrophotometer (Labman,India) at 600 nm before and after plate incubation at 37°C for 24 hours. The minimum inhibitory concentrations (MIC) of the gel that repressed the visible growth of *P.gingivalis and Fusobacterium nucleatum* was determined.

Minimum Bactericidal Concentration (MBC)

The MBC was decided by sub culturing of the wells that displayed no perceivable growth on a sterile agar plate. 100µL of the bacterial solutions that is considered as the MIC and higher concentrations was grown on tryptone soya agar plates. Six agar plates were used for each concentration. These plates were incubated for 24 hours at 37°C. Anaerobic conditions was generated through the use of a gas generating kit. Each plate was examined for growth at the conclusion of the incubation period both by the naked eye, by Colony forming units (CFUs) which were calculated on a grid. The MBC value was concluded as the lowest concentration that showed no apparent growth on agar subculture.

In-vitro degradation of probiotic gel:

Probiotic gel was placed in glass vials containing 10 mL PBS (pH 7.4) with 20 mg/mL lysozyme. The sealed vials were placed in a shaking water bath at 37˚C and shaken at a frequency of 15 rpm. Samples were withdrawn from the vials and replenished with a fresh medium every day for up to 12 weeks, the bacterium release was measured using spectrophotometer at 600 nm at regular intervals.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis includes expression of study parameters in terms of Mean & SD for continuous variables**.** One-way ANOVA Test followed by Tukey's post hoc test was used to compare the mean Zone of Inhibition, MIC, MBC values for *P. gingivalis* & *F. nucleatum* and OD values at 600nm for Invitro degradation of probiotic gel at different time intervals. The level of significance was set at P<0.05.

RESULTS

Porphyromonas Gingivalis

Zone of inhibition (Table 1)

Zone of inhibition of the probiotic gel was done at different concentrations such as 50mg/ml, 100mg/ml and were compared with 0.2% chlorhexidine. Probiotic gel at all concentrations showed antimicrobial activity. At 50mg/ml (Group 1) the mean zone of inhibition was 11.00±1.00 mm, at 100mg/ml (Group 2) the mean zone of inhibition was 16.00±1.00 mm. Zone of inhibition for chlorhexidine (Group 3) was 20.33± 2.08 mm. This difference in the mean Zone of Inhibition for *P. gingivalis* between 3 groups was statistically significant at p<0.001. Multiple comparison b/w groups revealed that Group 3 demonstrated significantly highest zone of inhibition as compared to Group 1 & Group 2 at p=0.001 & p=0.03. The mean zone of inhibition for *P. gingivalis* was significantly highest in Group 3, followed by Group 2 & least in Group 1.

Table 1: Comparison of Mean ZOI (in mm) for *P. gingivalis* b/w groups using One-way ANOVA Test followed by Tukey's Post hoc Test

Groups	N	Mean	SD	Min	Max	p-value a	Sig. Diff	p-value b
Group 1	3	11.00	1.00	10	12	<0.001*	G1 vs G2	0.01*
Group 2	3	16.00	1.00	15	17		G1 vs G3	0.001*
Group 3	3	20.33	2.08	18	22		G2 vs G3	0.03*

* - Statistically Significant

Note: Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel & Group 3 – CHX

Minimum inhibitory concentration (Table 2)

In order to determine the lowest concentration of probiotic that can inhibit the growth of *Porphyromonas gingivalis* MIC was performed. One way ANOVA was done to compare between different concentrations. The mean MIC for probiotic gel at 50 mg/ml (Group 1) was 0.1687±0.012. For group 2 at 100mg/ml concentration it was 0.1007±0.081, for chlorhexidine (Group 3) MIC was 0.0963±0.013. For group 4 that is control group MIC was 1.6910±0.0165. Group 2 showed the highest MIC when compared with group 1 and it showed a statistically significant difference. On comparing Group 2 and Group 3 there was no statistical significant difference.

Table 2: Comparison of mean MIC values for *P. gingivalis* b/w groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	0.1687	0.0122	0.158	0.182	<0.001*
Group 2	3	0.1007	0.0081	0.092	0.108	
Group 3	3	0.0963	0.0135	0.083	0.110	
Group 4	3	1.6910	0.0165	1.672	1.702	

* - Statistically Significant

Note: Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel, Group 3 – CHX & Group 4 - P. Gingivalis control without sample

Minimum bactericidal concentration (Table 3)

The minimum bactericidal concentration of probiotic on *Porphyromonas gingivalis* was determined by subculturing of wells that display no perceivable growth on sterile agar plate, and the MBC value was concluded as lowest concentration that showed no apparent growth on subculture. The mean MBC at 50mg/ml of probiotic gel was 6.67±1.53, at 100mg/ml of probiotic gel and chlorhexidine the MBC was 0.33±0.58. Probiotic gel at 50mg/ml concentration showed 90.06% reduction in CFU of *Porphyromonas gingivalis*, at 100mg/ml it showed 94.02% reduction in CFU of *Porphyromonas gingivalis* whereas Chlorhexidine showed 94.29% reduction. Intergroup comparision between groups was done using tukey's post hoc test. The difference was statistically significant when compared between group 1 and group 2, and between group 1 and group 3. There was no statistical significant difference seen when group 2 was compared with group 3.

Table 3: Comparison of mean MBC values for P. Gingivalis b/w groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	6.67	1.53	5	8	<0.001*
Group 2	3	0.33	0.58	0	1	
Group 3	3	0.33	0.58	0	1	
Group 4	3	12.67	2.52	10	15	

* - Statistically Significant

Note: Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel, Group 3 – CHX & Group 4 - P. Gingivalis control without sample

Fusobacterium nucleatum

Zone of inhibition (Table 4)

Zone of inhibition of the probiotic gel was done at different concentrations such as 50mg/ml (Group 1), 100mg/ml (Group 2), 200mg/ml (Group 3) and were compared with 0.2% chlorhexidine (Group 4). Probiotic gel at 200mg/ml concentration showed antimicrobial activity. At 200mg/ml the mean zone of inhibition was 12.33±1.53 mm. Zone of inhibition for chlorhexidine was 20.33± 2.08 mm. This difference in the mean Zone of Inhibition for *F. nucleatum* between 4 groups was statistically significant at p<0.001. Multiple comparison b/w groups revealed that Group 4 demonstrated significantly highest zone of inhibition as compared to Group 3.

Table 4: Comparison of mean ZOI values for *F. nucleatum* b/w groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	0.00	0.00	0	0	<0.001*
Group 2	3	0.00	0.00	0	0	
Group 3	3	12.33	1.53	11	14	
Group 4	3	20.33	2.08	18	22	

* - Statistically Significant

Note: Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel, Group 3 - 200 mg/ml of Probiotic Gel & Group 4 – CHX.

Minimum inhibitory concentration (Table 5)

In order to determine the lowest concentration of probiotic that can inhibit the growth of *Fusobacterium nucleatum* MIC was performed. One way ANOVA was done to compare between different concentrations. The mean MIC for probiotic gel at 50 mg/ml (Group 1) was 1.7853±0.0035. For group 2 at 100mg/ml concentration it was 1.6187±0.0162, at 200mg/ml (Group 3) the mean MIC was 0.4557±0.0040, and for chlorhexidine (Group 4) MIC was 0.0973±0.057. For group 5 that is control group MIC was 1.8863±0.0074. Group 3 showed the highest MIC when compared with group 1 and group 2 and the difference was statistically significant. On comparing group 3 and group 4 there was statistical significant difference in MIC against *F. nucleatum*.

Table 5: Comparison of mean MIC values for F. *nucleatum* b/w groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	1.7853	0.0035	1.782	1.789	<0.001*
Group 2	3	1.6187	0.0162	1.600	1.628	
Group 3	3	0.4557	0.0040	0.452	0.460	
Group 4	3	0.0973	0.0057	0.091	0.102	
Group 5	3	1.8863	0.0074	1.878	1.892	

* - Statistically Significant

Note: Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel, Group 3 - 200 mg/ml of Probiotic Gel & Group 4 – CHX.

Minimum bactericidal concentration (Table 6)

The minimum bactericidal concentration of probiotic on *Fusobacterium nucleatum*, was determined by sub culturing of wells that display no perceivable growth on sterile agar plate, and the MBC value was concluded as lowest concentration that showed no apparent growth on subculture. The mean MBC at 50mg/ml of probiotic gel was 11.67±1.16, at 100mg/ml the mean MBC was 5.67±0.58, for 200mg/ml probiotic gel and chlorhexidine MBC was 0.33±0.58. Probiotic at 50mg/ml concentration showed 5.35% reduction in CFU of *Fusobacterium nucleatum,* at 100mg/ml it showed 14.21% reduction in CFU of *Fusobacterium nucleatum*, at 200mg/ml concentration probiotic gel showed 75.87% whereas Chlorhexidine showed 94.85% reduction. Intergroup comparison between groups was done using tukey's post hoc test. The difference was statistically significant when compared between group 1, group 2 and group 3. There was no statistical significant difference seen when group 3 was compared with group 4.

Table 6: Comparison of mean MBC values for F. *nucleatum* b/w groups using One-way ANOVA Test

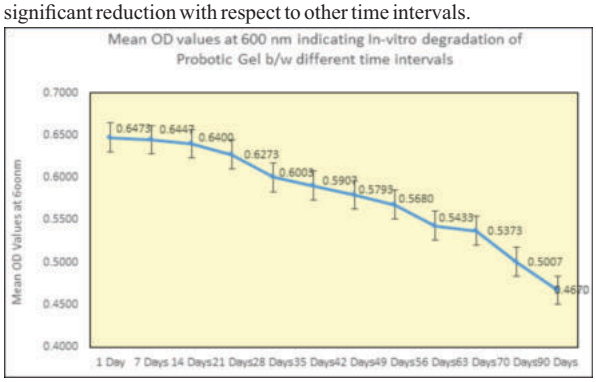
Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	11.67	1.16	11	13	<0.001*
Group 2	3	5.67	0.58	5	6	
Group 3	3	0.33	0.58	0	1	
Group 4	3	0.33	0.58	0	1	
Group 5	3	59.67	7.77	51	66	

* - Statistically Significant

Group 1 - 50mg/ml of Probiotic Gel; Group 2 - 100 mg/ml of Probiotic Gel, Group 3 - 200 mg/ml of Probiotic Gel, Group 4 – CHX & Group 5 – *F. nucleatum* control without sample.

In vitro degradation of probiotic gel (Graph 1)

The mean Optical Density (OD) values was significantly reduced from Day 21 to 90 days at p<0.001. However, the mean OD values in the initial time intervals from Day 1 to Day 14 did not show significant differences [p>0.05]. Further, the reduction in OD values was not statistically significant between Day 28 vs Day 35 [p=0.65], Day 42 [p = 0.41], and also between Day 42 & Day 49 [p=0.41] and further between Day 56 & Day 63 [p = 0.97]. The mean OD values showed significant reduction with respect to other time intervals.



Graph 1: Mean OD values at 600 nm indicating In-vitro degradation of Probiotic Gel b/w different time intervals

DISCUSSION

A dysbiosis in oral microbial population triggers the microbial derived periodontal disease.[12] Conventional therapies include professional removal of dental plaque and adjunctive use of antibiotic, but recolonization potential of oral pathogens and emerging antibiotic resistance has paved way to alternate therapies like the use of probiotics to combat periodontal diseases.

Bifidobacterium and Lactobacillus remains the most common probiotic strains used in dental health. In the present study an attempt was made to formulate the probiotic gel as a thermo reversible polymer with the use of poloxomer 188 to increase the stability and handling properties of the drug in the periodontal pocket. Thereby, overcoming the drawbacks caused due to the other forms of local drug delivery attempts like fibers, probiotic powders mixed in tragacanth and saline.[8] The thermo reversible gel when applied in liquid form has the benefit of reaching the entire depth of the pocket and it undergoes gelation at body temperature hence ensuring the stability of the drug at the target site for a longer period of time.

Before the in vivo use of drug in the periodontal pocket, an in vitro study was designed to test its efficacy against *Fusobacterium nucleatum* and *Porphyromonas gingivalis* and also an attempt was made to evaluate the in vitro degradation of the drug using spectrophotometry.

Our results showed that Bifidobacterium gel exerted a strong inhibitory effect against *Porphyromonas gingivalis* at 100mg/ml concentration, where the mean zone of inhibition was 16±1.00 mm, MIC was 0.1007±0.0081, and MBC was 0.33±0.58 and at this concentration when compared to chlorhexidine there was no statistically significant difference in all the parameters. The probiotic gel showed an antimicrobial activity against *Fusobacterium nucleatum* at 200mg/ml where the Zone of inhibition was 12.33±1.53mm, MIC was 0.4557±0.0040 and MBC was 0.33±0.58 and this effect was statistically significant. This result is in accordance with studies done by Zhu et al in 2010 and Jasberg et al in 2016 through biofilm assays. [13,11] This suggests that *Bifidobacterium animalis subsp. Lactis*, has the capacity to perfectly adhere with bacteria such as *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* and also

can co-aggregate with *Fusobacterium nucleatum*, [13] the biological bridge in biofilm formation. This Co aggregation might lead to reduced number of binding sites for red complex bacteria thereby reducing dysbiosis. [14]

The in vitro degradation of gel was measured through spectrophotometer at 600 nm [15] for every 7 days in triplicates. The drug did not degrade till 21 days. And a statistical significant degradation of the drug was observed from the 21st day to 90th day. This suggests the retention of the drug at the site for longer period of time and also ensures the slow release of drug for a longer period of time. This slow and sustained release of drug ensures the maintenance of periodontal health by inhibiting the recolonization of periodontopathogenic bacteria and allowing the symbiotic bacteria to recolonize in the pocket thereby disrupting the dysbiosis and leading to guided pocket recolonization [16]

After immense literature search and to the best of our knowledge, this is the first in vitro study to evaluate the degradation of the drug and antimicrobial efficacy of *Bifidobacterium animalis subsp lactis* thermo reversible gel against *P gingivalis* and *F nucleatum,* Hence there are no studies available to discuss our results from the present study.

Probiotics have been documented to modulate host immunity both systemically and locally. The antimicrobial efficacy of probiotics against periodontal pathogens can be exhibited by secretion of bacteriocins and probiotics also play an important role in biofilm formation leading to guided pocket recolonization. Probiotics stimulate dendritic cells (antigen presenting cells) resulting in expression of Th1 (T-helper cell 1) or Th2 (T-helper cell 2) response, which modulates immunity. Probiotics enhances innate immunity and modulate pathogen induced inflammation through "Toll-like receptors" on dendritic cell. [17] *Bifidobacterium* exhibit their effect against the various periodontal pathogens by their antimicrobial and host modulating effect and also activation of innate immunity. Recent molecular findings in animal studies have also shown their influence on RANKL/OPG ratio suggesting their important role in the inhibition of NFκβ pathway leading to bone formation. [18]

The limitations of the study are that we did not check the efficacy of the probiotic gel on plaque biofilm. The degradation of the gel should have been followed up after 90 days so that we could actually know when the drug completely degenerates.

As periodontitis is a major oral health concern, the antimicrobial efficacy of probiotic gel paves way to evaluate it as a therapeutic antimicrobial agent whose efficacy as an adjunct to non-surgical therapy can be checked in vivo on periodontitis patients which can prove to be beneficial and a viable alternative for antibiotics thereby reducing the risk of antibiotic resistance.

CONCLUSION

Bifidobacterium animalis subsp lactis gel has shown a great antimicrobial activity against *P.gingivalis* and *F. nucleatum*. The invitro degradation of the gel has shown that the gel is still present with bacterium release at the end of 90 days. This study is followed up by an in vivo study which has been designed to check the clinical and microbial efficacy of the drug in periodontitis patients.

Conflict Of Interest

No potential conflict of interest relevant to this article was reported

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REFERENCES

1. Gatej, S., Gully, N., Gibson, R., & Bartold, P. M. (2017). Probiotics and Periodontitis - A Literature Review. *Journal of the International Academy of Periodontology*, *19*(2), 42–50.
2. Slots J. (2013). Periodontology: past, present, perspectives. *Periodontology 2000*, *62*(1), 7–19.
3. How, K. Y., Song, K. P., & Chan, K. G. (2016). Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. *Frontiers in microbiology*, *7*, 53
4. Marsh P. D. (2010). Microbiology of dental plaque biofilms and their role in oral health and caries. *Dental clinics of North America*, *54*(3), 441–454.
5. Mira, A., Simon-Soro, A., & Curtis, M. A. (2017). Role of microbial communities in the pathogenesis of periodontal diseases and caries. *Journal of clinical periodontology*, *44 Suppl 18*, S23–S38.
6. Marsh, P. D., & Zaura, E. (2017). Dental biofilm: ecological interactions in health and disease. *Journal of clinical periodontology*, *44 Suppl 18*, S12–S22.
7. Schlagenhauf, U., & Jockel-Schneider, Y. (2021). Probiotics in the management of gingivitis and periodontitis. A review. *Frontiers in Dental Medicine*, *2*, 61.
8. Boyeena, L., Koduganti, R. R., Panthula, V. R., & Jammula, S. P. (2019). Comparison of efficacy of probiotics versus tetracycline fibers as adjuvants to scaling and root planing. *Journal of Indian Society of Periodontology*, *23*(6), 539–544..
9. Gaekwad, S. S., Gujjari, S. K., Kumar, T. P., & Devishree, S. (2015). Effect of subgingivally delivered simvastatin in postmenopausal women with chronic periodontitis: a randomized controlled trial. *Journal of Advanced Clinical and Research Insights*, *2*(6), 253-258.
10. Bansal, M., Mittal, N., Yadav, S. K., Khan, G., Gupta, P., Mishra, B., & Nath, G. (2018). Periodontal thermoresponsive, mucoadhesive dual antimicrobial loaded *in-situ* gel for the treatment of periodontal disease: Preparation, *in-vitro* characterization and antimicrobial study. *Journal of oral biology and craniofacial research*, *8*(2), 126–133.
11. Zhu, Y., Xiao, L., Shen, D., & Hao, Y. (2010). Competition between yogurt probiotics and periodontal pathogens in vitro. *Acta odontologica Scandinavica*, *68*(5), 261–268.
12. Kumar P. S. (2013). Oral microbiota and systemic disease. *Anaerobe*, *24*, 90–93.
13. Jäsberg, H., Söderling, E., Endo, A., Beighton, D., & Haukioja, A. (2016). Bifidobacteria inhibit the growth of Porphyromonas gingivalis but not of Streptococcus mutans in an in vitro biofilm model. *European journal of oral sciences*, *124*(3), 251–258.
14. Invernici, M. M., Furlaneto, F. A. C., Salvador, S. L., Ouwehand, A. C., Salminen, S., Mantziari, A., Vinderola, G., Ervolino, E., Santana, S. I., Silva, P. H. F., & Messora, M. R. (2020). Bifidobacterium animalis subsp lactis HN019 presents antimicrobial potential against periodontopathogens and modulates the immunological response of oral mucosa in periodontitis patients. *PloS one*, *15*(9), e0238425.
15. Garala, K., Joshi, P., Shah, M., Ramkishan, A., & Patel, J. (2013). Formulation and evaluation of periodontal in situ gel. *International journal of pharmaceutical investigation*, *3*(1), 29–41.
16. Teughels, W., Newman, M. G., Coucke, W., Haffajee, A. D., Van Der Mei, H. C., Haake, S. K., Schepers, E., Cassiman, J. J., Van Eldere, J., van Steenberghe, D., & Quirynen, M. (2007). Guiding periodontal pocket recolonization: a proof of concept. *Journal of dental research*, *86*(11), 1078–1082.
17. Sarkar, A., & Mandal, S. (2016). Bifidobacteria-Insight into clinical outcomes and mechanisms of its probiotic action. *Microbiological research*, *192*, 159–171.
18. Oliveira, L. F., Salvador, S. L., Silva, P. H., Furlaneto, F. A., Figueiredo, L., Casarin, R., Ervolino, E., Palioto, D. B., Souza, S. L., Taba, M., Jr, Novaes, A. B., Jr, & Messora, M. R. (2017). Benefits of Bifidobacterium animalis subsp. lactis Probiotic in Experimental Periodontitis. *Journal of periodontology*, *88*(2), 197–208.