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Research Article

EFFICACY OF REDOX AGENT ON SUBGINGIVAL MICROFLORA IN CHRONIC PERIODONTITIS- A MICROBIOLOGICAL EVALUATION

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ABSTRACT

Background: An alternative approach to conventional antimicrobial or antiseptic agents in the suppression of subgingival bacteria is to inhibit the growth by changing the subgingival ecology. The subgingival environment has been demonstrated to have a low redox potential which supports the growth and survival of subgingival anaerobic bacteria. Redox agents, such as methylene blue raise the redox potential of an ecosystem.

Aim: To study the effects of methylene blue incorporated into chitosan strips on clinical parameters and on Porphyromonas. gingivalis, Prevotella. intermedia, Fusobacterium. nucleatum and Capnocytophaga.

Materials & Methods: A total of 40 sites from 20 patients. were included in a split-mouth design. 40 sites were divided into Group A (experimental) and Group B (control). Clinical parameters - plaque index, gingival index, probing pocket depth were recorded. Microbiological evaluation was done by Gram's stain and culture. The clinical and microbiological parameters were recorded on days 0, 7, 15 and 30.

Results: The Groups A and B showed a reduction in clinical parameters plaque index, gingival index and probing pocket depth. Gram's stain revealed that there was a significant increase in gram positive and negative cocci and decrease in gram negative bacilli in both the groups. Group A showed highly significant changes towards the healthy flora. Culture analysis showed that all the three organisms were predominant except Porphyromonas. gingivalis in both the groups. At the end of the study, there was a decrease in F. nucleatum, Capnocytophaga and P. intermedia and absence of P. gingivalis in both the groups. But experimental sites showed highly significant reduction compared to control sites.

Conclusion: Methylene blue incorporated in chitosan stips can be used as an adjunctive treatment in adult periodontitis. Further long term studies are recommended to evaluate the known sustained effect of the redox agent.

INTRODUCTION

In chronic periodontitis there is an increase in the proportion of anaerobic motile bacteria in the pocket area including Porphyromonas. gingivalis, Prevotella intermedia, Fusobacterium. nucleatum, Wolinella. recta, Eubacterium species and spirochetes. Wolinella. recta and Capnocytophaga species which have also been associated with disease progression. The major aim is the removal or inhibition of subgingival plaque. Systemically administered antimicrobials have objections in their wide spread use in the treatment of chronic periodontal disease because of their adverse effects. Local administration of systemic antimicrobial agents offers a "site-specific" approach to periodontal therapy that has several benefits, primarily, it can be localized to infected sites at high concentrations while avoiding the potential adverse reactions inherent in the systemic use of these medications.(53) Antimicrobials are not the only means of approach in controlling the growth of bacteria. An alternative approach is to inhibit the microbial growth by changing the subgingival environment. It has been shown that the subgingival environment is highly anaerobic with a prevailing low oxygen tension. Survival and growth of such organisms in an ecosystem is, in part, dependent on the existence of a low redox potential (Eh). By raising the redox potential of the periodontal pocket, it should be possible to create an environment incompatible with the growth of anaerobic periodontal pathogens, thereby allowing control of these organisms. A variety of substances are available which, while not liberating oxygen can raise the redox potential of an ecosystem by acting as electron acceptors. Examples include redox dyes such as methylene blue and ferric ions.

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The effect of an increased Eh on anaerobes is to reduce the redox potential of their environment by shifting the redox couples to a more oxidized state, disrupting the redox dependent enzyme systems and consuming essential metabolic reducing power. Methylene blue has no major side effects and is used in the treatment of methaglobinaemia and urolithiasis (Ower, 1995). It has been demonstrated that methylene blue, in a subgingivally placed slow carrier has potential to change the ecosystem thereby enhancing the effects of mechanical therapy. Chitin is the second abundant compound next to cellulose on earth. Chitosan is a biodegradable natural polymer demonstrated to be non-toxic and non-immunogenic.

It can be produced in various forms including film, sponge and fiber and its degradation rate can be controlled (Madhavan, 1992). The aim of this study is to evaluate the effect of methylene blue incorporated in chitosan as a slow release biodegradable device for its antimicrobial efficacy and clinical utility in controlling anaerobic pathogens. It has been used as hemostatic agent, It can be used as surgical glove powder, as artificial skin, in wound dressings, as anticoagulants (Ito, 1991). It can be used as controlled drug delivery system (Madhavan, 1992; Bernkop and Schnurch, 2000; Jameela, 1998). It has also been used as a bone substitute material (Addy, 1988; Ito, 1991). Redox agents have been used in the treatment of Periodontitis (Addy, 1988; Gibson, 1992; Wilson, 1992).

The aims of the present study was to determine the in vitro release pattern of drug release at different time intervals, to evaluate the effect of methylene blue - redox agent incorporated in a slow release biodegradable device on clinical parameters like Plaque index (Silness and Loe), Gingival index (Loe and Silness) and probing pocket depth and to find out the efficacy of methylene blue as an antimicrobial agent on four known periodontal pathogens-Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and Capnocytophaga.

MATERIALS AND METHODS

Chitosan was weighed and taken in a beaker containing 1% glacial acetic acid. Distilled water was added and. heated on a water bath for 15-20 minutes, until chitosan dissolves into the solution. It was allowed to stand for 30 minutes for the entrapped air bubbles to escape and the consistency of the fluid to become viscous. 0.1% of methylene blue was then added to the polymer solution and stirred gently. It was allowed to stand for another 18 minutes.

This polymer was then poured onto the Tarsons disposable petri dishes and kept overnight for drying. Thus, uniform films of 0.1% methylene blue impregnated chitosan films were obtained. The films were cut into strips of 2 x 10mm strips and were treated with 25% aqueous glutaraldehyde for 5 minutes. Content uniformity studies were carried out to ascertain that methylene blue was uniformly distributed throughout the strips. Chitosan strips measuring 2x10mm were selected and transferred onto a 5oml volumetric flask and the volume was made up to 50m1 with distilled water. The volumetric flask was shaken thoroughly in order to leach out the drug. After 30 minutes, all the methylene blue was leached out and its absorbance was studied at 664nm.

Invitro dissolution studies

Static in vitro dissolution studies was carried Out in order to ascertain that there is a sustained release of methylene blue. For this, five methylene blue strips measuring 2x10mm weighing 2mgs were taken in a l0ml volumetric flask. The concentration of methylene blue being released was measured from day 0 to 15, and on day 30. 4m1 of the solution from each of the flask was withdrawn and replaced with 4m1 of fresh distilled Water in order to maintain static conditions. The absorbance was measured by a 1601 Shimadzu UV-Visible Spectrophotometer at 664nm.

Patient selection

The patients were selected from the Department of Periodontics, J.S.S. Dental College, Mysore. A total of 20 patients were selected including both males and females in the age range of 55 years were considered. Patients consent was obtained in a prescribed proforma. The Inclusion criteria included patients diagnosed with adult periodontitis, patients free from any systemic diseases, patients who had not undergone any form of non-surgical or surgical periodontal therapy in the last 6 months, patients who had probing pocket depth of ≤6mm. Exclusion criteria included patients with a history of use of antibiotic / antibacterial mouthwashes in the past 6 months, pregnant women / Nursing mothers, patients with a history of allergy. A split-mouth design was considered. In each patient, two sites were selected. Selected sites were showing a probing pocket depth of ≤ 6mm. A total of 40 sites were selected in this study. The selected patients were subject to basic therapy and oral hygiene instructions.

The selected sites were grouped as

Group A: (Test site) These sites received scaling and root planing along with a chitosan strip with methylene blue placed at baseline. Group B: (Control site) These sites received only scaling and root planing. The clinical and microbiological data collected were subjected to statistical analysis.

Clinical parameters

Each of the sites (both Test and Control) were assessed for:
Plaque Index (Silness and Loe, 1964), Gingival Index (Loe and Silness, 1963), Probing Pocket Depth (PPD) measured with William's Graduated periodontal probe at Day 0,7,15 days and 30. Pocket probing was done on mesial, distal facial, lingual or palatal areas around the tooth and the deepest area probed was used for placing the controlled release methylene blue inserts. After assessing the clinical parameters, scaling was performed on both test and control site.

Subgingival plaque samples were collected with sterile curettes and samples were transferred to a bottle containing thioglycollate transport media. Root planing was performed. The test sites were isolated with Cotton and a chitosan-methylene blue strip was inserted with a tweezer. A Periodontal dressing was placed. The control sites received only scaling and root Planing and the placement of a periodontal pack. The subgingival plaque samples were collected on days 0, 7, 15 and 30. Collected samples were incubated at 37°C for 30 minutes. A smear was prepared from the sample and was Gram's stained and cultured.

Organisms were provisionally identified on their colony characteristics and the colony count taken. Smears were prepared from the colonies, Gram's stained and the colony morphology noted. The colonies were further subjected to the following tests for identification of the specific organism: UV-light microscopy, agglutination of sheep erythrocytes, Indol test, sucrose and lactose fermentation tests —in accordance with the study done by Caroline *et al*. (Madhavan, 1992) The microbial days 0, 7, 15 and 30. The data collected were subjected to statistical analysis using one way ANOVA and Duncan multiple range test. Prior to the beginning of the study, drug release pattern was established using spectrophotometric analysis in vitro (Graph no. 11). The results showed maximum drug release on day 30.

RESULTS

Plaque index

The value obtained was statistically highly significant (.000) in both the groups. The 'p' value showed statistical significance (0.01 5) in both the groups from day 0 to 30 Table 1 .

Gingival index (Table 2)

On comparison of gingival index between each group, the 'p' value showed statistical significance (0.01 5) in both the groups from day 0 to 30.

Probing Pocket Depth (Table 3)

Comparison of probing pocket depth between each groups showed no significance on day 0, 15, the 'P' value showed statistical significance (.022) on day 30 between the 2 groups.

Microbiological analysis

Gram's Stain

Gram positive cocci (Table 4)

The value obtained was statistically highly significant (.000) in group A & B. Comparison of gram positive cocci between each groups showed statistically no significance on day 0. The 'p' value showed statistical significance (.002). Comparison between Group A and Group B on day 30 showed statistically highly significance (000).

Gram negative cocci: (Table 5)

The value obtained was statistically highly significantly (.000) in both the group. Comparison between Group A and Group B the 'p' value showed statistically no significance (.720). on day 0. On day 7, the 'p' value showed statistical significance (.006). On day 30, the 'p' value showed statistically high significance (.000).

Gram positive bacilli: (Table 6)

The value obtained was statistically highly significant (.000) in both the groups. Comparison between Group A and Group B on day 0 showed statistical high significance (.000) on day 30 in both the groups.

Table 1. Plaque Index (one way ANOVA)

	N	Mean	F value	p value
Group A				
Day 0	20	2.19±011	385.219	.000
Day 7	20	1.77±0.07		
Day 15	20	1.60±0.06		
Day 30	20	1.32±0.06		
Group B				
Day 0	20	2.21±0.11	358.689	.000
Day 7	20	1.84±0.06		
Day 15	20	1.66±0.04		
Day 30	20	1.45±0.05		

Duncan's TestTest

Days	Mean Significance level			
30	1.32	1.61	1.78	2.19
15				
7				
0				

Control

Days	Mean Significance level			
30	1.45	1.67	1.84	2.22
15				
7				
0				

T-Test

Day	Group	N	Mean	t	P
Day 0	A	20	2.19±0.11	-.543	.590
	B	20	2.21±0.11		
Day 7	A	20	1.77±0.07	-2.942	0.006*
	B	20	1.84±0.06		
Day 15	A	20	1.60±0.06	-3.244	.002**
	B	20	1.66±0.04		
Day 30	A	20	1.32±0.06	-6.725	.000***
	B	20	1.45±0.05		

Table 2. Gingival Index

	N	Mean	F value	p value
Group A				
Day 0	20	2.27±0.12	318.165	.000
Day 7	20	2.18±0.11		
Day 15	20	1.61±0.14		
Day 30	20	1.24±0.09		
Group B				
Day 0	20	2.26±0.12	285.349	.000
Day 7	20	2.18±0.13		
Day 15	20	1.70±0.08		
Day 30	20	1.28±0.13		

Duncan's Test

Test

Days	Mean Significance level			
30	1.24	1.61	2.19	2.27
15				
7				
0				

Control

Days	Mean Significance level			
30	1.28	1.70	2.18	
15				
7				
0				2.27

T-Test

Day	Group	N	Mean	t	P
Day 0	A B	20 20	2.27±0.12 2.21±0.11	.140	.889*
Day 7	A B	20 20	2.18±0.11 1.84±0.06	.219	.828*
Day 15	A B	20 20	1.61±0.14 1.70±0.08	-2.548	.015**
Day 30	A B	20 20	1.24±0.09 1.28±0.13	-1.081	.286*

Table 3. Probing pocket depth

	N	Mean	F value	p value
Group A				
Day 0	20	5.6±0.50	3.051	.034
Day 7	20	5.6±0.50		
Day 15	20	5.45±0.51		
Day 30	20	5.2±0.41		
Group B				
Day 0	20	5.6±0.50	.133	.915
Day 7	20	5.6±0.50		
Day 15	20	5.5±0.41		
Day 30	20	5.55±0.51		

Duncan's Test

Test

Days	Mean Significance level	
30	5.2	
15	5.45	5.45
7		5.6
0		5.6

T-Test

Day	Group	N	Mean	t	P
Day 0	A B	20 20	5.6±.50 5.6±.50	.000	1.000*
Day 7	A B	20 20	5.6±.50 5.6±.50	.000	1.000*
Day 15	A B	20 20	5.45±.51 5.55±.48	-1.265	.214*
Day 30	A B	20 20	5.2±.41 5.55±.51	-2.390	.022**

Table 3. Probing pocket depth

	N	Mean	F value	p value
Group A				
Day 0	20	5.6±0.50	3.051	.034
Day 7	20	5.6±0.50		
Day 15	20	5.45±0.51		
Day 30	20	5.2±0.41		
Group B				
Day 0	20	5.6±0.50	.133	.915
Day 7	20	5.6±0.50		
Day 15	20	5.5±0.41		
Day 30	20	5.55±0.51		

Duncan's Test

Test

Days	Mean Significance level	
30	5.2	5.45
15	5.45	5.6
7		5.6
0		

T-Test

Day	Group	N	Mean	t	P
Day 0	A B	20 20	5.6±.50 5.6±.50	.000	1.000*
Day 7	A B	20 20	5.6±.50 5.6±.50	.000	1.000*
Day 15	A B	20 20	5.45±.51 5.55±.48	-1.265	.214*
Day 30	A B	20 20	5.2±.41 5.55±.51	-2.390	.022**

Table 4. Gram positive Cocci

	N	Mean	F value	p value
Group A				
Day 0	20	17.15±1.0	263.012	.000
Day 7	20	22.65±1.3		
Day 15	20	26.80±1.6		
Day 30	20	31.75±2.4		
Group B				
Day 0	20	17.25±0.7	68.622	.000
Day 7	20	21.40±1.0		
Day 15	20	25±1.65		
Day 30	20	24.3±3.1		

Duncan's Test

Test

Days	Mean Significance level			
30	17.15			
15		22.65	26.8	
7				
0				31.75

Control

Days	Mean Significance level		
30	17.25		
15		21.4	
7			24.3
0			25

T-Test

Day	Group	N	Mean	t	P
Day 0	A B	20 20	17.15±1.04 17.25±.78	-.343	.733*
Day 7	A B	20 20	22.65±1.3 21.40±1.1	3.218	.003**
Day 15	A B	20 20	26.80±1.6 25.00±1.65	3.421	.002**
Day 30	A B	20 20	31.75±2.4 24.30±3.1	8.358	.000***

Table 5. Gram negative Cocci

	N	Mean	F value	p value
Group A				
Day 0	20	17.25±1.51	352.522	.000
Day 7	20	21.2±1.39		
Day 15	20	26.7±1.75		
Day 30	20	31.8±1.36		
Group B				
Day 0	20	17.5±1.67	316.010	.000
Day 7	20	21.35±1.22		
Day 15	20	25.4±.99		
Day 30	20	29.70±1.30		

Duncan's Test

Test

Days	Mean Significance level			
30	17.25			
15		21.2		
7			26.7	
0				31.8

Control

Days	Mean Significance level			
30	17.5			
15		21.35		
7			25.4	
0				29.7

T-Test

Day	Group	N	Mean	t	P
Day 0	A	20	17.15±1.51	-.495	.623*
	B	20	17.50±1.67		
Day 7	A	20	21.20±1.39	-.361	.720**
	B	20	21.35±1.22		
Day 15	A	20	26.70±1.75	2.888	.006**
	B	20	25.40±.99		
Day 30	A	20	31.80±1.36	4.986	.000***
	B	20	29.70±1.30		

Table 6. Gram Positive Bacilli

	N	Mean	F value	p value
Group A				
Day 0	20	25±2.33	250.375	.000
Day 7	20	18.15±1.42		
Day 15	20	12±1.16		
Day 30	20	8.85±2.71		
Group B				
Day 0	20	24.15±2.43	82.447	.000
Day 7	20	19.9±1.88		
Day 15	20	17.05±1.60		
Day 30	20	14.45±2.16		

Duncan's Test

Test

Days	Mean Significance level			
30	8.82			
15		12.0		
7			18.15	
0				25.0

Control

Days	Mean Significance level			
30	14.45			
15		17.05		
7			19.90	
0				24.15

T-Test

Day	Group	N	Mean	T	P
Day 0	A	20	25.0±2.33	1.126	.267*
	B	20	24.15±2.43		
Day 7	A	20	18.15±1.42	-3.308	.002**
	B	20	19.90±1.88		
Day 15	A	20	12.00±1.16	-11.37	.000***
	B	20	17.05±1.60		
Day 30	A	20	8.85±2.71	-7.206	.000***
	B	20	14.45±2.16		

Gram negative bacilli (Table 7)

The value obtained was statistically highly (.000) in both the groups. On comparison between Group A and Group B on day 7 showed statistically high significance (.000). Comparison between Groups on day showed the 'p' value showed statistically high significance (.000). Comparison between Group A and Group B on day 30 the 'p' value showed statistically high significance (.000).

Table 7. Gram Negative Bacilli

	N	Mean	F value	p value
Group A				
Day 0	20	21.95±1.14	413.277	.000
Day 7	20	17.65±1.08		
Day 15	20	14.0±1.45		
Day 30	20	8.75±1.20		
Group B				
Day 0	20	21.95±1.19	275.694	.000
Day 7	20	19.0±1.07		
Day 15	20	16.60±1.09		
Day 30	20	12.10±1.11		

Duncan's Test

Test

Days	Mean Significance level			
30	8.75			
15		14.0		
7			17.65	
0				21.95

Control

Days	Mean Significance level			
30	12.10			
15		16.60		
7			19.0	
0				21.95

T-Test

Day	Group	N	Mean	t	P
Day 0	A	20	21.95±1.14	.000	1.000*
	B	20	21.95±1.19		
Day 7	A	20	17.65±1.08	-3.943	.000***
	B	20	19.0±1.07		
Day 15	A	20	14.0±1.45	-6.396	.000***
	B	20	16.60±1.09		
Day 30	A	20	8.75±1.20	-9.095	.000***
	B	20	12.10±1.11		

Culture

Fusobacterium. nucleatum: (Table 8)

The value obtained was statistically highly significant (.000) in both the groups. Comparison between Group A and Group B on day 0 showed statistically no significance (.140). Comparison between Group A and Group B on day 15 showed statistical high significance (.000). Comparison between Group A and Group B on day 30 showed statistically high significance.

Capnocytophaga: (Table 9)

The mean Capnocytophaga score statistically highly significant (.000) in both the groups. On comparison between Group A and Group B on day 0 showed no difference between the groups on day 0. On day 7 showed statistical significance(.002), on day 15 showed statistically high significance (.000). On day 30 showed statistically high significance (.000).

Prevotella. intermedia: (Table 10)

The value obtained was statistically highly significant (.000) in both the groups. Comparison between Group A and Group B on

day 0 showed 'p' value showed statistically no significance on day 0, 7 &15. The 'p' value showed statistical significance

Porphyromonas. gingivalis: (Table 11)

P. gingivalis was not detected from any of the sites at any of the time intervals.

Table 8. Fusobacterium nucleatum

	N	Mean	F value	p value
Group A				
Day 0	20	71.75±16.56	82.854	.000
Day 7	20	57.25±14.46		
Day 15	20	32±7.67		
Day 30	20	17.25±6.17		
Group B				
Day 0	20	72.25±16.97	36.482	.000
Day 7	20	64.50±15.88		
Day 15	20	44.75±11.75		
Day 30	20	30.25±10.8		

Duncan's Test

Test

Days	Mean Significance level			
30	17.25			
15		32.0		
7			57.25	
0				71.75

Control

Days	Mean Significance level		
30	30.25		
15		44.75	
7			64.50
0			72.25

T-Test

Day	Group	N	Mean	t	P
Day 0	A	20	71.75±16.56	-.094	.925*
	B	20	72.25±16.97		
Day 7	A	20	57.25±14.46	-1.509	.140*
	B	20	64.50±15.88		
Day 15	A	20	32.00±7.67	-4.062	.000***
	B	20	44.75±11.75		
Day 30	A	20	17.25±6.17	-4.668	.000***
	B	20	30.25±10.81		

Table 9. Capnocytophaga

	N	Mean	F value	p value
Group A				
Day 0	20	131.05±6.84	1628.980	.000
Day 7	20	72.75±3.79		
Day 15	20	42.75±4.43		
Day 30	20	25.25±4.99		
Group B				
Day 0	20	132.35±6.91	370.607	.000
Day 7	20	80.25±9.52		
Day 15	20	68.5±9.88		
Day 30	20	35.75±20.54		

Duncan's Test

Test

Days	Mean Significance level			
30	25.25			
15		42.75		
7			72.75	
0				131. 05

Control

Days	Mean Significance level			
30	35.75			
15		68.50		
7			80.25	
0				132.35

Table 9. Capnocytophaga

	N	Mean	F value	p value
Group A				
Day 0	20	131.05±6.84	1628.980	.000
Day 7	20	72.75±3.79		
Day 15	20	42.75±4.43		
Day 30	20	25.25±4.99		
Group B				
Day 0	20	132.35±6.91	370.607	.000
Day 7	20	80.25±9.52		
Day 15	20	68.5±9.88		
Day 30	20	35.75±20.54		

Duncan's Test

Test

Days	Mean Significance level			
30	25.25			
15		42.75		
7			72.75	
0				131.05

Control

Days	Mean Significance level			
30	35.75			
15		68.50		
7			80.25	
0				132.35

T-Test

Day	Group	N	Mean	t	P
Day 0	A	20	131.05±6.84	-.598	.554*
	B	20	132.35±6.91		
Day 7	A	20	72.75±3.9	-3.271	.002**
	B	20	80.25±9.52		
Day 15	A	20	42.75±4.43	-10.633	.000***
	B	20	68.50±9.88		
Day 30	A	20	25.25±4.99	-4.024	.000***
	B	20	35.75±10.54		

Table 10. Prevotella, intermedia

	N	Mean	F value	p value
Group A				
Day 0	20	43±37.32	8.647	.000
Day 7	20	27.5±23.75		
Day 15	20	15.5±13.94		
Day 30	20	7.6±6.83		
Group B				
Day 0	20	43.25±37.34	4.046	.000
Day 7	20	35.5±31.61		
Day 15	20	24.75±22.12		
Day 30	20	14.75±13.42		

Duncan's Test

Test

Days	Mean Significance level		
30	7.60		
15	15.50		
7		15.50	
0		27.50	
			43.0

Control

Days	Mean Significance level		
30 15 7 0	14.75 24.75	24.75 35.50	35.50 43.25

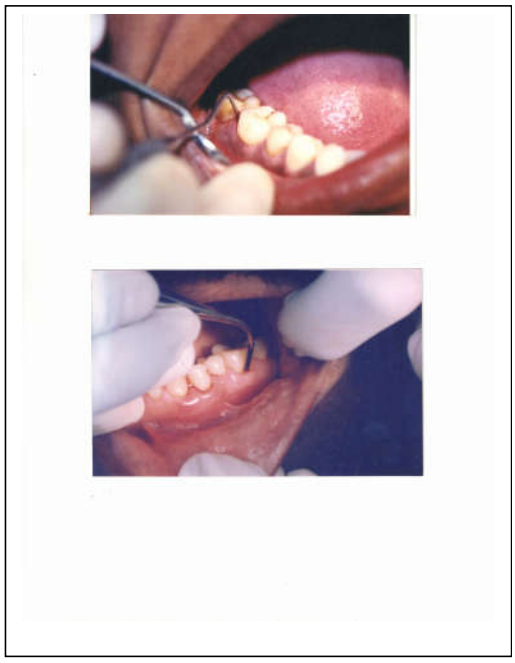
T-Test

Day	Group	N	Mean	t	P
Day 0	A B	20 20	43.0±37.32 43.25±37.35	-.021	.983*
Day 7	A B	20 20	27.50±23.75 35.50±31.61	-.905	.371**
Day 15	A B	20 20	15.50±13.94 24.75±22.15	-1.580	.122*
Day 30	A B	20 20	7.60±6.83 14.75±13.42	-2.122	.040**

Table 11. Porphyromonas, gingivalis

	N	Mean
Group A		
Day 0-30	20	0
Group B		
Day 0-30	20	0

P>0.05- Not significant* P<0.05- Significant ** P -.000 – Highly significant ***



Clinical photographs placement of chitosan-methylene blue strips

DISCUSSION

Microbial colonization in the gingival crevice brings about changes in the periodontium and is characterized by the presence of periodontal pocket and active bone resorption with inflammation. Studies have demonstrated that we are not dealing with a single disease, but with a set of diseases of different etiologies and host responses. As with all other form of periodontal disease, microorganisms play a crucial role in the initiation and progression of adult periodontitis.

Among the periodontopathic pathogens are species like Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans and Capnocytophaga species (Tanner *et al*. 1979, Slots and Genco 1980, Slots *et al* 1986, Tanner *et al*. 1992, Socransky and Haffajee 1994). Elimination or adequate suppression of periodontopathic microorganisms in the subgingival microbiota is essential for periodontal healing. Local drug delivery involves direct placement of antimicrobial agents into subgingival sites, minimizing the impact of agents on nonoral body sites. The most often cited initial attempt utilize controlled local drug delivery for the management of periodontitis was pioneered by Max Goodson *et al*. (1979) Sustained pocket delivery of local antimicrobial agents may be attained with the use of slow-release and controlled-release drug delivery systems.

Vehicles tested for sustained delivery include solutions, pastes, hollow fibers, acrylic strips, monolithic fibres, resorbable cellulose, collagen and biodegradable gel. In controlled-release drug delivery, the antimicrobial agent reservoir is protected from local removal mechanisms after placement, enabling a zero-order drug release kinetics, which maintain consistently elevated pocket considerations of the agent during its application (Rams and Slots, 1996). Chitin is a biodegradable natural polymer that is non-toxic and nonimmunogenic. Chitosan's availability in variety of forms including solutions, powder, flakes, gels and films together with its unique chemical and biological properties makes it a very versatile biomaterial[36]. Keeping in view these aspects of chitosan, it was used in this study as a delivery system for application of the redox agent to the periodontal pocket. Survival and growth of anaerobic organisms is dependent in part, on the existence of a low redox potential (Eh), which is a measure of reducing power of system. It has been shown that the redox potential pockets have a negative redox potential (-48mV). By raising the redox potential of the periodontal pocket, it should be possible to create an environment incompatible with the growth of anaerobic organisms.[42] A variety of substances including the redox dyes such as methylene blue can raise the redox potential of an ecosystem (Wilson *et al*. 1992).[68]

Increasing resistance and side effects of the anaerobic organisms to the currently available antibiotics initiates the need of other/alternative means of intervention to arrest the periodontopathic anaerobic organisms. Redox dye, methylene blue offer an alternative and adjunctive approach in arresting these organisms. Studies have concluded that methylene blue, especially in the controlled release mode was superior to a single thorough subgingival debridement in reducing subgingival microflora (Gibson *et al* 1992,[68] Ower P.c.1995).[42] The advantage of methylene blue and its clinical and microbiological efficacy as demonstrated by these studies has entailed us to use this agent as a 'modifier' of the subgingival environment. In this study methylene blue was incorporated in chitosan in the concentration of 0.1 which is in accordance with the study by Gibson *et al* (1992). Clinical observations revealed that in both the groups there was mean reduction in plaque score, gingival inflammation and probing pocket depth when compared to baseline. This is accordance with the study by Ower P.C. *et al* 1995,[42] which demonstrated clinical and microbiological improvements following methylene blue placement in a slow release device as compared to subgingival debridement alone.

This is also in accordance with the study by Addy *et al*. The resultant decrease pocket depth could be explained by the reduced inflammation following therapy (Addy et at 1988). (Ali, 1994) Bacteria have an affinity to basic dyes due to their acidic nature of their protoplasm. Gram's stain is one of the differential stain used to study the morphotypes of the bacteria (Caroline, 1994). Gram's staining was done to determine the morphotypes of the plaque, and to evaluate the effects of methyelene blue on these morphotypes. A mean increase in the gram positive cocci, gram negative cocci and grain positive bacilli was observed in both the groups when compared from day 0 to day 30. Comparison between the groups showed statistical significance in Group A on days 7, 15 and 30. A mean decrease in gram negative bacilli was observed in both the groups when compared from Day 0 to Day 30. Comparison between groups showed a statistical significant decrease in Group A on days 15 and 30. This is in accordance with the study by Addy *et al*. (1984).

The increase in proportion of gram negative bacilli in periodontal active sites appeared to be at the cost of gram positive bacilli. Bacterial culture is the 'Gold Standard' microbiological assay against which all other tests are compared and validated. No single microbiological method can identify all the bacterial species in a clinical sample, bacterial culture is capable of detecting the broadest of the bacterial periodontal pathogens. The range of cultivable organisms can be extended through the use of selective media targeted to specific antibiotic susceptibility and resistance of certain species, and the use of growth conditions and media nutrients.[67] There is a plethora of evidence available on periodontal microbiological assay, isolation of organisms being one of them. Culture is a gold standard for microbiological evaluation. The isolated organisms have to the further identified by different tests to recognise species and subspecies genera as done by Caroline et at. (l994). The outcome of the results also depends on the transport media and nutrient media. In our study, we have employed methodology as per the study conducted by Caroline *et al*. (1994).

Slots *et al*., 1986.[58] Petsios *et al*., 1995[44] demonstrated a statistically significant association of Capnocytophaga with moderate periodontitis with attachment loss of 4-6 mm. Au *et al* 1994 (Ananthanarayan and Paniker, ?) demonstrated that most patients were positive for Capnocytophaga. This study demonstrated a similar pattern. Our study is in contrast with the study by Savitt and Socransky 1984[50] where they demonstrated Capnocytophaga in gingivitis in comparison to adult and juvenile periodontitis respectively. The difference in data could be related to the experimental designs, microbiological methods, and statistics and to the nature and type of adult periodontitis. The efficiency of peptide utilization may be among the key determinants of the microbial ecology of periodontal pockets.[61] Amino acids may be used as both energy source and as building blocks for the formation of new cellular constituents. Effective use of amino acids and peptides require transport systems to transport these nutrients into the cell. Some of the periodontal bacteria, A. actinomycetemcomitans and peptides are important nutritional sources. F. nucleatum and P. gingivalis compete for amino acids and peptides. The efficiency of peptide utilization by various organisms may be influenced by their growth conditions as demonstrated by F. nucleatum when grown on the surface of blood agar.

The efficiency of peptide utilization may be among the key determinants of microbial ecology of periodontal pockets (Tang-Larsen *et al*., 1995).[61] Also, intergeneric co-aggregation is an important factor in the formation and maturation of dental plaque. F. nudeatum has been demonstrated to co-aggregate with streptococci. Murray *et al*. (1988).[38] demonstrated that the existence of galactose-sensitive adhesin of F. aucleatum which might participate in co-aggregation. It is suggested that several receptors are participating in the co-aggregation of F. nucleatum with treptococci. (Takemoto 1995).[62] Therefore, it is a great advantage for F. nucleatum to have several adhesin and to change the predominant adhesin according to the change in colonization on plaque and developing into subgingival plaque. However, the success of a specific organism may not be predicted unless the conditions of the periodontal pockets can be produced in the laboratory.

The lesser number of patients with P. intermedia found in this study is in accordance with the finding by Au *et al* 1994, (Ananthanarayan and Paniker, ?) who demonstrated a lower percentage of patients with P. intermedia in the Sudanese population compared to the Norwegians. A mean decrease in Capnocytophaga, F. nucleatum and P. intermedia was observed in both the groups when compared from day 0 to day 30. Comparison between the groups showed statistical significance in Group A on days 15 and 30 and statistically high significance at day 30. This is in accordance with the study by Gibson *et al*. (1992) Wilson *et al*. (1992) Ower *et al*. (1995). This study is an attempt to identify and culture the organisms and to determine the efficacy of methylene blue, redox agent on the bacterial populations in subgingival area.

In this study, we have demonstrated significant improvements in clinical parameters like reduction of plaque and gingival inflammation and decrease in probable pocket depth. The microbiological evaluation showed an increase in organisms which are present in a normal, healthy individual. This has been identified by Gram's stain and by culture. The results of this study demonstrate clinical and microbiological improvements following use of adjunctive chitosan strips containing methylene blue. Therefore, it can be concluded that methylene blue incorporated in chitosan stips can be used as an adjunctive treatment in adult periodontitis.

Conclusion

From this study it can be concluded that- Methylene blue, a redox agent is one of the potential agents that can be used to change the ecology of the periodontal pocket. Methylene blue can be used in a local drug delivery system in non-surgical periodontal therapy. Chitosan, a biodegradable and biocompatible polymer can be used as a vehicle for delivering the drug. Further long term studies are recommended to evaluate the known sustained effect of the redox agent.There was no conflict of interests in this study. The funding for this study was done under a loan sanctioned by the State Bank of Mysore, Mysore

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