Trends in Biomaterials and Artificial Organs
www.biomaterials.org.in/tibao

Original Article

A Novel Use of Tissue Conditioner as a Local Drug Delivery Medium for Microbial Control around Dental Implants: *In Vitro* Release Studies of Antibiotics and Curcumin

Vibha Shetty[1], R. Deveswaran[2], Beena Antony[3], Shrestha Shetty[4], K. Ranganath[5]

[1]*Department of Prosthodontics,* [5]*Department of Oral Surgery, Faculty of Dental Sciences,* [2]*Department of Pharmaceutics, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bangalore, India*
[3]*Professor, Department of Microbiology, Fr Muller Medical College & Research Centre, Mangalore, India.*
[4]*Department of Public Health Dentistry, AJ institute of Dental Sciences, Mangalore, India*

Received: *1 June 2023*
Accepted: *29 June 2023*
Published online: *30 June 2023*

Keywords: *amoxycillin, metronidazole, curcumin, tissue conditioner, peptostreptococcus, fusobacterium, porphyromonas*

Microbe mediated inflammation is one of the common causes of peri-implantitis soon after surgery. A medium that delivers antibiotics locally in a sustained manner may mitigate the common side effects of the antibiotic especially gastric irritation. To determine the effectiveness of tissue conditioner as a carrying medium to deliver antibiotics and curcumin as an alternative at an implant surgical site post operatively, retaining the properties of tissue conditioner. The antibiotics amoxycillin-clavulonic acid and metronidazole, and the antimicrobial agent curcumin are incorporated into tissue conditioner (TC) at various concentrations. The antimicrobial properties are studied using antimicrobial sensitivity tests against *Peptostreptococcus*, *Fusobacterium*, and *Porphyromonas*. In addition, the flow properties and viscosity of selected TC-antimicrobial combinations are analyzed. Relevant statistical analyses are carried out to assess the data. The tissue conditioner (TC) mixed with antibiotic and curcumin separately, showed a zone of inhibition that was greater than the control used. This showed that there was elution from the tissue conditioner mixed with the chosen drugs into artificial saliva The materials mixed in 1:1 ratio of metronidazole and different ratios of curcumin each with TC showed spreadability and viscosity similar to that of control. Differential Scanning Calorimetry showed no interaction between the drugs and tissue conditioner. Tissue conditioner can be used as an effective medium to carry antibiotics or curcumin with positive elution into artificial saliva. Treatment strategies could involve targeted pharmaceutical approach with drugs delivered locally.

Introduction

Tooth loss due to periodontitis or dental caries is a debilitating disease that has been successfully treated with a predictable treatment option of dental implant supported restorations. These restorations provide rehabilitation solutions for patients with a few or all missing teeth. Yet the procedure is prone to failure. Early failure is determined by microbial contamination, improper case selection, poor surgical protocols, and habits, whereas delayed failures are caused by systemic conditions, lack of oral hygiene maintenance, occlusal overload and prosthetic defects. The human mouth is the habitat of over 750 aerobic and anaerobic microbes, that have been identified, genetically mapped and named by the human oral microbiome project [1]. Therefore, the chances are high for the immediate post operative infection, which can lead to peri-implantitis and subsequent loss of implant.

Microbial colonization of the surgical site starts within a few hours to weeks after placing implants [2]. The microbial flora in mouth contain both aerobic and anaerobic bacteria, but the latter have largely been implicated in periodontitis and peri-implantitis [3]. Minimizing impact of microbial colonization has been achieved with pre-operative or post-operative antibiotic coverage [4]. Oral administration, namely the *per oral* (PO) route of antibiotics is associated with several side effects including gastrointestinal disturbances causing nausea, vomiting, diarrhea, pain and other symptoms [5]. Lack of patient compliance to complete the prescribed course is an equal challenge. Antibiotics are absorbed best through the intravenous route. However, antibiotics like

* *Coresponding author*
E-mail address: *vibhashetty.pr.ds@msruas.ac.in* (Prof. Vibha Shetty, MDS)

amoxicillin and metronidazole have good bioavailability even when administered through the PO route and are best absorbed though the small intestine. The addition of clavulanic acid to amoxycillin enhances the absorption of the antibiotic. In addition, due to the mode of normal distribution of antibiotics through the plasma and final delivery to the desired area of application, a large dose of antibiotic must be administered. This leads to the problem of high antibiotic resistance especially in the south Asian region [6]. Alternative therapeutics like curcumin have been explored to avoid the side effects of antibiotics [7].

Localized drug delivery has been identified as a good strategy to deliver higher doses to the site, rather than losing a large part of the administered drug to metabolism [8]. This could be presumably adopted to control the peri-implantitis, with two-fold advantage. A customized therapy is possible and the use of orally consumed broad spectrum antibiotics could be minimized, if not fully avoided. Currently, topically applicable antibiotic containing gels are available for local drug delivery in mouth, but they get washed away by saliva and demand constant re-application. For sustained drug delivery, it requires the use of a material that will remain *in situ* for a period of five to seven days, the normal duration of an antibiotic course or the time required for primary closure of the surgical site. A suitable carrying medium for the sustained delivery of antibiotic could solve the problem.

The present work proposes tissue conditioner (TC) or soft-liner materials as a potential media for sustained drug delivery to prevent or control peri-implantitis. Polymer based tissue conditioners are used often in Prosthodontics as a soft liner under dentures [9]. They have also been used as a medium to carry anti-fungal drugs to combat the problem of denture stomatitis [10]. Also, they are used to condition inflamed tissues when used with a denture. The use of tissue conditioner has not been assessed yet, as a medium to carry antibiotics to enable local drug delivery at an intra oral surgical site. It is worthwhile to explore the viability of tissue conditioner as a carrying medium of antibiotics and bacteriostatic agents like curcumin. Along with the good release characteristics ensuring the bioavailability of the drug, the addition of the drug must not change the well accepted handling properties of tissue conditioner.

The study is designed to determine the effectiveness of tissue conditioner (TC) as an alternative carrying medium to deliver antibiotics and curcumin at the implant surgical site post operatively, without changing its essential properties. Amoxycllin and metronidazole are commonly prescribed antibiotics as they are well absorbed through the PO route and administration with food or in a fasting state does not impact the absorption of either drug. The absorption of the drug is largely through the small intestine and has shown very good bioavailability [11]. Curcumin, on the other hand is proven to be a good anti-oxidant, anti-inflammatory, anti-bacterial, and anti-fungal agent [12-14].

The main objective of this study is to determine the release of amoxycillin-clavulonic acid, metronidazole and curcumin when incorporated in a tissue conditioner and the effectiveness against three common anaerobic microbes inhabiting the oral cavity. The tissue conditioner carrying effective ratios of antimicrobial agents were tested for physical properties like spreadability and viscosity, and the effectiveness of drug release was tested through the zone of inhibition method against *Peptostreptococcus*, *Fusobacterium*, and *Porphyromonas*.. The compatibility of each additive with tissue conditioner is assessed using Differential Scanning Calorimetry. Bare polymer-based TC, a well-accepted material in Prosthodontics, is taken as the control material. The potential out come is the possibility of using TC to control peri-implantitis, when applied along with an immediate post-surgical denture.

Materials and Methods

Polymethyl methacrylate (PMMA) based tissue conditioner (GC Soft-Liner, GC Europe N.V) was used as the Tissue Conditioner (TC) material to be tested as the drug carrying medium. The antibiotics chosen were Amoxycillin and Potassium Clavulanate (Co-amoxiclav; MOXCLAV DS, 457 mg manufactured by Sun Pharmaceutical India Ltd, Dewas, M.P., India), Pure metronidazole powder (Astitva Chemicals, Valsad, Gujarat, India), and pure medical grade curcumin (Sami Labs, Peenya, Bangalore, Karnataka, India).

Sample preparation and handling

The test samples were prepared by mixing co-amoxyclav, metronidazole and curcumin with the powder part of the tissue conditioner (TC) in different ratios of 1:1, 1:2. 1:3, 1:4 and 1:5 by weight. The powders were weighed on a digital weighing machine, added as per the proportioned required and mixed thoroughly using mortar-pestle to get an even distribution. The sample discs of respective TC-antimicrobial agent combinations were made by mixing the powder and liquid components according to manufacturer's instructions (at a ratio 1.0:1.1, thoroughly stirred for 45 secs). Samples for the microbiological study were prepared as discs of 6mm diameter and 2 mm height by filling the mixed tissue conditioner in custom made acrylic molds (figure 1). Elutes of the samples of drug loaded TC and control TC were taken in triplicate by placing the discs in 2ml artificial saliva for 6 hours in test tubes.

Antibiotic sensitivity test

Disc diffusion technique was used to evaluate the antibiotic sensitivity to TC samples incorporated with antimicrobial agents. A control plate was used for each of the microbes with control discs of a β-lactamase inhibitor (30 µg/disc), metronidazole (5 µg/disc), and curcumin (50 µg/disc). The elutes of TC-co-amoxiclav, TC-metronidazole and TC-curcumin discs were prepared in triplicate and collected aseptically. The samples were randomized and blinded with colour coding. The same was received by the second investigator who was blinded to the concentration and carried out the microbiological study.

Sterile filter paper discs were saturated with the elute samples and were placed over the Brucella blood agar plates inoculated with *Peptostreptococcus*, *Porphyromonas* and *Fusobacterium*. The antimicrobial activity was assessed by measuring the zone of inhibition (ZOI) around the sample discs.

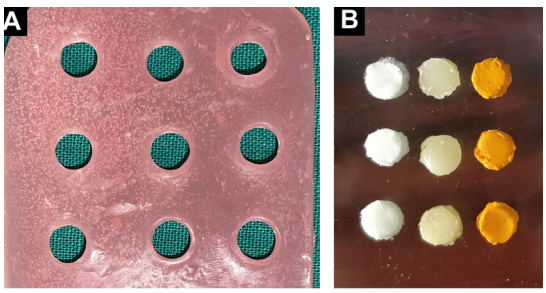

Figure 1: Sample preparation for microbiological study. Acrylic mold (A) and one set of sample discs (B)

The one-way analysis of variance (ANOVA) was used to determine any statistically significant differences between the means of each of the pharmaceutical agents used to create a zone of inhibition (dependent variable) measured in millimeters. The post hoc Tukey's test provides a deeper insight into the comparison between specific groups. A confidence interval of 95 % and error rate of 0.05 was set and a difference below 0.05 was considered to be statistically significant.

Testing physical properties

The characterization of relevant physical properties of the tissue conditioner was carried out to understand the potential changes in the relevant physical properties of tissue conditioner with the incorporation of antibiotics and antimicrobial agents.

(i) Spreadability: The spreadability testing apparatus contained two glass slides placed one over another on a horizontal platform. The lower glass plate is fixed to the platform and the upper one is free to slide over it. The test material (freshly mixed tissue conditioner) is placed in between the glass plates and the upper glass plate is pulled horizontally using a string running over a pulley fixed to the edge of the platform and loaded with a fixed weight. The time taken by the glass slide to travel the distance of 6 cm is measured with the help of a stop clock [15].

Combinations of the tissue conditioner loaded with Co-amoxyclav in the proportions of 4:1 and 5:1, metronidazole in the proportions of 1:1 and 4:1 and curcumin in in the proportions of 1:1, 3:1 and 4:1 were taken for the test, wherein the bare tissue conditioner (TC) served as control. In each case, 0.5g of sample was put in between the glass slides kept in aligned position and the upper slide was pulled horizontally with a fixed weight of 20g. The sliding time was taken as the indication of spreadability.

(ii) Viscosity: Viscosity of tissue conditioner incorporated with antibiotics and curcumin was analysed using Brookfield viscometer. The viscometer uses the principle of rotation of a cylinder or disc in a fluid sample that shows viscous resistance, and measures the torque needed to overcome that resistance to movement brought about by the rotation of the cylinder. The spindle is rotated with an electric motor through a beryllium-copper spring [16]. All measurements were carried out at room temperature to simulate clinical mixing conditions. The viscometer was positioned, and the No.1 spindle was suitably centered in the samples taken in a 10 ml test tube to standardize the testing environment. Each sample was tested thrice and the mean of three readings was considered. The spindle was rotated at a standard 100 rpm, and the readings were recorded in centipoise when the reading became stable. This implied that the materials setting process had initiated and was no longer amenable to manipulation [16].

(iii) Thermal phase transition: The crystallization and phase transformation of antimicrobial agents and their interactions with the tissue conditioner were analysed using Differential Scanning Calorimetry (STA 449 F5 Jupiter, NETZSCH Geratebau, GmbH). Approximately 2-5 mg of each sample was heated in an aluminum pan with lid from 30 to 200°C at a scanning rate of 10°C min^{-1} under a stream of nitrogen gas at a flow rate of 50mL min^{-1}. The DSC graphs of bare TC (control) and TC incorporated with Co-amoxiclav, metronidazole and curcumin were recorded.

Results

Microbial sensitivity through disc diffusion technique

The images of the plates of the anti-biotic sensitivity test are shown in figure 2. The zone diameter for each drug was interpreted using criteria published by Clinical and Laboratory Standards Institute. Our controls showed ZOI comparable with those cited in literature for both co-amoxyclav and metronidazole. No ZOI was seen around curcumin control disc.

(i) Zone of Inhibition values: A zone of inhibition (ZOI) of ≥ 21mm and ≥ 15 mm for Co-Amoxyclav (Amoxycillin- Clavulanic acid) and metronidazole respectively, is considered to be susceptible for gram negative anaerobe. A ZOI of ≥ 14 mm is considered resistant for co-amoxyclav. For metronidazole, ≥ 15mm is considered resistant [5]. For curcumin, 9 mm was considered resistant, 9-12 mm as intermediate and 13 as susceptible [6]. All three antimicrobial agents were sensitive to *Peptostreptococcus* (table 1). Co-amoxiclav was sensitive to all three bacteriae. Metronidazole was effective against *Peptostreptococcus* and *Porphyromonas*, while curcumin was effective against *Peptostreptococcus* (table 1).

(ii) Statistical comparison: As per the one-way Anova, there was a significant difference in the ZOI of Co-amoxyclav, metronidazole and curcumin against the growth of *Porphyromonas*, *Fusobacterium* ($P<0.001$) and *Peptostreptococcus* ($P<0.011$) (table 1). The post hoc Tukey's analysis of antimicrobial activity by ZOI measurement showed that metronidazole exhibited the most difference as compared to Co-amoxyclav and curcumin against the growth of *Peptostreptococcus* (table 2). All three pharmaceutical agents showed good results against *Porphyromonas* and against *Fusobacterium*. Co-amoxyclav and metronidazole showed better ZOI than curcumin.

(iii) Effectiveness of Co-amoxyclav, metronidazole and curcumin concentrations: A comparison of the five concentrations showed that 5:1 ratio of Co-amoxyclav, 1:1 of metronidazole and 1:1 of curcumin is the most effective in producing a zone of inhibition against *Fusobacterium*. The concentrations of 4:1 of co-amoxyclav, 1:1 of metronidazole and 3:1 of curcumin are most effective in producing a zone of inhibition against *Porphyromonas*. The concentrations 5:1 of co-amoxyclav, 4:1 of metronidazole and 4:1 of curcumin are found most effective in producing a zone of inhibition against *Peptostreptococcus* (figure 3).

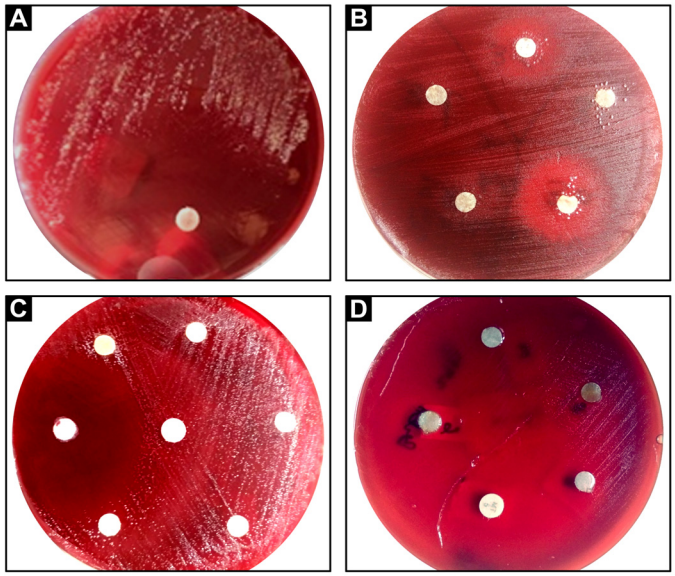

Figure 2: A) Zone of inhibition obtained with amoxicillin control against peptostreptococcus. B) Porphyromonas Zone of inhibition. C) Peptostreptococcus Zone of inhibition. D) Fusobacterium Zone of inhibition

Table 1: One-way ANOVA of zone of inhibition around three microbes by Amoxicillin - Clavulanic acid, Metronidazole, and Curcumin

Micro-organism	Material	N	Mean ZOI	SD	95% CI for Mean		F value	P value
					Lower Bound	Upper Bound		
Peptostreptococcus	Co-Amoxyclav	15	19.53	2.100	18.37	20.70	5.023	0.011*
	Metronidazole	15	24.00	6.448	20.43	27.57		
	Curcumin	15	20.20	2.455	18.84	21.56		
Porphyromonas	Co-Amoxyclav	15	24.87	2.560	23.45	26.28	20.280	0.001*
	Metronidazole	15	18.27	8.481	13.57	22.96		
	Curcumin	15	9.33	7.509	5.18	13.49		
Fusobacterium	Co-Amoxyclav	15	22.73	4.448	20.27	25.20	32.952	0.001*
	Metronidazole	15	10.40	4.852	7.71	13.09		
	Curcumin	15	11.00	4.766	8.36	13.64		

**Statistical significance P < 0.05*

Table 2: Post hoc Tukey's test of zone of inhibition around three microbes by Amoxicillin clavulanic acid, Metronidazole and Curcumin. Post Hoc Tukey Test.

Micro-organism	(I) group	(J) group	Mean Difference (I-J)	P value
Peptostreptococcus	Co-Amoxyclav	Metronidazole	4.467	0.014*
		Curcumin	0.667	0.900
	Metronidazole	Co-Amoxyclav	4.467	0.014*
		Curcumin	3.800	0.043*
	Curcumin	Co-Amoxyclav	0.667	0.900
		Metronidazole	3.800	0.043*
Porphyromonas	Co-Amoxyclav	Metronidazole	6.600	0.027*
		Curcumin	15.533	0.001*
	Metronidazole	Co-Amoxyclav	6.600	0.027*
		Curcumin	8.933	0.002*
	Curcumin	Co-Amoxyclav	15.533	0.001*
		Metronidazole	8.933	0.002*
Fusobacterium	Co-Amoxyclav	Metronidazole	12.333	0.001*
		Curcumin	11.733	0.001*
	Metronidazole	Co-Amoxyclav	12.333	0.001*
		Curcumin	0.600	0.935
	Curcumin	Co-Amoxyclav	11.733	0.001*
		Metronidazole	0.600	0.935

**Statistical significance P < 0.05*

Spreadability

The material was mixed for 45 secs and the time taken for the initiation of tests was standardized to 45 seconds equaling the mixing time of 1.5 minutes by the manufacturer. Spreadability was calculated as the time taken by one glass slab to slide over 6 cm, measured with the help of a stop clock. The statistical analyses of the results showed significant difference between control and all other groups. Tissue conditioner with Co-amoxiclav showed the maximum deviation from the control. Tukey Post hoc test revealed significant difference between control and both ratios of amoxicillin. The spreadability of metronidazole 1:1 and curcumin 4:1 ratio remained similar to that of the control (table 3,4).

Viscosity tests

The time taken by the sample to start setting (initial setting time) marked the end of the viscosity test. Viscosity was measured in centipoise units. There was a significant difference between viscosity of the control vs viscosity of Co-amoxiclav (Amox), and two ratios of curcumin mixtures. The 3:1 ratio of curcumin and either ratio of metronidazole showed nearly the same viscosity as the control (tables 5, 6 and 7).

Thermal phase transition and crystallinity

The DSC for the control showed a prolonged glass transition

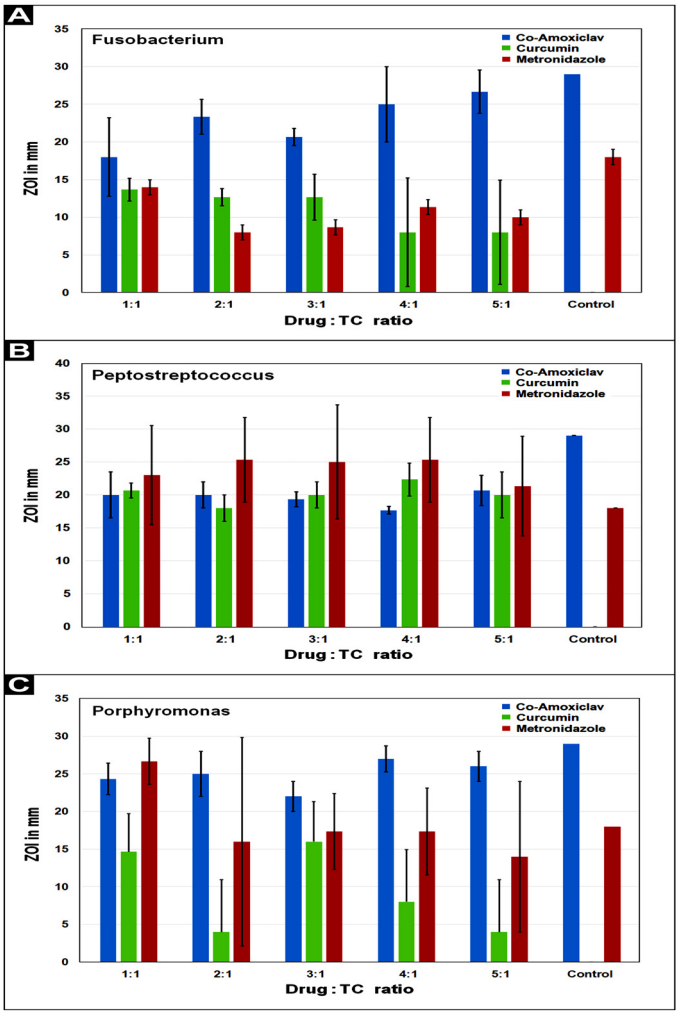

Figure 3: Comparison of effectiveness of various con centrations of Co-amoxyclav, Metronidazole and Curcumin against A) Fusobacterium; B) Peptostreptococcus and C) Porphyromonas

temperature similar to that of polymers (figure 4A). The DSC for the sample with amoxicillin 4:1 showed an endothermic reaction with melting point of around 190°C, corresponding to that of amoxicillin. The nature of the peak implies that amoxicillin is in an amorphous form. The apparent increase in the enthalpy could be due to the glass transition of the tissue conditioner (figure 4B). This confirms that there is no interaction between tissue conditioner and amoxicillin. The DSC for the sample with metronidazole 1:1 showed an endothermic peak at 161°C, corresponding to the melting point of metronidazole. The nature of the peak implies that metronidazole is in a crystalline form (figure 4C). The DSC for the sample with curcumin 1:1 showed an endothermic peak at 179°C, corresponding to the melting point of curcumin. The nature of the peak implies that curcumin is in a crystalline form (figure 4D). This confirms that the tissue conditioner has no interaction with metronidazole and curcumin.

Discussion

Microbial control plays a crucial role in progress of healthy osseointegration. Anaerobic bacteria have been particularly identified as causative organisms in both early and late healing phases. Chronic periodontitis and peri implantitis may have similar microbial etiology [17]. A meta-analysis cited 19% of patients had peri

Table 5: Descriptive statistics for viscosity

	Mean	SD
Control	61.267	3.1086
Amox4:1	43.333	0.9452
Amox 5:1	37.300	1.1533
Metro1:1	65.533	0.8083
Metro4:1	61.267	1.0066
Cur1:1	40.467	1.1372
Cur3:1	65.333	1.0263
Cur 4:1	88.200	1.0000
Total	57.838	16.1756

Table 3: Anova to determine significance between groups

	Sum of Squares	df	Mean Square	F	Sig.
Spreadability Between Groups	78246.049	7	11178.007	21.874	0.000

Table 6: Anova for significance between groups

	Sum of Squares	df	Mean Square	F	Sig.
Viscosity Between Groups	5984.156	7	854.879	404.677	.000

Table 4: Post hoc Tukey test of significance between specific groups

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Control	amox4:1	-143.097*	18.45765	0.000
	amox 5:1	-119.430*	18.45765	0.000
	metro1:1	1.57	18.45765	1.000
	metro4:1	4.47	18.45765	1.000
	cur1:1	-4.73	18.45765	1.000
	cur3:1	-4.53	18.45765	1.000
	cur 4:1	1.537	18.45765	1.000

Table 7: Post hoc Tukey test of significance between specific groups

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Control	amox4:1	17.9333*	1.1867	0.000
	amox 5:1	23.9667*	1.1867	0.000
	metro1:1	-4.2667*	1.1867	0.039
	metro 4:1	0	1.1867	1.000
	cur1:1	20.8*	1.1867	0.000
	cur3:1	-4.0667	1.1867	0.053
	cur 4:1	-26.9333*	1.1867	0.000

**The mean difference is significant at the 0.05 level*

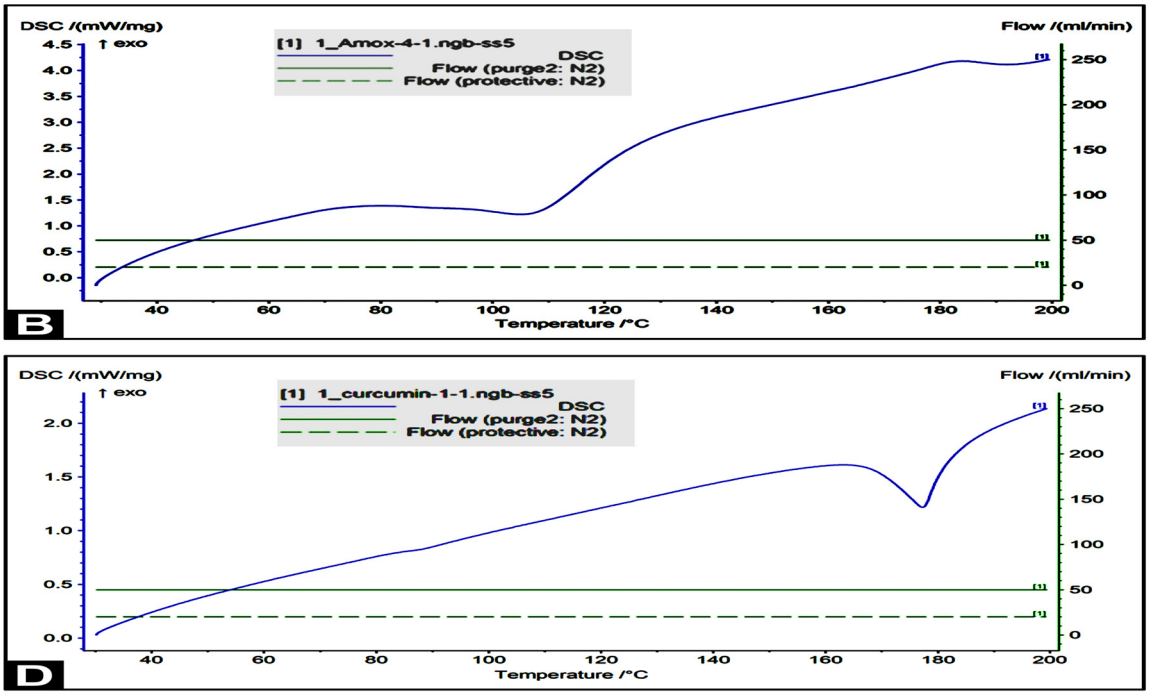

Figure 4: A) Control; B) Co-Amoxiclav 4:1; C)Metronidazole 1:1; D) Curcumin 1:1

implantitis and 46% of patients had peri implant mucositis [18].

The Disc Diffusion Method uses the principle of testing for antibiotic sensitivity against specific bacteria. The diameter of the zone of inhibition is directly proportional to the susceptibility of the microorganism to the tested drug [19]. As per the one-way Anova, there was a significant difference in the ZOI of Co-amoxyclav, metronidazole and curcumin against the growth of *Porphyromonas* and *Fusobacterium* ($P<0.001$) and *Peptostreptococcus* ($P<0.011$). The post hoc Tukey's analysis showed that metronidazole showed the most difference as compared to co-amoxyclav and curcumin against the growth of *Peptostreptococcus*. All three pharmaceutical agents showed good results against *Porphyromonas* and against *Fusobacterium*, Co-amoxyclav and metronidazole showed better ZOI than curcumin. This is in concurrence with a study that showed metronidazole displayed a 97% susceptibility rate when tested by the test method against anaerobe isolates from species like Bacteroides, Prevotella and *Fusobacterium* while Co-amoxyclav showed a 95.5% susceptibility rate just like imipenem [20].

The elute of curcumin obtained from the artificial saliva produced a zone of inhibition that was comparable with the recommended ZOI in literature [21]. While a minimum serum level of 16μg/ml of antibiotic is needed, the same is not known with curcumin. This is in concurrence with a study that showed the zone of inhibition of curcumin against gram positive aerobes, a bulk microbial component of the biofilm, was comparable to that of Ciprofloxacin. Another study showed minimum inhibitory concentrations achieved only in high concentrations of curcumin [22]. The study evaluated gram positive cocci and not anaerobic rods. This could explain why the control disc produced no zone of inhibition against anaerobic bacteria [23]. Further studies will be necessary to identify the strength required in serum levels to obtain bactericidal activities with curcumin or antibiotics and compare them with levels reached in saliva or Gingival Crevicular Fluid on local application.

In this study, the effectiveness of each of the elutes was tested in creating a zone of inhibition against obligate gram negative anaerobes (*Porphyromonas* and *Fusobacterium*) as well as gram positive anaerobes like *Peptostreptococcus*, accounting for the poly microbial oral environment. Therefore, it is imperative that pharmaceutical agents have a bactericidal effect on a range of microbes.

The advantages of the disc diffusion method include simplicity, providing definite results of clinical relevance and flexibility to test a variety of pharmaceutical agents. The disadvantage is that all procedures are manual thereby making error inclusion inadvertent. Results could be either "susceptible", "resistant" and "intermediate" [19].

Post hoc Tukey analyses of spreadability test results showed significant difference in spreadability between control and Co-amoxiclav. Metronidazole 1:1 and the curcumin groups showed similar spreadability properties to that of the control, implying that the consistency of the mixed material did not vary significantly from that of control. Co-amoxiclav exhibited the maximum deviation from the control indicating drastic change in the spreadability of the tissue conditioner. The incorporation of Metronidazole and curcumin did not cause drastic changes in the spreadability of tissue conditioner when compared to Co-amoxiclav. The difference in spreadability upon adding the antibiotics and antimicrobial agents has significant clinical implication, as the spreadability of the material will influence the thickness of the material as well as the ease of handling [15]. A runny material will be hard to handle and a very thick material will not flow adequately as this may cause pressure on a new surgical site.

Viscosity is an important viscoelastic property of tissue conditioners that allows for adequate flow of the material within the setting time. A fluid has an internal friction referred to as shear and is a function of the amount of force required to overcome the internal friction. Shear rate is the speed at which the fluids move. The force per unit area to produce a shearing movement is the shear stress. A material requiring a shear stress of one dyne per square centimeter to produce a shear rate of one reciprocal second has a viscosity of one poise (P), or 100 centipoises (MPa). The rheology of semi-solids is very much influenced by the microstructure changes. In addition, the viscosity of a semisolid formulation as in the present case will influence its application and handling properties while being used intraorally [16]. Tissue conditioner with Co-amoxiclav in either ratio and Curcumin at 1:1 and 4:1 ratios showed significant difference from the control group. Incorporation of metronidazole and curcumin at 3:1 concentration did not significantly alter the viscosity of the tissue conditioner.

Differential Scanning Calorimetry is a highly sensitive technique to study the thermotropic properties of many different biological macromolecules and extracts. DSC is also a very relevant tool for analyzing the thermodynamic properties as a function of time and temperature of various pharmaceutical products, such as, biopolymers, proteins, peptides, and lipid carriers [24]. We obtain qualitative and quantitative details such as the melting and degradation temperatures, glass transition temperature which is a second order transition often seen in polymeric materials, melt and crystallization enthalpy, specific and latent heats, polymorphism, and purity of the materials. In amorphous solids, glass transition temperature is associated with molecular movement and their relaxation time. The resultant thermogram shows four transitions identified from low to high temperature as: glass transition temperature (Tg), crystallization temperature (Tc), melting temperature (Tm), and degradation temperature (Td). The wide range of temperatures allows for easy analyzing of data of the transitions.

The DSC for the control showed a prolonged glass transition temperature like that of polymers. The powder of auto polymerizing acrylic based material consists of polyethyl methacrylate/copolymer, Polymethylmethacrylate/copolymer, benzoyl peroxide, pthalyl butyl glyconate, pigments, fillers. Liquid contains methyl methacrylate, ethylene glycol dimethacrylate, ester plasticizer mixture like dibutyl phthalate, butylpthalyl butylglycolate, benzylbutyl phthalate [16,24,25], dibutylsebacate, ethyl alcohol [25].

The nature of the peak for Co-amoxiclav implied that amoxicillin was in an amorphous form. An apparent increase in enthalpy was noted, which could be due to the glass transition of the tissue conditioner. This confirms that there is no interaction between tissue conditioner and amoxicillin [26,27]. The nature of peaks in test materials showed that the incorporated metronidazole and curcumin retained their crystalline form without interaction with the polymeric tissue conditioner [28,29]. The DSC of the tissue conditioner was similar to that of polymers. The incorporation of metronidazole or curcumin did not show any shift in the peak of the tested materials, indicating there was no interaction between the materials [28,29]. Therefore, they are compatible with each other, and tissue conditioner could be used a carrying medium.

Conclusion

This study proved definitively that polymer-based tissue conditioner can be used as an effective medium to carry drugs with positive elution. The elutes from tissue conditioner incorporated with antimicrobial agents showed a zone of inhibition around the anaerobic and aerobic cultures indicating the effectiveness of release of the drugs. The handling properties like spreadability and viscosity can be kept similar to that of bare tissue conditioner using appropriate concentrations of the antimicrobial agents. No evident interaction was observed between the tissue conditioner and the drugs in differential scanning calorimetry test. The properties of tissue conditioner were not altered by the addition of specific drugs like metronidazole and curcumin. Also, the potency of antibiotics was not affected by the tissue conditioner. The addition of Co-amoxyclav changed the properties of the TC carrier marginally. The drug eluting tissue conditioner can open new treatment modalities in the management of peri-implantitis, with reference to effectiveness of local drug delivery and reduction of oral administration of antibiotics.

Acknowledgements

We acknowledge the Ramaiah University of Applied Sciences, Bangalore, Ramaiah Institute of Technology, Father Mullers Hospital for the test facilities and Sami Labs of the Sami-Sabinsa Group Limited, Bangalore, India for supplying free the curcumin required for this study. Also, we are grateful to Dr. Eva C. Das for the final copy editing of the manuscript.

References

1. Chen T., Yu W.H., Izard J., Baranova O.V., Lakshmanan A., Dewhirst F.E., The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database (Oxford). 2010, baq013 (2010).
2. Van Brakel R., Cune M.S., Van Winkelhoff A.J., De Putter C., Verhoeven J.W., Van der Reijden W., Early bacterial colonization and soft tissue health around zirconia and titanium abutments: An in vivo study in man. Clin Oral Implants Res. 22(6), 571–7 (2011).
3. Aoki M., Takanashi K., Matsukubo T., et al., Transmission of perio-dontopathic bacteria from natural teeth to implants. Clin Implant Dent Relat Res., 14(3), 406–11 (2012).
4. Dar-Odeh N.S., Abu-Hammad O.A., Al-Omiri M.K., Khraisat A.S., Shehabi A.A., Antibiotic prescribing practices by dentists: a review. Ther Clin Risk Manag., 6, 301-6 (2010).
5. Mohsen S., Dickinson J.A., Somayaji R., Update on the adverse effects of antimicrobial therapies in community practice., Can Fam Physician. 66, 651–9 (2020).
6. Venable S.J., Principles of antimicrobial therapy. Drug Ther Nurs. 10(39), 805–14 (2012).
7. Najah A. Mohammed, Neama Y. Habil., Evaluation of antimicrobial activity of curcumin against two oral bacteria, Autom Control Intell Syst. Special Issue: Artificial Nano Sensory System., 3(2), 18-21 (2015).
8. Wen H., Jung H., Li X., Drug Delivery Approaches in Addressing Clinical Pharmacology-Related Issues: Opportunities and Challenges. AAPS J. 7(6), 1327-40 (2015).
9. Rathi S., Verma A., Resilient liners in prosthetic dentistry: An update. Int J Appl Den Sci., 4(3), 34–8 (2018).
10. Urban V.M., De Souza R.F., Galvao Arrais C.A., Borsato K.T., Vaz L.G., Effect of the association of nystatin with a tissue conditioner on its ultimate tensile strength. J Prosthodont. 15(5), 295–9 (2006).
11. Levison M.E., Levison J.H., Pharmacokinetics and pharmacodynamics of antibacterial agents. Infect Dis Clin North Am. 23(4), 791-815 (2009).
12. Adamczak A., O zarowski M., Karpinski T.M., Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals (Basel). 13(7), 153 (2020).
13. Ampicillin P. Statement 1996 CA-SFM Zone sizes and MIC breakpoints for non-fastidious organisms. Clin. Microbiol Infect., 2(1), S46-S49 (1996).
14. Bomdyal R.S., Shah M.U., Doshi Y.S., Shah V.A., Khirade S.P., Antibacterial activity of curcumin (turmeric) against periopathogens - An in vitro evaluation. J Adv Clin Res Insights., 4(6), 175–80 (2017).
15. Kryscio D.R., Sathe P.M., Lionberger R., et al. Spreadability measurements to assess structural equivalence (Q3) of topical formulations - A technical note. AAPS PharmSciTech., 9(1), 84–6 (2008).
16. Lubrizol Test Procedure TP N01004. Determination of Viscosity Using A Brookfield Viscometer for Conditioning Polymers. Lubrizol Adv Mater., 1–5 (2013).

17. De Boever A.L., De Boever J.A., Early colonization of non-submerged dental implants in patients with a history of advanced aggressive periodontitis. Clin Oral Implants Res., 17(1), 8–17 (2016).
18. Lee CT, Huang YW, Zhu L, Weltman R. Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. J Dent. 62, 1-12 (2017).
19. Giuliano C, Patel CR, Kale-pradhan PB. A Guide to Bacterial Culture Identification and Results Interpretation. Pharmacy and Therapeutics. 44(4), 192–200 (2019).
20. Shilnikova II, Dmitrieva N V. Evaluation of antibiotic susceptibility of Bacteroides, Prevotella and *Fusobacterium* species isolated from patients of the N. N. Blokhin Cancer Research Center, Moscow, Russia. Anaerobe. 31, 15–8 (2015).
21. Gul P, Bakht J. Antimicrobial activity of turmeric extract and its potential use in food industry. J Food Sci Technol., 52(4), 2272–9 (2015).
22. Gunes H, Gulen D, Mutlu R, Gumus A, Tas T, Topkaya AE. Antibacterial effects of curcumin: An in vitro minimum inhibitory concentration study. Toxicol Ind Health., 32(2), 246–50 (2016).
23. Jorgensen J.H., Ferraro M.J., Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin Infect Dis. 49(11), 1749–55 (2009).
24. Chiu M., Prenner E., Differential scanning calorimetry: An invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. J Pharm Bioallied Sci., 3(1), 39–59 (2011).
25. Anupama Prasad D., Rajendra Prasad B., Shetty V., Shastry C.S., Krishna Prasad D., Tissue conditioners: A review. Nitte Univ J Heal Sci., 4(2):152-7.
26. Swamivelmanickam M., Valliappan K., Reddy P.G., Madhukar A., Manavalan R. Preformulation studies for amoxicillin trihydrate and dicloxacillin sodium as mouth dissolve tablets. Int. J ChemTech Res., 1(4), 1032-1035 (2009).
27. Concannon J., Lovitt H., Ramage M., Tai L.H., McDonald C., Sunderland V.B., Stability of aqueous solutions of amoxicillin sodium in the frozen and liquid states. Am J Hosp Pharm., 43(12), 3027-30 (1986).
28. de Souza, N.A.B., de Souza, F.S., Basílio, I.D. et al. Thermal stability of metronidazole drug and tablets. J. Thermal Analysis and Calorimetry 72, 535–538.
29. Sayyar Z., Jafarizadeh H., Temperature effects on thermodynamic parameters and solubility of curcumin O/W nanodispersions using different thermodynamic models. Int. J. Food Engg, 15 (1-2), 20180311 (2019).