IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 1 Ser. 7 (January. 2025), PP 52-64
www.iosrjournals.org

"Anti-MicrobialEfficacyOfChlorhexidineHexametaphosphateCoatedElastomeric Modules.''

Dr.T.Kiran Kumar
Dept.of Orthodontics &Dentofacial Orthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur.

Dr.Ashok kumar Talapaneni *Professor*
Dept.of Orthodontics & Dentofacial Orthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur

Dr.RevathiPeddu
Professor&HOD
Dept.of Orthodontics&DentofacialOrthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur

Dr.Aruna Dokku Reader
Dept.of Orthodontics & Dentofacial Orthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur

Dr.Devikanth.Lanka *Professor*
Dept.of Orthodontics & Dentofacial Orthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur

Dr Sanjeev Jakati *Professor*

Dept.of Orthodontics&DentofacialOrthopedics
Sibar Institute of dental sciences, Takkellapadu, Guntur

Dr.Saravanan Pichai *Professor*
Dept.of Orthodontics &Dentofacial Orthopedics
SibarInstituteof dental sciences, Takkellapadu, Guntur

Abstract:
Background*: Orthodontic ligaturesarepotentialvectorthatmaybeusedforlocaldeliveryofantimicrobial agent to prevent WSL in orthodontic patients.Coatingelastomericligatures with antimicrobial CHX-HMP nanoparticles could provide asustaineddoseofanti-microbialdeliveryeliminatingdependenceonpatientcompliance. there is no literature evidence on anti-microbialeffect of CHX-HMP NPs coated ligatures in orthodontic patients.*
Aims & Objectives*: To evaluate and compare antimicrobial efficacy of elastomericmodules functionalized with*

CHXdg and CHX-HMP and elution ofCHX over a function of time inaqueousmedium

***Materials & Methodology**: Aneffectivetotalsampleof30wasobtained.F or each group (n=2) 15 participants were allocated. Oral prophylaxis was done in all patients before placing elastomericmodulesin1st visittobringbaselineplaqueindexof patientsto0.Archwiresweretiedwithdifferentlyfunctionalizedelastomericmodules. A total of 620 silver-colored polyurethane elastomeric ligatures(3M Unitek) were rinsed in DIW and allowed to air dry for 1 hour beforeuse. 310 ligatures were immersed in ethanol for 60 minutes underagitation.Immediatelyafterconditioning,ligatureswereimmersed 5mM CHX-HMP for 10 minutes under agitation. Another setof 310 ligatures were immersed 5mM CHXdg for 10 minutes underagitation. Followed by a final immersion in DIW for 10 seconds toremove any unbound material and air drying for at least 1 hour beforefurtheruse.*

Inthisway2setsof(n=300pergroupover8weeksperiod)elastomericligatureswerefunctionalizedwitheither CHXdg orCHX-HMP aqueous suspensions. Afterfunctionalization,twogroupsofligatures(n=10) wereplaced into individualUV-transparentcuvettessuitable for ultravioletspectrophotometry. AmountofreleasedCHXfromcoatedligatureswasstudiedforaperiodof56daysover12intervals. A singleligature was placed in an individually labeled cuvette and 2.5 ml ofdeionized water was added to submerge the ligature. Cuvettes werekept sealed at ambient room temperature (24°C) and medium wascollected for evaluation of CHX release on 12 intervals. Entirevolume was collected on each time point and then cuvette wasrefilledwith2.5mldeionizedwater.Collectedmediawaskeptinsealedcuvettesandstoredinfreezerat0°Funtilsampl ecollection was completed. Absorption at 260 nm was measured byspectrophotometry from 200 µL of collected samples to determineamountof releasedCHX. Standard solutions of0–50µmCHXwaspreparedasareferenceandtocalibrateCHXconcentrations[19].CumulativeCHXreleaseatconclusi onof8th weekperiodwasdetermined.

Microbial count was assessed at end of 1st week (T0), at end of 4th week (T1) and at end of 8th week (T2). Swab was inoculated into tube containing 2mlofBHIbroth for bacterial isolation and identification; it wasincubated at37°Cfor 2 hrs. After2 hours,10µl of broth wasinoculatedonto Blood agar for Streptococcus mutans isolation. Cultured plates were incubated at 37°C for 24 hours for Streptococcus mutans. Afterbacterialgrowth,colonymorphologiesevaluated,countedandmeasured inCFUpermL.

Results:

Highest CHX release in Chlorhexidine-dg group was observedonday1(63.13±0.81µmol/L)withaconsistentreductioninrelease at subsequent observations before the releasedecayed downtozeroon35thdayforCHX-dggroup. In CHX-HMP group highest release was observed on day 1 (111.74±1.76 µmol/L) witha consistent reduction in release at subsequent observations withleast mean values recorded on 56th day (16.21±0.52 µmol/L).

Conclusion:

CHX-HMPnanoparticlescoatedelastomericligaturesincubatedinwaterreleasedaqueousCHXbeyondaperiodof8weeks.C HXdgcoatedelastomericligaturesstoppedelutionofCHXby35thday ofincubationperiod.CHX-HMPnanoparticlecoatedelastomericligatureswerecapableofinhibitionofStreptococcusmutansgrowth.Significant reductionofPlaquescoreswasobservedwithCHX-HMPcoatedelastomericligatures.

***Keywords:**Chlorhexidine, elastomeric modules, anti-microbial*

Date of Submission: 20-01-2025 Date of Acceptance: 30-01-2025

I. Introduction

Fixed orthodontic appliance is composed of brackets or bandswhicharebondedtotoothsurfacewithcompositeorGlassIonomer Cement(GIC).Arch wiresare tied tobracketswith help of elastomeric ligature ties or stainless-steel ligature ties.Out ofseveraliatrogeniceffectsoforthodontictreatment, One most common effects is WSLs as the fixed appliance compromises oral hygiene andpromotes plaqueaccumulation.[1] Dental plaqueconsistsofavarietyofmicrobesandamongthem,*Streptococcusmutans* is most virulent and associated with white spotlesions, caries and gingival inflammation.[2] WSLsaredefinedas'subsurfaceenamelporosityfromcariousdemineralization.[3]WithWSLsdevelopingwithin4weeks ofappliance placement.[4]Althoughthereisevidenceofremineralisationafterremovalof fixedappliance,butbaselinepretreatmentlevelsarenotregained. Moreover,fixed orthodontic appliances lead to increase in number of pathogenic microbes, increasing the incidence of cariesdevelopment.[5] If WSLs are left untreated, may cause progressioninto dental caries. It was stated thatincidence of new dental carieslesions in patients who are undergoing fixed orthodontic treatment is45.8%.[3] Moreover, periodontal diseasesandgingival

diseasescandevelop if deposited plaque is not removed properly, and thesediseasesleadtolossoftoothinseverecases.[6]

Oral hygiene practice include mechanical and chemicalmethods are most important preventive method incontrollingWSLs.Mechanicalmethodsincludeproperbrushing,flossingetc. chemical methods include mouthwashes and fluoride toothpastes,fluoridegelsetc.Chemicalagentscouldbeusedinadditiontomechanical agents such as brushing and flossing during activephaseoforthodontictreatmenttoreducebacterialplaqueaccumulation,gingivitisandperiodontitis.[7]Themostco mmonmethodof managing WSLs by dental professionals is by fluoride mouthwash, to counsel patient about brushing habits and to maintainproper oral hygiene. This fluoride mouth wash causes additionalproblems as itcauses formation of fluorapatite crystals whichpreventsremineralisationofWSLs.Asanalternativetothese other mouthwashes are available[1] inmarket,chlorhexidinewashighlyeffectiveinreductionofpathogenic microorganisms like Streptococcus mutans thus reducingdentalplaque.[8]

Chlorhexidine (CHX)isa biguanide classofdrugsthat actsas antimicrobialagentagainstgramnegativeandgram-positivebacteria's and yeasts.[9] And isused in medicine and dentistry,asa mouthrinsesas CHX-digluconate(CHXdg).[9,10]CHXbeingcationicinnature,isattractedtonegativelychargedbacterialcellwallandbinds toinner membrane. This increases permeability of cell wall, therebycausing lossofcellcomponents,precipitationofbacterialcytoplasm,andcelldeath.[11]Thisisarapidprocess,occurringwithin20 seconds of exposure, causing most damage.[12] CHX acts bydamagingcellmembraneinvolvingphospholipid-bilayeratbothlow and high concentrations causing congealing of cytoplasm. CHXdoes not encourage development of bacterial resistance as CHX isabroad-spectrumantimicrobialandantifungalagent.[10,12]CommonlyusedformofCHXisChlorhexidine digluconate (CHXdg), which readily dissolves in water,andtherefore,easytoformulateintomouthrinsesandotheraqueous topical agents.[11]Main advantage of CHX is sustainedsubstantivity over longer periods results in prolongedantimicrobialeffectsduetocombinationofCHXwithhydroxyapatite of tooth enamel, oral mucosa, and oral bacteria, whichresults in prolonged release over 12– 24 hrs.[13,14] However, becausesaliva is continuously released into mouth, effects of suchproducts do not last long. If antimicrobial materials were able to remaininside oral cavity for prolonged periods, they wouldavoiddentaldiseasethroughoutorthodontictreatment.[6]StudiesconductedbyWoodetal[15]andBarbouretal[16]todev elopCHXreleasing materials utilizing hexametaphosphate (HMP) nanoparticles(NPs). Sodium HMP is a cyclic inorganic phosphate used infoodindustryanddentalfieldduetoitsabilitytoinhibitformationofdentalcalculusand preventformationofextrinsicstains.[17,18]ResearchindicatedthatChlorhexidinehexametaphosphate(CHX-HMP)nanoparticles(NPs)canbeaffixedto substrate materials to get prolonged release of antimicrobiallyactiveCHX.[16]

Orthodonticpatientsoftenvisitdentalofficetochangeelasticligaturesoffixedorthodontictreatment. Orthodontic ligaturesarepotentialvectorthatmaybeusedforlocaldeliveryofantimicrobial agent to prevent WSL in orthodontic patients. Ligaturesare close to enamel and are regularly changed during orthodontictreatment.Coatingelastomericligatures with antimicrobial CHX-HMP nanoparticles could provide asustaineddoseofanti-microbialdeliveryeliminatingdependenceonpatientcompliance.

Wood NJ et al[15] reported that titanium, glass, elastomericwound dressing and ethylene-vinyl acetate (EVA) polymer specimenswere coated with CHX-HMP nanoparticles that provided continuous release of soluble-CHX over 50 days without reaching aplateau.[15] Subramani et al reported that coating of Orthodonticelastomericchains(OEC)withantimicrobialCHX-HMPnanoparticles,serveasmeanstoreduceWSLsbyinhibitingmicrobescausingformationofWSL.[19]Yasmin etal[11]reportedasustainedCHXreleaseof200µMover8weekperiodfromCHX-HMP treatedelastomerswithoutchangeintheirmechanicalproperties andethanolconditioningenhancedCHX-HMPuptakebyelastomers.

However, there is no literature evidence on anti-microbialeffect of CHX-HMP NPs coated ligatures in orthodontic patients. Hencethis study was done to evaluate and compare antimicrobial efficacyofelastomericmodulesconditioned withCHXdgandCHX-HMPinadditiontoinsituevaluationofCHXelutionfromfunctionalizedelastomericligaturesover8-weekduration.

Aim: -

To evaluate and compare antimicrobial efficacy of elastomericmodules functionalized with CHXdg and CHX-HMP and elution ofCHX over a function of time inaqueousmedium.

Objectives: -

1. ToevaluateantimicrobialeffectofCHXdgfunctionalizedelastomeric modules at 1 week after appliance placement (T0), 4thweek(T1)and8thweek(T2)interval.

2. ToevaluateantimicrobialeffectofCHX-HMPfunctionalizedelastomeric modules at 1 week after appliance placement (T0), 4thweek(T1)and8thweek(T2)interval.
3. To compare antimicrobial efficacy between the CHXdg and CHX-HMPconditionedelastomericmodules.
4. ToevaluateelutionofCHXfromCHXdgfunctionalizedelastomericmodulesover56daysat12intervals(1,2,3,5,7,14, 21,28,35,42,49,56).
5. ToevaluateelutionofCHXfromCHX-HMPfunctionalizedelastomericmodulesover56daysat12intervals(1,2,3,5,7,14,21,28,35,42,49,56).
6. To compare elution of CHX over a function of time between theCHXdgandCHX-HMPconditionedelastomericmodules.

ThispresentclinicaltrialwasconductedinDepartmentofOrthodonticsandDentofacialOrthopedics,SIBARInstituteof DentalSciences, Guntur, & wasapprovedbyInstitutionalEthicsCommittee(RefNo:1/IEC-SIBAR/CIR/21).

***Inclusioncriteria*:**
1. Patientwithinagegroupof18–25years.
2. Patientswithpermanentdentition.
3. Orthodontic casestreated bynon-extractionmethod.

***Exclusioncriteria*:**
1. Subjectswhohaveusedantibiotics3monthspriortostudy.
2. Patientwithhistoryofsmokingandanyperiodontal dis
3. Patientwith anysystemic disorders.
4. Prioruseofanymouthwashfor10consecutivedaysinlast3months.
5. Patientwithcariouslesion,restoration,visiblecracksandenamelhypoplasia.
6. Pregnantwomen.

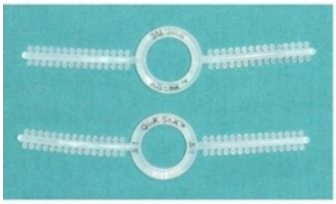
Samplesize:

Determined by G*Powerversion3.1.9.2softwarewithfollowingparameters:
Effectivesize-0.56
Confident Interval- 95%
Power-80%

Aneffectivetotalsampleof30wasobtained.Basedon1:1 dallocation, for each group (n=2) 15 participants were allocated. 30subjectsofbothgendersreceivingorthodontic treatment with pre-adjusted edgewise appliances and havingequal base line plaque index score were randomly included in study.Informed consent was procured from all patients who participated instudy.

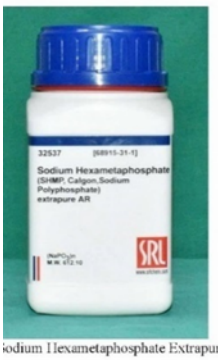
Patientswereassignedinto2groups: -
1. GroupI:BracketswillbeligatedwithelastomericmodulesfunctionalizedwithCHXdg.
2. GroupII:BracketswillbeligatedwithelastomericmodulesfunctionalizedwithCHX-HMP.

Materials:
1. 3M™AlastiK™QuiKStiK™-Elastomericmodules
2. Chlorhexidinegluconate-Central DrugHouse(P) Ltd.
3. SodiumHexametaphosphateExtrapurAR-SicsoResearchLimitedPvt.Ltd.
4. Ethanol- ChangsuHongshengFineChemicalsCo.Ltd.
5. Deionizedwater(DIW)
6. BrainHeartInfusion(BHI) broth medium



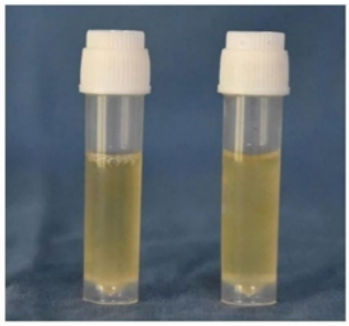
M™ AlastiK™ QuiK StiK™- Elastomeric modules.

Chlorhexidine gluconate - Central Drug House (P) Ltd.



Sodium Hexametaphosphate Extrapur AR – Sicso ResearchLimited Pvt. Ltd.

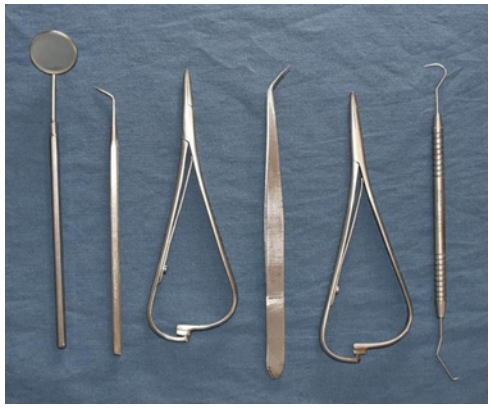
Ethanol - Changsu Hongsheng Fine Chemicals Co. Ltd.



Brain Heart Infusion (BHI) broth medium

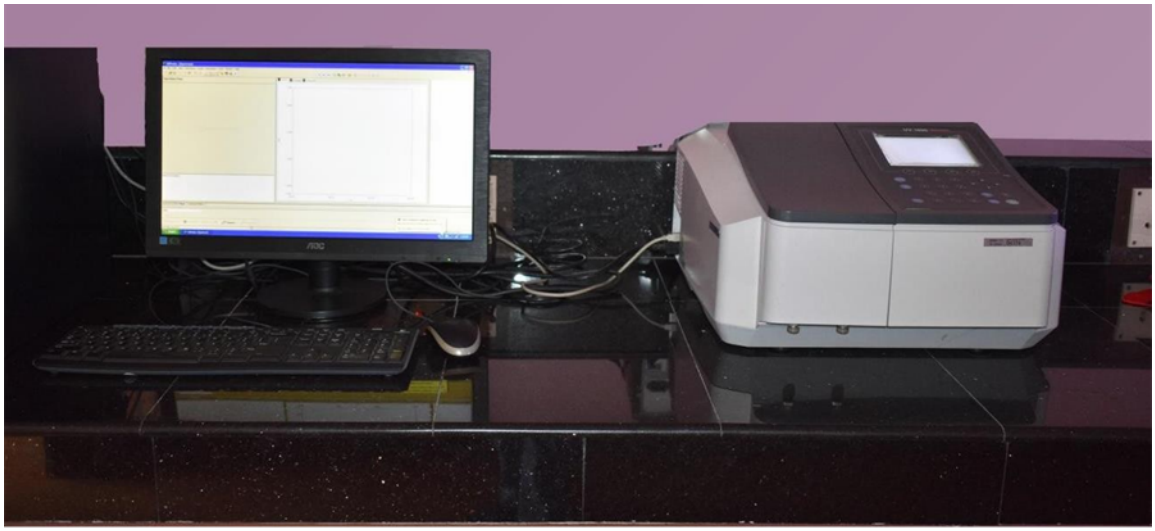
Armamentarium:

Mathewplier
Tweezers
Mouthmirror
Straightprobe



Armamentarium forelastomericmoduleplacement and removalofelastomericmodule.

***Equipmentused*:**
Spectrophotometricmachine- Shimadzu corp.



II. Methodology:

Oral prophylaxis was done in all patients before placing elastomericmodulesin1st visittobringbaselineplaqueindexof patientsto0.Standardoralhygieneinstructionsweregiventoallparticipants.Archwiresweretiedwithdifferentlyfunctionalizedelastomericmodules

***PreparationofCHX-HMPNPscoated elastomericligatures*: Flowchart 1**

100mL of 10mMaqueousNaHMPwasaddedto100mL of10mMaqueousCHXdgunderconstantstirringatroomtemperatureandpressure.Thisresultedinaqueoussuspensionof CHX–HMP,withCHXconcentrationof5mM. Forcomparison,a5mMaqueoussolutionofCHXdgwasalsoprepared.[16]

A total of 620 silver-colored polyurethane elastomeric ligatures(3M Unitek) were rinsed in DIW and allowed to air dry for 1 hour beforeuse. 310 ligatures were immersed in ethanol for 60 minutes underagitation.Immediatelyafterconditioning,ligatureswereimmersed 5mM CHX-HMP for 10 minutes under agitation. Another setof 310 ligatures were immersed 5mM CHXdg for 10 minutes underagitation. Followed by a final immersion in DIW for 10 seconds toremove any unbound material and air drying for at least 1 hour beforefurtheruse.[11]

Inthisway2setsof(n=300pergroupover8weeksperiod)elastomericligatureswerefunctionalizedwitheither CHXdg orCHX-HMP aqueous suspensions. Accordingly Group I sample of patientsreceived ligatures treated with CHXdg and Group II sample receivedligatures treated with CHX-HMP. Two sets of elastomeric ligatures (n=10pergroup)wereutilizedtocheckCHXelutionfromligatures.

***CHXelutionfromfunctionalizedligatures*:**

Afterfunctionalization,twogroupsofligatures(n=10)wereplacedintoindividualUV-transparent cuvettes suitable

forultravioletspectrophotometry.AmountofreleasedCHXfromcoatedligatureswasstudiedforaperiodof56daysover 12intervals (1, 2, 3, 5, 7, 14,21, 28, 35, 42, 49 & 56 days). A singleligature was placed in an individually labeled cuvette and 2.5 ml ofdeionized water was added to submerge the ligature. Cuvettes werekept sealed at ambient room temperature (24°C) and medium wascollected for evaluation of CHX release on 12 intervals. Entirevolume was collected on each time point and then cuvette wasrefilledwith2.5mldeionizedwater.Collectedmediawaskeptinsealedcuvettesandstoredinfreezerat0°Funtilsamplecollection was completed. Absorption at 260 nm was measured byspectrophotometry from 200 µL of collected samples to determineamountof releasedCHX. Standard solutions of0–50µmCHXwaspreparedasareferenceandtocalibrateCHXconcentrations[19].CumulativeCHXreleaseatconclusionof8th weekperiodwasdetermined.

EstimationofMicrobialcount: -

Microbial count was assessed at end of 1st week (T0), at end of 4th week (T1) and at end of 8th week (T2). For processof sample collection for microbial analysis, quadrants were isolated withcotton rolls to avoid saliva contamination before collecting sample.Plaquesampleswerecollectedaseptic allywithsterilecottonswabmoistened with sterile saline from around orthodontic attachments ofmaxillary pre-molars and lower incisors. Only one cotton swabwas used for both regions and only one sample was collected from eachpatient. Collectedswabwastransferredaseptically andimmediately into sterile tube containing 2 ml of BHI broth mediumanditwasdeliveredtomicrobiology lab.

Swab was then inoculated into tube containing 2mlofBHIbroth for bacterial isolation and identification; it wasincubated at37°Cfor 2 hrs. After2 hours,10µl of broth wasinoculatedonto freshBlood agar for Streptococcus mutans isolation. Cultured plates werethen incubated at 37°C for 24 hours for Streptococcus mutans. Afterbacterialgrowth,colonymorphologiesevaluated,countedandmeasured incolonyformingunitspermL(cfu/ml).[20]

RecordingofPlaqueindex: -

Plaque index scores will be recorded in each individual atend of 1st week (T0), at end of 4th week (T1) and at end of 8thweek(T2).

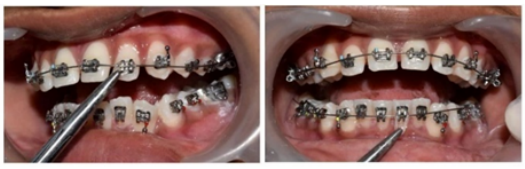
Criteriaforplaqueindexsystem:[21]

0=No plaque ingingivalarea.

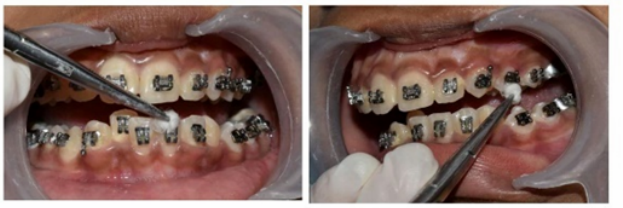
1 = A film of plaque adhering to free gingival margin and adjacentareaoftooth. P laquemayonlyberecognizedbyrunningaprobeacrosstooth surface.

2 = Moderate accumulation of soft deposits within gingival pocket,ongingivalmarginand/oradjacenttoothsurface,whichcanbeseenbynakedeye.

3 = Abundance of soft matter within gingival pocket and/or on gingivalmarginandadjacent toothsurface



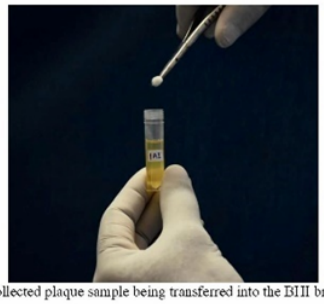
Functionalized module being placed in patient.



Plaque samples being collected from the incisor andpremolar area for microbiological colony count.

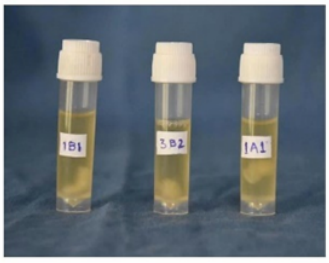
Prepared CHX-HMP solution concentration of 5mM

Prepared CHXdg solution with a CHX concentration of 5mM



Collected plaque sample being transferred into the BHI broth.

: Sample being placed in the spectrophotometric machine tocheck the release of CHX from the solution.



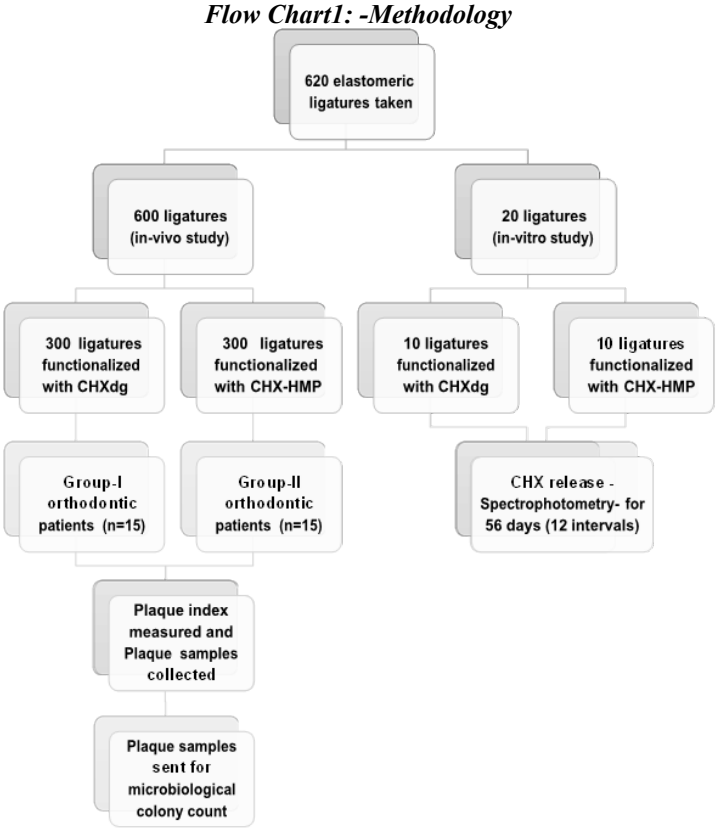
BHI broth with plaque samples sent to microbiologicallaboratory for microbiological colony count.

***InterpretationofPlaquescore*: -**

Rating	Score
Excellent	0
Good	0.1-0.9

Fair	1.0-1.9
Poor	2.0-3.0

Flow Chart1: -Methodology



***Statisticalanalysis*:**

Data were analyzed using IBM SPSS version-20 software(IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics,repeatedmeasuresanalysisofvariance(ANOVA),andindependentsamples t-testsweredonetoevaluatestudydata.

Participantflow

Thirty patients were invited to participate in this clinical trial(15 participants in CHXdg group, 15 participants in CHX-HMPgroup).

***Flow Chart 2: CONSORT flowchart of participants throughеachstageoftrial*.**

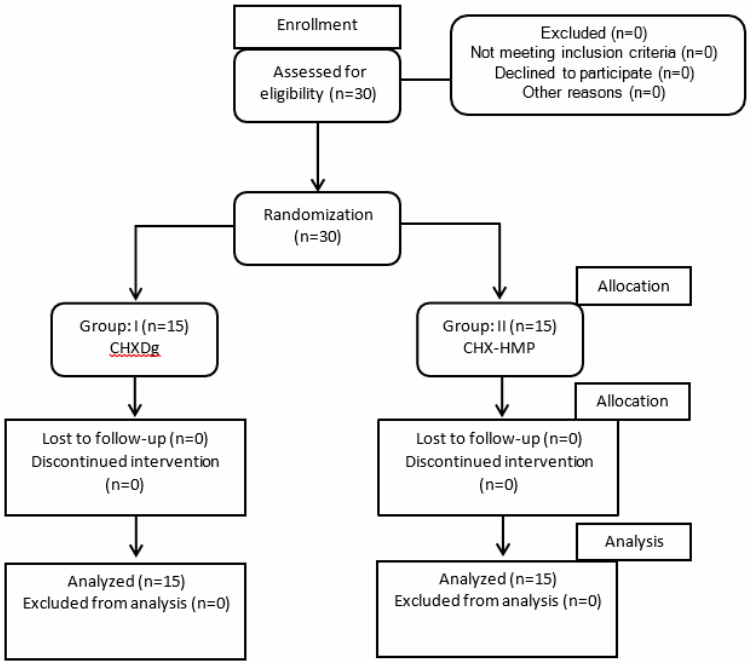


Table 1: Inter-groupcomparisonofchlorhexidinerelease (µmoles/L)atdifferent timepoints.

Day	Group		tvalue	Pvalue
	CHX-dg	CHX-HMP		
Day1	63.13±0.81	111.74±1.76	78.92	<0.001*
Day2	42.34±0.54	68.76±0.45	116.9	<0.001*
Day3	27.36±1.01	60.87±0.575	90.77	<0.001*
Day5	22.92±0.611	49.75±0.64	95.251	<0.001*
Day7	18.67±0.603	45.29±0.45	111.206	<0.001*
Day 14	14.67±0.816	41.51±0.53	86.973	<0.001*
Day 21	7.73±0.46	37.07±0.51	133.878	<0.001*
Day 28	0.757±0.286	31.15±0.26	244.48	<0.001*
Day 35	0±0	26.49±0.46	179.88	<0.001*
Day 42	0±0	21.78±0.44	154.298	<0.001*
Day 56	0±0	16.21±0.52	97.118	<0.001*

Independent samples t test; p≤0.05 - statistically significant; * denotesstatisticalsignificance

Graph1: Intra Group Comparisonof chlorhexidinerelease(µmoles/L) betweenthestudy groups.

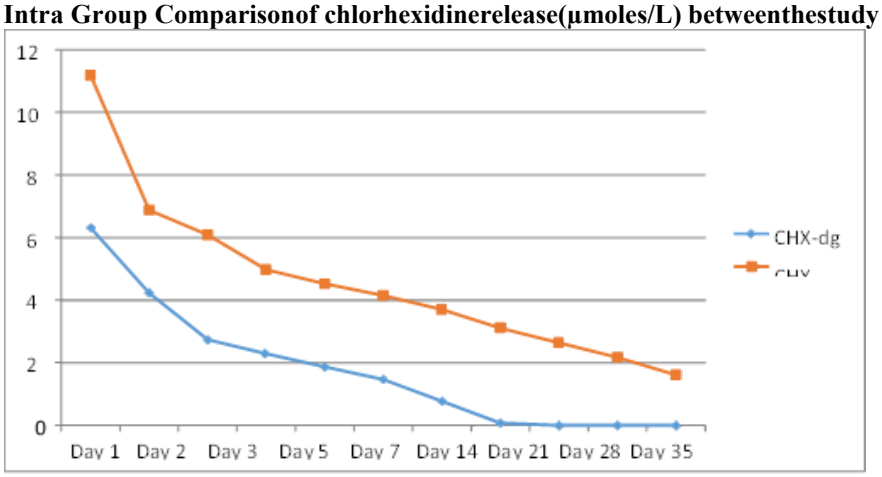


Table 2: Inter-group comparison of cumulative chlorhexidine release(µmoles/L).

Group	Mean	SD	SE	tvalue	Pvalue
CHX-dg	197.61	1.88	0.595	-279.85	<0.001*
CHX-HMP	510.66	2.99	0.947		

p≤0.05 considered statistically significant; * denotes statisticalsignificance

Graph 2:Comparisonof colonyformingunits(*10^5cfu/ml)betweenthestudy groups.

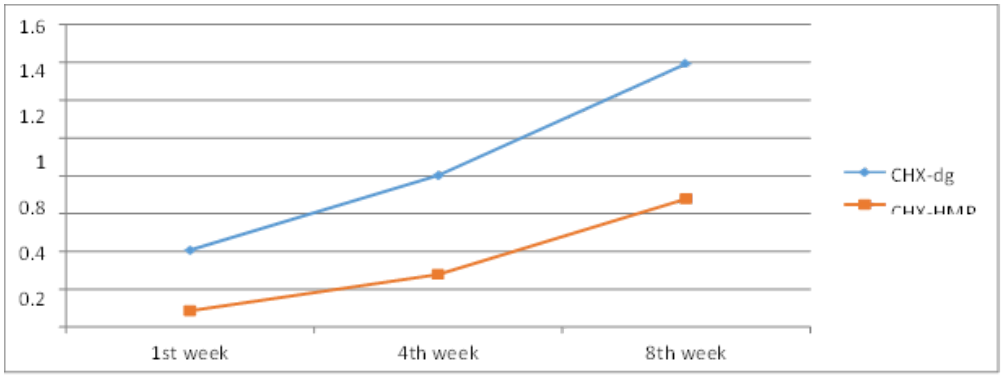


Table3: Inter-groupcomparisonof% decreaseinCHXrelease withreferenceto Day1atdifferenttimepoints.

Parameter	Group	N	Mean	Std.Deviation	Std.Errormean	Tvalue	Pvalue
%decreaseDay 2	CHX-dg	10	32.923111	1.0886500	.3442614	-11.68	<0.001*
	CHX-HMP	10	38.445165	1.0247380	.3240506		
%decreaseDay 3	CHX-dg	10	56.645668	1.7844717	.5642995	18.84	<0.001*
	CHX-HMP	10	45.516647	.5524280	.1746931		
%decreaseDay 5	CHX-dg	10	63.683537	1.0200849	.3225792	17.85	<0.001*
	CHX-HMP	10	55.457754	1.0400273	.3288855		
%decreaseDay 7	CHX-dg	10	70.408359	1.1165360	.3530797	25.58	<0.001*
	CHX-HMP	10	59.454148	.7657460	.2421501		
%decreaseDay 14	CHX-dg	10	76.748270	1.4876539	.4704375	27.49	<0.001*
	CHX-HMP	10	62.843284	.5875396	.1857963		
%decreaseDay 21	CHX-dg	10	87.752941	.7933392	.2508759	65.2	<0.001*
	CHX-HMP	10	66.818746	.6335895	.2003586		
%decreaseDay 28	CHX-dg	10	98.798463	.4546282	.1437661	147.18	<0.001*
	CHX-HMP	10	72.111693	.3493732	.1104815		
%decreaseDay 35	CHX-dg	10	100.000000	0	0	164.45	<0.001*
	CHX-HMP	10	76.289215	.4559445	.1441823		
%decreaseDay 42	CHX-dg	10	100.000000	0	0	104.72	<0.001*
	CHX-HMP	10	80.500173	.5888111	.1861984		
%decreaseDay 56	CHX-dg	10	100.000000	0	0	87.77	<0.001*
	CHX-HMP	10	85.485512	.5229380	.1653675		

Independentsamplesttest;p≤0.05consideredstatisticallysignificant;* denotesstatisticalsignificance

Table 4:Inter-groupcomparisonof colonyformingunits(*10^5cfu/ml)betweenthe studygroups.

Time	Group	n	Mean	SD	tvalue	Pvalue
1st week	CHX-dg	15	0.4073	0.08	13.806	<0.001*
	CHX-HMP	15	0.086	0.008		
4th week	CHX-dg	15	0.802	0.112	14.357	<0.001*
	CHX-HMP	15	0.278	0.084		
8th week	CHX-dg	15	1.392	0.324	8.106	<0.001*
	CHX-HMP	15	0.678	0.104		

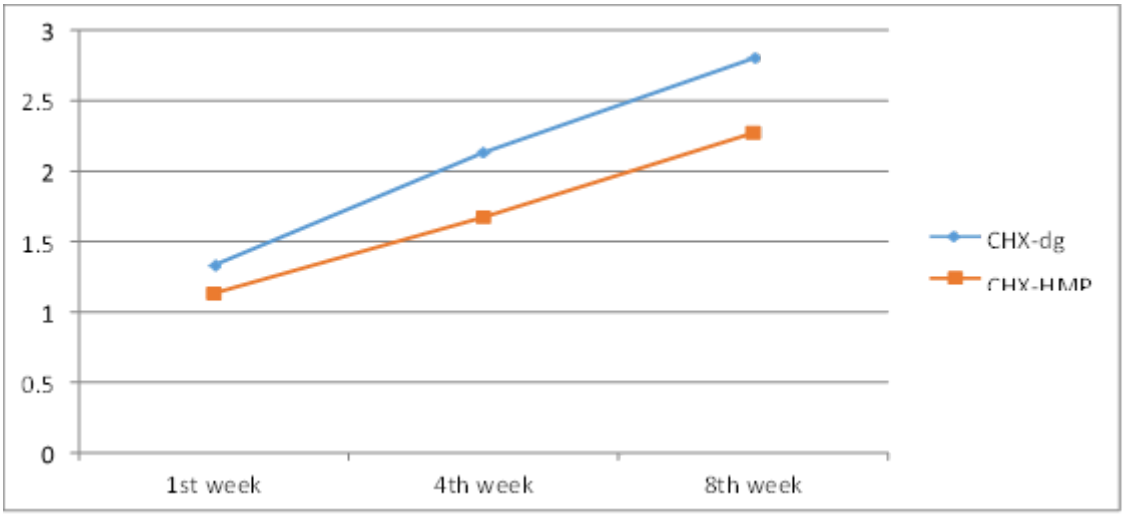
Independentsamplesttest;p≤0.05consideredstatisticallysignificant;
* denotesstatisticalsignificance

Table5:Inter-groupcomparisonofplaqueindex scoresbetweenthestudy groups.

Time	Group	n	Mean	SD	tvalue	Pvalue
1st week	CHX-dg	15	1.33	0.488	1.288	0.208
	CHX-HMP	15	1.13	0.352		
4th week	CHX-dg	15	2.13	0.516	2.54	0.017*
	CHX-HMP	15	1.67	0.488		
8th week	CHX-dg	15	2.8	0.414	3.347	0.002*
	CHX-HMP	15	2.27	0.458		

Independentsamplesttest;p≤0.05consideredstatisticallysignificant;
* denotesstatisticalsignificance

Graph3:Comparisonsfor plaqueindexscoresbetweenthestudygroups.



III. Results:

Highest CHX release in Chlorhexidine-dg group was observedonday1(63.13±0.81µmol/L)withaconsistentreductioninrelease at subsequent observations before the releasedecayed downtozeroon35thdayforCHX-dggroup.In CHX-HMP group highest release was observed on day 1 (111.74±1.76 µmol/L) witha consistent reduction in release at subsequent observations withleast mean values recorded on 56th day (16.21±0.52 µmol/L). Intra-group differences in both groups were statistically significantas analyzed with ANOVA (Tables 1). Table 2 shows inter-group comparison ofchlorhexidine release (µmoles/L) at different time points. At all-time pi in this study, CHX-HMP demonstrated significantlyhighercumulative releaseofchlorhexidinecomparedtoCHX-dggroup.Except at Day 2 Percentiledecreaserelativetoday1at11differenttimeintervalsbetweenCHX-dgandCHX-HMPgroups,fromDay2toDay56, percentage decrease was significantly higher inCHX-dg group compared to CHX-HMP group. (Table 3)

For CFU In both groups (Table 4, Graph 2), least mean values were documented at 1st week recording with a consistent increase in two subsequentrecordingsat4thand8thweeks,with significantly lesser colonies inthe CHX-HMP groupat each given time point.forPIscoresin both groups, least mean values were documentedat1stweekwithastatistically significant consistentincreaseintwosubsequentrecordingsat4thand8thweeks& significantly lesser mean PI scores in CHX-HMPgroupat 4thand8thweektimepoints.Therewasnosignificantdifferenceinmeanplaqueindexscoresbetweengroupsat1weektimepoint.(Table 5, Graph 3)

IV. Discussion:

CurrentpracticesinWSLmanagementbydentalprofessionalswereinvestigatedinearlierstudies.[22,23]Commonlyrecommendedmethodwas administration of fluoride mouth rinse after brushing.[22] Patients wereencouraged use fluoridemouth rinse by 85% of orthodontists,69% general dentists and 76% orthodontists suggested in-office fluoridetreatmentforsevereWSLsimmediatelyafterfixedorthodontic treatment.[23] This treatment cause additional problemssince use of fluoride treatment afterformation of WSL result in formation of fluorapatite crystals which prevents remineralizationofWSLs.Asanalternative,MIpastehasbeenrecommendedfortreatmentofWSL.Recaldent,theactiveingredientof MI Paste, is acomplex of casein phosphopeptides and amorphous calcium phosphate(CPP-ACP)thatincreaseslevelofcalciumphosphateindentalplaquetopromoteremineralizationofenamel.[24]

Butpreventionofmicrobialbuildupispreferredalternativeto treatmentofWSLs.Chlorhexidinewaswidelypreferredbydentistsand Orthodontistsforpreventionof WSLs in patients undergoing orthodontics treatment. CHX diacetatedemonstratedCHXreleaseover8dayswhen incorporated into some kind of material.[25] However, side effects such as unpleasanttaste, undesirable tooth discoloration,burning sensation and dryness inmouthdemotivatepatientstousethismouthwash.[26,27]

Treat posed by microbiologicalevolution of antibiotic resistance is of grave concern to internationalcommunity.Developmentofantimicrobialmethodsthatdonotsupportemergence of such resistance is strongly urged. It has been found that while specific populations of microbes canbecome less sensitive to CHX when exposed to rising environmentalconcentrations, these changes can be undone when CHX stimulus isremoved, indicating thatchanges are reversible and do not actuallyrepresent trueresistance.[40] Becauseof this,

CHXemployedasCHXdigluconatesalt,easilysolublein aqueoussolutions, has been regardedas promising candidate for creation of antimicrobial substances anddevices that do not increase need for antibiotics.[10]BysoakinginCHXdigluconatesolutions,biologicalmaterialshavebeentransformedintoantibacterialsubstances..[41,42]Itis also used in form of CHX-diacetate, and hasbeen introduced as dry crystalline powder to variety of materialswithaimofconferringthemantimicrobialcharacteristics.[26,27]

NaHMP is cyclic inorganic phosphate used infoodindustry and dental field due to its ability to inhibit formation of dentalcalculus and prevent formation of extrinsic stains.[17,18] A novel salt ofCHX,CHX-hexametaphosphate(CHX-HMP),hasbeenreportedasmaterial that provides sustained release of constituent CHX whenexposedtoaqueousenvironment.[16]and does not reach equilibrium in sealed system after 8weeks. An initial period of rapid CHX release was followed by slowerand gradual CHX release.[11] Attributing to physical andchemical properties of this salt, it can be employed as a component ofcompositematerials.Providedthecompositehasadegreeofwaterpermeability, substrate composite can provide sustained release of CHXunderaqueousconditions.Doseanddurationofreleaseareinfluencedbyvarietyoffactorssuchasdoping,localphysicochemicalconditions (such as flow, temperature, ionic strength, and other ions), andhost substrate.[11]Studies showed specimensofglass,titanium,elastomericwounddressingandethylenevinylacetate (EVA) polymerweresuccessfully coated with CHX-HMP NPsthat provided continuous elution of soluble CHX over a period of 50 dayswithout reaching a plateau.[15,16]Antimicrobial property of releasedsolubleCHXwasshownbygrowthinhibitionofmethicillin-resistantStaphylococcusaureus(MRSA),PseudomonasaeruginosaandStreptococcus gordonii.[16,28] These studies established that CHX-HMPnanoparticlecanbeaffixedtomaterialswithreleaseofantimicrobially active CHX.As CHX and Sodium HMP has been usedwidelyindentistryasantibacterial mouthwashandanticalculusagent andhas been effective against oral microbes causing WSLs, coating oforthodontic materials such as orthodontic elastomeric chains (OEC) withantimicrobial CHX-HMP nanoparticles provide means to reduceWSLs by inhibiting microbes[19]

Polyurethaneisapolymerusedformedicaldeviceproduction due to its exceptional physical and mechanical properties andgoodbiocompatibility.[29]Polyurethaneelastomericchainsarepreferredoptiontoclosespacesandcorrectrotationsinorthodonticmechanotherapy.[30,31]Orthodontictreatmentincreasesriskofcariesduetoaccumulationofplaqueaswellasbacterialfloramodification.[32]CHX-diacetate hasbeen associated for application on dental implant to minimizeriskofinfectioninearlierdaysfollowingintervention.[33,34]Antibacterial polyurethane nanocomposites using CHXdg have also beendeveloped.[35] Huynh TTN et al in their in-vitro research reportedpreparation,mechanical,andphysicochemicalcharacterizationofCHX-diacetateloadedpolyurethane biomaterialforlocaldeliveryofchlorhexidine.[36]Catalbas B et al[37] and Dalli M. et al[38] showedthat treating orthodonticelastomericchainswithCHXgeldidnotaffect mechanical properties of orthodontic elastomeric chainseither in-vitro nor in-vivo. Although gel formulation providedmomentary antibacterial action. Padois K et al, in their in-vitro study usedadvanceddrug delivery systembased onchlorhexidineloadinginto polyurethane elastomeric orthodontic chains for sustained release ofantimicrobialdrugduringorthodontictreatment.[39]CHX-salt could diffuse through polyurethane wall and elastomericorthodonticchainsshowedasustainedCHXreleaseoverlongerperiod suggesting it as good treatment modality. Subramani K.et al[19] concluded CHX-HMP nanoparticle coatedorthodonticelastomericchainsreleasedchlorhexidineovertimeperiodofatleast 28daysand thiselutioniscapable of antibacterialeffectthuspromisingclinicalapplicationsinorthodonticmechanotherapy. Kamarudin Y et al[11] concludedCHX-HMPconditionedelastomericligaturesshowedsustainedrelease of chlorhexidine up to 8 weeks proving sustained localizedantimicrobialdeliveryaroundorthodonticattachmentsthusreducingpatientcomplianceincontrollingWSLsandprovidinganeffectiveanticariogeniceffect.

There is no evidence in literature regardingefficacy of CHX-HMP functionalized ligatures; in-vivo conditions asCHXconcentrationinoralenvironmentinfluencedbyeffectofsalivaandfoodbeingconsumedbyorthodonticpatients.Henceourstudyaimedatevaluating antimicrobial efficacy ofCHX-HMP functionalized elastomeric ligatureswhilesimultaneouslycomparingitwithconcentrationsofCHX-elutionin;in-vitroconditionsoveradurationof8weeks.

IncurrentstudygreatestamountofCHXelutionoccurredon day1inbothtestgroups(CHXdg-63.13µmoles/L);(CHX-HMP-111.74µmoles/L).Bothgroupsshowedaconsistentreductionon subsequent observations with CHX-dg group exhibitingnoelutionfrom35thdayofobservation,whileCHX-HMPfunctionalized elastomeric ligatures showed elution beyond 8-weekperiod. Values ofCHXelutionin this study arein concurrence withvalues reported by Subramani K et al[19] with respect to 5mM CHX-dgand 5mM CHX-HMP functionalized elastomeric modules on day 1 andday 28 of observation period. Outcomes of current research

areinagreementwithoutcomesofSubramanietal[19]andKamarudin Y et al[11] who concluded there wasn't any CHX elution after28th day from 5mM CHXdgfunctionalized elastomeric ligatures andelution continued beyond 28th day and 8-week duration in 5mM CHX-HMP functionalized elastomeric ligatures. 5mM CHX-HMP demonstrated higherreleaseofCHXcomparedto5mMCHXdgfunctionalizedorthodonticligaturesalthoughtherewasdecreasingintensityofelutionasafunctionoftimeinbothgroups.Percentagedecreaseasafunctionoftimewashigherin CHX-dg group compared to CHX-HMP group reflecting sustainedreleaseofCHXovertimeinCHX-HMPfunctionalizedelastomericligatures. When coated with CHX-HMP-5, more CHX was bound toelastomericligaturesusingHMP-nanoparticlesandHMPnanoparticlespromoted slowandsteadyreleaseofCHXwhichextendstreatmentperiodwithantimicrobial.ThisshowsthatHMP-nanoparticle is effective as carrier for CHX coatings and for slow,long term continual release.[19]In current study functionalization oforthodonticligatureswith5mMCHX-HMPwasenhancedbysolventconditioningusingethanol.Literatureshownethanolasbestorganic solvent in impregnating biomedical polymers with antimicrobialnanoparticlescomparedtoacetoneconditioning.Ethanolsoftenssurface layer of ligature without effecting bulk thus enhancing uptake of CHX-HMP at surface and in near subsurface region.Ethanol conditioning showed a minimum degree of chemical degradationin terms of discoloration without effecting physical and mechanicalpropertiesoforthodonticligatures, whereas conditioningwithacetonecaused decrease inlumen size and swelling of elastomericligature and significant effect on force and extension of ligature atrupture.[11]

Microbialcolonycountsincurrentstudywereestimatedfollowing 1st week of start of orthodontic treatment after an initial oralprophylaxis in an attempt to bring baseline plaque scores to zero inbothtreatmentgroups.O utcomesofpresentclinicaltrialshowed S. mutans colonies increased over a function of time in both groups. Minimum values were documented at 1st week and therewas consistent increase at 4th and 8th weeks. Increase in number of CCU was significantlyhigherinCHX-dggroupwhencomparedtoCHX-HMPgroupat all time points. Results reflecteffect of HMP nanoparticles as effective carrier for chlorhexidinefrom elastomeric ligatures to local orthodontic environment. Microbialevaluationfromconfirmssufficientandextended CHX release to be effective against S. mutans, primary microbes thatcauseWSLsanddentaldecay.Resultsofthisstudyareinagreement with Subramani K et al[19] who concluded from their in-vitrostudy, CHX eluate is capable of inhibiting S. mutans and L. rhamnosus.While present study has utilized CFU, Subramani K et al[19] in theirstudyusedzoneofinhibitioncalculationforevaluationofantibacterialactivityofreleasedCHX.Sustainedantimicrobialefficacycanbeexpectedtobeeffectiveaslongastheirremains CHX-HMP nanoparticles to deliver soluble CHX. Since release mechanismandissolutionthiswillbeinherently affectedbymaximumnanoparticle coverage that can be applied to substrate used in oral environment be it either implants or orthodontic auxiliaries.It hasbeen proven in previousstudies primary riskperiod for colonizationof implant surface with microbes is soon afterplacement of implantandinsuchsituationsCHXofferseffectivetreatment ofperi-implantmucositissinceCHXreleaseforweeksofmonthsafterimplantsurgeryisofhighutility.

Thispropertyofpolymericbiomaterialtoexhibitsustainedrelease ofCHXoverperiodofprolongedduration hascreatedwidespread use in diverse applications such as intra-vitreal devices fortreatment of eye inflammation and disease as antibiotic coating forurethral catheters and in implants of various kinds for prevention andcontroloflocalinfection.[43,44]AbovemodalityofCHXnanoparticleimpregnation onpolymericorthodonticauxiliaries can beemployed to prevent iatrogenic effects of orthodontic treatment such asWSLsanddentalcaries.

***LimitationsOfTheStudy*:**

ShortcomingsofstudyincludefailuretoincludeSEMevaluation of elastomeric ligatures to study surface changes incoatedligatures.SEManalysisshowscoatingcharacteristicsofnanoparticle deposit either inhomogeneous or homogeneous. Patternof coating depends on conditioning of polymer wherein acetoneconditioningledtoinhomogeneity,whileethanolconditioningledtohomogeneity. Moreover, thisstudydidnotincludeadverseeffectsofconditioningpolymerswithorganicsolventsandfunctionalizationwithCHX-HMPnanoparticlesonchemicaldegradation,physicalpropertiesandmechanicalpropertiesof orthodonticelastomers.Primary strengthofelastomericligaturesistensilestrengthwhichretainsfullengagementofarchwirewithinbracketslot. Followingaresalientconclusionsfrompresentstudy.

V. Conclusion:

CHX-HMPnanoparticlescoatedelastomericligaturesincubatedinwaterreleasedaqueousCHXbeyondaperiodof8weeks.

CHXdgcoatedelastomericligaturesstoppedelutionofCHXby35thday ofincubationperiod.
CHX-HMPnanoparticlecoatedelastomericligatureswerecapableofinhibitionofStreptococcusmutansgrowth.
SignificantreductionofPlaquescoreswasobservedwithCHX-HMPcoatedelastomericligatures.

References

[1] SukontapatiparkWEtAl2001BacterialColonization AssociatedWithFixedOrthodonticAppliances.AScanningElectronMicroscopyStudy;EurJOrthod.23:475–84.
[2] BerkowitzRJ.MutansStreptococci:AcquisitionAndTransmission.PediatricDent.2006; 28:106–9.
[3] Sunderaraj D Et Al 2015 Critical Evaluation Of Incidence AndPrevalenceOfWhiteSpotLesionsDuringFixedOrthodonticApplianceTreatment:AMeta-Analysis;J.Int.Soc.Prev.CommunityDent.5:433–9.
[4] OgaardB,RollaG,ArendsJ.OrthodonticAppliancesAndEnamel Demineralization. Part 1. Lesion Development. Am JOrthod;1988; 94:68-73.
[5] Ireland AJ, Soro V, Sprague SV, Harradine NWT, Day C, Al-Anezi S, Et Al. The Effects Of Different Orthodontic AppliancesUponMicrobialCommunities.OrthodCraniofacRes2014; 17:115-23.
[6] JeonHS,ChoiCH,KangSM,KwonHK,KimBI.Chlorhexidine-ReleasingOrthodonticElastomeric.DentMaterJ2015; 28:2014-16.
[7] Lundstrom F, Lundstrom F-E, Hamp S, Nyman S. SystematicPlaqueControlInChildrenUndergoingLong-TermOrthodonticTreatment.EurJOrthod.1980; 2:27–39.
[8] Salehi P, Sh MD. Comparison Of The Antibacterial Effects OfPersicaMouthwashWithChlorhexidineOnStreptococcusMutansInOrthodonticPatients.DARUJPharmSci2006;14(4):178-82.
[9] McdonnellGAndRussellAD1999AntisepticsAndDisinfectants: Activity, Action, And Resistance Clin. Microbial.Rev.12147–79.
[10] Milstone A M, Passaretti C L And Perl T M 2008 Chlorhexidine:ExpandingTheArmamentariumForInfectionControlAndPreventionClin.Infect.Dis.46274–81
[11] KamarudinY,SkeatsMK,IrelandAJ,BarbourME.ChlorhexidineHexametaphosphateAsACoatingForElastomeric Ligatures With Sustained Antimicrobial Properties: ALaboratoryStudy.AmericanJournalOfOrthodonticsAndDentofacialOrthopedics.2020;1;158(5): E73-82.
[12] McdonnellG,RussellAD.AntisepticsAndDisinfectants:Activity,Action,AndResistance.ClinMicrobialRev.1999;12(1):147–179.
[13] Ten Cate JM, Van Loveren C, Buijs MJ, Gerardu VA, Van StrijpAJ.ChlorhexidineEfficacyInPreventingLesionFormationInEnamel And Dentine: An In-Situ Study. Caries Res 2008; 42:460-465.
[14] Davies A. The Mode Of Action Of Chlorhexidine. J PeriodontalResSuppl1973;12:68-75.
[15] Wood NJ, Maddocks SE, Grady HJ, Collins AM, Barbour ME.Functionalization Of Ethylene Vinyl Acetate With AntimicrobialChlorhexidineHexametaphosphateNanoparticles.IntJNanomed;2014; 9:4145.
[16] Barbour ME, Maddocks SE, Wood NJ, Collins AM. Synthesis,Characterization,AndEfficacyOfAntimicrobialChlorhexidineHexametaphosphateNanoparticlesForApplicationsInBiomedical Materials And Consumer Products. Int J Nano Med2013; 8:3507-19
[17] ConceicaoJMEtAl2015FluorideGelSupplementedWithSodium Hexametaphosphate Reduces Enamel Erosive Wear In-Situ J.Dent.431255–60.
[18] Vaara M And Jaakkola J 1989 Sodium HexametaphosphateSensitizes Pseudomonas Aeruginosa, Several Other Species OfPseudomonas,AndEscherichiaColiToHydrophobicDrugsAntimicrob.AgentsChemother.331741–7
[19] Subramani K, Seo HN, Dougherty J, Chaudhry K, Bollu P,Rosenthal KS, Zhang JF. In Vitro Evaluation Of AntimicrobialActivityOfChlorhexidineHexametaphosphateNanoparticle CoatingsOnOrthodonticElastomericChains.MaterialsResearchExpress.2020;1;7(7):075401.
[20] Abirami S, Jain RK, Girija AS. Effect OfTwo Different MouthRinsesOnS.MutansCountsInSubjectsUndergoingOrthodonticTreatment–APilotStudy,JournalOfPharmaceuticalResearch International. 2020;32(15):148-155.
[21] Loe H. The Gingival Index, The Plaque Index And The RetentionIndexSystems.JPerio;1967;38(6):610-6.
[22] Hamdan A M Et Al 2012 Preventing And Treating White-SpotLesionsAssociatedWithOrthodonticTreatment:ASurveyOfGeneral Dentists And Orthodontists. J. Am. Dent. Assoc 143777–83.
[23] Kerbusch A E Et Al 2012 Methods Used For Prevention Of WhiteSpotLesionDevelopmentDuringOrthodonticTreatmentWithFixedAppliancesActaOdontol.Scand.70564–8.
[24] Reynolds E C 1998 Anticariogenic Complexes Of AmorphousCalcium Phosphate Stabilized By Casein Phosphopeptides: AReview Spec.CareDentist18,8–16.
[25] Agarwal A, Nelson TB, Kierski PR, Et Al. Polymeric MultilayersThat Localize The Release Of Chlorhexidine From Biologic WoundDressings.Biomaterials.2012;33(28):6783–6792.
[26] BarrajoJ.L.,VarelaL.G.EfficacyOfChlorhexidineMouthrinsesWithAndWithoutAlcohol:AClinicalStudy.JPeriodont;2002;73:317-21.
[27] Bishara S.E., Damon P.L., Olsen M.E., Jakobsen J.R. Effect OfApplying Chlorhexidine Antibacterial Agent On The Shear BondStrengths Of Orthodontic Brackets. Angle Orthod1991; 66: 313-316.
[28] WoodNJEtAl2015ChlorhexidineHexametaphosphateNanoparticlesAsANovelAntimicrobialCoatingForDentalImplants.Mater.Sci.,Mater.Med.26201
[29] Chauvel-LebertDJ,AuroyP,Bonnaure-MalletM.BiocompatibilityOfElastomers.In:DumitriuS,Editor.PolymericBiomaterials.New York:MarcelDekker;2002.P.311–60.
[30] EliadesT.OrthodonticMaterialsResearchAndApplications:Part 2. Current Status And Projected Future Developments InMaterialsAndBiocompatibility.AmJOrthodDentofacialOrthop.2007;131(2):253–62.
[31] RenickMR,BrantleyWA,BeckFM,VigKWL,WebbCS.StudiesOfOrthodonticElastomericModules.Part1:GlassTransitionTemperaturesForRepresentativePigmentedProducts The As-Received Condition And After Orthodontic Use. Am JOrthodDentofacOrthop.2004;126(3):337–43.
[32] OpsahlVitalS,Haignere-RubinsteinC,LasfarguesJ-J,Chaussain C. Caries Risk And Orthodontic Treatment. Int Orthod.2010;8(1):28–45.
[33] Verraedt E, Pendela M, Adams E, Hoogmartens J, Martens

JA.ControlledReleaseOfChlorhexidineFromAmorphousMicroporousSilica.JControlRelease.2010;142(1):47–52.
[34] Verraedt E, Braem A, Chaudhari A, Thevissen K, Adams E,VanMellaertL,EtAl.ControlledReleaseOfChlorhexidineAntiseptic From Microporous Amorphous Silica Applied In OpenPorosityOfAnImplantSurface.IntJPharm.2011;419(1–2):28–32.
[35] FongN,SimmonsA,Poole-WarrenLA.AntibacterialPolyurethaneNanocompositesUsingChlorhexidineDiacetateAsAnOrganicModifier.ActaBiomater.2010;6(7):2554–61.
[36] Huynh TTN, Padois K, Sonvico F, Rossi A, Zani F, Pirot F, EtAl.CharacterizationOfAPolyurethane-BasedControlledRelease System ForLocalDelivery Of ChlorhexidineDiacetate.EurJPharmBiopharma.2010;74(2):255–64.
[37] CatalbasB,ErcanE,DalliM, GelgorIE,ErdemirA. DoesChlorhexidine Affect The Shear Bond Strengths Of OrthodonticBrackets?JDentSci.2011;6(2):76–81.
[38] DalliM,ErcanE,ZorbaYO,InceB,SahbazC,BahsiE,EtAl. EffectOf1%ChlorhexidineGelOnTheBondingStrengthToDentin.JDentSci.2010;5(1):8–13.
[39] Padois K, Bertholle V, Pirot F, Hyunh TT, Rossi A, Colombo P,Falson F, Sonvico F. Chlorhexidine Salt-Loaded PolyurethaneOrthodontic Chains: In Vitro Release And Antibacterial ActivityStudies.AAPSPharm Scitech.2012;13(4):1446-50.
[40] MeyerB,CooksonB.DoesMicrobialResistanceOrAdaptationToBiocidesCreateAHazardInInfectionPreventionAndControl?JHospInfect.2010;76(3):200–205.
[41] Barbour ME, Gandhi N, El-Turki A, O'Sullivan DJ, Jagger DC.Differential Adhesion OfStreptococcus Gordonii To Anatase AndRutileTitaniumDioxideSurfacesWithAndWithoutFunctionalization With Chlorhexidine. J Biomed Mater Res A.2009;90(4):993–998.
[42] Raad I, Mohamed JA, Reitzel RA, Et Al. Improved Antibiotic-Impregnated Catheters With Extended-Spectrum Activity AgainstResistantBacteria AndFungi.Antimicrobe Agents Chemother.2012;56(2):935–941.
[43] ParkJH,ChoYW,ChoYH,EtAl.Norfloxacin-ReleasingUrethral Catheter For Long-Term Catheterization. J Biomater SciPolymEd.2003;14(9):951–962.
[44] ChoYW,ParkJH,KimSH,EtAl.Gentamicin-ReleasingUrethral Catheter For Short-Term Catheterization. J Biomater SciPolymEd.2003;14(9):963–972.