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KLINIKINIS, MIKROBIOLOGINIS IR HISTOLOGINIS „CLEAN AND SEAL“ METODIKOS VERTINIMAS GYDANT II–III STADIJŲ, A/B LAIPSNIŲ PERIODONTITĄ

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CLINICAL, MICROBIOLOGICAL AND HISTOLOGICAL EVALUATION OF "CLEAN AND SEAL" METHOD IN THE TREATMENT OF STAGES II–III, GRADES A/B PERIODONTITIS

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TURINYS

SANTRUMPOS

A.a – *Aggregatibacter actinomycetemcomitans*
BOP – kraujavimas po zondavimo
CAL – klinikinis audinių prisitvirtinimo lygis
CEJ – cemento–emalio jungtis
CT – jungiamasis audinys
C&S – *Clean and Seal*
FMBOP – visos burnos kraujavimo po zondavimo indeksas
FMPI – visos burnos apnašo indeksas
HyA – hialurono rūgštis
JE – jungties epitelis
NaOCl – natrio hipochloritas
P.g – *Porphyromonas gingivalis*
P.i – *Prevotella intermedia*
PCR – polimerazės grandinės reakcija
PD – periodontologinės kišenės zondavimo gylis
PI – apnašo indeksas
REC – dantenų recesija
T.d – *Treponema denticola*
T.f – *Tanerella forsythia*
xHyA – didelio molekulinio svorio hialurono rūgštis

ĮVADAS

Periodontitas – lėtinė uždegiminė liga, siejama su burnos ertmės mikrobiomo disbalansu, pasireiškianti progresuojančiu dantis supančių audinių (dantenų, periodonto raiščio, alveolinio kaulo) netekimu [1]. Klinikiniai periodontito požymiai apima klinikinės jungties netekimą, radiologiškai stebimą alveolinio kaulo destrukciją, zonduojamas dantenų kišenes ir dantenų kraujavimą. Negydoma ar laiku nediagnozuota periodonto patologija lemia ankstyvą dantų netekimą, nors dauguma ligos atvejų gali būti gydomi, taip užkertant kelią dantų netekimui.

Remiantis epidemiologiniais duomenimis, periodontitas yra šešta pagal dažnumą liga pasaulyje, paveikusi daugiau nei 11,2 proc. populiacijos, t.y., 743 mln. žmonių [2], ir tai yra dažniausia dantų netekimo priežastis suaugusių žmonių populiacijoje [3]. Periodontitas turi neigiamą poveikį juo sergančių pacientų kramtymo funkcijai, gyvenimo kokybei, savivertei, įvairiems socio-ekonominiams faktoriams bei stipriai išaugusiems gydymo kaštams [3]. Populiacijai senstant, žmonių, sergančių periodontitu, skaičius nuolat auga [4].

Periodontitas yra daugiafaktorinė liga. Pagrindinis periodontito rizikos faktorius – bakterinio apnašo dantenų vagelėje kaupimasis, kuris ilgainiui sukelia uždegiminį destrukcinį imuninės sistemos atsaką [5]. Dantų apnašo biofilmas yra bakterinės kilmės sistema, palaikanti periodonto audinių homeostazę ir sveikatą bei užtikrinanti normalią sąveiką tarp mikroorganizmų ir imuninės sistemos gynybinių procesų. [6]. Imuninis organizmo atsakas į bakterinę infekciją priklauso nuo mikroorganizmų virulentiškumo ir pobūdžio. Sergant periodontitu, tam tikros patogeninių mikroorganizmų rūšys yra specifinės šiai ligai [7]. Tačiau vien tik mikroorganizmų ligai pasireikšti nepakanka. Ligą predisponuojantys veiksniai (genetika, rūkymas, nutukimas, sisteminės ligos, stresas, mityba ir kt.) tai pat nulemia gynybinių organizmo mechanizmų sutrikimus [8–12].

Pagrindinis periodontologinio gydymo tikslas – sustabdyti ligos progresavimą, atkurti sveikus, stabilius periodonto audinius ir, jei įmanoma, regeneruoti prarastus. Pagrindinė sėkmingo periodontito gydymo sąlyga – efektyvus podanteninio biofilmo pašalinimas. Efektyvaus periodontologinio gydymo atveju periopatogeninių mikroorganizmų rūšys, sukeliančios ar palaikančios periodonto audinių infekcijas, turėtų būti pašalinamos arba stipriai sumažinamas jų kiekis [13].

„Aukso standartas“ periodontito gydyme – kruopštus virš ir podanteninio apnašo ir konkrementų pašalinimas ultragarsu ir / ar rankiniais instrumentais. Tačiau šis gydymo būdas turi nemažai apribojimų – ligai pažengus, bakteri-

nį apnašą, susikaupusį giliose kišenėse, furkacijose, intrakauliniuose defektuose, anatominėse šaknų įdubose ir vagelėse mechaniškai pašalinti tampa sunku ar net neįmanoma. Todėl gydymo sėkmė labai priklauso nuo gydytojo manualinių įgūdžių ir patirties, o taip pat ir nuo su pacientu susijusių faktorių (rūkymas, sisteminės ligos ir kt.). Tyrimai rodo, kad, net ir po labai kruopštaus podanteninio konkrementų valymo, ant 30 proc. valytų šaknų paviršių lieka nenuvalytų dantų akmenų ir apnašo, o tai sąlygoja greitą mikroorganizmų rekolonizaciją į jau valytas sritis, o to rezultatas – sumažėjęs gydymo efektyvumas ir kliniškai stebimos negyjančios periodontologinės kišenės [14, 15]. Po pagrindinio periodontologinio gydymo išlikusios periodontologinės kišenės, kurių zondavimo gylis yra 4 mm ir kurios po zondavimo kraujuoja, blogina ilgalaikę danties prognozę, lemia tolimesnį ligos progresavimą bei ankstyvą danties netekimą [14]. Ilgalaikių klinikinių tyrimų duomenimis nustatyta, kad po pagrindinio periodontologinio gydymo išlikusi 5 mm kišenė didina priešlaikinio danties netekimo riziką 7 kartus, 6 mm – 11 kartų, o 7 mm kišenė – net 64 kartus [16].

Kadangi periodontitas yra mikroorganizmų sąlygota liga, logiška, kad kartu su mechaniniu instrumentavimu galima naudoti ir papildomas priemones, kurios padėtų eliminuoti ar inaktyvuoti patogeninę mikroflorą tose vietose, kur mašininiai ar rankiniai instrumentai fiziškai pasiekti negali [17]. Klinikinių tyrimų duomenimis papildomų priemonių kartu su podanteniniu instrumentavimu panaudojimas gali nulemti palankesnius gydymo rezultatus, pvz., mažesnį periodontologinių kišenių zondavimo gylį (PD) ar kraujavimą po zondavimo (BOP), didesnį klinikinį audinių prisitvirtinimo lygį (CAL) [17–19]. Analizuojant literatūrą, galima išskirti tris pagrindines papildomai naudojamų medžiagų grupes: sisteminiai antibiotikai, vietiniai antibiotikai ir antiseptikai [20–22].

Sisteminiai antibiotikai periodontito gydyme turėtų būti naudojami tik esant labai pažengusioms ligos formoms (IV ligos stadija, C laipsnis, labai gilios pūliuojančios kišenės). Dėl šių antibiotikų sukeliamų šalutinių poveikių pacientui, perteklinio jų skyrimo ir atsparių bakterijų padermių formavimosi, sisteminiai antibiotikai turėtų būti skiriami itin racionaliai ir laikantis optimalių protokolų [23].

Papildomas vietinių antibiotikų skyrimas taip pat turi trūkumų, nes vietiniai antibiotikai pasižymi siauru antibakteriniu veikimo spektru, rizika vystytis atsparioms bakterijų padermėms bei aukšta kaina [21].

Antiseptikai – medžiagos, kurios gali selektyviai sunaikinti mikroorganizmus „gyvuose“ audiniuose, nepadarydami jiems žalos. Antiseptikų pranašumas, lyginant juos su sisteminiais ir vietiniais antibiotikais, yra tas, kad jie pasižymi platesniu antimikrobiniu veikimo spektru ir dėl daugybinių intraląstelinių taikinių sumažėjusia atsparių bakterijų rūšių formavimosi rizika [21].

Nors papildomų priemonių šiuolaikinėje periodontologijoje labai daug, iki šiol nėra nė vieno aiškaus gydymo protokolo ar gairių, kurie padėtų gydyti periodontitą efektyviai, išvengiant chirurginių intervencijų ir ilgalaikėje perspektyvoje padėtų pacientams išsaugoti savus dantis.

Pastaruoju metu literatūroje aptinkama duomenų apie „*Clean and Seal*“ (C&S) metodiką kaip galimą alternatyvą periodontito gydyme. C&S metodas paremtas dviejų papildomų priemonių (natrio hipochlorito (NaOCl) /amino rūgščių gelio (Perisolv®, Regedent AG, Zürich, Switzerland) ir vidutiniškai stabilizuotos didelio molekulinio svorio hialurono rūgšties (xHyA) (Hyadent BG, Regedent AG Zürich, Switzerland) gelio) ir kruopštaus podanteninio instrumentavimo panaudojimo kartu. Iki šiol nebuvo atlikta nė vieno atsitiktinės imties kontroliuojamo klinikinio tyrimo, kuris leistų išsiaiškinti, ar papildomas šių preparatų panaudojimas kartu su podanteniniu instrumentavimu nulemtų geresnius klinikinius bei mikrobiologinius periodontito gydymo rezultatus, lyginant su standartiniu periodontito gydymu, kai naudojamas ultragarsas ir rankiniai instrumentai.

Darbo tikslas

Ištirti klinikinius, mikrobiologinius ir histologinius papildomo NaOCl/amino rūgščių ir xHyA gelių naudojimo kartu su podanteniniu instrumentavimu rezultatus, palyginti juos vien su podanteninio instrumentavimo rezultatis, gydant II–III stadijos A, B laipsnių periodontitą.

Darbo uždaviniai

1. Nustatyti, ar papildomas NaOCl/amino rūgščių ir xHyA gelių kartu su podanteniniu instrumentavimu panaudojimas lemia geresnius klinikinius periodontito gydymo rezultatus (PD sumažėjimą, CAL padidėjimą, BOP sumažėjimą), palyginus su vien podanteniniu instrumentavimu.
2. Nustatyti, ar papildomas NaOCl/amino rūgščių ir xHyA gelių kartu su podanteniniu instrumentavimu panaudojimas lemia geresnius mikrobiologinius periodontito gydymo rezultatus (nustatyti pagrindinių periopatogenų – *Aggregatibacter actinomycetemcomitans (A.a), Prevotella intermedia (P.i), Treponema denticola (T.d), Porphyromonas gingivalis (P.g), Tanerella forsythia (T.f)* aptikimo dažnį ir bendrą skaičių prieš ir po gydymo), palyginus su standartiniu podanteniniu instrumentavimu.
3. Atlikti histologinę analizę ir nustatyti NaOCl/amino rūgščių ir xHyA gelių poveikį periodonto audiniams.

Darbo mokslinis naujumas

1. Iki šiol nebuvo atliktas nė vienas atsitiktinės imties kontroliuojamas klinikinis tyrimas, kuriuo būtų vertintas C&S metodikos klinikinis efektyvumas. Klinikiniai mūsų tyrimo rezultatai atskleidė, kad taikant C&S metodiką konservatyviame periodontito gydyme stipriai viršijamos rinkoje esančių žinomų papildomų medžiagų, naudojamų periodontito gydymui, vidutinės išgaunamos naudos, todėl šios metodikos taikymas efektyviai sumažina chirurginės intervencijos būtinybę tolesniuose ligos gydymo etapuose. Dar daugiau, naudojant C&S metodiką, nustatytas statistiškai reikšmingas klinikinių rodiklių gerėjimas tarp 3 ir 6 mėnesių, todėl pirminis gydymo efektyvumas galėtų būti vertinamas ne, kaip buvo įprasta, po 3, o po 4 ar net 6 mėnesių po pradinio gydymo.
2. Šis tyrimas – pirmasis, vertinęs mikrobiologinius C&S metodikos gydymo rezultatus. Nustatytas svarbus rezultatas – reikšmingas *Aggregatibacter actinomycetemcomitans* bendro skaičiaus sumažėjimas praėjus 3 ir 6 mėnesiams po gydymo. Iki šiol atlikti klinikiniai tyrimai, nagrinėję įvairias konservatyvaus periodontito gydymo metodikas, nenustatė šio patogeno, ypač svarbaus periodontito etiologijoje, skaičiaus pokyčio.
3. Histologinė tyrimo dalis pirmą kartą literatūroje atskleidė, kad, periodontito gydymo metu taikant C&S metodiką, periodonto audinius (šaknies cementą, periodonto raištį, alveolinį kaulą) galima regeneruoti ir konservatyvaus gydymo metu.

1. LITERATŪROS APŽVALGA

1.1. Periodontitas

1.1.1. Periodontito etiologija

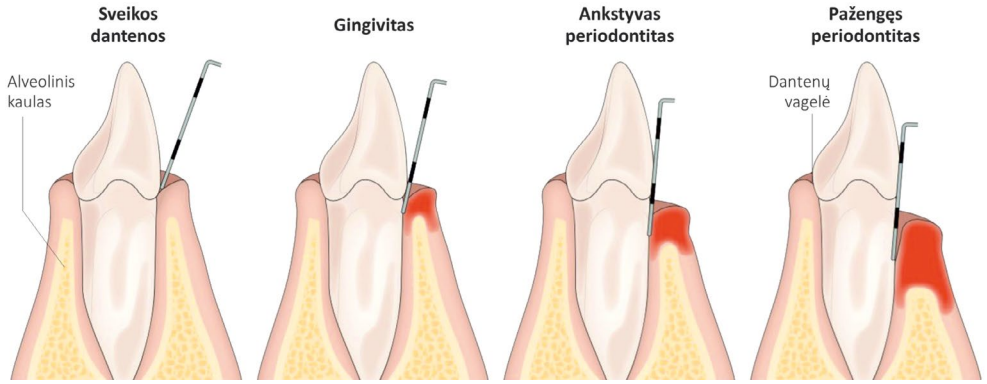
Periodontitas yra lėtinė uždegiminė liga, turinti mikrobinę etiologiją ir uždegiminę patogenezę. Pradinė periodontito stadija – gingivitas – vietinis uždegimas dantenose, kurį sukelia bakterijos, esančios dantų apnašose. Negydomas laiku gingivitas progresuoja į periodontitą, ir procesas tampa labiau išplitęs, apima periodonto raiščio, alveolinio kaulo netekimą, formuojasi gilios periodontologinės kišenės. Netaikant gydymo, tokia ligos eiga baigiasi ankstyvu danties netekimu (1.1.1.1 pav.).

Nors ant danties paviršiaus besiformuojantis bakterinis apnašas yra būtinas etiologinis faktorius, vien jo *per se* ligai inicijuoti nepakanka. Liga pasireiškia, kai sutrinka balansas tarp mikrobinio biofilmo ir imuninės sitemos mechanizmų. Šis disbalansas yra kompleksinis ir priklauso tiek nuo dantų apnašo savybių, tiek nuo paciento genetinių ir imuninės sistemos ypatybių, lemiančių uždegiminę audinių būklę būdingą periodontitui [24–26] (1.1.1.2 pav.).

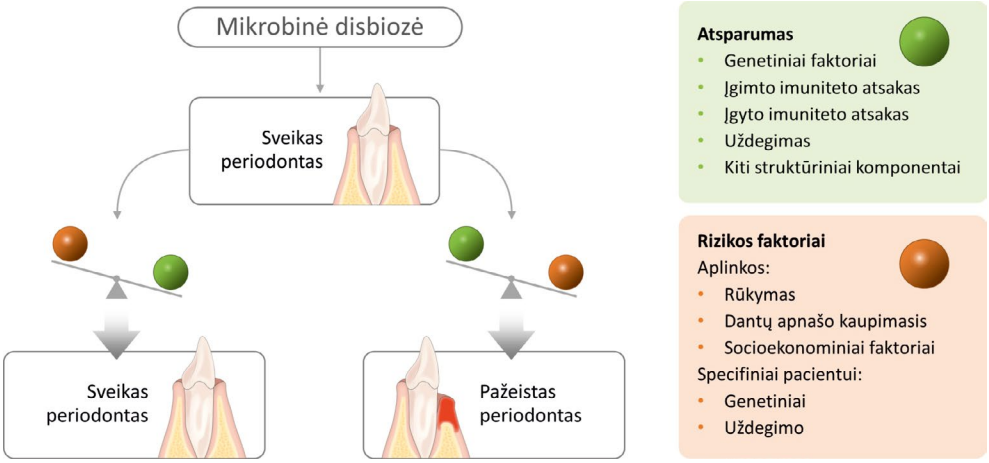
Rizikos faktoriai (genetika, gyvenimo būdas, stresas, sisteminės ligos ir kt.), inicijuojantys imunopatogenezinius mechanizmus ir virsmą iš sveikos būklės į ligą, yra būtini ligai pasireikšti [27]. Remiantis literatūra, galima išskirti šias keturias periodontito etiologijai įtaką darančias priežastis:

- *Bakterinė infekcija*. Bakterijos, ypač anaerobinės rūšys, tokios kaip *P.g, T.d, T.f, P.i* ir kt., yra pagrindinės bakterijų rūšys periodontito etiologijoje. Šios bakterijos formuoja dantų apnašas, vadinamas bioplėvele, kuri stimuliuoja uždegiminį organizmo atsaką ir sukelia audinių destrukciją [28–31];
- *Paciento imuninės sistemos atsakas.* Bakterijų buvimas periodonto kišenėje sukelia uždegiminį atsaką, kuris gali būti labai destruktyvus. Imuninė sistema neretai reaguoja per stipriai, todėl pacientams, turintiems silpną imuninę sistemą ar genetinį polinkį, pažeidžiamas jungiamasis audinys (CT) ir alveolinis kaulas [32–35];
- *Polimikrobinė disbiozė*. Periodontitas laikomas polimikrobinės disbiozės, t.y., bakterijų pusiausvyros sutrikimo, rezultatu. Šis disbalansas lemia patogeninių bakterijų rūšių gausėjimą, o tai dar labiau skatina būdingus šiai ligai uždegiminius procesus [36–39];
- *Genetiniai ir aplinkos veiksniai*. Kiti veiksniai, tokie kaip genetinis polinkis, sisteminės ligos, pvz., cukrinis diabetas, rūkymas, taip pat didina periodontito riziką ir progresavimą. Šie veiksniai gali pakeisti imuninį

atsaką ir paveikti burnos mikrobiotos sudėtį, dėl to liga tampa sunkesnė ar labiau tikėtina [40–42].



1.1.1.1 pav. *Sveikų dantenų, gingivito, ankstyvo ir pažengusio periodontito schematinis vaizdas*



1.1.1.2 pav. *Periodontitą lemiantys veiksniai*

1.1.2. Periodontito rizikos veiksniai

Periodontito rizikos veiksniai – tai įvairūs vietiniai ir sisteminiai veiksniai, galintys skatinti ligos vystymąsi. Pagrindiniai iš jų yra gyvenimo būdo pasirinkimas, sisteminės ligos, burnos higienos įpročiai. Šių veiksnių valdymas yra esminis periodontito prevencijos ir gydymo elementas [43]. Išskiriamos šios periodontito rizikos veiksnių grupės:

- *Rūkymas* – tai vienas pagrindinių periodontito rizikos veiksnių. Rūkantieji dažniau serga periodontitu, jų ligos eiga dažnai yra sunkesnė

[12, 44, 45]; Rūkantys pacientai, net ir gydant periodontitą, ilgalaikėje perspektyvoje netenka daugiau dantų nei nerūkantys, ir jų gydymo rezultatai mažiau prognozuojami. Rūkantiesiems dėl vazokonstrikcijos ir padidėjusios dantenų keratinizacijos dantenų uždegimo požymiai dažniau yra išreikšti silpniau nei nerūkantiesiems [46, 47];

- *Sisteminės ligos* – esami tyrimai rodo, kad yra daug bendrinių ligų, susijusių su periodontito vystymusi. Literatūros duomenimis, cukrinis diabetas, ypač blogai kontroliuojamas, ženkliai padidina periodontito riziką ir ligos sunkumą. Sergantiems diabetu kyla didenė rizika dėl kraujotakos sutrikimų ir silpnesnio imuninės sistemos atsako [8, 10, 48]. Egzistuoja glaudus ryšys tarp periodontito ir kardiovaskulinės patologijos. Yra tyrimų, rodančių, kad širdies ir kraujagyslių ligos sukelia organizme lėtinį uždegimą, kuris gali skatinti periodontito vystymąsi. Uždegimo mediatoriai, pvz., C-reaktyvusis baltymas, gali pabloginti periodonto audinių būklę, skatindami jų pažeidimą. Esant širdies ir kraujagyslių sistemos ligoms, pablogėjusi kraujotaka gali apsunkinti maistinių medžiagų, deguonies tiekimą į periodonto audinius, dėl to jie tampa labiau pažeidžiami ir mažiau atsparūs infekcijoms ir uždegimams [49–52]. Taip pat nustatytas glaudus reumatoidinio artrito ir periodontito ryšys. Reumatoidinis artritas didina periodontito riziką per bendrus uždegiminius mechanizmus, citrulinuotus autoantikūnus (reumatoidinio artrito pacientams būdingas specifinių autoantikūnų prieš citrulinuotą baltymą susidarymas, kurį skatina periodontitą sukeliančios bakterijos *P.g* ir *A.a*) ir mikrofloros disbiozę (reumatoidinis artritas gali paveikti burnos mikrobiotą, sukeldamas disbiozę, t.y., būklę, kurioje patogeninės bakterijos tampa dominuojančios) [53–55];
- *Stresas* – veiksnys, kuris gali skatinti periodontitą veikdamas imuninę sistemą (sumažina organizmo atsaką į bakterinę infekciją, skatinančią periodonto audinių uždegimą), hormonų veiklą (padidina kortizolio, hormono skatinančio uždegiminį atsaką, lygį) ir individualias paciento tendencijas (stresą patiriantys asmenys mažiau rūpinasi burnos higiena, yra linkę į žalingus įpročius) [56–59];
- *Hormoniniai pokyčiai* – hormonų veiklos pokyčiai, sąlygoti nėštumo, menopauzės, hormoninių kontraceptikų vartojimo, menstruacinio ciklo sutrikimų, didina periodontito riziką, skatina uždegiminius procesus ir mažina bendrą organizmo atsaką į bakterinę infekciją [60–62].

1.1.3. Periodontologinės kišenės formavimasis

Periodontologinė kišenė – pagrindinė klinikinė periodontito išraiška. Periodontologinė kišenė – tai patologiškai pagilėjusi dantenų vagelė, kurios vidu-

je stebimi uždegiminiai pokyčiai (jungties epitelio (JE) suardymas, osteoklastinio pobūdžio alveolinio kaulo rezorbcija) [63]. Periodonto sveikatos atveju dantenų kišenės zondavimo gylis yra nedidelis (1–2 mm) [64]. Sveikos dantenų kišenės vidų iškloja vagelės epitelis, kuris dantenų kišenės dugne pereina į jungiamąjį epitelį [65]. Jungiamojo epitelio ląstelės sudaro fizinę jungtį su danties paviršiumi ir tarnauja kaip sluoksnis, apsaugantis giliau esančius periodonto audinius nuo bakterinės infekcijos [66]. Pagrindinis periodontito formavimosi bruožas – jungties epitelio pokytis į kišenės epitelį [63].

Tokie patogenai kaip *P.g* ir *P.i* pradeda kolonizuoti dantenų vagelę, suformuodami bakterinį biofilmą, kuris prilimpa prie danties paviršiaus. Šiame biofilme dominuoja anaerobinės gramneigiamos bakterijos, kurios dauginasi podanteninėje srityje esant mažam deguonies kiekiui [67]. Imuninė sistema reaguoja į šiuos patogenus ir inicijuoja uždegiminį atsaką, kurį lydi imuninių ląstelių (neutrofilų, T-ląstelių, B-ląstelių, makrofagų) antplūdis [34, 68–70]. Toks atsakas, siekiantis kontroliuoti infekciją, sukelia destrukcinių fermentų (kolagenazių, matricos metaloproteinazių, elastazių) išsiskyrimą, kurie ardo aplinkinius periodonto audinius ir skatina granuliacinio audinio formavimąsi [71, 72]. Bakterijų apykaitos produktai ir uždegimo mediatoriai silpnina jungiamojo audinio struktūrą, leisdami bakterijoms prasiskverbti giliau į kišenę ir dar labiau skatina audinių irimą bei kišenės gilėjimą. Stipri lėtinė uždegiminė reakcija aktyvuoja osteoklastus, lemiančius alveolinio kaulo rezorbciją. Procesas tęsiasi tol, kol pašalinamas dantis, arba gydymo metu pašalinamas mikrobinis biofilmas ir uždegiminės granuliacijos.

1.1.4. Klinikinė periodontito diagnozė / ligos atvejis

Remiantis 2017 m. Pasaulio periodonto ir peri-implanto ligų ir būklių klasifikacija [1], periodontitas pacientui diagnozuojamas, kai:

- klinikinės jungties netekimas nustatomas daugiau nei dviejuose tarpdančiuose;
- bukaliai ar oraliai stebimas ≥ 3 mm klinikinės jungties netekimas ir aptinkamas > nei 3 mm kišenės zondavimo gylis daugiau nei dvejuose dantyse [1, 73].

Diagnozavus periodontitą, individualus kievieno paciento atvejis toliau apibūdinamas pagal stadijų ir laipsnių sistemą [1].

1.1.5. Klinikinė periodontito diagnostika

Tikslios diagnostinės priemonės yra būtinos norint: (1) nustatyti pacientus, turinčius riziką ligai išsivystyti, (2) aptikti ligą (pageidautina pradinėse ligos stadijose), (3) suklasifikuoti ligą, t. y., priskirti tam tikrai kategorijai, (4) nustatyti diagnozę ir sudaryti gydymo planą, (5) įvertinti gydymo efektyvumą,

(6) stebėti ligos stabilumą, recidyvą ar progresavimą, (7) nustatyti pacientus / sritis, turinčius riziką ligos progresavimui, (8) palengvinti komunikaciją tarp gydytojo ir pacientų bei individualizuoti palaikomojo gydymo programas [74, 75].

Pradinėse ligos stadijose yra pažeidžiamas jungiamasis audinys, o ligai progresuojant pažeidžiami periodonto raištis ir alveolinis kaulas. Kliniškai šie procesai išreikšti kaip klinikinės jungties netekimas cemento-emalio jungties (CEJ) atžvilgiu, uždegimine reakcija dantenose (kraujavimu, paburkimu, paraudimu). Papildomi klinikiniai simptomai apima periodontolginės kišenės formavimąsi, dantenų recesijas (REC), tarpšaknio pažeidimus daugiašakniuose dantyse, radiologiškai stebimą alveolinio kaulo rezorbciją. Pacientai dažniausiai skundžiasi padidėjusiu dantų paslankumu, dantų migracija, sunkumu sukramtyti maistą, nemaloniu kvapu iš burnos ertmės [63, 76]. Klinikinė periodontito diagnozė nustatoma remiantis objektyviai išmatuotais ir įvertintais prieš tai minėtais klinikiniais, radiologiniais duomenimis, tuo pačiu įvertinant ir ligos išplitimo laipsnį bei sunkumą [74, 75].

1.1.5.1. Kišenių zondavimo gylio įvertinimas

Dantenų kišenės zondavimo gylis (PD) – atstumas nuo kišenės dugno iki laisvo dantenų krašto. Dantenų kišenės matuojamos šešiuose taškuose (disto-bukaliniame, vidurio-bukaliniame, medio-bukaliniame, disto-oraliniame, vidurio-oraliniame, medio-oraliniame) standartizuotais, sugraduotais periodontologiniais zondais. PD – svarbus diagnostinis kriterijus, registruojamas specialiose periodontologinio paciento ištyrimo formose [74].

PD atspindi periodonto audinių pažeidimo lygį/sunkumą ir išplitimo laipsnį [77]. PD reikšmė yra tiesiogiai priklausoma nuo uždegiminių procesų audiniuose. Edema gali sukelti patinimą ir dantenų krašto migraciją vainikine kryptimi. Kita vertus, dantenų krašto atsitraukimas gali būti sėkmingo periodontologinio gydymo rezultatas, siejamas su uždegimo sumažėjimu. Norint tiksliai įvertinti periodonto būklę, PD matavimas turėtų būti atliekamas kartu su CAL vertinimu.

1.1.5.2. Klinikinio audinių prisitvirtinimo lygio įvertinimas

Klinikinis audinių prisitvirtinimo lygis (CAL) atspindi periodonto audinių pažeidimo apimtį ir sunkumą. Klinikinės jungties praradimas – mikrobinės disbiozės, sukeliančios imuninės sistemos atsaką ir lemiančios klinikinės jungties praradimą padarinys [78]. Klinikinės jungties įvertinimui svarbus atskaitos taškas yra cemento-emalio jungtis (CEJ). Nesant periodonto patologijai, jungiamojo audinio epitelinė jugtis sutampa su CEJ riba. Tokiu atveju

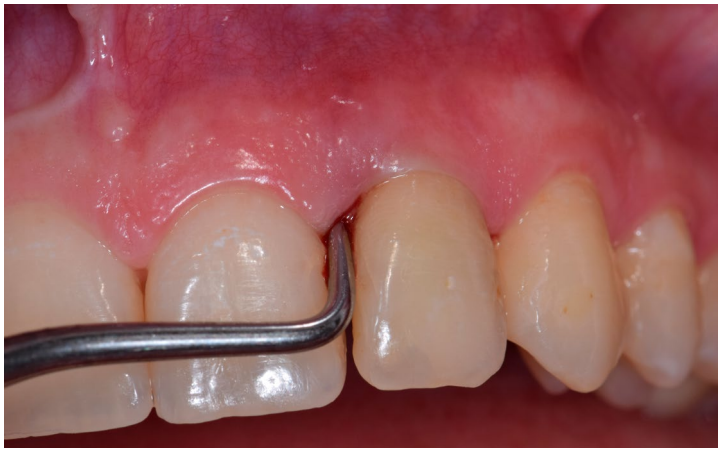
CAL matuojamas nuo CEJ ribos iki išzonduojamos dantenų / periodontologinės kišenės dugno.

Norint tiksliai išmatuoti CAL, yra svarbūs du klinikiniai matavimai: atstumas nuo laisvo dantenų krašto iki CEJ ir klinikinis PD. CAL apskaičiuojamas atimant atstumą nuo laisvo dantenų krašto iš PD (kai CEJ dengia minkštieji audiniai). Esant dantenų recesijai (REC), dantenų kraštas įgauna neigiamą reikšmę CEJ atžvilgiu, todėl tokiu atveju CAL apskaičiuojamas sudedant atstumą nuo laisvo dantenų krašto iki CEJ su PD verte.

1.1.5.3. Kraujavimo po zondavimo įvertinimas

Kraujavimas po zondavimo (BOP) – diagnostinis klinikinis testas, naudojamas įvertinti uždegiminę dento-gingivalinio komplekso būklę. BOP nebuvimas – patikimas periodonto sveikatą prognozuojantis faktorius [79]. Tyrimai rodo, kad egzistuoja tiesioginis ryšys tarp kliniškai pasireiškiančio BOP ir histologiškai stebimų uždegiminių pokyčių periodonte, todėl šio klinikinio rodiklio vertinimas – svarbus periodontito diagnostikos kriterijus [72].

BOP vertinimui naudojamas periodontologinis zondas, kuris įvedamas į kišenę lengvu 0,2–0,3 N spaudimu [80] (1.1.5.3.1 pav.). Jei dantena kraujuoja praėjus 10 s po zondo ištraukimo, BOP reikšmė vertinama teigiamai. BOP vertinamas kiekviename dantyje, šešiuose taškuose ir apskaičiuojama vidutinė procentinė kraujuojančių sričių reikšmė. Kuo didesnė BOP reikšmė, tuo labiau generalizuotas uždegiminis procesas. Remiantis 2017 m. Periodonto ligų klasifikacija, procesas laikomas generalizuotu tuomet, kai apima > 30 proc. sričių burnoje.



1.1.5.3.1 pav. *Klinikinio kišenės zondavimo gylio ir kraujavimo po zondavimo įvertinimas. Graduotas Mičigano zondas įvedamas iki periodontologinės kišenės dugno lygiagrečiai išilginei danties ašiai*

1.1.5.4. Tarpšaknio pažeidimo vertinimas

Periodontologinės kišenės apibrėžiamos kaip vienos, dviejų ar trijų sienų kaulinės kišenės, ketvirtą sieną sudaro danties paviršius. Visų krūminių dantų ir pirmųjų viršutinio žandikaulio kaplių furkacijos turėtų būti zonduojamos lenktu, graduotu furkacijų zondu, turinčiu 3 ir 5 mm žymas. Horizontalus zondavimo komponentas yra skirstomas į laipsnius nuo 0 iki 3 atsižvelgiant į šiuos kriterijus:

0 – furkacija nezonduojama;
1 – furkacijos pažeidimas, kai furkacija horizontaliai išzonduojama ≤ 3 mm;
2 – furkacijos pažeidimas, kai furkacija horizontaliai išzonduojama >3 mm;
3 – furkacijos pažeidimas kai furkacija zonduojama kiaurai [81].

1.1.5.5. Dantų paslankumo vertinimas

Norint įvertinti dantų paslankumą, dantis laikomas tvirtai tarp dviejų instrumentų ir judinamas pirmyn atgal. Danties paslankumas vertinamas skalėje nuo 0 iki 3, kurioje 0 reiškia, kad paslankumo nėra (fiziologinis danties paslankumas < 0,2 mm), 1 – šiek tiek didesnis nei fiziologinis danties paslankumas, 2–1 mm danties judesys bukolingvaline kryptimi, 3 – didesnis nei 1 mm danties paslankumas bukolingvaline kryptimi bei vertikalus danties judesys [82].

1.1.6. Periodontito klasifikacija

2017 m. Pasaulio periodonto ir peri-implanto ligų ir būklių klasifikacijos dirbtuvių antrosios darbo grupės konsensuso atsakaitoje buvo paskelbta nauja periodontito klasifikavimo sistema [1]. Prieš tai visuotinai priimtoje Armitage 1999 periodonto ligų klasifikacijoje išskirtos „lėtinė“ ir „agresyvi“ ligos formos buvo sujungtos į vieną kategoriją „*periodontitas*“, kuri klasifikuojama pagal stadijų ir laipsnių sistemą [1, 83].

- *Periodontito klasifikavimas pagal stadijas* priklauso nuo ligos sunkumo laipsnio (atsižvelgiant į tarpdantinį klinikinės jungties, radiologinį kaulo ir dantų netekimą), ligos sudėtingumo bei išplitimo. Iš viso išskiriamos I–IV ligos stadijos (1.1.6.1 lentelė).
- *Periodontito klasifikavimas pagal laipsnius* atskleidžia biologinius ligos požymius, įskaitant periodontito progresavimo ir rizikos ligai vystytis toliau įvertinimą, numatomą atsaką į gydymą ir poveikį bendrai organizmo sveikatai. Periodontito laipsnis naudojamas kaip pagrindinis indikatorius ligos progresavimo greičiui nusakyti. Pirminis kriterijus yra tiesioginis arba netiesioginis progresavimo įrodymas. Kai tik įmanoma, naudojamas tiesioginis įrodymas. Jeigu tiesioginio įrodymo nėra, netie-

sioginis vertinimas atliekamas naudojant kaulo netekimą kaip amžiaus funkciją prie labiausiai pažeisto danties (radiologinis kaulo netekimas išreikštas procentais (šaknies ilgio atžvilgiu), padalintas iš paciento amžiaus). Pirminiam pacientui paprastai daroma prielaida, kad yra B laipsnio periodontitas, ieškant įrodymų, galinčių priskirti pacientą prie A ar C laipsnio klasės. Kai periodontito laipsnis nustatomas pagal ligos progresavimo greitį, jis gali būti modifikuojamas pagal rizikos faktorių buvimą (1.1.6.2 lentelė).

1.1.6.1 lentelė. *Periodontito klasifikavimas pagal stadijas*

Periodontito stadija		**I stadija**	**II stadija**	**III stadija**	**IV stadija**
Sunkumas	**Tarpdantinis CAL didžiausio netekimo vietoje**	Nuo 1 iki 2 mm	Nuo 3 iki 4 mm	≥ 5 mm	≥ 5 mm
	Radiologinis kaulo netekimas	Vainikinis šaknies trečdalis (< 15 proc.)	Vainikinis šaknies trečdalis (nuo 15 proc. iki 33 proc.)	Siekia vidurinį ar viršūninį šaknies trečdalį	Siekia vidurinį ar viršūninį šaknies trečdalį
	Dantų netekimas	Nėra dantų netekimo dėl periodontito		≤ 4 dėl periodontito prarasti dantys	≥ 5 dėl periodontito prarasti dantys
Kompleksiškumas	**Vietinis**	• didžiausias zondavimo gylis ≤ 4 mm • vyrauja horizontalus kaulo netekimas	• didžiausias zondavimo gylis ≤ 5 mm • vyrauja horizontalus kaulo netekimas	II stadijos kompleksiškumas ir: • zondavimo gylis ≥ 6 mm • vertikalus kaulo netekimas ≥ 3 mm • II ar III klasės furkacijų pažeidimai • vidutinio sunkumo alveolinės ataugos defektas	III stadijos kompleksiškumas ir: • sudėtingos reabilitacijos poreikis dėl: – kramtymo funkcijos sutrikimo – sunkaus alveolinės ataugos defekto – antrinės okliuzinės traumos (dantų paslankumo laipsnis ≥ 2) – mažiau nei 20 likusių dantų (10 antagonistų) – sąkandžio griūties, išplatėjimo, poslinkio
Išplitimas ir pasiskirstymas	**Pridėti prie stadijos kaip apibūdinimą**	Kiekvienai stadijai apibūdinti proceso išplitimo laipsnį: • lokalus (paveikta < 30 proc. dantų) • generalizuotas (paveikta > 30 proc. dantų) • kandžių / krūminių dantų modelis			

CAL – klinikinis audinių prisitvirtinimo lygis; PD – kišenės zondavimo gylis.

1.1.6.2 lentelė. *Periodontito klasifikavimas pagal laipsnius*

Periodontito laipsnis			**A laipsnis: lėtas progresavimas**	**B laipsnis: lėtas progresavimas**	**C laipsnis: lėtas progresavimas**
Pirminiai kriterijai	Tiesioginis progresavimo įrodymas	Išilginiai duomenys (radiologinis kaulo arba CAL netekimas)	Jokio netekimo per pastaruosius 5 metus	< 2 mm per pastaruosius 5 metus	≥ 2 mm per pastaruosius 5 metus
	Netiesioginis progresavimo įrodymas	proc. kaulo netekimas / amžius	< 0,25	Nuo 0,25 iki 1,0	> 1,0
		Atvejo fenotipas	Gausu apnašo, nedidelis destrukcijos lygis	Destrukcija proporcinga apnašo kiekiui	Destrukcija viršija bakterinio apnašo kiekį; specifiniai klinikiniai požymiai įspėjantys apie itin greitą ligos progresavimą ir / ar ankstyvą ligos pradžią (kandžių / moliarų modelis, nepakankamas atsakas į gydymą)
Laipsnį modifikuojantys faktoriai	Rizikos faktoriai	Rūkymas	Nerūko	Rūko < 10 cigarečių per dieną	Rūko ≥ 10 cigarečių per dieną
		Diabetas	Normoglikemija, nediagnozuotas diabetas	HbA1c < 7 proc. pacientams, sergantiems cukriniu diabetu	HbA1c ≥ 7 proc. pacientams, sergantiems cukriniu diabetu

CAL – klinikinis audinių prisitvirtinimo lygis; HbA1c – glikuotas hemoglobinas.

1.1.7. Periodontito gydymas

Sėkmingai išgydytas, po gydymo stabilios periodontologinės būklės pacientas priskiriamas dantenų sveikatos turinčiai sumažėjusį periodontą kategorijai. Kliniškai dantenų sveikata išreiškiama mažu BOP indeksu (< 10 proc.) ir sekliu PD (≤ 4 mm, nekraujuoja po zondavimo) [64]. Jei po periodontologinio gydymo pacientas atitinka šiuos kriterijus, tačiau BOP indeksas yra > 10 proc., tuomet jam diagnozuojamas stabilus periodontitas su išreikštu dantenų uždegimu [64]. Jei po gydymo aptinkama periodontologinių kišenių, kurių gylis yra ≥ 4 mm ir kurios kraujuoja po zondavimo, tokiam pacientui diagnozuojama nestabili periodonto būklė, indikuojanti apie ligos progresavimą ir tolimesnio gydymo reikiamybę [64]. Svarbu tai, kad sėkmingai išgydyti ir stabilūs periodontologiniai pacientai vis tiek išlieka didesnėje rizikoje dėl ligos atsinaujinimo, todėl jie turėtų būti nuolat sekami, kad ligos atsinaujinimo būtų išvengta [43].

2020 m. Europos Periodontologų Federacija paskelbė I–III stadijų periodontito klinikinės praktikos gydymo gairių gidą, kuriame nurodė, kad periodontito gydymas yra daugiapakopis procesas, kurio metu, priklausomai nuo ligos stadijos, palaipsniui būtų atliekamos konkrečiam klinikiniam atvejui reikalingos procedūros [43]. Apibendrinant, ligos gydyme galima išskirti keturis žingsnius [43].

Pirmo gydymo žingsnio tikslas – motyvuoti pacientą, padėti jam suprasti jo indėlį į gydymo sėkmę (gerinant asmeninės burnos higienos įgūdžius bei kontroliuojant kitus tam atvejui reikšmingus rizikos faktorius). Šio etapo metu atliekamos gydymo procedūros apima:

- viršdanteninio apnašo kontrolę;
- priemones, padedančias suformuoti geresnius asmeninės burnos higienos įgūdžius (motyvacija, burnos higienos instruktažas);
- papildomas priemones, padedančias sumažinti dantenų uždegimą;
- profesionalią burnos higieną (viršdanteninio apnašo ir akmenų šalinimas, faktorių, sąlygojančių apnašo kaupimąsi, šalinimas);
- rizikos faktorių kontrolę (metimas rūkyti, metabolinės kontrolės gerinimas diabeto atveju, fizinio krūvio didinimas, mitybos konsultacija, paskatinimas mesti svorį nutukimo atveju).

Pirmas žingsnis turėtų būti atliekamas visiems be išimties periodontologiniams pacientams, nepriklausomai nuo jiems diagnozuoto periodontito stadijos ar laipsnio.

Antro gydymo žingsnio tikslas – kontroliuoti (sumažinti / visiškai pašalinti) podanteninį apnašą ir akmenis podanteniniu instrumentavimu. Šis gydymo etapas turėtų būti atliekamas be išimties visiems periodontologiniams pacientams, nepriklausomai nuo to, kokia ligos stadija jiems diagnozuota. Antrame

gydymo žingsnyje kartu su kruopščiu podanteniniu instrumentavimu galima naudoti ir šias papildomas priemones: fizines, chemines medžiagas, vietinius ar sisteminius imunitetą modifikuojančius preparatus, vietinius ar sisteminius antibiotikus. Individualus atsakas į gydymą vertinamas po 8–12 savaičių [84]. Jei gydymo tikslas (PD ≤ 4 mm, nėra BOP) nepasiekiamas, būtina svarstyti apie trečią gydymo žingsnį. Jei gydymo tikslą įgyvendinti pavyko, įvertinus individualų rizikos ligai atsinaujinti profilį, pacientui skiriamos reguliaraus palaikomojo gydymo programos [85, 86].

Trečio gydymo žingsnio tikslas – gydyti sritis, nereaguojančias į gydymą antrame žingsnyje (PD ≥ 4 mm, yra BOP arba PD ≥ 6 mm). Trečio etapo metu galimos šios procedūros: pakartotinis podanteninis instrumentavimas su arba be papildomomų priemonių, lopo operacija, rezekcinis arba regeneracinio pobūdžio periodontologinis gydymas. Gydymo efektyvumas po chirurginių procedūrų paprastai vertinamas po 6 mėnesių. Idealiu atveju, pasiekus gydymo tikslą, pacientui skiriamos reguliaraus palaikomojo gydymo programos.

Ketvirtas gydymo žingsnis – tai reguliarus palaikomasis periodontologinis gydymas, kurio tikslas – palaikyti stabilią periodonto audinių būklę. Jo metu, priklausomai nuo paciento būklės, derinamos gydymo koncepcijos, taikomos pirmame ir antrame gydymo žingsniuose.

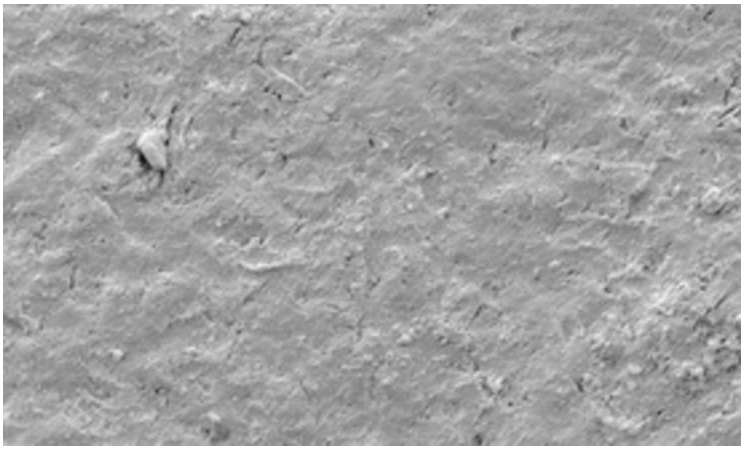
1.2. Natrio hipochlorito / amino rūgščių gelis

NaOCl/amino rūgščių gelis (Perisolv®) susideda iš dviejų komponentų – 0,95 proc. NaOCl ir trijų amino rūgščių (lizino, leucino ir glutaminės rūgšties). Sumaišius šiuos komponentus tarpusavyje gaunami chloraminai (pH = 12), kurie susiformuoja susijungus NaOCl sudedamajai daliai chlorinui su amino rūgščių amino funkcijomis [87]. Trys Perisolv® amino rūgštys (leucinas, lizinas ir glutaminė rūgštis) pasižymi skirtingomis elektrostatinėmis savybėmis – rūgštine, šarmine ir hidrofobine. Aukšto pH aplinkoje NaOCl chlorinas pernešamas į amino rūgščių amino funkcijas – taip sumažinamas aukštas oksidacinis preparato reaktyvumas ir nepadaroma žala sveikiems audiniams [88–90].

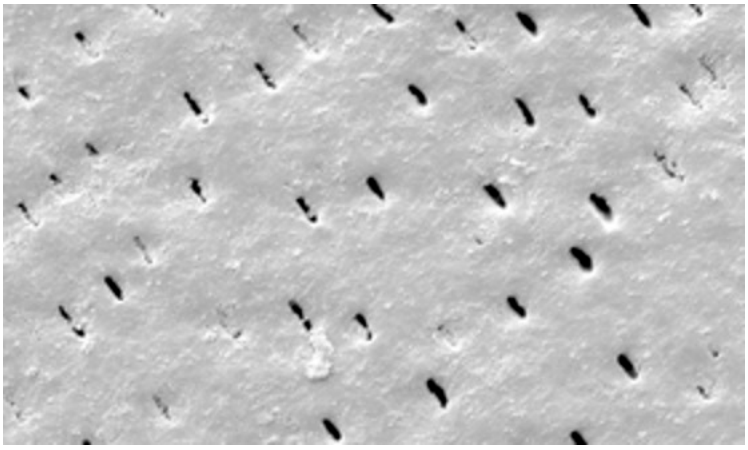
Susiformavę chloraminai oksiduoja nekrotinius audinius [87]. Podanteninio instrumentavimo metu fizinės ir cheminės reakcijos veikdamos kartu padeda suardyti bakterinio apnašo plėvelę ir pašalinti granuliacinius audinius. Kadangi medžiaga selektyviai veikia tik suardytus baltymus, toks chemo-mechaninis metodas nedaro neigiamo poveikio nei sveikam dentinui, nei šaknies cementui [91, 92]. Aukštas pH turi dantų akmenis minkštinantį poveikį, todėl podanteninį instrumentavimą galima atlikti lengviau.

1.2.1. Ikiklinikiniai natrio hipochlorito / amino rūgščių gelio tyrimai

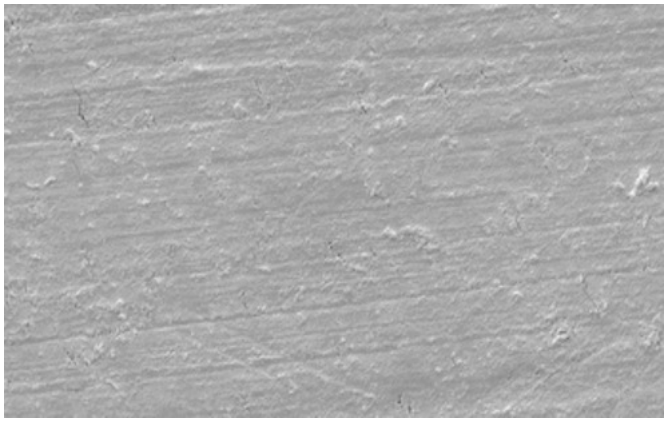
Shmidlin P.R. ir bendraautoriai laboratorijoje ištyrė NaOCl/amino rūgščių gelio poveikį dentinui ir periodonto raiščio ląstelių išgyvenamumui, prisitvirtinimui ir dauginimuisi, taip pat palygino jį su klasikinėmis medžiagomis, naudojamomis periodontito gydyme – natrio bikarbonato ir glicino milteliais [93]. Eksperimento metu testuojamos medžiagos buvo aplikuojamos ant paruoštų dentino diskų, vėliau jie buvo nuplaunami fiziologiniu tirpalu, ir sėjamos periodonto raiščio ląstelių kultūros. Ląstelių išgyvenamumas, prisitvirtinimas ir morfologinės variacijos buvo tiriamos panaudojant skenuojantį elektroninį mikroskopą (SEM). Tiriant morfologinius dentino pokyčius mikroskopu pastebėta, kad natrio bikarbonato milteliai sukelia žymius paviršiaus pokyčius (stebimas šiurkštus, su daugybe mikro įtrūkimų paviršius) (1.2.1.1 pav.). Panašus mikroskopinis vaizdas stebėtas ir po glicino miltelių aplikacijos – nustatyta, kad ši medžiaga turi potencialo atverti dentino tubules (kliniškai tai pasireikštų didesniu pooperaciniu jautrumu) (1.2.1.2 pav.). Dentino diskuose, kurie buvo paveikti NaOCl/amino rūgščių gelio, morfologinių pokyčių nustatyta nebuvo (1.2.1.3 pav.). Panašus periodonto raiščio ląstelių išgyvenamumas pastebėtas visose tiriamosiose grupėse, tačiau ląstelių prisitvirtinimas ir dauginimasis – ant tų dentino diskų, kurie buvo paveikti glicino milteliais ir NaOCl/amino rūgščių geliu, buvo reikšmingai didesnis.



1.2.1.1 pav. *Dentino disko SEM vaizdas jį paveikus natrio bikarbonato milteliais*



1.2.1.2 pav. *Dentino disko SEM vaizdas jį paveikus glicino milteliais*



1.2.1.3 pav. *Dentino disko SEM vaizdas jį paveikus NaOCl/amino rūgštimis*

Lupse I. ir bendraautoriai eksperimente *in vitro* tyrė NaOCl/amino rūgščių gelio poveikį dantenų mezenchimos stromos ląstelėms ir nustatė, kad paveikus dantų šaknų paviršių šia medžiaga, ląstelės išliko gyvybingos ir gebėjo repopuliuoti paveiktus paviršius [94].

Jurczyk K. ir bendraautoriai *in vitro* tyrė NaOCl/amino rūgščių gelio poveikį bakterijoms, susijusioms su periodontito etiologija (*P.g*, *T.f*, *A.a*, *Parvimonas micra* ir kt.) [95]. Eksperimento metu tyrėjai pastebėjo, kad NaOCl/amino rūgščių gelis labiausiai veikia gramneigiamas bakterijų rūšis (svarbias periodontito etiologijoje), o didžiausias antibakterinis preparato aktyvumas stebėtas ketvirtąją apnašo formavimosi dieną. Taip pat, pastebėta, kad NaOCl/amino rūgščių gelis pasižymėjo baktericidiniu poveikiu ir suardė biofilmo matricą.

2023 m. Tomina D.C. ir bendraautoriai tyrime su laboratorinėmis žiurkėmis įvairiomis modifikacijomis gydė eksperimentinį periodontitą [96]. Tyrimo rezultatai atskleidė, kad histologinis gijimas geriausiai vyko grupėje, kuriai podanteninis instrumentavimas buvo atliekamas kartu su NaOCl/amino rūgščių geliu.

1.2.2. Klinikiniai natrio hipochlorito / amino rūgščių gelio tyrimai

2024 m. Wallin-Bengtsson V. ir bendraautoriai tyrė papildomą NaOCl/amino rūgščių gelio poveikį klinikiniams rodikliams periodontito gydyme [97]. 12 mėn. trukusiame atsitiktinės imties kontroliuojamame klinikiniame tyrime pastebėtas statistiškai reikšmingas BOP sumažėjimas bei didesnių nei 6 mm kišenių skaičiaus sumažėjimas abiejose tiriamosiose grupėse. Statistiškai reikšmingo skirtumo tarp tiriamųjų grupių nustatyta nebuvo.

2022 m. Radulescu V. ir bendraautoriai atsitiktinės imties kontroliuojamame klinikiniame tyrė papildomą Perisolv® poveikį klinikiniams rodikliams gydant prieš tai į taikytą gydymą nereagavusias periodontologines kišenes ir lygino gydymo rezultatus su podanteniniu instrumentavimu [98]. Po 12 mėn. abiejose tyrimo grupėse buvo stebimas statistiškai reikšmingas vidutinio PD sumažėjimas ir CAL padidėjimas, tačiau reikšmingo skirtumo tarp grupių nenustatyta. Tačiau, po 12 mėn. Perisolv® grupėje nustatytas statistiškai reikšmingai didesnis kraujuojančių sričių proprocijos sumažėjimas, palyginus su kontroline grupe ($p = 0,010$).

2021 m. Iorio-Siciliano V. ir bendraautoriai 6 mėn. trukmės atsitiktinės imties kontroliuojamame klinikiniame tyrime tyrė papildomą klinikinį Perisolv® poveikį III/IV stadijos, A/B laipsnio periodontito gydyme, taikant minimaliai invazyvią, konservatyvią metodiką [99]. Tyrimas parodė statistiškai reikšmingą skirtumą tarp grupių PD sumažėjimo ($p = 0,001$) ir CAL padidėjimo ($p = 0,001$) tiriamosios (Perisolv®) grupės naudai. Po 6 mėn. Perisolv® grupėje buvo stebimas statistiškai reikšmingai didesnis kišenių, kurių gylis buvo ≥ 5 mm ir kurios po zondavimo kraujavo, skaičiaus sumažėjimas, palyginus su kontroline grupe ($p = 0,001$).

2019 m. Megally A. ir bendraautoriai tyrė papildomą Perisolv® poveikį klinikiniams rodikliams kartu su ultragarsiniu podanteniniu instrumentavimu gydant prieš tai į gydymą nereagavusias ≥ 5 mm gylio periodontologines kišenes [99]. Po 12 mėn. nenustatyta statistiškai reikšmingo skirtumo tarp kontrolinės ir tiriamosios grupių, tačiau Perisolv® grupėje buvo stebimas didesnis gilių kišenių skaičiaus sumažėjimas gilių periodontologinių kišenių kategorijoje (kai pradinis PD ≥ 7 mm). Konkrečiai, kontrolinėje grupėje po gydymo buvo likusios šešios gilios kišenės, o tuo tarpu Perisolv® grupėje – viena gili kišenė.

Klinikiniai NaOCl/amino rūgščių gelio tyrimai pateikti 1.2.2.1 lentelėje.

1.2.2.1 lentelė. *Klinikiniai natrio hipochlorito / amino rūgščių gelio tyrimai*

Autorius, metai	**Tyrimo dizainas**	**Tiriamųjų skaičius kontrolės ir testo grupėse (n)**	**Tyrimo trukmė**	**Gydymo protokolas kontrolės ir testo grupėse**	**Vidutinis PD pokytis kontrolės ir testo grupėse (mm (SD))**	**Vidutinis CAL pokytis kontrolės ir testo grupėse (mm (SD))**	**Vidutinis BOP pokytis kontrolės ir testo grupėse (proc. (SD))**	**Išvada**
Wallin-Bengtsson et al., 2024 [96]	RCT, paralelinės grupės	Kontrolė n = 18 Testas n = 20	12 mėn	Kontrolė Ultragarsas+rankiniai instrumentai Testas Ultragarsas+rankiniai instrumentai+Perisolv®	–	–	Kontrolė 35,5 (16,7) Testas 45,2 (17,8)	Nėra statistiškai reikšmingo skirtumo tarp grupių BOP pokytyje (p = 0,527)
Radulescu et al., 2022 [97]	RCT, paralelinės grupės	Kontrolė n = 18 Testas n = 20	12 mėn	Kontrolė Ultragarsas + oro abrazija Testas Ultragarsas + oro abrazija +Perisolv®	Kontrolė 0,75 (0,58) Testas 0,81 (0,38)	Kontrolė 0,57 (0,50) Testas 0,70 (0,40)	–	Nėra statistiškai reikšmingo skirtumo tarp grupių PD (p = 0,356)ir CAL pokyčiuose (p = 0,095)
Iorio-Siciliano et al., 2021 [98]	RCT, paralelinės grupės	Kontrolė n = 19 Testas n = 18	6 mėn	Kontrolė Ultragarsas Testas Ultragarsas+ Perisolv®	Kontrolė 1,98 (0,8) Testas 2,49 (0,76)	Kontrolė 2,01 (1,83) Testas 2,84 (2,09)	Kontrolė 28,6 (10,0) Testas 26,5 (13,2)	Statistiškai reikšmingas skirtumas tarp testo ir kontrolės kontrolinės grupės naudai PD sumažėjime (p = 0,001) ir CAL padidėjime (p = 0,001)
Megally et al., 2019 [99]	RCT, paralelinės grupės	Kontrolė n = 16 Testas n = 16	12 mėn	Kontrolė Ultragarsas Testas Ultragarsas+ Perisolv®	Kontrolė 0,87 (1,25) Testas 0,44 (0,74)	Kontrolė 0,82 (1,33) Testas 1,02 (1,49)	–	Nėra statistiškai reikšmingo skirtumo tarp grupių PD pokytyje (p = 0,73)

BOP – kraujavimas po zondavimo; CAL – klinikinis audinių prisitvirtinimo lygis; p – reikšmingumo lygmuo; PD – kišenės zondavimo gylis; RCT – atsitiktinės imties kontroliuojamas klinikinis tyrimas, SD – standartinis nuokrypis.

1.3. Hialurono rūgštis

Hialurono rūgštis (HyA) – natūraliai sintetinamas ilgos grandinės gliko-zaminglikanas, aptinkamas daugelio audinių (odos, sąnarių, akių, periodonto raiščio) ekstraceliuliniame matrikse. Tai svarbi ekstraceliulinio matrikso medžiaga, reguliuojanti ląstelių funkcijas ir užtikrinanti komunikaciją tarp skirtingų ląstelių rūšių [101]. Žmogaus organizme HyA sintetina hialurono sintezės baltymai [102]. Ši rūgštis pasižymi unikaliomis fizikocheminėmis ir biologinėmis savybėmis – yra hidroskopiška, viskoelstiška, pasižymi bakteriostatiniu, priešuždegiminiu, antiedeminiu poveikiu, turi pro-angiogenezinių ir osteoindukcinių savybių [103–106]. Kadangi HyA yra svarbi molekulė, dalyvaujanti uždegiminiuose procesuose, epitelio formavimesi ir audinių remodeliacijoje, nepaneigiama didžiulė jos įtaka ir periodonto žaizdos gijime [107]. Rinkoje HyA gali būti stabilizuojama – kelios glikozaminoglikanų grandinės sujungiamos tarpusavyje 1,4 butanediolio diglicidilo eterio pagalba [108].

Produkto stabilizacijos laipsnis priklauso nuo sujungtų grandinių skaičiaus – pavienė glikozaminoglikano grandinė (nestabilizuota HyA) suardoma hialuronidazių per 24 val. po susintetinimo. Stipriai stabilizuota, didelio molekulinio svorio HyA (xHyA) grandinė gali tarnauti kaip užpildas, kadangi jos degradacija trunka ilgiau nei 6 mėn. [108]. Odontologijoje naudojama vidutinės stabilizacijos laipsnio, butanediolio diglicidilo eterio pagalba stabilizuota xHyA, kuri aplikacijos vietoje geba išlikti stabili 4–6 sav. bei turi teigiamą poveikį minkštųjų ir kietųjų audinių regeneracijai [109, 110].

Klinikines HyA savybes nusako jos molekulinis svoris. Didelio molekulinio svorio HyA (> 1000000 Da) pasižymi imunosupresinėmis ir anti-angiogenezinėmis savybėmis, vidutinio molekulinio svorio HyA (nuo 2×10^4 iki 1000000 Da) – daro įtaką embriogenezei, žaizdų gijimui, regeneracijai, mažo molekulinio svorio HyA (nuo 6×10^3 iki 2×10^4) – skatina uždegiminius, angiogenezinius procesus ir genų ekspresiją. Odontologijoje paprastai naudojama vidutiniškai stabilizuota didelio molekulinio svorio hialurono rūgštis (xHyA) [111].

1.3.1. Ikiklinikiniai stabilizuotos hialurono rūgšties tyrimai

2018 m. Asparuhova M. ir bendraautoriai *in vitro* eksperimento metu tyrė xHyA poveikį žmogaus dantenų fibroblastų metabolinėms, proliferacinėms ir migracinėms funkcijoms [112]. Eksperimentiniais tyrimais buvo nustatyta, kad xHyA yra ne tik biosuderinama ir neturi neigiamo poveikio ląstelių gyvybingumui, priešingai, pastebėta, kad ji skatina šių ląstelių proliferaciją ir migraciją. Nustatyta, kad xHyA skatina indikatorių (COL 3A1, TGFß3),

atsakingų už gijimą be randų, sintezę, reguliuoja genų, koduojančių augimą, faktorius (PDGFB, FGF-2), prouždegiminių citokinų ekspresiją, inicijuoja uždegiminį ląstelių atsaką bei turi poveikį ekstraceliulinio matrikso remodeliacijai.

2020 m. Asparuhova M. ir bendraautoriai tyrė *in vitro* xHyA poveikį mezenchimos stromos ląstelėms ir pre-osteoblastams [106]. Tyrimo duomenys atskleidė, kad xHyA stipriai stimuliuoja osteoprogenitorinių ląstelių proliferaciją bei skatina genų, koduojančių kaulinio audinio matricos proteinus, ekspresiją.

xHyA poveikį cementoblastų migracijai, diferenciacijai, mineralizacijai *in vitro* eksperimente 2024 m. tyrė Hakki S. ir bendraautoriai. Tyrėjai nustatė, kad HyA turėjo teigiamą poveikį mineralizuotų audinių markerių ir cementoblastų specifinių genų ekspresijai, todėl xHyA tenka esminis vaidmuo cemento regeneracijos procese [113].

Histologinį xHyA naudojimo pamatą periodontologijoje paklojo Shirakata Y. ir bendraautoriai, tyrę jos panaudojimo galimybes gydant intrakaulinius defektus, furkacijų pažeidimus bei dantenų recesijas [114–116]. Histologinės analizės, nepaisant operacijos ar defekto tipo, atskleidė, kad ten, kur papildomai buvo aplikuojama xHyA, susidarė naujas periodonto raištis, naujas cementas ir naujas alveolinis kaulas. Kontrolinėse grupėse periodonto audiniai neregeneravo, gijimas vyko antriniu būdu, t. y., formuojantis ilgai epitelinei jungčiai.

1.3.2. Klinikiniai stabilizuotos hialurono rūgšties tyrimai

Ioro-Siciliano V. ir bendraautoriai atsitiktinės imties kontroliuojamame klinikiniame tyrime nagrinėjo papildomos xHyA aplikacijos poveikį klinikiniams rodikliams konservatyvaus periodontologinio gydmo metu ir lygino juos su klasikiniu podanteniniu instrumentavimu. Autoriai nustatė, kad nors PD sumažėjimas ir CAL padidėjimas tiriamojoje grupėje buvo didesnis, statistiškai reikšmingas skirtumas tarp grupių nustatytas nebuvo [117].

Priešingus rezultatus tokio paties dizaino tyrime gavo Olszewska-Czyz I. ir bedraautoriai [118]. Konkrečiai, statistiškai reikšmingas CAL padidėjimas ir BOP sumažėjimas nustatytas tiriamojoje grupėje, kurioje kartu su podanteniniu instrumentavimu buvo naudojama xHyA ($p < 0,05$).

Klinikiniai xHyA tyrimai pateikti 1.3.2.1 lentelėje.

1.3.2.1 lentelė. *Klinikiniai xHyA tyrimai ir gauti rezultatai*

Autorius, metai	**Tyrimo dizainas**	**Tiriamųjų skaičius kontrolinėje ir tiriamojoje grupėse (n)**	**Tyrimo trukmė**	**Gydymo protokolas kontrolinėje ir tiriamojoje grupėse**	**Vidutinis PD pokytis kontrolinėje ir tiriamojoje grupėse (mm (SD))**	**Vidutinis CAL pokytis kontrolinėje ir tiriamojoje grupėse (mm(SD))**	**Vidutinis BOP pokytis kontrolinėje ir tiriamojoje grupėse (proc. (SD))**	**Išvada**
Iorio-Siciliano et al., 2024 [117]	RCT, Paralelinės grupės	Kontrolė n = 19 Testas n = 19	6 mėn.	Kontrolė Ultragarsas + rankiniai instrumentai Testas Ultragarsas + rankiniai instrumentai + xHyA	Kontrolė 2,6 (1,1) Testas 2,7 (1,5)	Kontrolė 1,9 (1,6) Testas 2,8 (2,1)	_	Nėra statistiškai reikšmingo skirtumo tarp grupių PD (p = 0,435), CAL (p = 0,563)
Olszewska-Czyz et al., 2021 [118]	RCT, Paralelinės grupės	Kontrolė n = 50 Testas n = 50	3 mėn.	Kontrolė Podanteninis instrumentavimas Testas Podanteninis instrumentavimas + xHyA	Kontrolė 0,75 (1,57) Testas 1,25 (1,56)	Kontrolė 1,0 (1,81) Testas 2,37 (1,56)	Kontrolė 10,5 (27,63) Testas 20,5 (29,05)	Nėra statistiškai reikšmingo skirtumo PD sumažėjime tarp grupių (p = 0,7), statistiškai reikšmingi skirtumai tarp grupių CAL padidėjime (p < 0,001) ir BOP sumažėjime (p < 0,001) tiriamosios grupės naudai

BOP – kraujavimas po zondavimo; CAL – klinikinis audinių prisitvirtinimo lygis; p – reikšmingumo lygmuo; PD – kišenės zondavimo gylis; RCT – atsitiktinės imties kontroliuojamas klinikinis tyrimas, SD – standartinis nuokrypis.

2. TYRIMO METODIKA

Klinikinė tyrimo dalis buvo atlikta LSMU Dantų ir burnos ligų klinikoje ir Laboratorinės medicinos katedroje, histologinė tyrimo dalis atlikta Kagošimos universiteto Periodontologijos skyriuje, Japonijoje.

2.1. Tyrimo struktūra

Buvo atliktas 6 mėn. prospektyvus, aklas, paralelių grupių atsitiktinės imties kontroliuojamas klinikinis tyrimas. Tyrimas atliktas remiantis CONSORT gairėmis atsitiktinės imties kontroliuojamiems klinikiniams tyrimams [119]. Tyrimo protokolą patvirtino LSMU Kauno regioninis biomedicininių tyrimų etikos komitetas (Nr. BE-2-87). Prieš dalyvavimą tyrime visi dalyviai buvo informuoti apie tyrimo struktūrą ir tikslus. Iš visų tiriamųjų buvo gauta informuoto asmens sutikimo forma. Asmens duomenys (vardas, pavardė, gimimo data, lytis, medicininės sveikatos istorija) buvo koduojama ir neviešinama. Tyrimas buvo atliekamas nuo 2019 m. rugsėjo mėn. iki 2022 m. sausio mėn. Tyrimo protokolas papildomai buvo registruotas ClinicalTrials.gov, NCT04662216.

Histologinės tyrimo dalies protokolą patvirtino Kagošimos universiteto Gyvūnų mokslo centras (Nr. D22017, 2023 m., sausio 23 d.). Tyrimas atliktas remiantis ARRIVE gairėmis, skirtomis ikiklinikiniams tyrimams su gyvūnais [120].

2.1.1. Klinikinė tyrimo dalis

2.1.1.1. Tiriamasis kontingentas

Į tyrimą buvo įtraukti į LSMU Dantų ir burnos ligų kliniką periodontologiniam gydymuisi kreipęsi pacientai.

2.1.1.2. Tyrimo dalyvių / pacientų atrankos ir atmetimo kriterijai

Tinkamų tyrimui dalyvių atrankos kriterijai buvo šie:

- vyresni nei 18 metų vyrai ir moterys;
- pacientai, kuriems diagnozuotas II–III stadijos, A/B laipsnių generalizuotas periodontitas;
- burnoje buvo likę bent 20 dantų (neskaitant protinių);
- nenešiojo išimamų protezų;
- suprato tyrimo eigą ir galintys dalyvauti 6 mėn. trukmės tyrime ir kontroliniuose vizituose.

Dalyvių atmetimo kriterijai buvo šie:

- dalyvavo kituose klinikiniuose tyrimuose;
- buvo rūkantys;
- per pastaruosius 12 mėn. buvo atliktas periodontologinis gydymas;
- per pastaruosius 3 mėn. vartojo antibiotikus;
- gydymui buvo reikalinga antibiotikų profilaktika;
- buvo nėščios, žindančios moterys;
- turėjo alergiją NaOCl.

2.1.1.3. Imties dydžio skaičiavimas

Imties dydžio skaičiavimas buvo atliekamas naudojant SPSS/W 27,0 programinę įrangą („Statistical Package for the Social Sciences for Windows, Inc.“, Čikaga, IL, JAV). Imties skaičiavimas atliktas remiantis kito tyrimo rezultatais [121]. Apskaičiuota, kad, norint aptikti 1 mm PD skirtumą su 1 mm standartiniu nuokrypiu ir mažiausiai 0,8 tyrimo jėga (1-α), kai reikšmingumo lygis $\alpha = 0,05$, reikėtų 20 pacientų vienoje tiriamojoje grupėje. Norint apsidrausti nuo galimo pacientų atkryčio, imties dydis kiekvienoje grupėje buvo padidintas iki 24. Tyrimo jėgos apskaičiavimas tyrimo pabaigoje su esamu tiriamųjų skaičiumi nurodė 99,6 proc. tyrimo galią.

2.1.1.4. Klinikiniai matavimai

Klinikiniai matavimai buvo atliekami naudojant Williams periodontologinį zondą (LM 51 ES, LM-Dental™, Suomija). Kiekvienas dantis buvo matuotas šešiuose taškuose / srityse (mezio-bukaliniame, viduriniame-bukaliniame, disto-bukaliniame, mezio-oraliniame, viduriniame-oraliniame, disto-oraliniame) per tris skirtingus eksperimentinio gydymo laiko tarpsnius, t. y., tyrimo pradžioje (T0), po 3 mėn. (T1) ir po 6 mėn. (T2). Klinikiniais matavimais buvo vertinami šie parametrai:

- BOP – sričių, kraujuojančių 10 s po zondavimo, skaičius, išreikštas procentais; vertintas gydytų sričių BOP (kai PD ≥ 4 mm) ir visos burnos BOP.
- Apnašo indeksas (PI) – sričių, turinčių matomo apnašo, skaičius, išreikštas procentais; vertintas gydytų sričių PI (kai PD ≥ 4 mm) ir visos burnos PI.
- PD – atstumas nuo dantenų krašto iki išzonduojamos kišenės dugno, išreikštas milimetrais.
- REC – atstumas nuo dantenų krašto iki CEJ, išreikštas milimetrais.
- CAL – apskaičiuotas sudėjus PD ir REC vertes.

2.1.1.5. Mikrobiologinių mėginių ėmimas

Podanteninio apnašo mėginiai buvo renkami tyrimo pradžioje, prieš pradedant gydymo procedūras, ir praėjus 3 ir 6 mėn. po gydymo. Mikrobiologiniai ėminiai buvo renkami iš kiekviename ketvirtyje esančios giliausios periodontologinės kišenės. Procedūrą atliko tyrėjas, nesusijęs su gydymo procedūromis. Po kruopštaus viršdanteninio apnašo ir akmenų pašalinimo ir danties paviršiaus nuvalymo steriliu vatos tamponėliu dantis buvo izoliuojamas ir nusausinamas vatos rulonėliais. Į periodontologinę kišenę buvo įvedamas sterilus endodontinis sauskaištis ISO # 30 (Dentsply Sirona; Bensheim, Vokietija) ir laikomas joje 20 s. Kiekvieno paciento keturi sauskaiščiai buvo patalpinti į sterilų užkoduotą Eppendorf mėgintuvėlį ir transportuoti į LSMU Laboratorinės medicinos katedrą. Mėginiai buvo šaldomi -20^{o} C temperatūroje iki tolimenės analizės (vienai dienai), po to – -80^{o} C temperatūroje iki galutinės mikrobiologinės analizės (ne daugiau nei 30 d.). Molekulinė podanteninio apnašo analizė atlikta trijų etapų metu:

- DNR išskyrimas;
- Multipleksinė amplifikacija su biotiniluotais praimeriais;
- Atbulinė hibridizacija.

2.1.1.5.1. DNR išskyrimas

DNR išskyrimas atliktas naudojant DNR išgryninimo iš tepinėlių rinkinį (Swab, 0517 versija, A&A Biotechnology; Gdynia, Lenkija). Į Eppendorf mėgintuvėlius su podanteninio apnašo mėginiais pilta 700 µl lizės tirpalo ir 20 µl K proteinazės. Mėgintuvėlio turinys kruopščiai išmaišomas, centrifuguojamas ir inkubuojamas 20 min. 37 °C temperatūroje. Po inkubacijos mėginiai vėl maišomi, centrifuguojami, ir gautas skystis išpilstomas į sukimo kolonas, kurios centrifuguojamos 1 min. 12000 apsukų/min greičiu pirmą ir 2 min. antrą kartą. Išcentrifuguotų kolonų turinys perkeliamas į naujus 1,5 ml mėgintuvėlius, į kuriuos buvo įpilama 150 µl 75 °C elutavimo buferio. Visas turinys buvo inkubuojamas 3 min. kambario temperatūroje, po to centrifuguojamas 1 min 12000 apsukų/min greičiu. Gauti DNR mėginiai toliau buvo laikomi –80 °C temperatūroje iki galutinės analizės.

2.1.1.5.2. Multipleksinė DNR amplifikacija

Šiame tyrime DNR mėginiai analizuoti panaudojant molekulinius genetinius tyrimus, skirtus identifikuoti penkias periopatogeninių bakterijų rūšis (*A.a, P.g, P.i, T.f, T.d*) (micro-IDent VER 2,0 Hain Lifescience; Nehren, Vokietija). Prieš testuojant DNR buvo ruošiamas amplifikacijai skirtas mišinys (*master mix*), į kurio sudėtį įeina visi komponentai reikalingi polimerazės

grandinės reakcijai (PCR) atlikti. Toliau, 45 µl *master mix* buvo sumaišoma su 5 µl DNR mėginio arba negatyvia kontrole (molekulinės biologijos vandeniu). Negatyvi kontrolė naudota su kiekviena 24 mėginių partija. PCR buvo atliekama šiluminiame cikleryje pagal gamintojo rekomendacijas. Amplifikacijos produktai buvo laikomi 2–4 °C temperatūroje iki tolimesnės analizės.

2.1.1.5.3. Atvirkštinė hibridizacija

Pagal gamintojo reikalavimus, prieš pradedant procedūrą, reagentai buvo šildomi iki 45 °C temperatūros ir paruošiami reikiami skiestiniai tirpalai. Tarpusavyje sumaišyti 20 µl denatūruoto ir 20 µl amplifikuoto DNR mėginio tirpalai 5 min. buvo inkubuojami kambario temperatūroje. Vėliau buvo pridedama 5 ml hibridizacijos buferio ir į kiekvieną šulinėlį, turintį denatūruotos DNR, talpinamos testo juostelės. Po inkubacijos hibridizacijos buferis buvo aspiruojamas ir į kiekvieną šulinėlį įpilama 1 ml griežtojo tirpalo. Šulinėliai inkubuojami 15 min. 45 °C temperatūroje vandeninėje purtyklėje. Tuomet griežtasis tirpalas buvo pašalinamas, kiekviena testo juostelė plaunama 1 ml skalavimo tirpalu. Po to, į kiekvieną šulinėlį buvo įpilta 1 ml praskiesto konjugatinio tirpalo ir inkubuota 30 min. kambario temperatūroje vandeninėje purtyklėje. Kito etapo metu konjugatas šalinamas, kiekviena juostelė plaunama 1 min. 3 kartus: 2 kartus su skalavimo tirpalu, 1 kartą su distiliuotu vandeniu. Toliau į kiekvieną šulinėlį buvo pilama 1 ml praskiesto substrato tirpalo ir inkubuojama 15 min. Kai substrato juostelės tapdavo aiškiai matomos, jos buvo plaunamos distiliuotu vandeniu, nusausinamos dvejais sluoksniais absorbuojančio popieriaus ir dedamos ant vertinimo lapo, apsaugant jas nuo tiesioginės šviesos.

2.1.1.5.4. Mikrobiologinių rezultatų interpretacija ir vertinimas

Pirmiausia buvo atliekama testo juostelių patikrinimo procedūra stebint tris (konjugato, hibridizacijos ir amplifikacijos) kontrolines juosteles. Įsitikinus, kad visos juostelės išvystytos teisingai, specifinės juostelės, skirtos penkioms bakterijų rūšims identifikuoti, tirtos pusiau kiekybiniu vertinimu. Priklausomai nuo išsivysčiusio šviesos intensyvumo, kiekvienai juostelei priskirtas balas nuo 0 iki 4. Skaičiai 0, 1, 2, 3 ir 4 atitinka kolonijas formuojančių vienetų skaičių (2.1.1.5.4.1 lentelė).

2.1.1.5.4.1 lentelė. *Pusiau kiekybinis tyrimo rezultatų vertinimas*

Bakterijų rūšys	Testo juostelių spalvos intensyvumo balas, CFU/ml				
	0	**1**	**2**	**3**	**4**
Aggregatibacter actinomycetemcomitans	$< 10^3$	10^3	$< 10^4$	$< 10^5$	$> 10^6$
Porphyromonas gingivalis	$< 10^4$	10^4	$< 10^5$	$< 10^6$	$> 10^7$
Prevotella intermedia					
Tannerella forsythia					
Treponema denticola					

CFU – koloniją formuojantys vienetai, ml – mililitras.

2.1.1.6. Tyrimo baigties kintamieji

Klinikinei analizei atlikti periodontologinės kišenės buvo suskirstytos į dvi kategorijas pagal PD – vidutines (4–6 mm) ir gilias (≥ 7mm). Pirminis baigties kintamasis buvo PD sumažėjimo pokytis tarp tyrimo pradžios ir praėjus 6 mėn. po gydymo vidutinėje kišenių gylio kategorijoje. Antriniai nagrinėjami klinikiniai baigties rodikliai buvo PD pokytis gilioje kategorijoje, CAL pokytis tarp gydymo pradžios ir 6 mėn. po gydymo vidutinių ir gilių kišenių kategorijose. BOP, PI pokyčiai buvo vertinti atskirai – gydytose srityse (visos kišenės, kurių PD ≥ 4 mm ir kurios kraujavo po zondavimo) ir bendrai visoje burnoje.

Pirminis mikrobiologinio tyrimo baigties rodiklis – *A.a, P.g, P.i, T.f* ir *T.d* aptikimo dažnis praėjus 6 mėn. po gydymo. Antrinis baigties rodiklis – bendras minėtų periopatogenų skaičiaus pokytis praėjus 6 mėn. po gydymo.

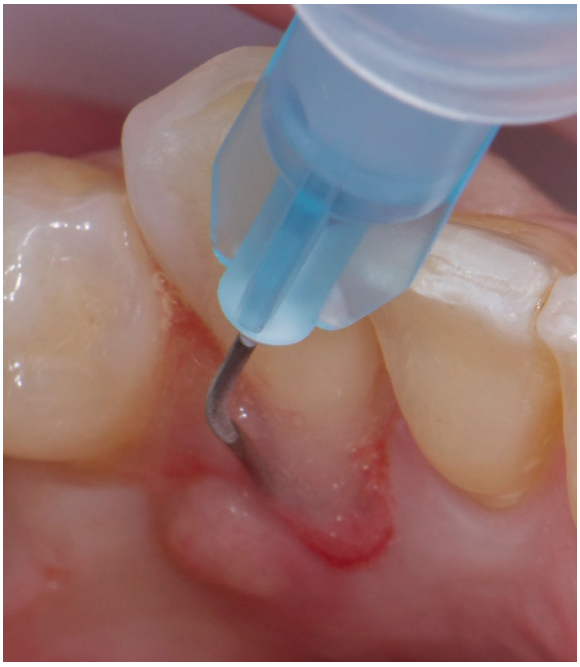
2.1.1.7. Periodontologinis gydymas

Pradiniai klinikiniai rodikliai ir podanteninio apnašo mėginių paėmimas vyko 2 sav. prieš pagrindinę gydymo procedūrą. Šio vizito metu visiems į tyrimą įtrauktiems pacientams buvo atliekamas profesionalus viršdanteninio apnašo šalinimas, duodamos tikslios asmens burnos higienos instrukcijos. Pacientai buvo aprūpinti dantų šepetėliais (CS 5460, Curaprox, Curaden, Šveicarija), tinkamo dydžio tarpdančių šepetėliais (TePe, TePe Mundhugienprodukten, Švedija) ir dantų pasta (Elmex Enamel Protection, Gaba GmbH, Vokietija). Burnos higienos instruktažas ir pacientų motyvacija buvo atliekama kiekvieno kontrolinio vizito metu. Kontrolinių vizitų metu (3 ir 6 mėn. po gydymo) jokios papildomos gydymo procedūros atliekamos nebuvo.

Po 2 sav., taikant vietinę nejautrą, kontrolinės grupės pacientams buvo atliktas kruopštus podanteninis instrumentavimas ultragarsu (Satelec/Acteon Suprasson Newtron ultragasiniu skaleriu) ir rankiniais instrumentais

(LM Sharp Diamond 1/2, 7/8, 11/12, 13/14 mini Gracey ir Gracey kiuretėmis, LM Dental™, Suomija). Įsitikinus, kad podanteninis instrumentavimas baigtas, dantų paviršiai buvo poliruojami mažai abrazyvia dantų poliravimo pasta (Lunos Super Soft, RDA < 5, Dürr Dental, Vokietija). Vidutinis vieno paciento podanteninio instrumentavimo vizitas truko apytikriai 3,5 val.

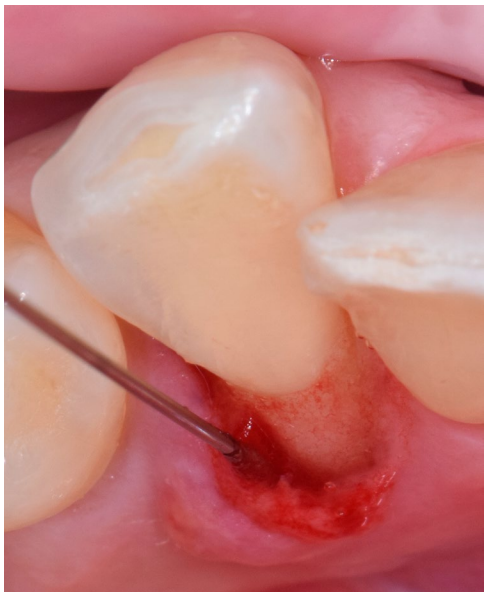
Tiriamosios grupės pacientams į visas periodontologines kišenes, kurių PD buvo ≥ 4 mm ir kurios kraujavo po zondavimo, 60 sekundžių prieš pradedant podanteninį instrumentavimą, buka adata buvo įvestas NaOCl/amino rūgščių gelis (Perisolv®, Regedent AG, Ciurichas, Šveicarija). Podanteninis instrumentavimas atliktas tais pačiais ultragarsiniais ir rankiniais instrumentais kaip ir kontrolinėje grupėje. Perisolv® gelio aplikacija į kišenę buvo kartojama 2–3 kartus tol, kol buvo jaučiama, kad podanteninis instrumentavimas yra pakankamas ir šaknų paviršiai švarūs (2.1.1.7.1 pav.).



2.1.1.7.1 pav. *Perisolv® įvedimas į periodontologinę kišenę prieš podanteninį instrumentavimą*

Visos gydymo procedūros buvo atliekamos su didinamaisiais akiniais (4,5X – Ergo Advanced, Univet, Rezzato BS, Italija). Podanteninis instrumentavimas vertintinas pakankamu tuomet, kai zonduojant zondu ieškikliu (Explorer-Periodontal Probe, 8-520B, LM Dental™, Suomija) buvo jaučiamas švelnus ir lygus šaknų paviršius.

Baigus podanteninį instrumentavimą ir nupoliravus dantų vainikų paviršius, į visas gydytas kišenes buka adata buvo suvedama xHyA (susidedanti iš stabilizuotos (1000 kDA HA monomerų) ir nestabilizuotos (2500 kDA) HA, santykiu 8:1) (Hyadent BG, Regedent AG, Ciurichas, Šveicarija) (2.1.1.7.2 pav.).



2.1.1.7.2 pav. *Hyadent BG® įvedimas į periodontologinę kišenę po podanteninio instrumentavimo*

2.1.1.8. Maskavimas

Klinikinius matavimus, mikrobiologinio apnašo paėmimą ir pradinę burnos higieną vykdė sukalibruotas tyrėjas, nesusijęs su gydymo procedūromis (U.M.D.). Visi matavimai buvo atliekami be prieigos prie ankstenių matavimų, norint išvengti šališkumo. Gydymo procedūras atliko patyręs tyrėjas (E.R.). Trečiasis tyrėjas (L.P.), nieko nežinodamas apie gydymo procedūras, atliko koduotų duomenų mikrobiologinę analizę. Ketvirtasis tyrėjas, nesusijęs su gydymo procedūromis, atliko koduotų duomenų statistinę analizę (I.N.).

Tyrimo dalyviai / pacientai nežinojo, kuriai gydymo grupei jie buvo priskirti. Gydymo procedūros buvo atliekamos uždengus paciento veidą steriliomis veido uždangomis, kad pacientas neturėtų galimybės stebėti procedūrą.

2.1.1.9. Atsitiktinė atranka ir pasiskirstymo slėpimas

48 tyrimo dalyviai atsitiktine tvarka buvo priskirti į tiriamąją ir kontrolinę grupes. Pirmiausia buvo sukurta kompiuterio sugeneruota randomizacijos (atsitiktinės atrankos) lentelė. Kiekvienam pacientui buvo priskirtas dalyvavimo tyrime numeris (nuo 1 iki 48) ir sukurti du atsitiktinių skaičių rinkiniai (24 kontrolinei ir 24 tiriamajai grupėms), kurie buvo patalpinti į du atskirus, nepermatomus vokus. Pasiskirstymo nuslėpimas buvo atliekamas pacientui atsitiktine tvarka pasirenkant voką ir ištraukiant numerį. Šį etapą atliko tyrėjas, nesusijęs su gydymo procedūromis ar klinikiniais matavimais (F.Y.).

2.1.1.10. Kalibracija

Kalibracijos sesijoje dalyvavo penki nedalyvaujantys tyrime pacientai, kuriems buvo diagnozuotas II–III stadijos, A/B laipsnio periodontitas [1]. Kalibruotas, klinikinius matavimus atlikęs tyrėjas, nuo kurio buvo nuslėptos gydymo procedūros, turėjo išmatuoti kiekvieno paciento PD, REC, CAL PI ir BOP šešiuose taškuose kiekviename dantyje dviejų skirtingų vizitų, vykusių 48 val. intervalu, metu. Kalibracija buvo laikoma tinkama, kai pradiniai matavimai su matavimais po 48 val. sutapo > 90 proc. lygmenyje.

2.1.1.11. Statistinė analizė

Statistinė analizė buvo atliekama naudojant SPSS/W 27,0 programinę įrangą („Statistical Package for the Social Sciences for Windows, Inc.“, Čikaga, IL, JAV). Duomenų analizė buvo atliekama naudojant pacientą kaip statistinį vienetą.

Klinikiniai rodikliai

Vidutinės visų klinikinių rodiklių vertės buvo skaičiuojamos atskirai kiekvienam pacientui kiekvieno vizito metu. Konkrečiai, vidutinis PD ir CAL vidutinių kišenių kategorijoje buvo gaunamas apskaičiuojant kiekvieno paciento PD ir CAL vidurkių reikšmes T0, T1 ir T2 tyrimo etapuose. Tokiu pačiu principu buvo skaičiuojamas kiekvieno paciento PD ir CAL vidurkis gilių kišenių kategorijoje T0, T1 ir T2 tyrimo etapais. Kiekvieno paciento BOP ir PI buvo apskaičiuojamas procentais pagal kraujuojančias sritis ir matomą apnašą vidutinio sunkumo ir gilių kišenių kategorijose, gydytose srityse (visos kišenės, kurių PD ≥ 4 mm) ir bendrai visoje burnoje.

Shapiro-Wilk testas buvo naudojamas norint patikrinti, ar klinikiniai periodonto rodikliai atitiko normalųjį pasiskirstymą. Jei duomenys atitiko normalųjį pasiskirstymą, norint palyginti periodontologinių rodiklių reikšmes prieš ir po gydymo grupių viduje, buvo atliekamas porinių imčių *t* testas. Jei duo-

menys normalaus pasiskirtstymo neatitiko, gydymo reikšmių pokytis prieš ir po gydymo grupių viduje buvo atliekamas Wilcoxon rangų sumų testas priklausomoms imtims. Palyginimai tarp grupių buvo atliekami nepriklausomų imčių *t* testu (jei duomenys atitiko normalųjį pasiskirstymą) arba Mann-Whitney testu (jei duomenys neatitiko normalaus pasiskirstymo). Testais buvo nustatytas 0,05 reikšmingumo lygmuo.

Mikrobiologiniai rodikliai

Periopatogenų aptikimo dažnio (0 – neaptikta, 1 – aptikta) skirtumai tarp tiriamosios ir kontrolinės grupių pradiniame tyrimo etape bei po 3 ir 6 mėn. buvo analizuojami naudojant χ^2 testą. Pokyčiai grupių viduje vertinti McNemar testu.

Bendro periopatogenų skaičiaus pokyčio įvertinimui rezultatai buvo suskirstyti į kategorijas pagal balus: 0 (periopatogenų neaptinkama), 1, 2, 3 ir 4 (2.1.1.5.4.1 lentelė). Kiekybinis periopatogenų pokytis grupės viduje pradiniame etape ir po 3 ir 6 mėn. buvo analizuojamas atliekant Wilcoxon rangų sumų testą priklausomoms imtims. Palyginimas tarp grupių kiekviename tyrimo etape buvo analizuojamas atliekant Mann-Whitney testą; nustatytas 0,05 reikšmingumo lygmuo.

2.1.2. Histologinė tyrimo dalis

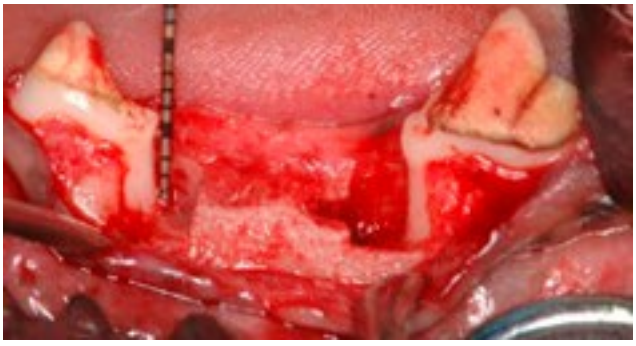
2.1.2.1. Gyvūnai

Tyrime dalyvavo keturi sveiki Biglio veislės patinai, kurių amžius buvo 26–38 mėn., svoris – 9–15 kg. Tyrimo metu gyvūnų lokacija ir kasdieninis stebėjimas vyko Shin Nippon eksperimentų su gyvūnais laboratorijoje, Kagošimoje, Japonijoje. Kiekvienas gyvūnas buvo laikomas atskirame narve, 20–26 °C temperatūroje, 30–70 proc. santykinėje drėgmėje, 12 val. šviesos / tamsos režime. Apytikriai 300 g kieto maisto ir neribotas kiekis vandens kasdien buvo skirtas kiekvienam tyrimo gyvūnui.

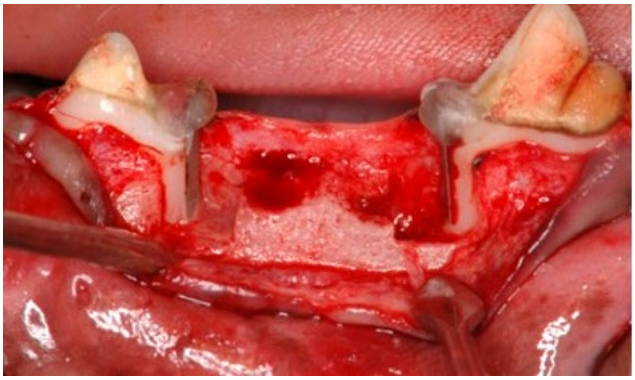
2.1.2.2. Eksperimentinis periodontitas

Vienas patyręs chirurgas (Yo.S.) atliko visas chirurgines procedūras, taikant bendrinę ar vietinę nejautrą. Prieš chirurginę intervenciją į raumenis buvo leidžiami antibiotikai (0,05 ml/kg; Mycillin Sol Meiji veterinarinei panaudai, Meiji Seika Pharma Co. LTD, Tokijas, Japonija). Bendrinė nejautra buvo sukeliama į raumenis suleidžiant medetomidino hidrochlorido (Domitor®, 0,08 ml/kg; Orion Corporation, Espoo, Suomija), 0,08 ml/kg midazolamo (Dormicum®, IM; Maruichi Pharmaceutical, Osaka, Japonija) ir 0,02 ml/kg butopranolio tartrato (Vetorphale® 5 mg, Meiji Seika Pharma, To-

kijas, Japonija). Po sedacijos, anestezija buvo palaikoma sevoflurano inhaliacija (0,5 proc.–5,0 proc., Mylan Pharma Co., Ltd. Osaka, Japonija) ir nitrogeno/deguonies (santykiu 2:1) mišiniu. Vietinė nejautra atlikta lidokaino HCl/epinefrinu (2 proc., 1:80.000; Xylocaine; Fujisawa Inc., Osaka, Japonija). Pirmiausia atsargiai bilateraliai buvo pašalinti apatinio žandikaulio pirmieji ir tretieji premoliarai. Po 8 sav. gijimo periodo, medialiai ties apatinio žandikaulio ketvirtaisiais premoliarais (P4) ir distaliai ties apatinio žandikaulio antraisiais moliarais (P2) bilateraliai buvo sukurti dviejų sienų (5 mm gylio ir 5 mm pločio) intrakauliniai defektai (keturi defektai kiekvienam gyvūnui). Po pilno storio gleivinės-antkaulio lopo atkėlimo, defektai buvo sukuriami naudojant deimantinius grąžtus (2.1.2.2.1 pav.). Cementas buvo pašalintas Gracey kiuretėmis. Atskaitos taškai buvo pažymėti apvaliu deimantiniu grąžtu ant šaknies paviršiaus žymint CEJ ir danties vainiką. Norint išvegti spontaninio gijimo ir užtikrinti apnašo kaupimąsi, kompozitu prie danties paviršiaus, intrakauliniuose defektuose buvo įtvirtinamos metalinės juostelės (2.1.2.2.2 pav.). Tuomet gleivinės-antkaulio lopas buvo repozicionuojamas į pradinę padėtį, viskas susiūta 4-00 šilko siūlais. Pooperacinio skausmo malšinimui buvo skiriamas ketoprofenas (Capisten IM 50 mg, 2mg/lg, 0,1 ml/kg; Kissei Pharmaceutical Co. Ltd, Masumoto, Japonija) ir antibiotikai (Mycillin Sol) kasdien 2 d. po operacijos.

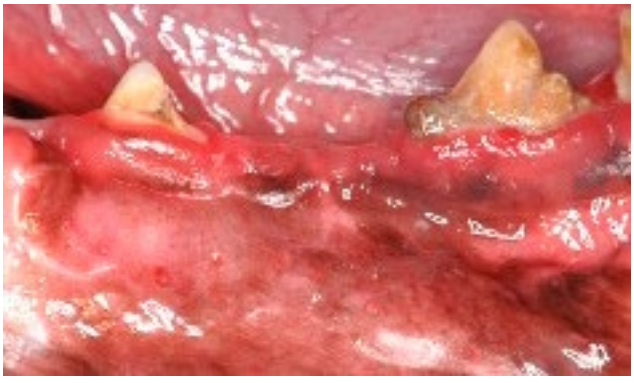


2.1.2.2.1 pav. *Chirurginiu būdu sukurti dvisieniai intrakauliniai defektai*

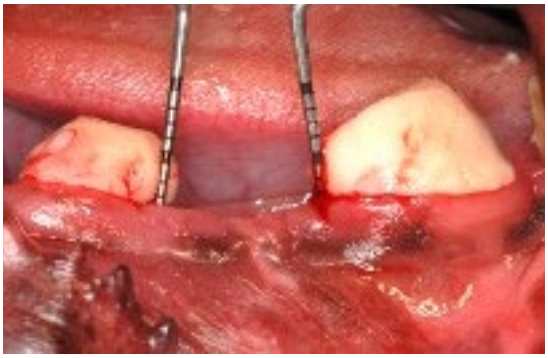


2.1.2.2.2 pav. *Metalinių juostelių fiksavimas prie šaknų paviršių kompozitu*

Iškart po operacijos buvo atliekamos intraoralinės, periapikalinės rentgeno nuotraukos ties P2 ir P4 sritimis. Siūlių šalinimas buvo atliekamas praėjus 14 d. po operacijos. Norint užtikrinti apnašo kaupimąsi, gyvūnai buvo šeriami minkštu maistu (2.1.2.2.3 pav.). Po 4 sav. buvo atliekamos pakartotinės periapikalinės rentgeno nuotraukos ir, patvirtinus alveolinio kaulo netekimą, šalinamos metalinės juostelės neatkeliant gleivinės-antkaulio lopo. Klinikinių rodiklių matavimui prie dantų buvo tvirtinami akriliniai stentai (2.1.2.2.4 pav.).



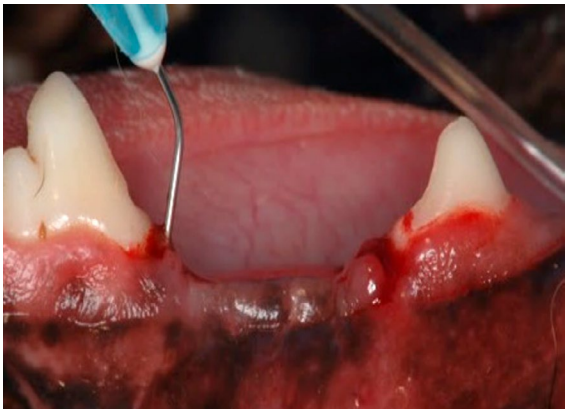
2.1.2.2.3 pav. *Apnašo kaupimasis praėjus keturioms savaitėms*



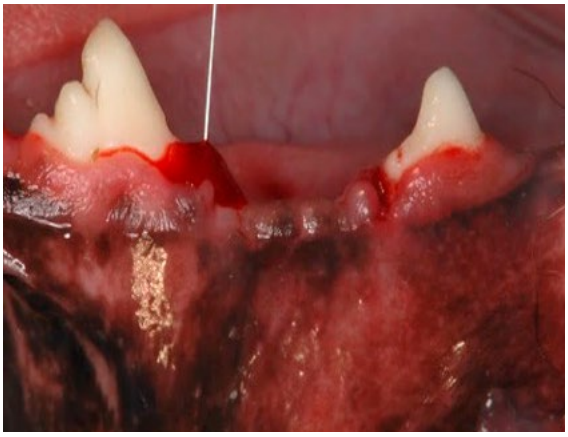
2.1.2.2.4 pav. *Klinikinių rodiklių matavimas tyrimo pradžioje*

2.1.2.3. Periodontologinis gydymas

Apnašo kontrolei 2 sav. prieš gydymo procedūrą burna buvo skalaujama 2 proc. chlorheksidino digliukonato tirpalu 3 kartus per savaitę. Tiriamoji ir kontrolinės sritys kiekvienoje burnoje priešingose žandikaulio pusėse buvo nusprendžiamos metant monetą. Prieš pradedant podanteninį instrumentavimą, pirmiausia buvo atliekamas viršdanteninio apnašo šalinimas. Kontrolinėje pusėje buvo atliekamas podanteninis šaknų paviršių valymas ultragarsu (ENAC 10 WA, Osada, Tokijas, Japonija) ir rankiniais instrumentais (LM Sharp Diamond Mini gracey 11/12, 13/14, LM Dental ™, Suomija). Tiriamojoje pusėje 30 s. prieš podanteninį instrumentavimą į kišenes buka adata buvo aplikuojama NaOCl/amino rūgščių gelio (Perisolv®, Regedent AG, Ciurichas, Šveicarija). (2.1.2.3.1 pav.). Podanteninis instrumentavimas atliktas taip pat, kaip ir kontrolinėje pusėje – ultragarsu ir rankiniais instrumentais. Valytos sritys iriguotos fiziologiniu tirpalu ir į kišenes suvesta xHyA (susidedanti iš stabilizuotos (1000 kDA HA monomerų) ir nestabilizuotos (2500 kDA) HA, santykiu 8:1) (Hyadent BG, Regedent AG, Ciurichas, Šveicarija) (2.1.2.3.2 pav.). Po gydymo nebuvo skiriami antibiotikai ar analgetikai, buvo grįžta prie kieto ėdalo. Burnos ertmės skalavimo režimas buvo toks pats, kaip ir prieš operaciją.



2.1.2.3.1 pav. *NaOCl/amino rūgščių gelio įvedimas į periodontologinę kišenę prieš podanteninį instrumentavimą*



2.1.2.3.2 pav. *xHyA gelio įvedimas į periodontologinę kišenę po podanteninio instrumentavimo*

2.1.2.4. Histologinis paruošimas

Praėjus 8 sav. po periodontologinio gydymo, atlikus intraoralines rentgeno nuotraukas, gyvūnai buvo eutanuoti perdozuojant natrio tiopentalio.

Dantys buvo pašalinti kartu su juos supančiais minkštaisiais ir kietaisiais audiniais. Audinių blokai fiksuoti 10 proc. buferiniame formalino tirpale, supjaustyti pagal intraoralines rentgenografijas ir atskaitos taškus, nuskalauti fosfatiniu buferiniu tirpalu. Mėginiai buvo dekalcifikuojami, dehidratuojami ir fiksuojami parafine. Tuomet meziodistalinėje plokštumoje paruoštos serijinės 6 µm storio sekcijos, kurios buvo dažomos hematoksilinu ir eozinu.

2.1.2.5. Histomorfometrinė analizė

Visi mėginiai buvo analizuojami naudojant šviesinį mikroskopą (BX51; Olympus Corp., Tokijas, Japonija) su kompiuterizuota vaizdo sistema (WinROOF2015; Mitani Corporation, Tokijas, Japonija). Histomorfometrinei analizei iš kiekvieno dvisienio defekto centrinės srities buvo pasirinkti trys pjūviai, tarp kurių buvo maždaug 90 μm tarpai, nustatyti pagal šaknies kanalo ilgį ir atskaitos taškus. Kiekvieno histomorfometrinio parametro vidutinė reikšmė buvo skaičiuojama kiekvienai sričiai atskirai. Siekant užtikrinti intra-ekspertinį atkartojamumą, 16 pjūvių iš visų vietų buvo vertinami vieno nešališko eksperto 48 val. skirtumu. Eksperto (T.I.) kalibracija buvo priimta, kai sutapo > 90 proc. matavimų. Matuoti šie parametrai:

- Defekto aukštis – atstumas tarp apikalinio šaknies ploštumos taško iki CEJ;
- JE ilgis – atstumas tarp JE apikalinės dalies iki CEJ;
- CT (be šaknies cemento) – atstumas tarp JE apikalinės dalies iki naujai susiformavusio cemento vainikinės dalies;
- Naujo kaulo ilgis – atstumas tarp naujai susiformavusio kaulo vainikinės iki šakninės dalių;
- Naujo kaulo sritis – naujai susiformavęs trabekulinis kaulas (5×5 plokštumoje, atitinkančioje defekto dydį);
- Naujo cemento ilgis – naujai susiformavusio cemento atstumas tarp vainikinės ir apikalinės apnuogintos šaknies sričių;
- Naujos jungties ilgis – linijinis šaknies, padengtos nauju cementu šalia naujai susiformavusio kaulo, ilgis su funkcionaliai orientuotomis kolageno skaidulomis.

2.1.2.6. Klinikinis vertinimas

Periodontolginiu zondu (UNC 15 Hu-Friedy, Čikaga, Jungtinės Valstijos) viename taške, vienam dančiui tyrimo pradžioje ir 6 sav. po gydymo buvo vertinti šie klinikiniai rodikliai:

- PD;
- CAL;
- BOP.

2.1.2.7. Statistinė analizė

Pirminis tyrimo baigties kintamasis – naujos jungties ilgis, išmatuotas abejoms tyrimo grupėms po 8 sav. Dėl etinių sumetimų pasirinkta absoliučiai minimali tyrimo, kuriame gyvūnas buvo pasirinktas statistiniu vienetu, imtis (4 gyvūnai). Kiekvieno histomorfometrinio parametro vidurkiai ir standarti-

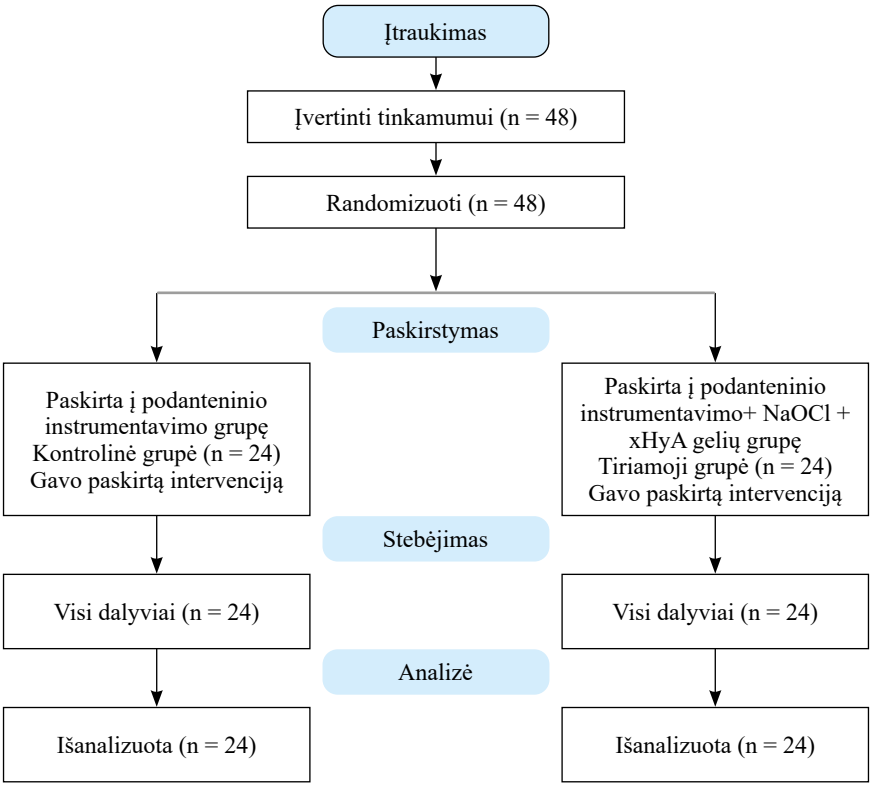
niai nuokrypiai skaičiuoti kiekvienai gydymo grupei atskirai. Mann-Whitney U testas naudotas palyginti histomorfometriniams rodikliams tarp tiriamosios ir kontrolinės grupių. Nustatyta statistiškai reikšminga $p < 0,05$ reikšmė. Statistinė analizė buvo atliekama naudojant Bell-Curve for Excel, Social Survey Research, Tokijas, Japonija, statistinę programinę įrangą.

3. REZULTATAI

3.1. Klinikiniai tyrimo tezultatai

3.1.1. Dalyvių srautas

Visi 48 pacientai baigė dalyvavimą 6 mėn. trukmės tyrime. Kiekvieną gydymo grupę (podanteninio instrumentavimo ir podanteninio instrumentavimo + NaOCl/amino rūgščių + xHyA) sudarė 24 atsitiktine tvarka į grupes priskirti pacientai. Tyrimo eiga yra pavaizduota CONSORT srauto diagramoje (3.1.1.1. pav.). Visų, į tyrimą įtrauktų, pacientų gijimas vyko be komplikacijų, nebuvo registruota pašalinių NaOCl/amino rūgščių ar xHyA gelių poveikių.



3.1.1.1 pav. *CONSORT pacientų srauto diagrama*

3.1.2. Pradinės charakteristikos

Klinikinių ir demografinių 48 dalyvių pradinių charakteristikų duomenys (vidurkiai ir standartiniai nuokrypiai) pateikti 3.1.2.1. lentelėje.

Pradinio tyrimo rezultatai parodė, kad abi tiriamosios grupės turėjo panašias charakteristikas pagal PD, CAL, BOP ir visos burnos BOP, išskyrus PI ir visos burnos PI.

3.1.2.1 lentelė. *Klinikinės ir demografinės visų tiriamųjų charakteristikos tyrimo pradžioje*

	Kontrolinė grupė (n = 24)	**Tiriamoji grupė (n = 24)**	***p* reikšmė**
Amžius (metai)	49,3 (11,2)	47,3 (10,7)	0,53[a], n.s.
Lytis, n (proc.) vyrai moterys	 7 (29,2) 17 (70,8)	 6 (25) 18 (75)	0,745[b], n.s.
Periodontito stadija, n (proc.) II stadija III stadija	 16 (66,7) 8 (33,3)	 17 (70,8) 7 (29,2)	0,134[b], n.s.
PD (mm)	5,3 (0,6)	5,2 (0,4)	0,592[c], n.s.
CAL (mm)	5,5 (0,5)	5,6 (0,6)	0,546[c], n.s.
PI (proc.)	38,8 (26)	60,6 (10,9)	**0,002**[c]
BOP (proc.)	81,8 (16,2)	83,2 (15,5)	0,687[c], n.s.
FMPI (proc.)	35,7 (23,7)	52,9 (11,4)	**0,003**[c]
FMBOP (proc.)	68,9 (20,3)	76,5 (18,2)	0,184[c], n.s.

BOP – kraujavimas po zondavimo; CAL – klinikinis audinių prisitvirtinimas; FMBOP – visos burnos kraujavimo indeksas; FMPI – visos burnos apnašo indeksas; *p* – reikšmingumo lygmuo; PD – kišenių zondavimo gylis; PI – apnašo indeksas.
n.s. – nėra statistiškai reikšmingo skirtumo tarp grupių;
[a] nepriklausomų imčių *t* testas;
[b] Fisher's tikslusis testas 2×2 lentelei, lytis pagal grupę (kontrolinė, tiriamoji);
c Mann-Whitney U testas dviems nepriklausomoms imtims.

3.1.3. Poveikis klinikiniams rodikliams

3.1.3.1. Kišenių zondavimo gylis

PD pokytis kontrolinėje ir tiriamojoje grupėse buvo vertintas vidutinių (4–6 mm) ir gilių (≥ 7 mm) kišenių kategorijose (3.1.3.1.1 lentelė).

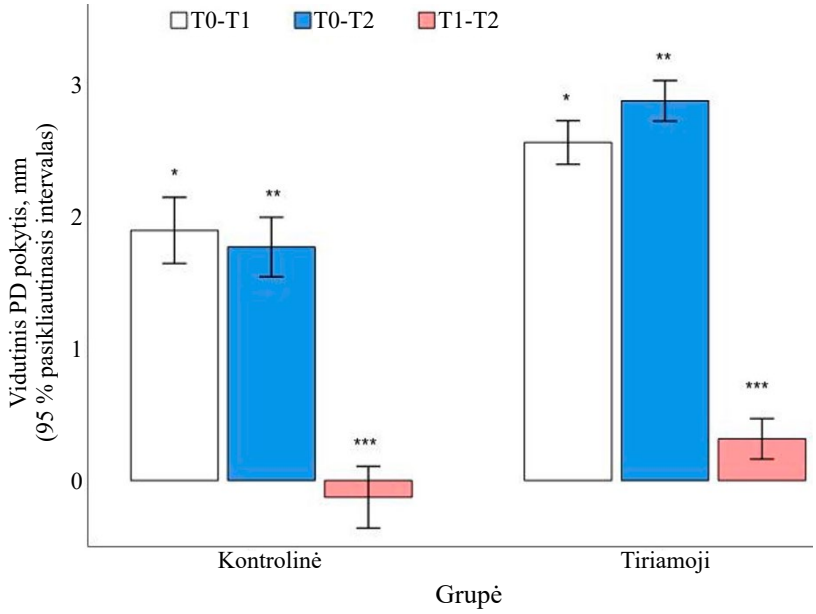
Vidutinio gylio kišenių kategorijoje pradinės vertės neatskleidė statistiškai reikšmingo skirtumo tarp kontrolinės ir tiriamosios grupių (atitinkamai 4,8 (0,2) mm ir 4,7 (0,2 mm), $p = 0,417$). Nors abi grupės parodė statistiškai reikšmingą pagerėjimą po 3 ir 6 mėn., palyginus su pradinėmis vertėmis ($p < 0,001$), statistiškai reikšmingai didesni sumažėjimai buvo stebimi tiriamojoje grupėje abiem laikotarpiais, palyginus su kontroline ($p < 0,001$)

(3.1.3.1.1 lentelė). PD pokytis stebėtas tarp 3 ir 6 mėn. statistiškai reikšmingai skyrėsi tarp grupių tiriamosios grupės naudai (p = 0,002) (3.1.3.1.1 pav.).

3.1.3.1.1 lentelė. *Vidutinis PD (vidurkis(standartinis nuokrypis)) vidutinio gylio (4–6 mm) ir gilių (≥ 7 mm) kišenių kategorijose kontrolinėje ir tiriamojoje grupėse skirtingais tyrimo etapais*

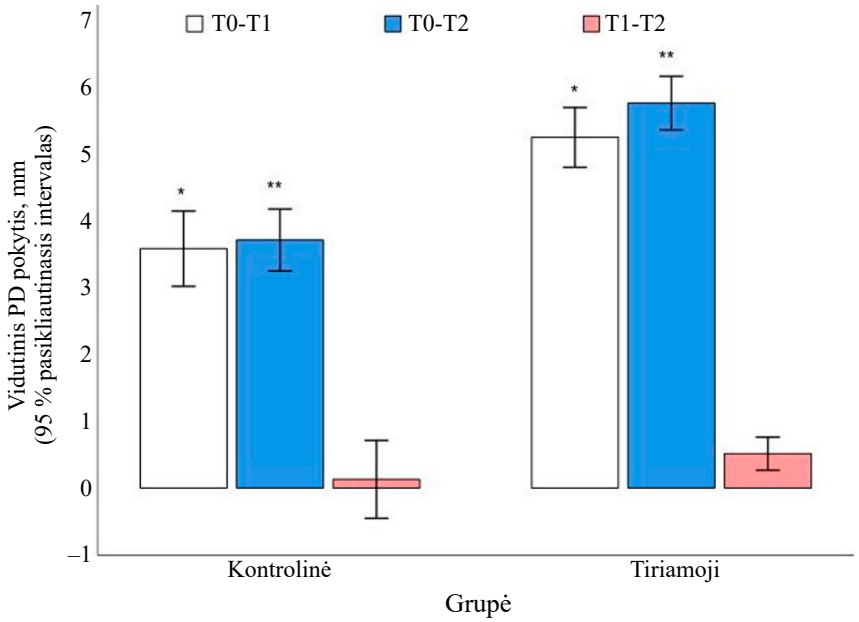
	Kontrolinė grupė (n = 24)	**Tiriamoji grupė (n = 24)**	***p* reikšmė**
Vidutinio gylio kišenės (4–6 mm)			
Prieš gydymą	4,8 (0,2)	4,7 (0,2)	0,417[a]
Po 3 mėn.	2,9 (0,7)	2,2 (0,4)	< 0,001[a]
Prieš gydymą vs. 3 mėn.	< 0,001[b]	< 0,001[b]	
Po 6 mėn.	3,0 (0,6)	1,8 (0,4)	< 0,001[a]
Prieš gydymą vs. 6 mėn.	< 0,001[b]	< 0,001[b]	
Gilios kišenės (≥ 7mm)			
Prieš gydymą	8,0 (0,7)	8,2 (0,9)	0,443[a]
Po 3 mėn.	4,4 (1,4)	2,9 (1,1)	< 0,001[a]
Prieš gydymą vs. 3 mėn.	< 0,001[b]	< 0,001[b]	
Po 6 mėn.	4,3 (1,0)	2,4 (1,0)	< 0,001
Prieš gydymą vs. 6 mėn.	< 0,001[b]	< 0,001[b]	

p – reikšmingumo lygmuo.
[a] Student'o t testas dviems nepriklausomoms imtims; [b] porinis priklausomų imčių t testas.



3.1.3.1.1 pav. *Vidutiniai kišenių zondavimo gylio pokyčiai vidutinių kišenių kategorijoje*

Pradinės PD vertės gilių kišenių kategorijoje tarp kontrolinės ir tiriamosios grupių statistiškai reikšmingai nesiskyrė (atitinkamai 8,0 (0,7) mm ir 8,2 (0,9) mm, $p = 0{,}443$). Abi grupės pasiekė statistiškai reikšmingą pagerėjimą po 3 ir 6 mėn., palyginus su pradinėmis vertėmis ($p < 0{,}001$), tačiau PD sumažėjimas tiriamojoje grupėje buvo statistiškai reikšmingai didesnis, palyginus su kontrole, abiejuose stebėjimo laikotarpiuose ($p < 0{,}001$) (3.1.3.1.1 lentelė). Pokyčiai stebėti tarp 3 ir 6 mėn. tarp grupių nesiskyrė ($p = 0{,}096$) (3.1.3.1.2 pav.)



3.1.3.1.2. pav. *Vidutiniai kišenių zondavimo gylio pokyčiai gilių kišenių kategorijoje*

3.1.3.2. Klinikinis audinių prisitvirtinimo lygis

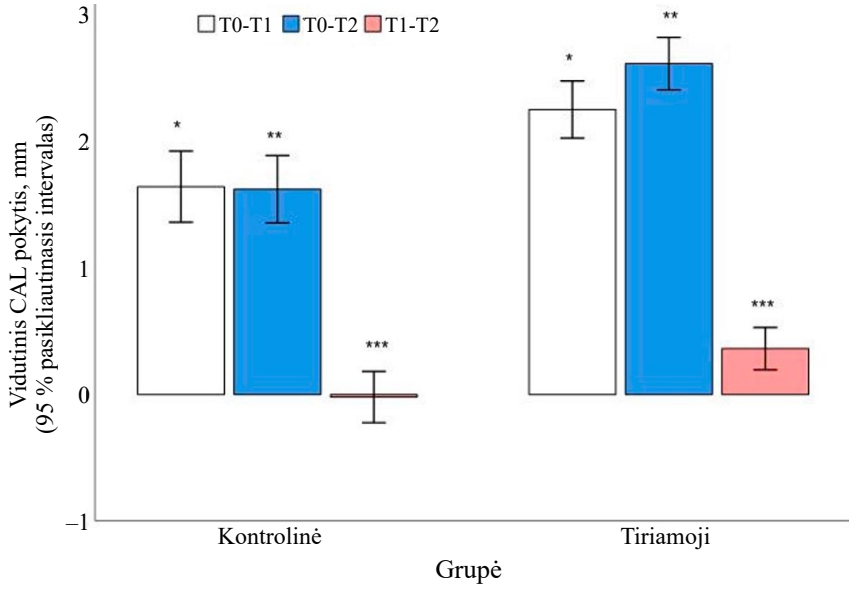
CAL pokyčiai vidutinio ir gilaus zondavimo gylio kategorijose pateikti 3.1.3.2.1 lentelėje.

Tyrimo pradžioje CAL vertės buvo šiek tiek didesnės kontrolinėje grupėje (4,8 (0,3) mm) nei tiriamojoje grupėje (4,6 (0,2) mm) ($p = 0{,}026$). Abi grupės pasiekė reikšmingą pagerėjimą po 3 ir 6 mėn., palyginti su pradinėmis vertėmis ($p < 0{,}001$), tačiau abiem tyrimo laikotarpiais buvo stebimas statistiškai reikšmingas skirtumas tarp grupių tiriamosios grupės naudai ($p < 0{,}001$) (3.1.3.2.1 lentelė). Vidutinio CAL pokytis tarp 3 ir 6 mėn. tarp grupių skyrėsi reikšmingai tiriamosios grupės naudai ($p < 0{,}001$) (3.1.3.2.1 pav.).

3.1.3.2.1 lentelė. *Vidutinis klinikinis audinių prisitvirtinimas (vidurkis (standartinis nuokrypis)) vidutinio gylio (4–6 mm) ir gilių (≥ 7 mm) kišenių kategorijose, kontrolinėje ir tiriamojoje grupėse skirtingais tyrimo etapais.*

	Kontrolinė grupė (n = 24)	**Tiriamoji grupė (n = 24)**	***p* reikšmė**
Vidutinės kišenės (4–6 mm)			
Prieš gydymą	4,8 (0,3)	4,6 (0,2)	0,026[a]
Po 3 mėn.	3,1 (0,8)	2,4 (0,6)	< 0,001[a]
Prieš gydymą vs. 3 mėn	< 0,001[b]	< 0,001[b]	
Po 6 mėn.	3,1 (0,7)	2,0 (0,5)	< 0,001[a]
Prieš gydymą vs. 6 mėn	< 0,001[b]	< 0,001[b]	
Gilios kišenės (≥ 7mm)			
Prieš gydymą	7,9 (0,6)	8,1 (0,7)	0,412[a]
Po 3 mėn.	4,5 (1,2)	3,2 (1,4)	0,002 [a]
Prieš gydymą vs. 3 mėn	< 0,001[b]	< 0,001[b]	
Po 6 mėn.	4,6 (1,0)	2,8 (1,3)	< 0,001 [a]
Prieš gydymą vs. 6 mėn	< 0,001[b]	< 0,001[b]	

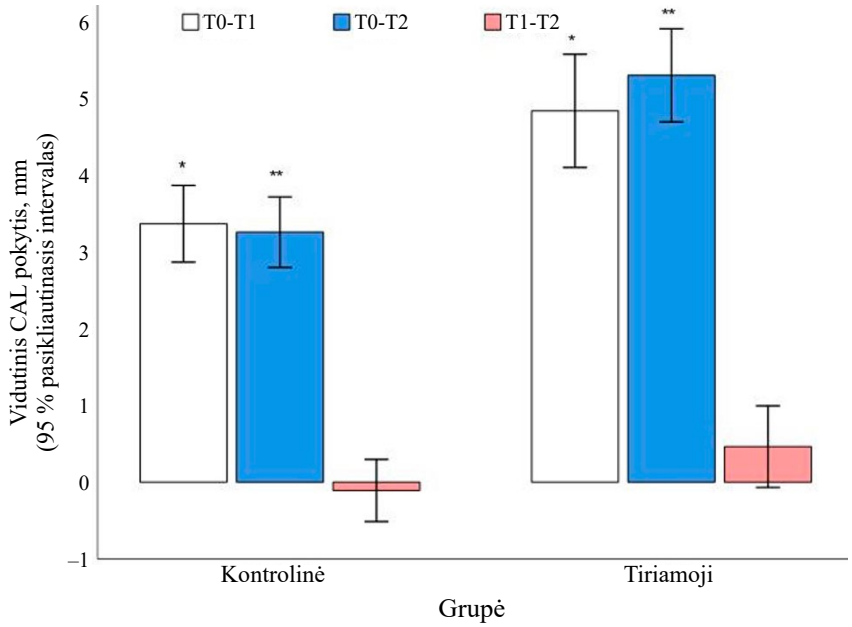
p – reikšmingumo lygmuo.
[a] Student'o *t* testas dviems nepriklausomoms imtims; [b] Porinis priklausomų imčių *t* testas.



3.1.3.2.1 pav. *Vidutinio audinių prisitvirtinimo pokyčiai vidutinių kišenių kategorijoje*

Gilių kišenių kategorijoje vidutinis CAL tyrimo pradžioje tarp grupių nesiskyrė ir buvo 7,9 (0,6) mm kontrolinėje ir 8,1 (0,7) mm tiriamojoje grupėse ($p = 0{,}412$). Po 3 ir po 6 mėn. abi grupės pasiekė statistiškai reikšmingą

pagerėjimą, palyginti su pradinėmis vertėmis ($p < 0,001$), tačiau statistiškai reikšmingai didesnis pagerėjimas buvo stebimas tiriamosios grupės naudai ($p < 0,001$) (3.1.3.2.1 lentelė). Vidutiniai CAL pokyčiai tarp 3 ir 6 mėn. stebėjimo laikotarpių neparodė statistiškai reikšmingo skirtumo tarp grupių ($p = 0,077$) (3.1.3.2.2 pav.)



3.1.3.2.2 pav. *Vidutinio audinių prisitvirtinimo pokyčiai gilių kišenių kategorijoje*

3.1.3.3. Kraujavimas po zondavimo

Analizuoti visos burnos (FMBOP) ir gydytų sričių (BOP) (kai PD≥ 4mm ir kraujuoja po zondavimo) kraujavimo po zondavimo pokyčiai.

Visos burnos FMBOP analizė neatskleidė statistiškai reikšmingo skirtumo tarp kontrolinės ir tiriamosios grupių tyrimo pradžioje (atitinkamai 68,9 (20,3) proc., 76,5 (18,2) proc., $p = 0,184$). Po 3 mėn. nenustatyta statistiškai reikšmingo skirtumo tarp grupių kraujavimo sumažėjime ($p = 0,06$), tačiau po 6 mėn. šis skirtumas tapo reikšmingas tiriamosios grupės naudai ($p < 0,001$) (3.1.3.3.1 lentelė).

Gydytų sričių BOP duomenys tyrimo pradžioje tarp grupių nesiskyrė ($p = 0,687$). Nors abiejose grupėse kraujavimas, palyginus su pradinėmis

vertėmis, reikšmingai sumažėjo abejų kontrolinių vizitų metu ($p < 0,001$), palyginimas tarp grupių atskleidė statistiškai reikšmingą pokytį tiriamosios grupės naudai tiek po 3, tiek po 6 mėn. po gydymo (atitinkamai $p = 0,018$ ir $p < 0,001$) (3.1.3.3.1 lentelė).

3.1.3.3.1 lentelė. *Kraujavimas po zondavimo gydytose srityse (BOP (proc.), (PD ≥ 4mm, BOP+)) ir visoje burnoje (vidurkis (standartinis nuokrypis))*

	BOP		***p* reikšmė**	**FMBOP**		***p* reikšmė**
	Kontrolinė grupė (n = 24)	**Tiriamoji grupė (n = 24)**		**Kontrolinė grupė (n = 24)**	**Tiriamoji grupė (n = 24)**	
Gydymo pradžia	81,8 (16,2)	83,2 (15,5)	0,687[a]	68,9 (20,3)	76,5 (18,2)	0,184[a]
Po 3 mėn	39,1 (15,9)	28,3 (14,6)	**0,018[a]**	33,3 (13,7)	25,9 (12,3)	0,06[a]
Pradžia vs 3 mėn	**< 0,001[b]**	**< 0,001[b]**		**< 0,001[b]**	**< 0,001[b]**	
Po 6 mėn	48,9 (14,5)	17,6 (11,5)	**< 0,001[a]**	40,8 (13,8)	15,6 (9,9)	**< 0,001[a]**
Pradžia vs 6 mėn	**< 0,001[b]**	**< 0,001[b]**		**< 0,001[b]**	**< 0,001[b]**	

BOP – kraujavimas po zondavimo; FMBOP – visos burnos kraujavimas po zondavimo, p – reikšmingumo lygmuo, proc. – procentai.
[a] Student'o *t* testas dviems nepriklausomoms imtims; [b] porinis priklausomų imčių *t* testas.

3.1.3.4. Seklaus, vidutinio ir gilaus zondavimo gylio kišenių dažnio pasiskirstymo analizė

Buvo atlikta seklių (1–3 mm), vidutinio gylio (4–6 mm) ir gilių (≥ 7 mm) kišenių dažnio pasiskirstymo analizė gydymo pradžioje ir po gydymo praėjus 3 ir 6 mėn. (3.1.3.4.1 lentelė).

Gydymo pradžioje kontrolinėje grupėje buvo 1518 (41,2 proc.), o tiriamojoje – 1803 (48,6 proc.) vidutinio gylio kišenės ($p = 0,041$). Po 6 mėn. šis skaičius sumažėjo iki 803 (22,6 proc.) kontrolinėje ir 234 (7,7 proc.) tiriamojoje grupėse, pasiekdamas statistiškai reikšmingą skirtumą tarp grupių tiriamosios grupės naudai ($p < 0,001$).

Gilių kišenių skaičius pakito iš 277 (7,6 proc.) į 35 (1,0 proc.) kontrolinėje ir iš 298 (8,7 proc.) į 4 (0,1 proc.) tiriamojoje grupėje, pasiekdamas statistiškai reikšmingą pokytį tarp grupių tiriamosios grupės naudai ($p = 0,003$).

3.1.3.4.1 lentelė. *Seklių (1–3 mm), vidutinių (4–6 mm) ir gilių (≥ 7 mm) kišenių skaičius kontrolės ir tiriamosiose grupės skirtingais tyrimo etapais*

	1–3 mm			**4–6 mm**			**≥ 7 mm**		
	Kontrolė	**Testas**	***p* reikšmė**	**Kontrolė**	**Testas**	***p* reikšmė**	**Kontrolė**	**Testas**	***p* reikšmė**
Prieš gydymą	1916 (51,2 proc.)	1603 (42,7proc.)	**0,05***	1518 (41,2 proc.)	1803 (48,6 proc.)	**0,041***	277 (7,6 proc.)	298 (8,7 proc.)	0,52
Po 3 mėn	2938 (78,6 proc.)	3284 (88,2 proc.)	**0,013***	728 (20,3 proc.)	402 (11,5 proc.)	**0,018***	39 (1,1 proc.)	12 (0,3 proc.)	0,053
Po 3 mėn	2859 (76.4 proc.)	3398 (92,2 proc.)	**0,006***	803 (22,6 proc.)	234 (7,7 proc.)	**< 0,001***	35 (1,0 proc.)	4 (0,1 proc.)	**0,003***

*Student'o t testas dviems nepriklausomoms imtims, p – reikšmingumo lygmuo, proc. – procentai.

3.2. Mikrobiologiniai rezultatai

3.2.1. Mikrobiologinių ėminių sričių pasiskirstymas

Abiejose grupėse mikrobiologinių ėminių sričių pasiskirstymas pagal dantų grupes buvo vienodas (išskyrus šoninius kandžius) (3.2.1.1 lentelė).

3.2.1.1 lentelė. *Mikrobiologinių ėminių sričių pasiskirstymas pagal dantų grupes*

Gydymas	Antrieji krūminiai	Pirmieji krūminiai	Antrieji prieškrūminiai	Pirmieji prieškrūminiai	Iltys	Šoniniai kandžiai	Centriniai kandžiai
Kontrolės grupė (n)	10	5	9	10	16	25	21
Tiriamoji grupė (n)	9	9	14	11	21	13	19
p	0,621	0,244	0,503	0,152	0,327	**0,021**	0,504

Mann-Whitney U testas dviems priklausomoms imtims.

3.2.2. Periodontopatogenų aptikimo dažnio analizė

3.2.2.1 lentelėje pateikiami kiekvieno periodontopatogeno aptikimo dažniai skirtingais tyrimo etapais tiriamojoje ir kontrolinėje grupėse. Rezultatai išreikšti kaip pacientų, kuriems aptiktas tam tikras patogenas, procentinė dalis.

3.2.2.1 lentelė. *Periodonto patogenų aptikimo dažnis*

Periodonto-patogenas	Gydymo grupė	Prieš gydymą	Po 3 mėnesių	Po 6 mėnesių
A.a	Kontrolinė grupė	42,5	54,2	58,3
	Tiriamoji grupė	45,8	29,2	33,3
P.g	Kontrolinė grupė	75,0	58,3	$75,0^{*}$
	Tiriamoji grupė	$87,5^{ab}$	$41,7^{a}$	$41,7^{*b}$
T.f	Kontrolinė grupė	$91,7^{a}$	$62,5^{a}$	79,2
	Tiriamoji grupė	$83,3^{ab}$	$54,2^{a}$	$58,3^{b}$
T.d	Kontrolinė grupė	$87,5^{a}$	$58,3^{a}$	$79,2^{*}$
	Tiriamoji grupė	$95,8^{ab}$	$41,7^{a}$	$33,3^{*b}$
P.i	Kontrolinė grupė	58,3	29,2	$45,8^{*}$
	Tiriamoji grupė	$45,8^{ab}$	$20,8^{a}$	$8,3^{*b}$

$^{*}p < 0,05$, skirtumas tarp grupių (χ^2 testas);
$^{a}p < 0,05$, skirtumas tarp tyrimo pradžios ir 3 mėn. po gydymo (McNemar testas);
$^{b}p < 0,05$, skirtumas tarp tyrimo pradžios ir 6 mėn. po gydymo (McNemar testas).

Kontrolinėje grupėje po 3 mėn. buvo nustatytas reikšmingas *T.f* ir *T.d* sumažėjimas ($p < 0,05$), tačiau po 6 mėn. šių bakterijų aptikimo dažnis vėl padidėjo iki lygio, buvusio prieš gydymą ($p > 0,05$). Tiriamojoje grupėje reikšmingas *P.g*, *T.d* ir *P.i* sumažėjimas buvo pastebėtas po 3 ir 6 mėn. ($p < 0,05$). Tiriamosios ir kontrolinės grupių palyginimas atskleidė reikšmingus aptikimo dažnio skirtumus *P.g* ($p = 0,034$), *T.d* ($p < 0,01$) ir *P.i* ($p = 0,02$) po 6 mėn. tiriamosios grupės naudai.

3.2.3. Bendro periodontopatogenų skaičiaus pokyčio analizė

3.2.3.1 lentelėje pavaizduotas bendro *A.a, P.g, T.f, T.d* ir *P.i* skaičiaus pokytis kontrolinėje ir tiriamojoje grupėse skirtingais tyrimo etapais.

3.2.3.1 lentelė. *Bendro A.a, P.g, T.f, T.d ir P.i skaičiaus (vidurkis (standartinis nuokrypis)) pokytis kontrolinėje ir tiriamojoje grupėse skirtingais tyrimo etapais*

Bakterijos rūšis	**Tyrimo etapas**	**Skaičius**	**Viso, n (proc.)**	**Kontrolinė grupė, n (proc.)**	**Tiriamoji grupė, n (proc.)**	***p* reikšmė**[**]
A.a	Prieš gydymą	0	22 (45,8)	9 (37,5)	13 (54,2)	0,174
		1	2 (4,2)	1 (4,2)	1 (4,2)	
		2	4 (8,3)	2 (8,3)	2 (8,3)	
		3	8 (16,7)	4 (16,7)	4 (16,7)	
		4	12 (25,0)	8 (33,3)	4 (16,7)	
	Po 3 mėnesių	0	28 (58,3)	11 (45,8)	17 (70,8)	**0,044**
		1	2 (4,2)	1 (4,2)	1 (4,2)	
		2	6 (12,5)	4 (16,7)	2 (8,3)	
		3	6 (12,5)	2 (8,3)	4 (16,7)	
		4	6 (12,5)	6 (25,0)	0	
	[*]p reikšmė		**0,013**	0,231	**0,011**	
	Po 6 mėnesių	0	26 (54,2)	10 (41,7)	16 (66,7)	**0,028**
		1	4 (8,3)	1 (4,2)	3 (12,5)	
		2	4 (8,3)	3 (12,5)	1 (4,2)	
		3	6 (12,5)	3 (12,5)	3 (12,5)	
		4	8 (16,7)	7 (29,2)	1 (4,2)	
	[*]p reikšmė		0,085	0,064	**0,016**	
P.g	Prieš gydymą	0	9 (18,8)	6 (25,0)	3 (12,5)	0,884
		1	1 (2,1)	-	1 (4,2)	
		2	2 (4,2)	1 (4,2)	1 (4,2)	
		3	11 (22,9)	4 (16,7)	7 (29,2)	
		4	25 (52,1)	13 (54,2)	12 (50,0)	

3.2.3.1 lentelės tęsinys

Bakterijos rūšis	**Tyrimo etapas**	**Skaičius**	**Viso, n (proc.)**	**Kontrolinė grupė, n (proc.)**	**Tiriamoji grupė, n (proc.)**	***p* reikšmė**[**]
P.g	Po 3 mėnesių	0 1 2 3 4	24 (50,0) 3 (6,3) 6 (12,5) 8 (16,7) 7 (14,6)	10 (41,7) 1 (4,2) 3 (12,5) 4 (16,7) 6 (25,0)	14 (58,3) 2 (8,3) 3 (12,5) 4 (16,7) 1 (4,2)	0,099
	[*]p reikšmė		**< 0,001**	**0,013**	**< 0,001**	
	Po 6 mėnesių	0 1 2 3 4	20 (41,7) 7 (14,6) 8 (16,7) 6 (12,5) 7 (14,6)	6 (25,0) 4 (16,7) 4 (16,7) 3 (12,5) 7 (29,2)	14 (58,3) 3 (12,5) 4 (16,7) 3 (12,5) -	**0,006**
	[*]p reikšmė		**< 0,001**	**0,039**	**< 0,001**	
T.f	Prieš gydymą	0 1 2 3 4	6 (12,5) 3 (6,3) 5 (10,4) 18 (37,5) 16 (33,3)	2 (8,3) 3 (12,5) 3 (12,5) 8 (33,3) 8 (33,3)	4 (16,7) - 2 (8,3) 10 (41,7) 8 (33,3)	0,846
	Po 3 mėnesių	0 1 2 3 4	20 (41,7) 8 (16,7) 8 (16,7) 10 (20,8) 2 (4,2)	9 (37,5) 1 (4,2) 5 (20,8) 7 (29,2) 2 (8,3)	11 (45,8) 7 (29,2) 3 (12,5) 3 (12,5) -	0,088
	[*]p reikšmė		**< 0,001**	**0,007**	**< 0,001**	
	Po 6 mėnesių	0 1 2 3 4	15 (31,3) 10 (20,8) 9 (18,8) 12 (25,0) 2 (4,2)	5 (20,8) 3 (12,5) 4 (16,7) 10 (41,7) 2 (8,3)	10 (41,7) 7 (29,2) 5 (20,8) 2 (4,2) -	**0,004**
	[*]p reikšmė		**< 0,001**	**0,048**	**< 0,001**	
T.d	Prieš gydymą	0 1 2 3 4	4 (8,3) 10 (20,8) 22 (45,8) 12 (25,0) -	3 (12,5) 4 (16,7) 11 (45,8) 6 (25,0) -	1 (4,2) 6 (25,0) 11 (45,8) 6 (25,0) -	0,878
	Po 3 mėnesių	0 1 2 3 4	24 (50,0) 13 (27,1) 10 (20,8) 1 (2,1) -	10 (41,7) 6 (25,0) 7 (29,2) 1 (4,2) -	14 (58,2) 7 (29,2) 3 (12,5) - -	0,125
	[*]p reikšmė		**< 0,001**	**0,003**	**< 0,001**	

3.2.3.1 lentelės tęsinys

Bakterijos rūšis	Tyrimo etapas	Skaičius	Viso, n (proc.)	Kontrolinė grupė, n (proc.)	Tiriamoji grupė, n (proc.)	*p* reikšmė**
T.d	Po 6 mėnesių	0 1 2 3 4	21 (43,8) 13 (27,1) 12 (25,0) 2 (4,2) -	5 (20,8) 6 (25,0) 11 (45,8) 2 (8,3) -	16 (66,7) 7 (29,2) 1 (4,2) - -	**< 0,001**
	*p reikšmė		**< 0,001**	0,083	**< 0,001**	
P.i	Prieš gydymą	0 1 2 3 4	23 (47,9) 5 (10,4) 4 (8,3) 12 (25,0) 4 (8,3)	10 (41,7) 4 (16,7) - 8 (33,3) 2 (8,3)	13 (54,2) 1 (4,2) 4 (16,7) 4 (16,7) 2 (8,3)	0,413
	Po 3 mėnesių	0 1 2 3 4	36 (75,0) 2 (4,2) 7 (14,6) 3 (6,3) -	17 (70,8) 1 (4,2) 3 (12,5) 3 (12,5) -	19 (79,2) 1 (4,2) 4 (16,7) - -	0,399
	*p reikšmė		**< 0,001**	**0,012**	**0,014**	
	Po 6 mėnesių	0 1 2 3 4	35 (72,9) 4 (8,3) 5 (10,4) 4 (8,3) -	13 (54,2) 3 (12,5) 4 (16,7) 4 (16,7) -	22 (91,7) 1 (4,2) 1 (4,2) - -	**0,003**
	*p reikšmė		**< 0,001**	0,091	**0,003**	

n – dažnis, p – reikšmingumo lygmuo.

* Wilcoxon testas palyginimui grupių viduje skirtingais tyrimo etapais;

**Mann-Whitney testas palyginimui tarp grupių skirtingais tyrimo etapais.

Tyrimo pradžioje bendras periodontopatogenų skaičius kontrolinėje ir tiriamojoje grupėse nesiskyrė ($p > 0,05$).

Po 3 mėn. kontrolinėje grupėje aptiktas statistiškai reikšmingas *P.g* ($p = 0,013$), *T.f* ($p = 0,007$), *T.d* ($p = 0,003$) ir *P.i* ($p = 0,012$) kiekio sumažėjimas, palyginus su jų skaičiumi prieš pradedant gydymą. Po 6 mėn. statistiškai reikšmingas sumažėjimas, palyginus su vertėmis prieš gydymą, išliko šiems periodontopatogenams: *P.g* ($p = 0,039$) ir *T.f* ($p = 0,048$).

Bendro periodontopatogenų skaičiaus palyginimas prieš gydymą ir praėjus 3 mėn. po gydymo tiriamojoje grupėje atskleidė statistiškai reikšmingus skirtumus visoms tirtoms bakterijų rūšims: *A.a* ($p = 0,011$), *P.g* ($p < 0,001$), *T.f* ($p < 0,001$), *T.d* ($p < 0,001$) ir *P.i* ($p = 0,014$). Šie pokyčiai reikšmingi išliko ir praėjus 6 mėn. po gydymo: *A.a* (p = 0,016), *P.g* ($p < 0,001$), *T.f* ($p < 0,001$), *T.d* ($p < 0,001$) ir *P.i* ($p = 0,003$).

Palyginamoji analizė tarp grupių atskleidė statistiškai reikšmingą *A.a* ($p = 0{,}044$) bendro skaičiaus sumažėjimą po 3 mėn. ir *A.a* ($p = 0{,}028$), *P.g* ($p = 0{,}006$), *T.f* ($p = 0{,}004$), *T.d* ($p < 0{,}001$) ir *P.i* ($p = 0{,}003$) po 6 mėn. tiriamosios grupės naudai.

3.3. Histologinio tyrimo rezultatai

3.3.1. Klinikiniai stebėjimai

Pooperacinis gijimas buvo sklandus visais 16 atvejų (8 sritys / grupei) kontrolinėje ir tiriamojoje grupėse. Nebuvo stebima pašalinių reakcijų (pūliavimo, absceso formavimosi, padidėjusio dantų paslankumo).

3.3.2. Klinikiniai matavimai

Klinikinių matavimų vertės prieš gydymą ir 6 sav. po gydymo pateikti 3.3.2.1 lentelėje.

3.3.2.1 lentelė. *Klinikiniai kontrolinės ir tiriamosios grupių palyginimai 8 sav. po gydymo (vidurkis (standartinis nuokrypis); n = 4 gyvūnai*

Klinikinis rodiklis	Kontrolė	Tiriamoji
PD (mm)		
Prieš gydymą	5,46 (0,82)	5,50 (0,57)
6 sav po gydymo	2,12 (0,32)	1,25 (0,50)
PPD sumažėjimas	3,34 (0,54)	4,25 (0,50)†
CAL (mm)		
Prieš gydymą	5,71 (0,84)	5,59 (0,47)
6 sav po gydymo	2,93 (0,23)	1,68 (0,89)
CAL padidėjimas	2,78 (0,79)	3,90 (0,82)
BOP (+) n (proc.)		
Prieš gydymą	8 (100)	8 (100)
6 sav po gydymo	6 (75,0)	1 (12,5)*†

BOP – kraujavimas po zondavimo; CAL – klinikinis audinių prisitvirtinimo lygis; PD – kišenės zondavimo gylis;
*statistiškai reikšmingas skirtumas grupės viduje ($p < 0{,}01$);
†statistiškai reikšmingas skirtumas tarp grupių ($p < 0{,}05$).

Pradinių klinikinių matavimų vertės kontrolinėje ir tiriamojoje grupėse nesiskyrė. Abejose grupėse stebėtas klinikinių rodiklių pagerėjimas 6 sav. po gydymo, palyginus su jų vertėmis prieš gydymą. Vidutinis PD sumažėjimas nuo gydymo pradžios iki 6 sav. po gydymo statistiškai reikšmingai didesnis buvo tiriamosios grupės naudai ($p < 0{,}05$). Nors CAL pagerėjimas po 6 sav. buvo didesnis tiriamojoje grupėje, neaptikta statistiškai reikšmingo skirtu-

mo tarp grupių. Kraujuojančių sričių (BOP+) skaičius statistiškai reikšmingai maženis po 6 sav. buvo tiriamojoje grupėje, palyginus su kontroline grupe ($p < 0,05$)

3.3.3. Histomorfometrinė analizė

Histomorfometrinės analizės duomenys pateikti 3.3.3.1 lentelėje. Nenustatyta statistiškai reikšmingo skirtumo tarp grupių šiems parametrams: DH, JE ir CT. Nustatytas naujo cemento ilgis statistiškai reikšmingai didesnis tiriamojoje (2,46 ± 0,77 mm) nei kontrolinėje grupėje (0,85 ± 0,84 mm) ($p < 0,01$). Tiriamojoje grupėje aptiktas statistiškai reikšmingai didesnis naujos jungties (NA) (1,75 ± 0,65 mm), (linijinio naujo cemento, susiformavusio šalia naujai susiformavusio kaulo su funkciškai orientuotomis kolageno skaidulomis), formavimasis, palyginus su kontroline grupe (0,48 ± 0,79 mm) ($p < 0,05$). Susiformavusio naujo kaulo kiekis statistiškai reikšmingai skyrėsi tarp tiriamosios (3,01 ± 0,64 mm) ir kontrolinės (2,26 ± 0,64 mm) grupių tiriamosios grupės naudai ($p < 0,05$).

Histologiniai kontrolinės ir tiriamosios grupių vaizdai pateikti 3.3.3.1 ir 3.3.3.2 pav.

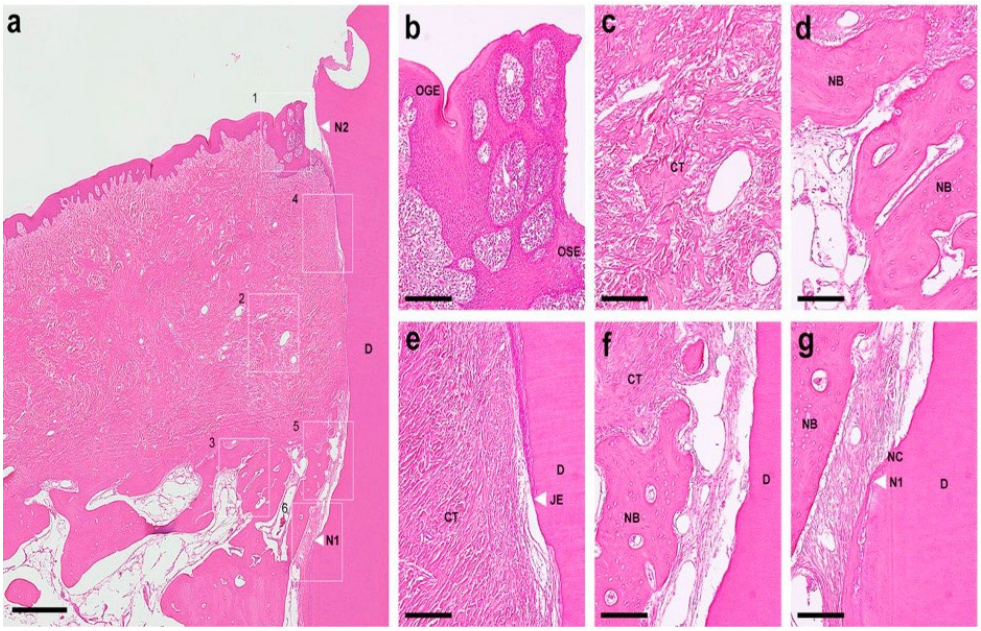
3.3.3.1 lentelė. *Histomorfometriniai kontrolinės ir tiriamosios grupių palyginimai 8 sav. po gydymo (vidurkis(standartinis nuokrypis)); n = 4 gyvūnai*

Parametrai	Kontrolė	Tiriamoji
DH (mm)	5,60 (0,42)	5,77 (0,52)
JE (mm)	1,22 (0,45)	1,03 (0,31)
CT (mm)	2,84 (1,33)	1,86 (1,00)
NC (mm)	0,85 (0,84)	2,46 (0,77)**
NA (mm)	0,48 (0,79)	1,75 (0,65)*
NB (mm)	2,26 (0,64)	3,01 (0,64)*

DH – defekto aukštis; JE – jungties epitelio ilgis; CT – jungiamojo audinio jungtis (be cemento); mm – milimetrai; NB – naujo kaulo ilgis; NC – naujo cemento ilgis; NA – naujos jungties ilgis;

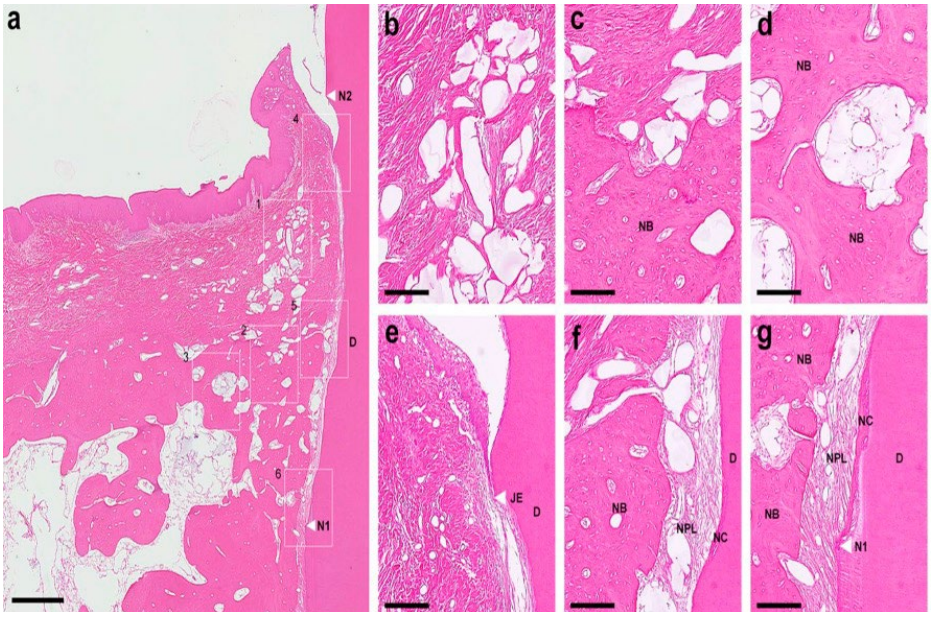
* statistiškai reikšmingas skirtumas, palyginus su kontroline grupe ($p < 0,05$);

** statistiškai reikšmingas skirtumas, palyginus su kontroline grupe ($p < 0,01$).



3.3.3.1 pav. *Histologinis kontrolinės grupės defekto vaizdas*

Pastaba: a – bendras histologinis vaizdas; b – pirmo kvadratėlio padidintas vaizdas; c – antro kvadratėlio padidintas vaizdas; d – trečio kvadratėlio padidintas vaizdas; e – ketvirto kvadratėlio padidintas vaizdas; f – penkto kvadratėlio padidintas vaizdas; g – šešto kvadratėlio padidintas vaizdas. D – danties šaknies dentinas; N_1 – apikalinė defekto dalis; N_2 – vainikinė defekto dalis; OGE – burnos dantenų epitelis; OSE – dantenų vagelės epitelis; JE – jungties epitelio apikalinė dalis; CT – jungiamojo audinio jungtis; NB – naujas kaulas; NC – naujas cementas.



3.3.3.2 pav. *Histologinis tiriamosios grupės defekto vaizdas*

Pastaba: a – bendras histologinis vaizdas; b – pirmo kvadratėlio padidintas vaizdas; c – antro kvadratėlio padidintas vaizdas; d – trečio kvadratėlio padidintas vaizdas; e – ketvirto kvadratėlio padidintas vaizdas; f – penkto kvadratėlio padidintas vaizdas; g – šešto kvadratėlio padidintas vaizdas. D – danties šaknies dentinas; N_1 – apikalinė defekto dalis; N_2 – vainikinė defekto dalis; JE – jungties epitelio apikalinė dalis; CT – jungiamojo audinio jungtis; NB – naujas kaulas; NC – naujas cementas; NPL – naujas periodonto raištis.

4. REZULTATŲ APTARIMAS

4.1. Klinikiniai rezultatai

Atlikto atsitiktinės imties kontroliuojamo klinikinio tyrimo tikslas – įvertinti klinikinius rodiklius, kai II–III stadijos periodontito gydyme papildomai naudojami NaOCl/ amino rūgščių ir xHyA geliai. Tai yra pirmasis toks atsitiktinės imties kontroliuojamas klinikinis tyrimas. Tyrimo rezultatai atskleidė, kad papildomas šių medžiagų panaudojimas kartu su podanteniniu instrumentavimu lėmė statistiškai reikšmingus gydymo rezultatus, išreikštus PD, BOP sumažėjimu ir CAL padidėjimu, palyginus su podanteniniu instrumentavimu *per se*.

Įdomus šio tyrimo pastebėjimas yra susijęs su PD ir CAL pokyčiais tarp 3 ir 6 mėn. stebėjimo laikotarpių vidutinio gylio kišenių kategorijoje. Konkrečiai, kontrolinėje grupėje statistiškai reikšmingo pokyčio tarp 3 ir 6 mėn. rezultatų nenustatyta, tuo tarpu tiriamojoje grupėje šis pokytis pasiekė statistiškai reikšmingą skirtumą. Šis pastebėjimas leidžia manyti, kad tiriamojoje grupėje vyko laipsniškas gijimas, rodantis klinikinių rodiklių gerėjimus tarp 3 ir 6 mėn., nors papildomos gydymo procedūros šiuo laikotarpiu nebuvo atliktos. Panašius rezultatus gavo Benyei L. ir bendraautoriai, ta pačia metodika atsitiktinės imties kontroliuojamame klinikiniame tyrime gydę prieš tai į gydymą nereagavusias periodontologines kišenes [122]. Pastebėję laipsnišką klinikinių parametrų gerėjimą tiriamojoje grupėje, autoriai padarė išvadą, kad ši gydymo metodika gali lemti periodonto audinių regeneraciją. Palaipsnis gijimas galėtų būti paaiškintas xHyA poveikiu. Didelis molekulinis produkto, naudoto šiame tyrime, svoris ir vidutinis stabilizacijos laipsnis lėmė jo gebėjimą išlikti stabiliu aplikacijos vietoje nuo 4 iki 6 sav. laikotarpiu.

Analizuojant klinikinius tyrimo pokyčius, turėtų būti pabrėžiama, kad galutinis konservatyvaus periodontologinio gydymo tikslas – PD ≤ 4 mm su neigiama BOP reikšme [64]. Mūsų tyrimo rezultatai atskleidė, kad tolimesnio gydymo reikiamybė statistiškai reikšmingai mažesnė buvo tiriamojoje grupėje. Konkrečiai, vidutinių kišenių (4–6 mm) skaičius sumažėjo nuo 1518 iki 803 kontrolinėje ir nuo 1803 iki 234 tiriamojoje grupėje ($p < 0{,}001$). Panašiai, gilių kišenių (≥ 7 mm) kategorijoje skaičius pakito nuo 277 iki 35 kontrolinėje ir nuo 298 iki 4 tiriamojoje grupėse ($p = 0{,}003$).

Iš literatūros žinoma, kad konservatyviai gydant periodontologines kišenes, kurių pradinis PD yra 4–6 mm, galima tikėtis vidutinio 1–1,5 mm kišenių sumažėjimo, tuo tarpu gydant gilias kišenes (≥ 7 mm) ši vertė siekia 2–2,5 mm [123]. Panašiai ir su CAL padidėjimu – vidutinio gylio kišenėse galima tikėtis vidutinio 0,5 mm, giliose – 1,5 mm naujos klinikinės jungties susidarymo [123]. Mūsų tyrime gauti rezultatai šiuos duomenis patvirtina –

vidutinėse kišenėse po 6 mėn. gautas 1,7 mm PD sumažėjimas kontrolinėje ir 2,9 mm tiriamojoje grupėje; giliose kišenėse šios vertės atitinkamai siekė 3,7 mm ir 5,8 mm. Kalbant apie CAL padidėjimą, vidutinėse kišenėse po 6 mėn. stebėtas 1,6 mm kontrolinėje ir 2,6 mm tiriamojoje grupėje, giliose kišenėse šios vertės atitinkamai siekė 3,2 mm ir 5,8 mm.

Tyrimai, nagrinėjantys papildomą antiseptikų naudojimą, rodo, kad galima išgauti papildomą 0,3–0,9 mm PD sumažėjimą ir 0,3–0,5 mm CAL padidėjimą kartu su podanteniniu instrumentavimu naudojant antiseptikus [17, 18, 124, 125]. Šie rezultatai atsispindi ir mūsų tyrime, indikuodami, kad tiriamojoje grupėje išgautas klinikinių rodiklių pagerėjimas stipriai viršija jau žinomus literatūroje duomenis, todėl galima daryti prielaidą, kad papildomą naudą tiriamojoje grupėje suteikė būtent NaOCl ir xHyA gelių panaudojimas.

Interpretuojant gautus rezultatus gali kilti klausimas, kokia yra kiekvieno komponento įtaka gydymo rezultatams atskirai. Norima pabrėžti, kad mūsų tyrimo tikslas buvo naudoti šias dvi medžiagas kaip vieną konceptą, taip sujungiant NaOCl/amino rūgščių gelio poveikį mechaniniam biofilmo pašalinimui su gerai žinomu xHyA poveikiu žaizdų gijimui ir regeneracijai [95, 114–116].

Neseniai atlikta retrospektyvinė 29 atvejų analizė vertino papildomą klinikinį NaOCl/amino rūgščių ir xHyA gelio poveikį konservatyviai gydant II–IV stadijų periodontitą ultragarsu ir rankiniais instrumentais [126]. Autoriai nurodė didesnį nei 2 mm PD sumažėjimą ir 2,02 mm CAL pagerėjimą. Reikia paminėti, kad tyrimas buvo atliekamas su periodontologiniais pacientais, įtrauktais į reguliarias palaikomojo periodontologinio gydymo programas, be to, buvo gydomi ir IV stadijos periodontito atvejai, todėl tiesioginiai palyginimai su mūsų tyrimo rezultatais nebūtų tikslūs. Dar vienoje retrospektyvinėje 21 atvejo analizėje nagrinėta ta pati metodika gydant II–III stadijos, A/B laipsnio periodontitą [127]. Šiame tyrime stebėtas vidutinis PD sumažėjimas po 3 ir 6 mėn. buvo atitinkamai 2,6 mm ir 2,9 mm, CAL – atitinkamai 2,3 ir 2,6 mm. Paminėti rezultatai atspindi mūsų tyrimo metu stebėtas tendencijas, patvirtindami naujo gydymo metodo svarbą šiuolaikinėje periodontologijoje.

Papildomos NaOCl gelio aplikacijos klinikinis poveikis tirtas atskiruose atsitiktinės imties klinikiniuose tyrimuose. Iorio-Siciliano V. ir bendraautoriai, tyrę papildomą NaOCl gelio panaudojimą kartu su podanteniniu instrumentavimu, nustatė tiriamojoje grupėje statistiškai reikšmingą PD sumažėjimą ir CAL padidėjimą, palyginus su kontroline grupe ($p < 0,05$) [99]. Kito klinikinio tyrimo, trukusiame 12 mėn., pabaigoje buvo nustatytas statistiškai reikšmingai maženis kraujuojančių sričių skaičius, palyginus su placebu ar chlorkesidinu [98].

Klinikinių tyrimų, nagrinėjančių papildomą vidutiniškai stabilizuotos xHyA poveikį, duomenys nenuoseklūs. Vienose jų nustatyta statistiškai reikš-

minga xHyA panauda kartu su podanteniniu instrumentavimu PD sumažėjime ir CAL padidėjime [117, 118], kitose – nors gautas statistiškai reikšmingas klinikinių parametrų pagerėjimas, palyginus su pradinėmis vertėmis, reikšmingo skirtumo tarp tyrimo grupių nustatyta nebuvo [128].

Interpretuojant šio tyrimo metu gautus rezultatus svarbu atsižvelgti į keletą aspektų – santykinai mažą tyrimo imtį bei trumpą tyrimo trukmę. Į tyrimą buvo įtraukti tik sistemiškai sveiki ir nerūkantys pacientai, kurių asmeninė burnos higiena buvo labai gera. Ateityje reikėtų ilgesnės trukmės atsitiktinės imties klinikinių tyrimų, nagrinėjančių prieš tai minėtus aspektus.

4.2. Mikrobiologiniai rezultatai

Remiantis gautais tyrimo rezultatais, abi gydymo metodikos (podanteninis instrumentavimas ir podantenininis instrumentavimas + NaOCl/amino rūgščių + xHyA geliai) lėmė statistiškai reikšmingus periodontopatogeninių bakterijų pokyčius. Šie pokyčiai kontrolinėje ir tiriamojoje grupėse skyrėsi. Konkrečiai, po 3 mėn. abiejose grupėse nustatytas statistiškai reikšmingas *T.f* ir *T.d* aptikimo dažnis ($p < 0,05$), papildomai, tiriamojoje grupėje reikšmingas jis buvo ir *P.i* ir *P.g* bakterijų rūšims ($p < 0,05$). Po 6 mėn. *T.f* ir *T.d* aptikimo dažnis kontrolinėje grupėje tapo panašus į buvusį prieš gydymo pradžią ($p > 0,05$), tuo tarpu tiriamojoje grupėje po 6 mėn. aptikimo dažnis išliko statistiškai reikšmingas šioms bakterijų rūšims: *T.f*, *T.d*, *P.i* ir *P.g*. Svarbu paminėti, kad *A.a* aptikimo dažniui įtakos neturėjo nė viena gydymo metodika ($p > 0,05$).

Panašūs rezultatai buvo gauti ankstesniuose klinikiniuose tyrimuose, nagrinėjančiuose podanteninio instrumentavimo poveikį periodonto patogenams, naudojant tokias molekulines technologijas kaip dNR zondai ar PCR amplifikacija [129–131]. Po konservatyvaus periodontologinio gydymo reikšmingai sumažėjo *P.g*, *T.f* ir *T.d* lygiai [129–131], tačiau *A.a* sumažėjimui periodontologinis gydymas įtakos neturėjo [130, 131]. Šie rezultatai dar kartą patvirtina ankstesnių tyrimų išvadas, kurios neparodė reikšmingo *A.a* lygio sumažėjimo po periodontologinio gydymo [132]. Be to, keli tyrimai parodė, kad statistiškai reikšmingi *P.g*, *T.f* ir *T.d* aptikimo dažnio sumažėjimai yra sėkmingo periodontologinio gydymo rezultatas [133]. Mūsų stebėjimai sutampa su šiuo teiginiu, nes po 3 mėn. buvo nustatytas statistiškai reikšmingas *P.g*, *T.f* ir *T.d* aptikimo dažnis tiriamojoje grupėje ($p < 0,05$), o kontrolinėje – tik *T.f* ir *T.d* bakterijų rūšims ($p < 0,05$). Po 6 mėn. sumažėjimai išliko stabilūs tik tiriamojoje grupėje šioms bakterijų rūšims: *P.g*, *T.f* ir *T.d* ($p < 0,05$).

Praėjus 3 mėn. po gydymo, bendras bakterijų skaičiaus pokytis statistiškai reikšmingas buvo tiek tiriamojoje, tiek kontrolinėje grupėse šioms bakterijų rūšims: *T.f*, *T.d*, *P.g* ir *P.i* ($p < 0,05$), tačiau reišmingas *A.a* pokytis stebėtas

tik tiriamojoje grupėje ($p = 0,001$). Po gydymo praėjus 6 mėn., kontrolinėje grupėje statistiškai reikšmingas bendro bakterijų skaičiaus pokytis išliko tik *P.g* ir *T.f* bakterijų rūšims ($p < 0,001$), o tiriamojoje grupėje – visoms tirtoms bakterijų rūšims, įskaitant *A.a* ($p < 0,05$). Šie rezultatai sutampa su neseniai atlikto atsitiktinės imties kontroliuojamo klinikinio tyrimo, nagrinėjusio papildomo NaOCl gelio įtaką penkiems pagrindiniams periodontopatogenams, rezultatais [98]. Autoriai nustatė statistiškai reikšmingus *P.g* ($p = 0,015$) *ir T.f* ($p = 0,004$) skaičiaus pokyčius NaOCl grupėje, palyginus su vertėmis prieš gydymą, o *A.a* skaičius išliko nepakitęs ($p = 0,098$). Dar kitu atsitiktinės imties klinikiniu tyrimu, nagrinėjusiu NaOCl klinikinį ir mikrobiologinį poveikį, buvo nustatytas statistiškai reikšmingas *T.d* ($p < 0,05$) ir *T.f* ($p < 0,05$) skaičiaus sumažėjimas tiriamojoje grupėje praėjus 12 mėn. po gydymo [100].

Statistiškai reikšmingi mikrobiologiniai skirtumai tarp tiriamosios ir kontrolinės grupių, nustatyti šiame tyrime, galėtų būti priskirti antibakterinėms NaOCl/amino rūgščių ir xHyA savybėmis [95, 134]. Remiantis žinomais *in vitro* ir tyrimų su gyvūnais duomenimis, gali būti daroma prielaida, kad NaOCl/amino rūgščių gebėjimas palengvinti mechaninį instrumentavimą ir biofilmo pašalinimą gali suteikti papildomą naudą bakteriostatinėms ir gijimą skatinančioms xHyA savybėms [95, 114–116, 134]. Iš tiesų, kaip rodo daugybė klinikinių tyrimų, vien podanteninis periodontologinių kišenių instrumentavimas turi ribotas galimybes, kai norima pašalinti kišenėse reziduojančias periodontopatogeninių bakterijų rūšis, atsižvelgiant ir į tai, kad bakterijos gali reziduoti ir minkštuosiuose audiniuose, šaknies paviršiaus nelygumuose, dentino kanalėliuose [15, 135–138].

Mikrobiologiniai šio tyrimo rezultatai sutampa su prieš tai aprašytais klinikiniais duomenimis. 3 mėn. po gydymo etape abiejose tyrimo grupėse buvo stebimas statistiškai reikšmingas PD, BOP sumažėjimas bei CAL padidėjimas, tačiau šie pokyčiai reikšmingai didesni buvo tiriamojoje grupėje. Įdomus pastebėjimas, kad tiriamojoje grupėje stebėtas statistiškai reikšmingas klinikinių rodiklių gerėjimas tarp 3 ir 6 mėn., kai tuo tarpu kontrolinėje grupėje tuo pačiu laikotarpiu reikšmingų pokyčių neįvyko.

Apibendrinant, šio tyrimo rezultatai įrodo, kad mikrobiologinė papildomo NaOCl/ amino rūgščių ir xHyA panaudojimo nauda išliko viso tyrimo stebėjimo metu (6 mėn.), su ilgalaikio mikrobiologinio efekto tendencija. Dar daugiau, buvo rastas ryšys tarp klinikinės ir mikrobiologinės ligos išraiškų, nors nėra aišku, ar podanteninio biofilmo sumažinimas lėmė klinikinių rodiklių gerėjimą, ar atvirkščiai. Svarbu pabrėžti, kad šio tyrimo esmė – abiejų medžiagų panaudojimas kartu, todėl ateityje reikalingi klinikiniai tyrimai, kurie nagrinėtų minėtų komponentų klinikinį ir mikrobiologinį poveikius kartu ir atskirai.

4.3. Histologinė analizė

Šis tyrimas – pirmasis, pateikęs histologinius periodonto audinių (periodonto raiščio, alveolinio kaulo, šaknies cemento) regeneracijos įrodymus, pasitelkiant papildomą podanteninę NaOCl/amino rūgščių ir xHyA aplikaciją. Histologiniai tyrimo duomenys sutapo su klinikinių matavimų reikšmėmis – statistiškai reikšmingas PD, BOP sumažėjimas, CAL padidėjimas tiriamojoje grupėje, palyginus su kontroline, pabrėžia potencialią šių išvadų klinikinę svarbą.

Šio tyrimo su gyvūnais metu gauti klinikiniai duomenys sutampa su klinikinių tyrimų rezultatais, rodančiais, kad podanteninis instrumentavimas kartu su NaOCl/ amino rūgščių ir xHyA gelių aplikacija statistiškai reikšmingai pagerino PD, CAL ir BOP vertes, turėtas prieš gydymą, [126, 127], ir buvo efektyvesnis nei gydymas vien tik podanteniniu instrumentavimu [139]. Lygiagrečiai klinikiniams duomenims, histologinė analizė atskleidė statistiškai reikšmingą naujo kaulo, naujo cemento ir naujos jungties formavimąsi tiriamojoje grupėje, palyginus su kontroline.

Analizuojant šio tyrimo rezultatus, reikia pabrėžti, kad šiame tyrime naudojamos dvi skirtingos medžiagos tarnavo kaip viena metodika, kai iš kiekvieno komponento sudedamosios dalies buvo tikimasi tam tikro papildomo poveikio atliekant podanteninį instrumentavimą. Terminas „*Clean and Seal“* (angl.) reiškia išvalyti ir užsandarinti. Valymo efektas šioje metodikoje išgaunamas chloraminų, susiformuojančių susijungus NaOCl chlorinui ir amino rūgščių amino funkcijoms. Chloraminai turi stiprų antibakterinį poveikį biofilmui, bet nedaro neigiamo poveikio minkštiesiems audiniams, cementui, dentinui [94, 95, 100, 140]. Taip pat, yra klinikinių ir *in vitro* tyrimų įrodžiusių, kad NaOCl/amino rūgščių gelis geba suardyti biofilmą, palengvina granuliacinio audinio pašalinimą iš periodontologinės kišenės ir turi akmenis minkštinantį poveikį [95, 98, 100, 141]. Teigiamo poveikio gijimui buvo tikimasi iš vidutiniškai stabilizuotos xHyA. Keletas *in vitro* eksperimentų dokumentavo, kad HyA, kaip biologinis moduliatorius, skatinantis periodonto žaizdos gijimą / regeneraciją, stimuliuoja kraujo krešulio formavimąsi [140, 142], skatina angiogenezę [103, 142], osteogenezę [105, 142]. Klinikiniai tyrimai rodo, kad, gydant intrakaulinius defektus, papildoma xHyA aplikacija padeda statistiškai reikšmingai sumažinti PD ir padidinti CAL [143].

Svarbu paminėti, kad šiame tyrime nebuvo tiriamųjų grupių, kuriose būtų tiriamas kiekvienos medžiagos poveikis atskirai, todėl neaišku, iki kokio lygmens kiekviena iš medžiagų prisidėjo prie sėkmingo gydymo rezultatų. Nepaisant to, pastaruoju metu atliktas tyrimas su gyvūnais, nagrinėjęs NaOCl/ amino rūgščių poveikį kartu su podanteniniu instrumentavimu, nustatė, kad papildomas šios medžiagos panaudojimas lėmė ne tik geresnius klinikinius

tyrimo rezultatus, bet ir histologinį gijimą [96]. Megally A. ir bendraautoriai paskelbė, kad, nors papildoma NaOCl/amino rūgščių gelio aplikacija kartu su podanteniniu instrumentavimu reikšmingai pagerina PD bei CAL, reikšmingo skirtumo tarp šios gydymo modifikacijos ir kontrolinės grupės nepastebėta [100]. Panašaus pobūdžio rezultatai gauti ir Pilloni A. ir bendraautorių tyrime, nagrinėjusiame klinikinį ir mikrobiologinį xHyA panaudojimą periodontito gydyme [128]. Konkrečiau, nors xHyA grupėje klinikinių parametrų pokytis buvo geresnis, statistinė analizė neparodė reikšmingų skirtumų tarp kontrolinės ir tiriamosios grupių. Šie rezultatai galėtų paaiškinti, kodėl abi medžiagos buvo naudotos vienos gydymo procedūros metu.

Dar vienas svarbus šio tyrimo atradimas, reikalaujantis dėmesio, yra faktas, kad šios gydymo metodikos metu išgautas naujo cemento ir naujos jungties formavimasis (atitinkamai 2,46 (0,77) mm ir 1,75 (0,65) mm) atitinka rezultatus, gautus tokio paties dydžio tyrimo, nagrinėjusio xHyA poveikį intrakaulinių defektų gydyme (atitinkamai 3,20 (1,29) mm ir 2,43 (1,29) mm) [114]. Šie rezultatai parodo, kad papildoma xHyA aplikacija yra kliniškai naudinga, o didelio molekulinio svorio produktas gali išlikti stabilus aplikacijos vietoje nuo 4 iki 6 sav.

Iš klinikinės perspektyvos, histologinio tyrimo duomenys rodo, kad C&S metodikos taikymas suteikia papildomos naudos konservatyviame periodontito gydyme ir sumažina chirurginio gydymo reikiamybę.

Įvertinant teigiamus klinikinius ir histologinius šio tyrimo rezultatus, reikėtų atkreipti dėmesį ir į tai, kad gydymas buvo atliekamas eksperimentiškai sukeltuose defektuose ir mažam gyvūnų kiekiui. Norint pagrįsti šio metodo pritaikymą klinikinėje praktikoje, reikia daugiau tolimesnių klinikinių tyrimų.

IŠVADOS

4. Papildoma natrio hipochlorito / amino rūgščių ir vidutiniškai stabilizuotos didelio molekulinio svorio hialurono rūgšties gelių kartu su podanteniniu instrumentavimu panaudojimas lėmė statistiškai reikšmingą kišenių zondavimo gylio, kraujavimo po zondavimo sumažėjimą, klinikinio audinių prisitvirtinimo lygio padidėjimą, palyginus vien su podanteniniu instrumentavimu.
5. Mikrobiologiniai tyrimo rezultatai atskleidė papildomo natrio hipochlorito / amino rūgščių ir vidutiniškai stabilizuotos didelio molekulinio svorio hialurono rūgšties gelių kartu su podanteniniu instrumentavimu panaudojimo pranašumą, palyginus vien tik su podanteniniu instrumentavimu, nustatant pagrindinių periopatogenų aptikimo dažnį ir bendrą skaičių prieš ir po gydymo – *A. Actinomycetemcoms, P. Intermedia, T. Denticola, P. Gingivalis, T. Forsythia*.
6. Histologinė tyrimo dalis atskleidė histologinį papildomo natrio hipochlorito / amino rūgščių ir vidutiniškai stabilizuotos didelio molekulinio svorio hialurono rūgšties gelių kartu su podanteniniu instrumentavimu pranašumą – konservatyviai regeneruoti visi periodonto audiniai – šaknies cementas, alveolinis kaulas, periodonto raištis.

PRAKTINĖS REKOMENDACIJOS

Klinikinės rekomendacijos

Nors kruopštus podanteninis bakterinio biofilmo pašalinimas ultragarsu ir / ar rankiniais instrumentais iki šiol yra „aukso standartas“ gydant periodontitą, šis gydymo metodas turi nemažai trūkumų – bakterinį biofilmą labai sunku pašalinti iš gilių kišenių, furkacijų, anatominių įdubų. Po gydymo likęs bakterinis apnašas sąlygoja greitą periopatogeninių bakterijų rekolonizaciją į jau gydytas sritis, kliniškai tai stebimos kaip aktyvios periodontologinės kišenės, kurios blogina ilgalaikę danties prognozę ir reikalauja tolesnio gydymo.

Atsižvelgiant į atlikto tyrimo rezultatus, rekomenduojama:

1. Pacientui diagnozavus II–III stadijos, A, B laipsnių periodontitą, kaip standartinį antro periodontito žingsnio gydymo protokolą, kartu su kruopščiu podanteniniu instrumentavimu, papildomai naudoti natrio hipochlorito / amino rūgščių ir vidutiniškai stabilizuotos hialurono rūgšties gelius.
2. Natrio hipochlorito/amino rūgščių gelis turėtų būti įvedamas į periodontologinę kišenę 1 min prieš pradedant podanteninį instrumentavimą ir kartojamas tol, kol zondu ieškikliu zonduojamas švelnus ir lygus gydytų šaknų paviršius.
3. Baigus podanteninį instrumentavimą į periodontologinę kišenę buka adata suvesti vidutiniškai stabilizuotą didelio molekulinio svorio hialurono rūgštį, pradedant nuo kišenės dugno, iki tol, kol preparatas pilnai užpildo periodontologinę kišenę.
4. Pradinis vertinimas po gydymo (klinikinių rodiklių matavimas) turėtų būti atliekamas po 4–5 mėnesių, kadangi šis gydymo metodas pasižymi regeneraciniu poveikiu periodonto audiniams.

Mokslinės rekomendacijos

1. Ateityje reikėtų ilgesnės trukmės, didesnę pacientų imtį turinčių kontroliuojamų klinikinių tyrimų, norint įvertinti šios gydymo metodikos efektyvumą ilgalaikėje perspektyvoje.
2. Į tyrimą buvo įtraukti tik sistemiškai sveiki ir nerūkantys pacientai, todėl būsimi tyrimai turėtų nagrinėti, ar C&S metodika tokia pat efektyvi ir kitose pacientų grupėse – pacientams, kuriems diagnozuota sisteminė patologija (cukrinis diabetas, kardiovaskulinė patologija ir kt.), rūkantiems pacientams.
3. Nagrinėti kiekvienos medžiagos (natrio hipochlorito / amino rūgščių gelio ir vidutiniškai stabilizuotos didelio molekulinio svorio hialurono rūgšties) poveikį atskirai klinikiniams periodontologiniams parametrams konservatyviai gydant periodontitą.

SUMMARY

INTRODUCTION

Periodontitis is a chronic inflammatory disease associated with microbial dysbiosis and characterized by progressive destruction of the tooth-supporting apparatus [1]. Primary clinical features of periodontitis include the loss of periodontal tissue support, which manifests through the losses of clinical attachment and radiographically assessed alveolar bone along with gingival bleeding and periodontal pockets. Untreated periodontitis leads to early tooth loss, though most cases can be managed and treated successfully.

According to epidemiologic data, periodontitis is one of the sixth most common diseases in a global burden of diseases; it affects more than 11.2 % of the population (743 million people) [2]. It is the main cause of teeth loss in adult population [3]. Periodontitis negatively affects the masticatory function, the patient's overall quality of life, self-esteem, different socio-economic factors, and high treatment costs [3]. As the population ages, the number of patients diagnosed with periodontitis increases [4].

Periodontitis is a multifactorial disease. The main risk factor for developing periodontitis is dental plaque that accumulates in the gingival crevice, and provokes the destructive inflammatory response of the host agent [5]. Dental plaque is a well-organized system of bacterial origin that causes homeostasis of periodontal tissues and the regular communication between the microorganisms and the defensive processes of the immune system [6]. The host agent's immune response is defined by the virulence of the bacteria and other specific factors where specific species of bacteria are associated with periodontitis [7]. However, only bacteria *per se* are not enough to cause periodontitis. Predisposing factors such as genetics, smoking, systemic conditions, obesity, stress, dietary habits, etc., influence the reduced response of the immune system [8–12].

The main treatment goal is arresting its progression and maintaining healthy and stable periodontal tissues. Eliminating biofilm is of key importance for successfully treating periodontitis. Periodontal therapy should also reduce or remove the accumulation of pathogenic species that cause and/or sustain periodontal diseases [13]. According to the cause-related therapy concept, a meticulous subgingival debridement (SD) is the basics of periodontal therapy. However, this therapy is technically demanding and has limitations, resulting in some bacterial plaque residue in the deep pockets, intrabony defects, or furcation areas. The efficacy of the treatment also depends on various other factors, such as the operators' manual skills, patients' smoking status and/or systemic diseases. Residual calculus may cover up to 30 % of the total

subgingivally debrided root surface area, and periodontal pockets that persist after the periodontal treatment impair the long-term prognosis of a tooth [14, 15]. The 6 mm residual probing depth poses a risk factor for tooth loss with ratio odds of 6 and 8, respectively, at site and tooth levels; the bigger the residual probing depth, the greater the chance of losing a tooth is [16].

As periodontitis is infectious in nature, it is logic to use adjunctive materials with antmicrobial activity in order to eliminate or deactivate pathogenic microflora in sites where using mechanical instruments is invidious [17]. Based on recent studies, adjunctive agents, when combined with subgingival debridement, might improve the clinical outcomes of non-surgical periodontal therapy [17–19]. These adjunctive aids could be systemic or local antibiotics or antiseptics [20–22].

Due to the emerging global public health issue of bacterial resistance, the number of warnings on the unrestricted antibiotics used in treating periodontal disease has increased. Therefore, specific patients with certain periodontal conditions, e.g., disease stages III–IV, grade C, "active," "refractory," and "recurrent" forms, should be treated with systemic antibiotics rationally and following optimal protocols [23]. Local antibiotics lack effectiveness in periodontal disease treatment due to several potential problems, including insufficient antimicrobial activity, risks of producing an antibiotic–resistant microbiota, and high acquisition costs [21]. Antiseptics, being chemical agents, can destroy microorganisms on live tissues since they feature several beneficial properties compared to systemic or local antibiotics. Notably, they have a more extensive activity spectrum with multiple intracellular targets that allow for reducing the possibility of resistance formation [21].

Recently, a novel concept termed *"Clean and Seal"* (C&S), in conjunction with subgingival instrumentation has been suggested to enhance the outcomes of non-surgical periodontal therapy. The two components of C&S are sodium hypochlorite/amino acids (NaOCl) (Perisolv®, Regedent AG, Switzerland) and cross-linked hyaluronic acid (high molecular weight) (xHyA) gels (Hyadent® BG, Regedent AG, Switzerland). At present no randomized controlled clinical trials have evaluated the potential clinical, histologic relevance and microbiological outcomes of this novel concept as compared to subgingival instrumentation alone.

Study aims and objectives

Aim:

To examine the clinical, microbiological, and histologic outcomes received with either SD combined with NaOCl /amino acids containing gel with a subsequent application of xHyA or with SD alone.

Objectives of the study:

1. 1. To compare the clinical outcomes–changes in pocket PDs, CAL, and BOP obtained with either SD in conjunction with a NaOCI/amino acid containing gel followed by subsequently applied xHyA gel or SD alone.
2. 2. To compare the microbiological changes in terms of detection frequency and changes of total bacterial counts for the principle periodontopathogenic bacteria (*A. Actinomycetemcoms (A.a), P. Intermedia (P.i), T. Denticola (T.d), P. Gingivalis (P.g), T. Forsythia (T.f)*) obtained with either SD in conjunction with a NaOCI/amino acid containing gel followed by subsequently applied xHyA gel or SD alone.
3. 3. To evaluate the periodontal wound healing following SD in conjunction with NoOCl/amino acids and xHyA gels in dogs.

MATERIAL AND METHODS

1. Clinical part

Design

The study was conducted as a 6-month prospective, examiner-blind, randomized controlled clinical trial with a parallel arms design. The trial was conducted according to the CONSORT guidelines (http://www.consort-statement.org/) [119] and under the ethical approval – Permit No. BE-2-87, issued by the Regional Biomedical Research Ethics Committee. Before participation, all subjects signed an informed consent form. They were allocated randomly to the control or test groups with a ratio of 1:1. The study was conducted between September 2019 and January 2022, and its protocol was registered at ClinicalTrials.gov, NCT04662216.

Study subjects

All subjects enrolled in this clinical trial were treated at the Dental and Oral Pathology Department of the Lithuanian University of Health Sciences in Kaunas, Lithuania.

Participant inclusion criteria:

- males and females ≥ 18 years old;
- diagnosed with periodontitis stages II–III, grades A/B, generalized [1];
- good overall health (i.e., without systemic diseases, no medication intake, which may affect the periodontal status of the patient);
- had at least 20 teeth (wisdom teeth excluded);
- with no removable dentures;
- were willing to sign an informed consent form and being able to complete the 6-month duration study.

Patient exclusion criteria:
- smokers;
- underwent periodontal treatment within the last 12 months;
- used antibiotics 3 months before the start of the study;
- antibiotic prophylaxis was needed;
- used medication that could affect the clinical properties of periodontitis;
- were pregnant/lactating;
- had allergies to sodium hypochlorite.

Sample size calculation

Before starting the study, calculations of the minimum number of necessary cases (at least 20 subjects per group) were performed. The following parameters were set: a significance level ($\alpha = 0.05$), a relevant average difference in PD (1 mm between the study groups with a standard deviation of 1 mm) and a power (1-α of at least 0.8). Considering the possibility of participant dropouts during the study period, the number of subjects in each group was increased to 24. A power calculation at the end of the study with the given number of cases yielded a power of 99.6 %.

Periodontal treatment

Two weeks before the treatment, the baseline periodontal examination was assessed. Then, all included patients received professional oral hygiene and were instructed for correct teeth and interdental cleaning techniques. All subjects received the same type of toothpaste (Elmex Enamel Protection, Gaba GmbH, Germany), toothbrush (CS 5460, Curaprox, Switzerland), and interdental brushes of the right size (TePe, Tepe Mundhygienprodukten, Sweden). The instructions for oral hygiene were reinforced during every follow-up visit.

After two weeks, participants in the control group under local anesthesia received a full-mouth SD by using the ultrasonic scaler (Satelec/Acteon Suprasson Newtron) and hand instruments (mini Gracey, Gracey Sharp Diamond Curettes (1/2, 7/8, 11/12, 13/14), LM Dental, TM, Finland).

Following the instrumentation, the teeth were polished with a low-abrasive polishing paste (Lunos Super Soft, RDA < 5, Dürr Dental, Germany). The subgingival instrumentation took approximately 3.5 hours per patient.

Study subjects in the test group under local anesthesia received a full-mouth SD complemented by sodium hypochlorite/amino acids (Perisolv®, Regedent AG, Switzerland) and cross-linked hyaluronic acid (high molecular weight) (xHyA) gels (Hyadent® BG, Regedent AG, Switzerland). In detail, all pockets with PDs ≥ 4 mm that bled on probing were filled up with Perisolv® 30–60 sec prior to instrumentation. The SD was carried out in the same manner as in the control group. Perisolv® was re-applied for a total of 2–3 times until the instrumentation was considered sufficient (Fig. 1).

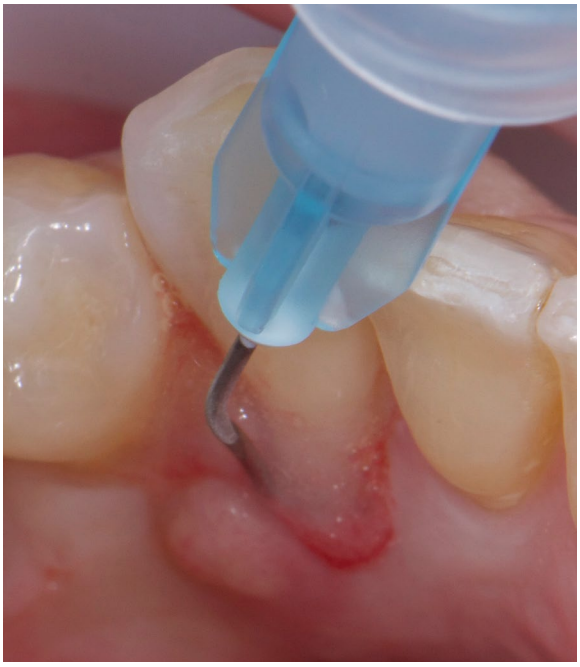


Fig. 1. *Application of Perisolv® to the periodontal pocket before subgingival instrumentation.*

After the SD polishing, a mixture gel (Hyadent BG, Regedent AG, Switzerland), consisting of 0.2 % native and 1.6 % cross-linked hyaluronic acid (high molecular weight), was applied into all treated sites by using a blunt needle (Fig. 2).

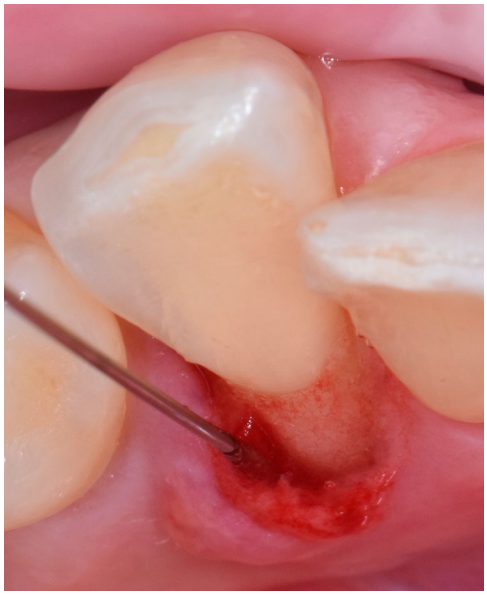


Fig. 2. *The application of Hyadent® BG to the treated site following the subgingival instrumentation.*

Clinical parameters

The clinical measurements were obtained by the Williams periodontal probe (LM 51 ES, LM-DentalTM, Finland) on all teeth at six sites per tooth (mesio-buccal (mb), mid-buccal (b), disto-buccal (db), mesio-oral (mo), mid-oral (o) and disto-oral (do)). Assessments were done before treatment – at baseline (T0), and at 3 months (T1) and 6 months (T2) after treatment. Clinical evaluation allowed to assess the following:

- BOP, defined by the positive to bleeding within 10 s after probing site percentage (%); BOP was assessed in the treated sites (PD ≥ 4 mm and BOP+) and full mouth (FMBOP);
- PI, defined by the percentage of sites with a visual plaque on the tooth surface (%), assessed in the treated sites (PD≥ 4 mm and BOP+) and full mouth (FMPI);
- PD, the distance from the gingival margin to the probed pocket bottom, measured in mm;
- REC, the distance from the gingival margin to the cementoenamel junction or the margin of a cervical restoration, measured in mm;
- CAL, calculated by adding PD and REC, measured in mm;

During every follow-up, complications or adverse occurrences had to be reported.

Outcomes

Clinical

For statistical evaluation, probing depths (PDs) were classified into two groups: moderate (PD 4–6 mm) and deep (PD ≥ 7mm). The average PD change in moderate sites after comparing the baseline and the 6-month results was the primary outcome variable. The secondary outcome variables encompassed the PD change for deep pockets at 6 months, along with the shifts of CAL in moderate and deeper sites. The mean BOP and PI variations were also analyzed by comparing the baseline and 6-month outcomes across treated sites (PD ≥ 4 mm and BOP +) and the entire mouth.

Microbiological

The primary outcome variable was a change in the detection frequency of *A.a, P.g, T.f, T.d,* and *P.i* from the baseline to 6 months. The secondary outcome variables included alterations in the corresponding bacterial detection scores, comparing the baseline with the 6-month follow-up findings.

Microbial sampling

Microbial sampling was performed from the deepest site per quadrant four samples per study subject at T0, T1 and T2. Before sampling, the surfaces of the teeth were meticulously cleaned with Gracey currettes and sterile cotton pellets. Afterwards, the site was dried and isolated with cotton rolls.

A sterile endodontic paper point ISO #30 (Dentsply Sirona, USA) was placed and retained for 20 s. The paperpoints were collected in coded sterile Eppendorf tubes and delivered to the Department of Laboratory Medicine (Lithuanian University of Health Sciences) for further analysis. The samples were stored at –20 °C until additional processing at the facility for one day, and then at –80 °C until the microbiological testing was conducted (not exceeding 30 days). The molecular assessment of the subgingival plaque samples was manually carried out in the following steps:

1. deoxyribonucleic acid (DNA) extraction;
2. simultaneous amplification with biotin-labeled primers;
3. reverse hybridization.

Deoxyribonucleic acid extraction

The isolation of DNA was carried out using a purification process with a swab-based kit (Swab, version 0517, A&A Biotechnology, Poland) by incorporating lysis solution (700 µl) and proteinase K (20 µL) into the initial Eppendorf tubes containing subgingival plaque samples. The tube contents were then thoroughly blended, briefly subjected to centrifugation, and maintained at at 37 °C for 20 min while being agitated at 500 revolutions per

minute (rpm). After incubation, the samples were mixed and then spun down. The supernatant was transferred to the filtration columns and subjected to centrifugation (1 min at 12000 rpm). Two rounds of washing were conducted using fresh 2 mL tubes and 500 μL of wash buffer per cycle; the wash solution was spun at 12000 rpm for 1 min initially, followed by a second round for 2 minutes. The cleaned and processed columns were relocated into fresh 1.5 mL tubes, where 150 μL of elution buffer, preheated to 75 °C, was added. The tubes were then left to incubate (for 3 min at ambient temperature) and subsequently spun down (1 min at 12000 rpm). The analyzed DNA samples were preserved at –80 °C until future examination.

Simultaneous amplification with biotin-labeled primers

The DNA samples were examined using a molecular genetic assay for the simultaneous identification of five periodontopathogenic bacterial species (micro-IDent VER 2.0, Hain Lifescience GmbH, Germany), including *A.a, P.g*, *P.i, T.f, and T.d.* A master mix of the amplification enzymes was freshly prepared before testing each batch of DNA samples. A volume of 45 μL of master mix and 5 μL of DNA samples or a negative control (molecular biology-grade water) were prepared and combined in separately designated laboratory areas. A negative control was included with each individual batch. The polymerase chain reaction for DNA amplification was executed using a thermal cycler, follwing the guidelines provided by the diagnostic kit supplier. The amplified products were preserved at 2–4 °C until subsequent processing.

Reverse hybridization

Before initiating the testing process and adhering to its instructions, the reagents were maintained at room temperature (20–25 °C) or heated up to 45 °C, and the required diluents were prepared. Initially, 20 μL of denaturation solution and 20 μL of amplified DNA sample were combined and left to incubate at room temperature for 5 minutes. Subsequently, the test strips containing 1 mL of pre-warmed hybridization buffer were placed into each well containing the denatured DNA samples. The wells were then incubated for 30 minutes at 45 °C in the shaking water bath. Following this incubation period, the hybridization buffer was removed, and 1 mL of stringent wash solution was introduced into each well. The incubation process lasted for 15 minutes at 45 °C in the shaking water bath. Once the stringent wash solution was eliminated, each strip underwent washing with rinse solution (1 mL) for 1 minute on a shaking platform. After that, 1 mL of diluted conjugate solution was introduced into each well and incubated for 30 minutes at room temperature while being agitated on a shaking platform. Once the conjugate was removed, each strip was washed three times for 1 minute on a shaking

platform: twice using a rinse solution and once with distilled water. Following this step, 1 mL of diluted substrate solution was added to each well and incubated while being shielded from light and kept stationary for 15 minutes. As soon as the test strip bands became distinct, they were briefly rinsed twice with distilled water, dried between absorbent paper layers, pasted onto the provided evaluation sheet, and stored in a light-protected environment.

Evaluation of microbiological results

To begin, the prepared test strips were examined to ensure accuracy and proper execution of the testing methods by monitoring three control bands (conjugate control, hybridization control, and amplification control). After verifying that all three control bands were properly formed, five bacterial species-specific bands were assessed through a semi-quantitative technique. The intensity of the developed band color was measured using a point scale ranging from 0 to 4. A semi-quantitative analysis of the band color intensity, expressed in colony-forming units per milliliter (CFU/mL), is provided in Table 1.

Table 1. *Semi-quantitative interpretation of the test results*

Bacterial species	**Test strip band color intensity, CFU/mL**				
	0	**1**	**2**	**3**	**4**
A.a	$< 10^3$	10^3	$< 10^4$	$< 10^5$	$> 10^6$
P.g	$< 10^4$	10^4	$< 10^5$	$< 10^6$	
P.i					
T.f					
T.d					

A.a – Aggregatibacter actinomycetemcomitans; CFU – colony forming units; *P.g – Porphyromonas gingivalis*; *P.i – Prevotella intermedia*; *T.d – Treponema denticola*; *T.f – Tannerella forsythia.*

Blinding

The clinical assessments, initial supragingival plaque removal, and microbial collection were conducted by a masked, calibrated examiner (U.M.D.) who was unaware of the type of intervention assigned to each case. To minimize bias, all records were entered without access to prior measurements.

To maintain blinding, an experienced periodontist carried out the procedures (E.R.). The participants were unaware of their assigned group, and those receiving periodontal treatment had facial drapes to prevent them from viewing the procedure.

Without knowledge of the assigned treatment, a third investigator handled the encrypted data for statistical evaluation (I.N.). A fourth researcher, who

was also unaware of the treatment assignments or clinical parameters, conducted the microbiological analysis (L.P.).

Randomization and allocation concealment

Forty-eight individuals enrolled in the clinical trial were randomly assigned to two treatment groups. The generated computer-based randomization table distributed unique participant numbers from 1 to 48 and divided them into two sets of randomized numbers (24 in the control group and 24 in the experimental group). The sealed envelopes (to be opened immediately before periodontal therapy) ensured the masking of participant assignment.

The creation of the random sequence allocation and the assignment of participants to interventions were conducted by the investigator (F.Y.), separate from the clinical examiner and the clinician administering the treatment.

Calibration

Five individuals not involved in the study, each diagnosed with periodontitis stages II–III [1], took part to ensure the examiner's calibration. The examiner was instructed to assess PD, REC, CAL, BOP, and PI at six locations per tooth across two separate visits, with a 48-hour interval between them. The calibration was considered valid if the measurements taken at the initial assessment and after 48 hours differed by no more than one millimeter in over 90 % of cases, and the examiner remained unaware of the procedure being conducted.

Statistical analysis

The IBM SPSS 27 software package ("Statistical Package for the Social Sciences for Windows, Inc.", Chikago, IL, USA) was used to perform a statistical analysis. In the data analysis, a study participant indicated a statistical unit. The mean values for all clinical parameters were calculated per group subject and follow-up visit. In particular, the PD and CAL of moderate pockets at the baseline and 3 and 6-month follow-ups were calculated by defining average PDs and CALs in moderate sites at the baseline, as well as 3 and 6-month time points for each participant. Similarly, per-patient PD and CAL of deep pockets at the baseline and 3 and 6-month time points were obtained by averaging PD and CAL values in the deep sites for each study group subject at the baseline and 3 and 6-month time points. The BOP and PI per group subject were obtained by calculating each participant's percentage share of tooth sites with BOP and plaque by classifying pockets by the baseline PD (all treated sites with PD $\geq$ 4 mm and full mouth).

The Shapiro–Wilk test was used to evaluate whether clinical periodontal parameters followed a normal distribution. A paired-sample *t* test, if the data

followed a normal distribution, and if it was out of a normal distribution range, the Wilcoxon signed rank test on the related samples was performed to evaluate the groups‘ prior and post-treatment comparisons. The between-group comparisons of the parameters were obtained by either the independent-sample *t* test (if a parameter was under a normal distribution) or the Mann–Whitney test (if a specific parameter did not follow a normal distribution). The differences in detection frequency (where 0 = undetected, 1 = detected) between the control and test groups at the baseline and 3 and 6-month follow-ups were analyzed using the x^2 test. The group changes were evaluated using the McNemar test.

The results for changes in the detection frequency scores were recorded and classified into one of the following categories: 0 – non-detectable, 1, 2, 3, and 4 (Table 1). The Wilcoxon signed rank test allowed the intergroup‘s baseline and 3 and 6-month results‘ evaluations of detected period pathogen species scores to be compared. The Mann-Whitney test was used to compare the inter-group detection scores at each study test time point. The significance level was set at 0.05.

2. Histologic part of the study

Animals

Four healthy male Beagle dogs, aged between 26 and 38 months and weighing between 9 and 15 kg, were included in this research. The housing conditions and daily monitoring of the animals throughout the study were carried out at Shin Nippon Biomedical Laboratories, Ltd., in the Animal Testing Facility in Japan. They were housed in separate enclosures under a controlled temperature range of 20–26°C, with a relative humidity of 30–70 % and a regulated light/dark cycle. Each dog was provided with approximately 300 g of solid feed daily (NVE-10, Nippon Pet Food, Co., Ltd., Japan) and had unrestricted access to water. All animal-related procedures during the experimental period were approved by the Ethics Committee of the Animal Research Center at Kagoshima University, Japan (Project Approval No. D22017; issued on January 23, 2023). This research adhered to the ARRIVE Guidelines for Preclinical Animal Studies.

Experimental periodontitis

An experienced surgeon carried out all surgical procedures under both general and local anesthesia while adhering to aseptic protocols. Before the surgical interventions, antibiotics (dihydrostreptomycin sulfate aqueous suspension for injection, 0.05 mL/kg; Mycillin Sol Meiji for veterinary use,

Meiji Seika Pharma Co. Ltd., Japan) were administered via intramuscular injection.

General anesthesia was induced via intramuscular injection using medetomidine hydrochloride (Domitor®, 0.08 mL/kg IM; Orion Corp., Finland), midazolam (Dormicum®, IM; Maruichi Pharmaceutical, Japan) at a dose of 0.08 mL/kg, and butorphanol tartrate (Vetorphale® 5 mg, Meiji Seika Pharma, Japan) at 0.02 mL/kg. Once sedation was achieved, anesthesia was maintained through inhalation of sevoflurane (0.5 %–5.0 %, Mylan Pharma Co., Ltd., Japan) combined with a nitrogen/oxygen (2:1) mixture delivered via an intracircuit vaporizer to facilitate spontaneous breathing.

Local anesthesia was administered using lidocaine HCl/epinephrine (2 %, 1:80,000; Xylocaine; Fujisawa Inc., Japan). The bilateral mandibular first and third premolars were extracted to ensure sufficient space for the formation of intrabony defects. After an 8-week recovery period, two-wall intrabony defects (measuring 5 mm in width and 5 mm in depth) were surgically created on both sides at the mesial region of the mandibular fourth premolars (P4) and the distal section of the mandibular second premolars (P2), resulting in four defects per dog.

Following the lifting of the mucoperiosteal flap, the defects were shaped using fissure burs while applying sterile saline coolant (Fig. 3)

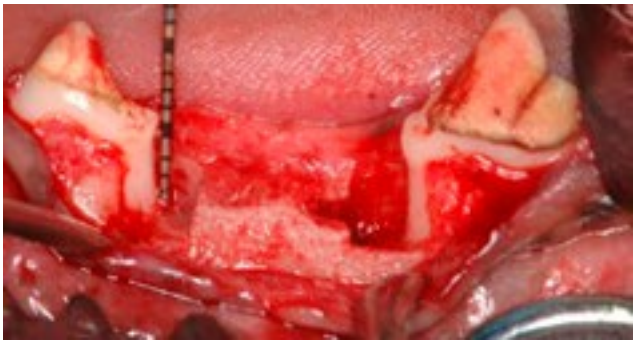


Fig 3. *Surgically created two-wall intrabony defects in the mandible.*

Gracey curettes and a chisel were utilized to eliminate the tissue. Reference notches on the root surface were created using a No. 1 round bur at the cementoenamel junction (CEJ) and the crown surface to mark a precise midline of the intrabony defects, facilitating an optimal histomorphometric assessment.

Metallic strips were attached to the tooth surface within the intrabony defects using a self-curing dental adhesive resin cement (Super Bond C&B,

Sun Medical Co., Ltd., Japan) (Fig. 4) to inhibit spontaneous healing and promote plaque accumulation.

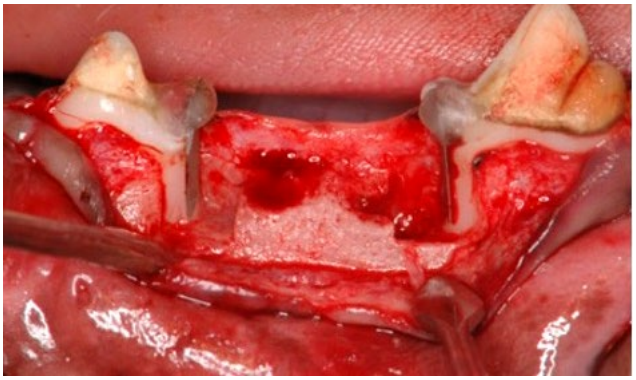


Fig. 4. *Placement of the metal strips on the denuded root surfaces.*

To ensure proper repositioning and securing of the flaps, 4-0 silk sutures were used (removed 14 days post-procedure). Ketoprofen for pain relief (Capisten IM 50 mg, 2 mg/kg, 1 mL/kg; Kissei Pharmaceutical Co. Ltd., Japan) and an antibiotic (Mycillin Sol) were administered daily for two days after the surgeries.

Intraoral periapical radiographs of the designated areas, including the premolars (P2 and P4), were obtained immediately following the procedure. The animals were provided a soft-textured diet during induction to encourage plaque accumulation (Fig. 5).

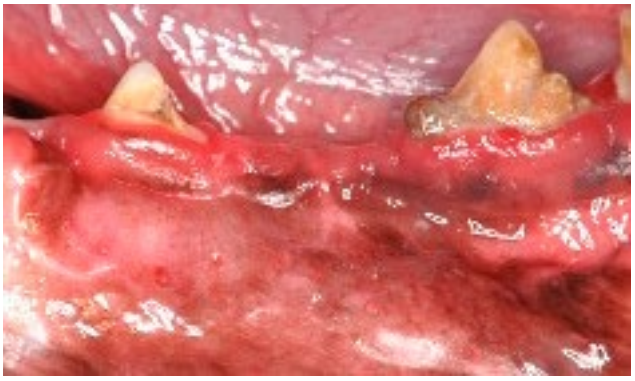


Fig. 5. *Plaque accumulation after 4 weeks.*

After four weeks, the radiographs verified the progression of bone loss, and the metallic strips were taken out without reflecting the flap. Acrylic stents featuring indentations on the mid-proximal root surfaces, where the

deepest periodontal pockets were identified, were crafted to standardize the clinical assessments in this study (Fig. 6).

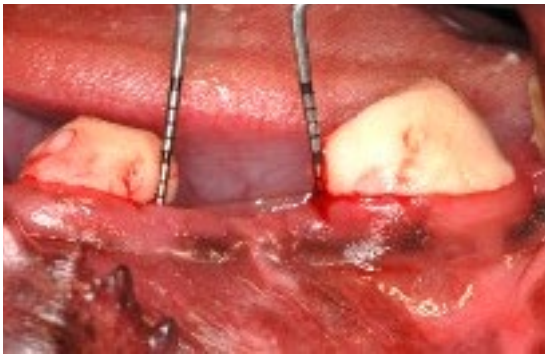


***Fig. 6**. Baseline clinical measurements.*

Periodontal treatment

Plaque management was carried out regularly (three times per week), irrigating the oral cavity with a 2 % chlorhexidine gluconate solution for two weeks prior to treatment. A split-mouth design was implemented in this study to avoid cross-contamination of gel-type agents between sites on the same mandibular side. The selected 16 experimental teeth, specifically bilateral P2 and P4, were designated as test and control sites using a coin flip. Prior to non-surgical periodontal therapy, the subgingival surfaces underwent professional cleaning. On one side, the teeth were managed with SD, followed by the application of a sodium hypochlorite/amino acid gel and a cross-linked hyaluronic acid (xHyA) gel (test group). Conversely, the teeth on the opposite side were subjected to SD as part of the control group, conducted by the same experienced dental specialist (T.N.). The SD protocol for the test group included the use of a sodium hypochlorite/amino acid gel (Perisolv®), along with an alkaline 0.95 % sodium hypochlorite solution. Additionally, a mildly viscous alkaline gel comprising glutamic acid, leucine, lysine, carboxymethyl cellulose, and titanium dioxide (Regedent AG, Switzerland) was inserted into the periodontal pockets using a blunt needle for 30 seconds (Fig. 7).

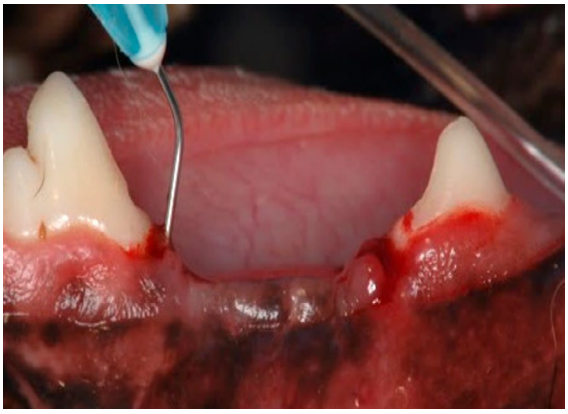


Fig. 7. *Sodium hypochlorite/amino acid gel application to the periodontal pocket.*

Prior to saline irrigation and instrumentation, an ultrasonic device (ENAC 10WA, Osada, Japan) and an ultrasonic tip (ST35, Osada, Japan) for 15 sec, followed by hand instrumentation with manual curettes (LM Sharp Diamond Mini Gracey 11/12, 13/14 SD curettes, LM DentalTM, Finland) through 5 traction movements in the buccal and interproximal area were applied and the same process was repeated. Following the final saline irrigation, the xHyA (Hyadent BG®, a gel formulation containing butanediol diglycidyl ether-cross-linked HA (1000 kDA HA monomers) and non-cross-linked HA (2500 kDA) in a ratio 8:1, made from biotechnologically produced synthetic HA, Regedent AG, Switzerland) gel (0.1 mL/tooth) was instilled in the pockets using a blunt needle (Fig. 8).

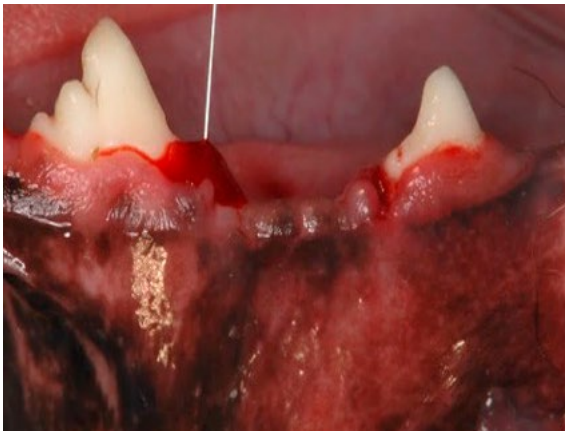


Fig. 8. *Application of a xHyA gel to the periodontal pocket.*

The control group animal teeth underwent the identical procedure except for the sodium hypochlorite/amino acid and xHyA application. After the treatments, no antibiotics or analgesics were administered, the animals were fed a solid diet, and the previously mentioned oral hygiene regimen was performed daily for 8 weeks to reduce plaque formation.

Histomorphometric analysis

All specimens were examined using a light microscope (BX51; Olympus Corp., Japan) equipped with a computerized imaging system (HF2015; Mitani Corp., Japan). The three sections (approximately 90 μm apart) from the most central region of each two-wall defect, identified by the root canal length and the reference notches, were chosen for histomorphometric assessment. The mean value of each histomorphometric parameter was determined for each site.

A blinded evaluator analyzed sixteen sections from all sites at two different time intervals (48 hours apart) to assess intra-examiner reproducibility, and the examiner's inter-calibration was confirmed at a 90 % level.

The researcher (T.I.) recorded the following parameters:

1. Defect height (DH) – the distance between the apical extent of root planing and the cemento-enamel junction.

2. Junctional epithelium length (JE) – the span between the junctional epithelium expansion and the cemento-enamel junction.

3. Connective tissue adhesion (CT) – the coronal extent of newly developed cementum, including the distance between the apical extent of the junctional epithelium.

4. New bone length (NB) – the span from the apical extent of root planing to the coronal edge of newly regenerated alveolar bone along the root surface.

5. New bone area (NBA) – newly formed trabecular bone within a 5×5 mm template, serving as a standardized reference for the defect location; the template was aligned parallel to the root surface, which interacted with the root planing apical extension.

6. New cementum length (NC) – the distance between the apical region of root planing and the coronal boundary of the newly developed cementum on the denuded root surface.

7. New attachment length (NA) – the linear segment of the root surface covered by the NC adjacent to newly regenerated bone, with functionally aligned collagen fibers.

Statistical analysis

The primary histomorphometric outcome, measured for the treatment groups at 8 weeks, was new attachment (NA). The clinical parameters

were evaluated as secondary ones. However, due to the limited number of preclinical studies (with a comparative design and primary outcome) in dogs, a power analysis of sample size calculation could not be performed. For ethical reasons, the sample size was set to an absolute minimum, i.e., four animals, and an animal was a statistical analysis unit. Each parameter's means and standard deviations were calculated for each treatment group. The clinical parameters between the baseline and at the 6-week follow-up were compared using the Wilcoxon signed-rank test, the clinical and histological values between the control and test groups – the Mann-Whitney U test, and the proportions of sites showing BOP-Fisher's exact test. A p value of < 0.05 was considered statistically significant. The statistical software (BellCurve for Excel; Social Survey Research Information Co., Ltd., Japan). was used to perform all study calculations.

RESULTS

Clinical results

Participant flow

All 48 individuals successfully completed the clinical trial. Each study group (SD or SD + NaOCl/amino acid + xHyA) consisted of 24 randomly assigned subjects. A flowchart illustrating the study enrollment process is presented in the CONSORT flow diagram (Fig. 9). The healing process in all trial participants proceeded without complications. No adverse reactions to sodium hypochlorite/amino acid and xHyA were detected throughout the trial period.

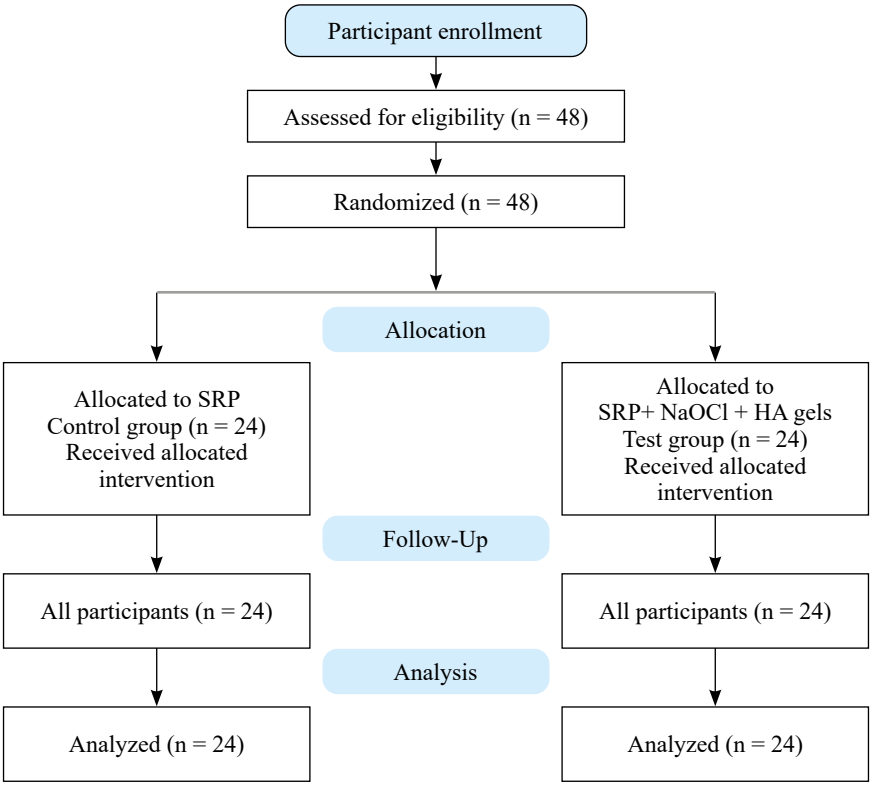


Fig. 9. *CONSORT Participant flow diagram*

Baseline characteristics

The initial clinical and demographic baseline characteristics of 48 study participants are summarized in Table 2. The initial assessment revealed comparable values for PD, CAL, BOP and FMBOP, and plaque scores (with no significant differences apart from PI and FMPI) between the two study groups.

***Table 2**. Characteristics of sample population at the baseline*

	Control group (n = 24)	**Test group (n = 24)**	***p* value**
Age (years)	49.3 (11.2)	47.3 (10.7)	0.53[a], n.s.
Gender, n (%) Males Females	 7 (29.2) 17 (70.8)	 6 (25) 18 (75)	 0.745[b], n.s.
Periodontitis stage, n (%) Stage II Stage III	 16 (66.7) 8 (33.3)	 17 (70.8) 7 (29.2)	 0.134[b], n.s.
PD (mm)	5.3 (0.6)	5.2 (0.4)	0.592[c], n.s.
CAL (mm)	5.5 (0.5)	5.6 (0.6)	0.546[c], n.s.
PI (%)	38.8 (26)	60.6 (10.9)	**0.002[c]**
BOP (%)	81.8 (16.2)	83.2 (15.5)	0.687[c], n.s.
FMPI (%)	35.7 (23.7)	52.9 (11.4)	**0.003[c]**
FMBOP (%)	68.9 (20.3)	76.5 (18.2)	0.184[c], n.s.

BOP – bleeding on probing; CAL – clinical attachment level; FMBOP – full-mouth bleeding on probing; FMPI – full-mouth plaque index; PD – probing depth; PI – plaque index.
n.s. not significant.
[a] independent-sample *t* test; [b] Fisher's exact test for the 2×2 table, gender by group (SRP, SRP+NaOCl+xHyA); [c] Mann-Whitney U test for two independent groups.

Effect on clinical parameters

The PD changes throughout the study duration were assessed for different pocket categories: moderate (4–6 mm) and deep (PD ≥ 7 mm). The data is presented in Table 3.

***Table 3.** PD (mean (SD)) at sites with moderate (4–6 mm) and deep (≥ 7mm) pockets*

	Control group (n = 24)	**Test group (n = 24)**	***p* value**
Moderate pockets (4–6 mm)			
T0	4.8 (0.2)	4.7 (0.2)	0.417[a]
T1	2.9 (0.7)	2.2 (0.4)	< 0.001[a]
T0 vs. T1 months	< 0.001[b]	< 0.001[b]	
T2	3.0 (0.6)	1.8 (0.4)	< 0.001[a]
T0 vs. T2 months	< 0.001[b]	< 0.001 [b]	
Deep pockets (≥ 7mm)			
T0	8.0 (0.7)	8.2 (0.9)	0.443[a]
T1	4.4 (1.4)	2.9 (1.1)	< 0.001 [a]
T0 vs. T1 months	< 0.001[b]	< 0.001[b]	
T2	4.3 (1.0)	2.4 (1.0)	< 0.001
T0 vs. T2 months	< 0.001[b]	< 0.001[b]	

[a] statistical analysis by Student's *t* test for two independent groups; [b] paired Student's *t* test for two dependent groups.

The baseline values in moderate pockets between the control and test groups revealed no statistically significant difference (4.8(0.2) mm and 4.7(0.2) mm, respectively, $p = 0.417$). When compared to the baseline, both study groups exhibited statistically significant improvements at 3 and 6-month intervals ($p < 0.001$). Nevertheless, the test group showed notably greater reductions at both evaluation time points ($p < 0.001$) (Table 3). The change in PD between 3 and 6-month assessment periods differed statistically significantly between groups, favoring the test group ($p = 0.002$) (Fig. 10).

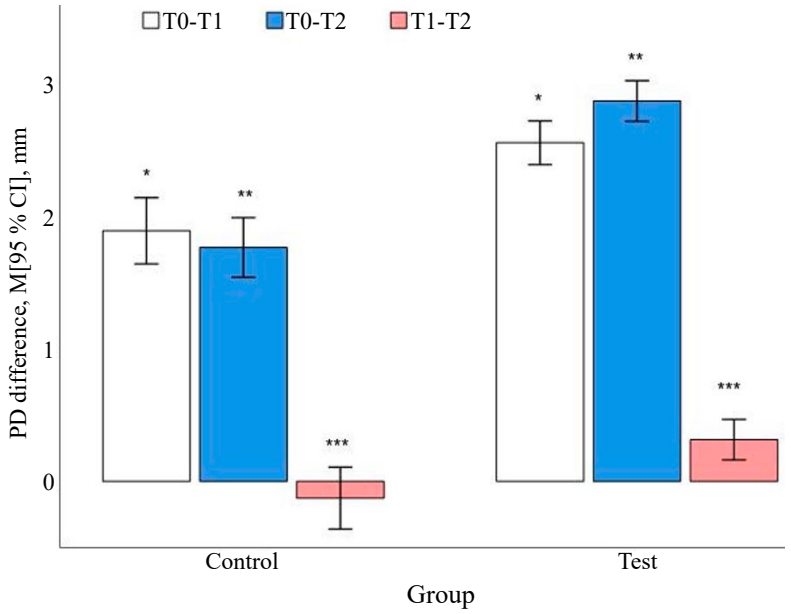


Fig. 10. *Mean changes in PD in moderate pockets (4–6 mm) at different study time points.*

$^{*,**}p < 0.001$, $^{***}p = 0.002$ by Student's *t* test for two independent groups
T0 – baseline; T1 – 3-month follow-up; T2 – 6-month follow-up.

The initial PD values in the deep pocket category did not exhibit a statistically significant difference between the control and test groups (8.0(0.7) mm and 8.2(0.9) mm, respectively, $p = 0.443$), and both groups achieved statistically significant levels of improvement at 3 and 6-month assessment intervals compared to the baseline ($p < 0.001$). However, the PD reduction in the test group was statistically significant and more pronounced compared to the control group at both evaluation points ($p < 0.001$) (Table 3). At the same time, the change between 3 and 6 months did not differ between the groups ($p = 0.096$) (Fig.11).

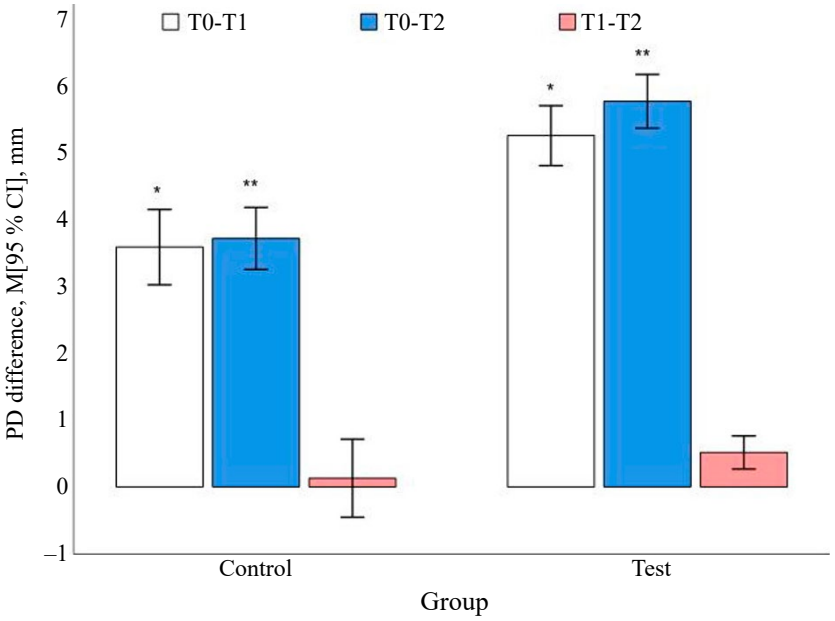


Fig. 11. *Mean changes in PD in deep pockets (≥ 7mm) at different study time points.*

*, **$p < 0.001$, by Student's *t* test for two independent groups

T0 – baseline; T1 – 3-month follow-up; T2 – 6-month follow-up.

CAL changes in moderate and deep pockets are reported in Table 4.

Table 4. *CAL (mean (SD)) at the sites with moderate (4–6 mm) and deep (≥ 7 mm) pockets*

	Control group (n = 24)	**Test group (n = 24)**	***p* value**
Moderate pockets (4–6 mm)			
T0	4.8 (0.3)	4.6 (0.2)	0.026[a]
T1	3.1 (0.8)	2.4 (0.6)	< 0.001[a]
T0 vs. T1 months	< 0.001[b]	< 0.001[b]	
T2	3.1 (0.7)	2.0 (0.5)	< 0.001[a]
T0 vs. T2 months	< 0.001[b]	< 0.001[b]	
Deep pockets (≥ 7mm)			
T0	7.9 (0.6)	8.1 (0.7)	0.412[a]
T1	4.5 (1.2)	3.2 (1.4)	0.002 [a]
T0 vs. T1 months	< 0.001[b]	< 0.001[b]	
T2	4.6 (1.0)	2.8 (1.3)	< 0.001 [a]
T0 vs. T2 months	< 0.001[b]	< 0.001[b]	

[a] statistical analysis by Student's *t* test for two independent groups; [b] paired samples *t* test for two dependent groups.

At the baseline, in the moderate pocket category, the control group demonstrated slightly higher CAL values (4.8(0.3) mm) than the test group (4.6(0.2) mm), with $p = 0.026$. Both groups exhibited significant improvements at 3 and 6 months compared to the initial values ($p < 0.001$). However, a statistically significant difference between groups was detected, favoring the test group at both evaluation points ($p < 0.001$) (Table 4). A mean CAL change between the 3 and 6-month time points was statistically significantly different between the groups in favor of the test group ($p = 0.004$) (Fig. 12).

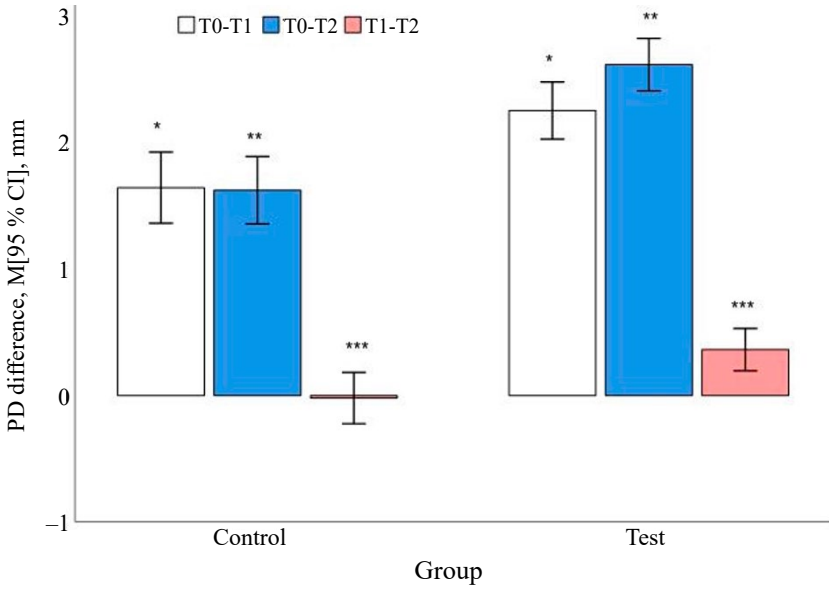


Fig. 12. *Mean changes in CAL in moderate pockets (4–6mm) at different study time points.*

*, **$p < 0.001$, ***$p = 0.004$ by Student's *t* test for two independent groups;
T0 – baseline; T1 – 3-month follow-up; T2 – 6-month follow-up.

In the deep pocket category at baseline, the CAL values were not statistically significantly different and measured 7.9(0.6) mm in the control group and 8.1(0.7) mm in the test group ($p = 0.412$), respectively. Both groups showed statistically significant progress at both follow-up intervals compared to the initial assessment ($p < 0.001$). However, notably superior improvements were observed in the test group ($p < 0.001$) (Table 4). The mean CAL change between 3 and 6-month time points did not show a statistically significant difference between the groups ($p = 0.077$) (Fig. 13).

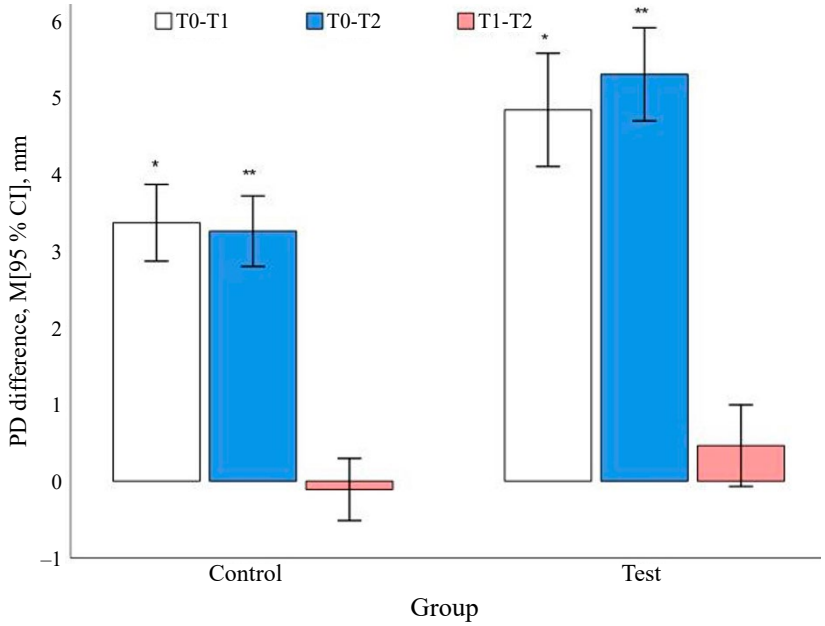


Fig. 13. *Changes in CAL in deep pockets (≥ 7 mm) at different study time points.*

*$p = 0.002$, **$p < 0.001$, by Student's *t* test for two independent groups;
T0 – baseline; T1 – 3-month follow-up; T2 – 6-month follow-up.

Bleeding on probing changes were assessed for treated sites (BOP) (PD ≥ 4 mm and BOP+) and full mouth (FMBOP).

The initial FMBOP values were comparable between the test (76.5(18.2) %) and control (68.9(20.3) %) groups, $p = 0.184$. Both groups showed significant improvement at 3 and 6 months compared to the baseline ($p < 0.001$). However, no statistically significant difference between the groups was detected at the 3-month evaluation point ($p = 0.06$), but the test group reached a statistically significant difference at the 6-month time point ($p < 0.001$) (Table 5).

The analysis of the treated pockets (PDs ≥ 4 mm and BOP+) did not indicate a statistically significant difference in the initial BOP values between the groups ($p = 0.687$). Although both groups demonstrated statistically significant improvements at 3 and 6-month follow-up intervals compared to the baseline ($p < 0.001$), the reduction in BOP was notably more pronounced in the test group than in the control group at both assessment periods ($p = 0.018$ and $p < 0.001$, respectively) (Table 5).

Table 5. *BOP (%) at treated sites (PD≥ 4mm) and full mouth (mean (SD))*

	BOP		*p* value	FMBOP		*p* value
	Control (n = 24)	**Test (n = 24)**		**Control (n = 24)**	**Test (n = 24)**	
T0	81.8 (16.2)	83.2 (15.5)	0.687[a]	68.9 (20.3)	76.5 (18.2)	0.184[a]
T1	39.1 (15.9)	28.3 (14.6)	**0.018**[a]	33.3 (13.7)	25.9 (12.3)	0.06[a]
T0 vs T1	**< 0.001**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	
T2	48.9 (14.5)	17.6 (11.5)	**< 0.001**[a]	40.8 (13.8)	15.6 (9.9)	**< 0.001**[a]
T0 vs T2	**< 0.001**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	

[a] statistical analysis by Student's *t* or Mann-Whitney test for two independent groups;
[b] Wilcoxon signed ranks test for two dependent groups.

Distribution of shallow, medium, and deep pockets

Additionally, the frequency distribution analysis of shallow (1–3 mm), moderate (4–6 mm), and deep (≥ 7 mm) pockets at the initial assessment, as well as at 3 and 6 months, was conducted (Table 6). At baseline, the control group subjects had 41.2 % sites with moderate, and the test group had 48.6 % sites, respectively. By the 6-month assessment, this number decreased to 22.6 % in the control and 7.7 % in the test group, and a statistically significant difference was observed between the two groups ($p < 0.001$). Similarly, based on the 6-month evaluation, the deep pocket number changed from 7.6 % to 1.0 % in the control group and from 8.7 % to 0.1 % in the test group, with a statistically significant difference between the groups ($p = 0.003$) (Table 6).

Table 6. *Number of sites with shallow (1–3 mm), medium (4–6 mm) and deep (≥ 7 mm) pockets in test and control groups at different study timepoints.*

	1–3 mm			4–6 mm			≥ 7 mm		
	Control	**Test**	***p* value**	**Control**	**Test**	***p* value**	**Control**	**Test**	***p* value**
T0	1916 (51.2 %)	1603 (42.7 %)	**0.05**[*]	1518 (41.2 %)	1803 (48.6 %)	**0.041**[*]	277 (7.6 %)	298 (8.7 %)	0.52
T1	2938 (78.6 %)	3284 (88.2 %)	**0.013**[*]	728 (20.3 %)	402 (11.5 %)	**0.018**[*]	39 (1.1 %)	12 (0.3 %)	0.053
T2	2859 (76.4 %)	3398 (92.2 %)	**0.006**[*]	803 (22.6 %)	234 (7.7 %)	**< 0.001**[*]	35 (1.0 %)	4 (0.1 %)	**0.003**[*]

[*] Data in bold represents statistically significant differences between test and control groups.

Microbiologic results

Detection frequency of periodontopathogens

Detection frequencies for *A.a*, *P.g*, *T.f*, *T.d*, and *P.i* at different time intervals in the study groups are summarized in Table 7. The findings were presented

as the proportion of the study participants (%) testing positive for a particular pathogen.

Table 7. *Detection frequencies sorted by periodontopathogen (%)*

Periodonto-pathogen	Protocol	Baseline	3 months	6 months
A.a	Control group	42.5	54.2	58.3
	Test group	45.8	29.2	33.3
P.g	Control group	75.0	58.3	75.0*
	Test group	87.5ab	41.7a	41.7*b
T.f	Control group	91.7a	62.5a	79.2
	Test group	83.3ab	54.2a	58.3b
T.d	Control group	87.5a	58.3a	79.2*
	Test group	95.8ab	41.7a	33.3*b
P.i	Control group	58.3	29.2	45.8*
	Test group	45.8ab	20.8 a	8.3*b

*p < 0.05, between-group differences (by χ^2 test); ap < 0.05, baseline and month 3 (by McNemar test); b*p* < 0.05, baseline and month 6 (by McNemar test).

In the control group, after 3 months, notable reductions were detected for *T.f* and *T.d* ($p < 0.05$). However, at 6 months, the detected frequencies of these bacteria rebounded to their initial levels, showing values similar to the baseline ($p > 0.05$).

In contrast, the test group exhibited significant reductions for *P.g*, *T.f*, *T.d*, and *P.i* after 3 and 6 months ($p < 0.05$).

After six months, a comparison between test and control groups revealed significant differences in detection frequency of *P.g* ($p = 0.034$), *T.d* ($p < 0.01$), and *P.i* ($p = 0.02$), in favor for the test group.

Changes of the detection scores of periodontopathogens

Table 8 depicts the detection frequency scores for *A.a, P.g, T.f, T.d*, and *P.i* at the baseline and 3 and 6-month follow-ups.

At the baseline, the control and test groups regarding detection frequency scores of the investigated periodontal pathogenic species showed no statistically significant differences ($p > 0.05$). In the control group at 3 months, a statistically significant decrease in detection scores from the baseline was found for *P.g* ($p = 0.013$), *T.f* ($p = 0.007$), *T.d* ($p = 0.003$), and *P.i* ($p = 0.012$). At 6 months, significant reductions from the baseline remained for *P.g* ($p = 0.039$) and *T.f* ($p = 0.048$). The test group at 3 months demonstrated a significant decrease in detection scores from the baseline for all investigated periopathogenic species: *A.a* ($p = 0.011$), *P.g* ($p < 0.001$), *T.f* ($p < 0.001$),

T.d ($p < 0.001$) and *P.i* ($p = 0.014$). These results were maintained after 6 months: *A.a* ($p = 0.016$), *P.g* ($p < 0.001$), *T.f* ($p < 0.001$), *T.d* ($p < 0.001$) and *P.i* ($p = 0.003$). The inter-group analysis exhibited statistically significant differences in detection scores between the control and test groups for *A.a* ($p = 0.044$) at 3-month and for *A.a* ($p = 0.028$), *P.g* ($p = 0.006$), *T.f* ($p = 0.004$), *T.d* ($p < 0.001$) and *P.i* ($p = 0.003$) at 6-month evaluation in favor of the test group.

Table 8. *Detection frequency scores for A.a, P.g, P.i, T.f, T.d at the baseline, 3 and 6-month time points*

Species	Time point	Detection score	Total, n (%)	Control group, n (%)	Test group, n (%)	*p* value**
A.a	Baseline	0 1 2 3 4	22 (45.8) 2 (4.2) 4 (8.3) 8 (16.7) 12 (25.0)	9 (37.5) 1 (4.2) 2 (8.3) 4 (16.7) 8 (33.3)	13 (54.2) 1 (4.2) 2 (8.3) 4 (16.7) 4 (16.7)	0.174
	Month 3	0 1 2 3 4	28 (58.3) 2 (4.2) 6 (12.5) 6 (12.5) 6 (12.5)	11 (45.8) 1 (4.2) 4 (16.7) 2 (8.3) 6 (25.0)	17 (70.8) 1 (4.2) 2 (8.3) 4 (16.7) 0	**0.044**
	*p value		**0.013**	0.231	**0.011**	
	Month 6	0 1 2 3 4	26 (54.2) 4 (8.3) 4 (8.3) 6 (12.5) 8 (16.7)	10 (41.7) 1 (4.2) 3 (12.5) 3 (12.5) 7 (29.2)	16 (66.7) 3 (12.5) 1 (4.2) 3 (12.5) 1 (4.2)	**0.028**
	*p value		0.085	0.064	**0.016**	
P.g	Baseline	0 1 2 3 4	9 (18.8) 1 (2.1) 2 (4.2) 11 (22.9) 25 (52.1)	6 (25.0) - 1 (4.2) 4 (16.7) 13 (54.2)	3 (12.5) 1 (4.2) 1 (4.2) 7 (29.2) 12 (50.0)	0.884
	Month 3	0 1 2 3 4	24 (50.0) 3 (6.3) 6 (12.5) 8 (16.7) 7 (14.6)	10 (41.7) 1 (4.2) 3 (12.5) 4 (16.7) 6 (25.0)	14 (58.3) 2 (8.3) 3 (12.5) 4 (16.7) 1 (4.2)	0.099
	*p value		**< 0.001**	**0.013**	**< 0.001**	
	Month 6	0 1 2 3 4	20 (41.7) 7 (14.6) 8 (16.7) 6 (12.5) 7 (14.6)	6 (25.0) 4 (16.7) 4 (16.7) 3 (12.5) 7 (29.2)	14 (58.3) 3 (12.5) 4 (16.7) 3 (12.5) -	**0.006**
	*p value		**< 0.001**	**0.039**	**< 0.001**	

Table 8 cont.

Species	Time point	Detection score	Total, n (%)	Control group, n (%)	Test group, n (%)	*p* value**
T.f	Baseline	0 1 2 3 4	6 (12.5) 3 (6.3) 5 (10.4) 18 (37.5) 16 (33.3)	2 (8.3) 3 (12.5) 3 (12.5) 8 (33.3) 8 (33.3)	4 (16.7) - 2 (8.3) 10 (41.7) 8 (33.3)	0.846
	Month 3	0 1 2 3 4	20 (41.7) 8 (16.7) 8 (16.7) 10 (20.8) 2 (4.2)	9 (37.5) 1 (4.2) 5 (20.8) 7 (29.2) 2 (8.3)	11 (45.8) 7 (29.2) 3 (12.5) 3 (12.5) -	0.088
	*p value		**< 0.001**	**0.007**	**< 0.001**	
	Month 6	0 1 2 3 4	15 (31.3) 10 (20.8) 9 (18.8) 12 (25.0) 2 (4.2)	5 (20.8) 3 (12.5) 4 (16.7) 10 (41.7) 2 (8.3)	10 (41.7) 7 (29.2) 5 (20.8) 2 (4.2) -	**0.004**
	*p value		**< 0.001**	**0.048**	**< 0.001**	
T.d	Baseline	0 1 2 3 4	4 (8.3) 10 (20.8) 22 (45.8) 12 (25.0) -	3 (12.5) 4 (16.7) 11 (45.8) 6 (25.0) -	1 (4.2) 6 (25.0) 11 (45.8) 6 (25.0) -	0.878
	Month 3	0 1 2 3 4	24 (50.0) 13 (27.1) 10 (20.8) 1 (2.1) -	10 (41.7) 6 (25.0) 7 (29.2) 1 (4.2) -	14 (58.2) 7 (29.2) 3 (12.5) - -	0.125
	*p value		**< 0.001**	**0.003**	**< 0.001**	
	Month 6	0 1 2 3 4	21 (43.8) 13 (27.1) 12 (25.0) 2 (4.2) -	5 (20.8) 6 (25.0) 11 (45.8) 2 (8.3) -	16 (66.7) 7 (29.2) 1 (4.2) - -	**< 0.001**
	*p value		**< 0.001**	0.083	**< 0.001**	
P.i	Baseline	0 1 2 3 4	23 (47.9) 5 (10.4) 4 (8.3) 12 (25.0) 4 (8.3)	10 (41.7) 4 (16.7) - 8 (33.3) 2 (8.3)	13 (54.2) 1 (4.2) 4 (16.7) 4 (16.7) 2 (8.3)	0.413

Table 8 cont.

Species	Time point	Detection score	Total, n (%)	Control group, n (%)	Test group, n (%)	*p* value**
P.i	Month 3	0 1 2 3 4	36 (75.0) 2 (4.2) 7 (14.6) 3 (6.3) -	17 (70.8) 1 (4.2) 3 (12.5) 3 (12.5) -	19 (79.2) 1 (4.2) 4 (16.7) - -	0.399
	p value*		**< 0.001**	**0.012**	**0.014**	
	Month 6	0 1 2 3 4	35 (72.9) 4 (8.3) 5 (10.4) 4 (8.3) -	13 (54.2) 3 (12.5) 4 (16.7) 4 (16.7) -	22 (91.7) 1 (4.2) 1 (4.2) - -	**0.003**
	p value*		**< 0.001**	0.091	**0.003**	

n – frequencies. * corresponding to the Wilcoxon tests for intra-group comparison of pathogen detection scores between successive time points; ** corresponding to the Mann-Whitney tests for inter-group comparisons of pathogen detection scores for each time point.

Histologic study

Clinical observations

Postsurgical clinical healing was uneventful at all 16 sites (8 sites/group) in the control and test groups. None of adverse reactions such as suppuration, abscess formation, or increased tooth mobility, were observed throughout the clinical trial period. Visual gingival redness remained longer or recurred in the control group after the treatment.

Clinical measurements

The values for clinical parameters at the baseline and 6-week examinations in both study groups are shown in Table 9. The baseline examination revealed that both groups demonstrated similar characteristics for the PPD, CAL, and BOP scores with no significant differences between the groups. Both groups showed clinical improvements at 6 weeks compared to the baseline results. The mean PPD reduction between the baseline and 6 weeks follow-up was statistically significantly different between the groups in favor of the test group ($p < 0.05$). The test group showed better mean CAL gain results than the control group. However, between the groups there was no statistically significant difference. The number of BOP + sites was markedly reduced in the test group at 6 weeks compared to the baseline. Similarly, the scores between the groups showed a statistically significant difference.

Table 9. *Clinical parameters for each treatment at baseline and 6 weeks (means (SD)), n = 4 animals*

Parameters	Control	Test
PD (mm)		
Baseline	5.46 (0.82)	5.50 (0.57)
6 weeks	2.12 (0.32)	1.25 (0.50)
PPDreduction	3.34 (0.54)	4.25 (0.50)†
CAL (mm)		
Baseline	5.71 (0.84)	5.59 (0.47)
6 weeks	2.93 (0.23)	1.68 (0.89)
CAL gain	2.78 (0.79)	3.90 (0.82)
BOP (+) n (%)		
Baseline	8 (100)	8 (100)
6 weeks	6 (75.0)	1 (12.5)*†

PD – probing pocket depth; CAL – clinical attachment level; BOP – bleeding on probing.
* Significantly different from baseline within each group ($p < 0.01$); †Significantly different from control group ($p < 0.05$).

Histomorphometric analysis

The results of histomorphometric analysis are shown in Table 10. There were no statistically significant differences between the DH, JE, and CT measurements between the groups. However, the CT length (without cementum formation) in the test group was smaller than in the control group. The length of the new cementum was statistically significantly greater ($p < 0.01$) in the test group (2.46 ± 0.77 mm) than in the control group (0.85 ± 0.84 mm). The test group (1.75 ± 0.65 mm) yielded a statistically significant ($p < 0.05$) and greater formation of new attachment (i.e., a linear length of NC adjacent to newly formed bone, with functionally oriented collagen fibers) compared with the control group (0.48 ± 0.79 mm). Moreover, the test group had the statistically significantly higher ($p < 0.05$) PDL scores (2.87 ± 1.59) than the control group (1.00 ± 0.94). The amount of newly formed bone (e.g., the length of NB and the area of NB) in the test group (3.01 ± 0.64 mm and 5.75 ± 2.21 mm^2, respectively) was statistically significantly greater ($p < 0.05$) than in the control group (2.26 ± 0.64 mm and 3.14 ± 1.94 mm^2, respectively).

The histologic images of the control and test groups are depicted in Fig. 14, 15.

Table 10. *Histomorphometric comparisons between the test and control groups 8 weeks after treatment (means (SD)); n = 4 animals)*

Parameters	Control	Test
DH (mm)	5.60 (0.42)	5.77 (0.52)
JE (mm)	1.22 (0.45)	1.03 (0.31)
CT (mm)	2.84 (1.33)	1.86 (1.00)
NC (mm)	0.85 (0.84)	2.46 (0.77)**
NA (mm)	0.48 (0.79)	1.75 (0.65)*
PDL score (1–5)	1.00 (0.94)	2.87 (1.59)*
NB (mm)	2.26 (0.64)	3.01 (0.64)*
NBA (mm^2)	3.14 (1.94)	5.75 (2.21)*

DH – defect height; JE – junctional epithelium length; CT – connective tissue adhesion (without cementum); NB – new bone length; NBA – new bone area; NC – new cementum length; NA – new attachment length; PDL score – periodontal ligament score.
* significantly different from control group ($p < 0.05$); ** significantly different from control group ($p < 0.01$).

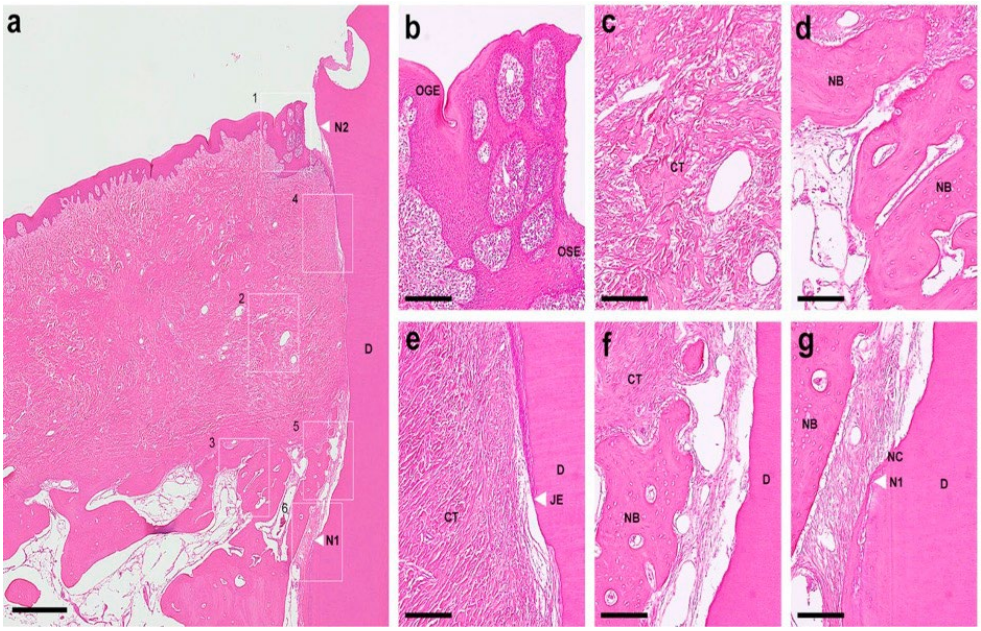


Fig. 14. *Histologic picture of a defect in control group.*

(a) Histologic overview of defect treated with subgingival debridement alone (control group), (scale bar, 1 mm; hematoxylin and eosin stain); (b) Higher magnification of the box 1 area; (c) Higher magnification of the box 2 area; (d) Higher magnification of the box 3 area; (e) Higher magnification of the box 4 area. (f) magnification of the box 5 area; (g) Higher magnification of the box 6 area, (scale bar, 200 μm; hematoxylin and eosin stain). D – root dentin; N1 – apical end of root planing; N2m – cementoenamel junction; OGE – oral gingival epithelium; OSE – oral sulcular epithelium; JE – apical end of junctional epithelium; CT – Higher gingival connective tissue; NB – new bone; NC – new cementum.

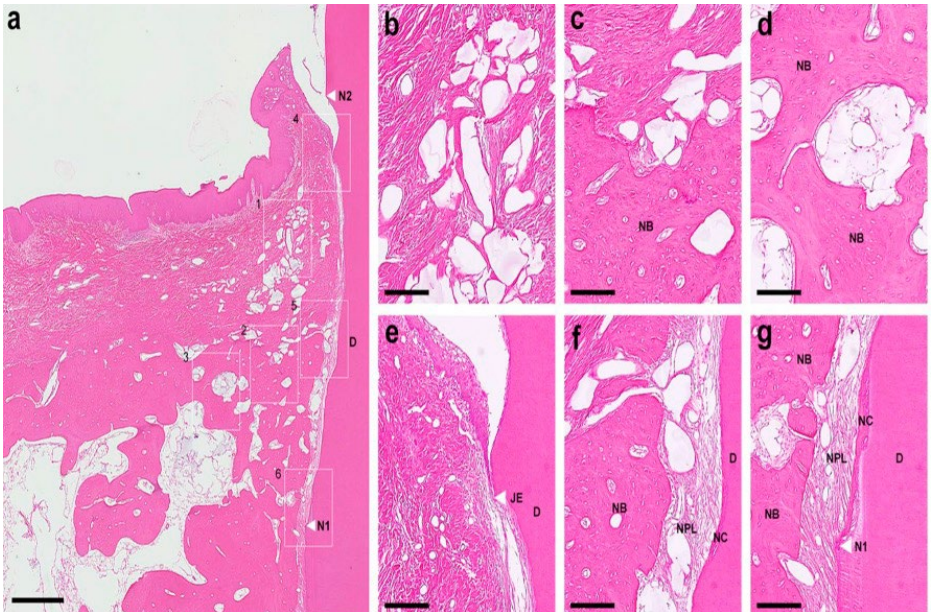


Fig. 15. *Histologic picture of a defect in test group.*

(a) Histologic overview of defect treated with SRP with a sodium hypochlorite and amino acids gel and a cross-linked hyaluronic acid gel (xHyA) gel (test group), (scale bar, 1 mm; hematoxylin and eosin stain); (b) Higher magnification of the box 1 area; (c) Higher magnification of the box 2 area; (d) Higher magnification of the box 3 area; (e) Higher magnification of the box 4 area; (f) Higher magnification of the box 5 area; (g) Higher magnification of the box 6 area, (scale bar, 200 μm; hematoxylin and eosin stain). D – root dentin; N1 – apical end of root planing; N2 – cementoenamel junction; JE – apical end of junctional epithelium; CT – gingival connective tissue; NB – new bone; NC – new cementum; NPL – new periodontal ligament.

CONCLUSIONS

1. The adjunctive subgingival application of sodium hypochlorite/amino acid gel and xHyA to subgingival debridement resulted in significantly higher reduction of probing depth, bleeding on probing, gain in clinical attachment, compared to subgingival debridement alone.
2. The adjunctive subgingival application of sodium hypochlorite/amino acid gel and xHyA to subgingival debridement resulted in significantly better improvements in terms of detection frequency and change in total bacterial counts for the main periodontopathogenic bacteria (*A.a, P.i, T.d, P.g, T.f*)
3. The histological part of the study reveals that by combining sodium hypochlorite/amino acid gel and xHyA with subgingival instrumentation, we can regenarate periodontal tissues (new cementum, new bone, new periodontal ligament) when treating periodontitis non-surgically.

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PUBLIKACIJOS DISERTACIJOS TEMA

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2. **Ramanauskaite E**, Machiulskiene V, Shirakata Y, Dvyliene UM, Nedzelskiene I, Sculean A (2023) Clinical evaluation of sodium hypochlorite/amino acids and cross-linked hyaluronic acid adjunctive to non-surgical periodontal treatment: a randomized controlled clinical trial. Clinical oral investigations. doi: 10.1007/s00784-023-05271-0
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Konferencijų, kuriose skelbti disertacijos metu gauti rezultatai, sąrašas:

1. Skaitytas mokslinis pranešimas „Hialurono rūgšties panaudojimo galimybės odontologijoje“ tarptautiniame kongrese „Odontologijos Kompasas“, kuriame pristatyti preliminarūs doktorantūros darbo rezultatai, Druskininkai, Lietuva, 2022 05 27;
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RESEARCH

Clinical evaluation of sodium hypochlorite/amino acids and cross-linked hyaluronic acid adjunctive to non-surgical periodontal treatment: a randomized controlled clinical trial

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Abstract

Objectives To compare the clinical outcomes obtained with either mechanical subgingival debridement in conjunction with a sodium hypochlorite and amino acids containing gel followed by subsequent application of a cross-linked hyaluronic acid gel (xHyA) gel, or with mechanical debridement alone.

Materials and Methods Fourty-eight patients diagnosed with stages II-III (Grades A/B) generalised periodontitis were randomly treated with either scaling and root planing (SRP) (control) or SRP plus adjunctive sodium hypochlorite/amino acid and xHyA gels (test). The primary outcome variable was reduction of probing depth (PD), while changes in clinical attachment level (CAL), bleeding on probing (BOP) and plaque index (PI) were secondary outcomes. The outcomes were assessed at baseline, at 3 and 6 months following therapy.

Results All patients completed the 6 months evaluation. At 6 months, the test group showed statistically significantly better results in terms of mean PD reduction (2.9 ± 0.4 vs 1.8 ± 0.6 mm, $p < 0.001$). Similarly, mean CAL gain was statistically higher in the test group compared to the control one (test: 2.6 ± 0.5 vs control: 1.6 ± 0.6 mm, $p < 0.001$). Mean BOP decreased from $81.8 \pm 16.2\%$ to $48.9 \pm 14.5\%$ in control ($p < 0.001$) and from $83.2 \pm 15.5\%$ to $17.6 \pm 11.5\%$ in test ($p < 0.001$) groups with a statistically significant difference favouring the test group ($p < 0.001$). Mean PI scores were reduced statistically significantly in both groups (from $38.8 \pm 26\%$ to $26.5 \pm 20.5\%$ in control ($p = 0.039$) and from $60.6 \pm 10.9\%$ to $12.7 \pm 8.9\%$ in test group ($p < 0.001$)), with a statistically significant difference between the groups ($p < 0.001$). The number of moderate pockets (4–6 mm) were reduced from 1518 (41.2%) to 803 (22.6%) in the control and from 1803 (48.6%) to 234 (7.7%) in the test group with a statistically significant difference between the groups ($p < 0.001$), while the number of deep pockets (≥ 7 mm) changed from 277 (7.6%) to 35 (1.0%) in the control and from 298 (8.7%) to 4 (0.1%) in test group ($p = 0.003$).

Conclusion Within their limits the present data indicate that: a) both treatments resulted in statistically significant improvements in all evaluated clinical parameters, and b) the adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA to SRP yielded statistically significantly higher improvements compared to SRP alone.

Clinical relevance The combination of sodium hypochlorite/amino acid and xHyA gels to subgingival mechanical debridement appears to represent a valuable approach to additionally improve the outcomes of non-surgical periodontal treatment. Clinical Trial Registration Number NCT04662216 (ClinicalTrials.gov).

Keywords Periodontitis · Non-surgical periodontal therapy · Cross-linked hyaluronic acid · Sodium hypochlorite/amino acids

Introduction

Periodontitis is a chronic multifactorial inflammatory disease caused by dysbiotic dental plaque biofilms with the formation of an inflammatory infiltrate that contributes to destruction of connective tissue attachment to the tooth, alveolar bone resorption and may result in tooth loss [1–5]. In case of periodontitis a disruption of the normal function of the healthy subgingival plaque biofilm with concomitant disruption to its functional properties in relation to innate defense surveillance and tissue maintenance, leading to excessive, deregulated inflammation and tissue destruction is observed [6, 7].

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Primary clinical features of periodontitis include the loss of periodontal tissue support, which manifests through clinical attachment loss and radiographically assessed alveolar bone loss with the presence of gingival bleeding and periodontal pockets [5]. The recently published clinical practice guidelines for treating stage I–III periodontitis concluded that cause-related therapy is aimed at reducing/eliminating the subgingival biofilm and calculus by means of subgingival instrumentation, which may include the adjunctive application of physical or chemical agents [8].

Recent systematic reviews have provided some evidence indicating that adjunctive aids, in conjunction with mechanical debridement, might enhance the outcomes of non-surgical periodontal therapy [9–12]. More recently, findings from *in vitro* experiments have shown, that a sodium hypochlorite gel has a softening effect on the extracellular biofilm matrix which in turn, may facilitate its mechanical removal. It has been shown that the effect of sodium hypochlorite/amino acid gel is due to its active part, the chloramine, which forms following the chlorine transfer of sodium hypochlorite to the amine functions of the added amino acids [13]. Amino acids act like a buffer and provide protection to soft tissues. The high pH (11) of this formulation has a softening effect on the calculus, which makes the cleaning process easier [14]. Therefore, it may be anticipated that during subgingival debridement treatment, both the mechanical and chemical components act synergistically to disrupt the hard and soft biofilm which in turn, may facilitate granulation tissue removal [13, 14]. In this respect, positive clinical effects of a sodium hypochlorite gel were reported in studies treating residual periodontal pockets [15, 16], peri-implant mucositis [17] and peri-implantitis [14].

HA is a naturally occurring biodegradable polymer that is responsible for several structural properties of tissues as a component of the extracellular matrix [18]. Several studies have provided evidence indicating that HA plays an important role in wound healing, supports scarless wound-healing, promotes angiogenesis and has a bacteriostatic effect in surgical wounds [19–22]. When used during periodontal surgery, HA has been shown to promote periodontal regeneration in intrabony, recession and furcation defects [23–25]. Clinical studies revealed that HA may represent a valuable constituent to mechanical debridement (i.e., scaling and root planing), thus resulting in statistically significant clinical improvements, evidenced by reduction in probing depth (PD), gain of clinical attachment (CAL) and improved bleeding on probing (BOP) values, compared to scaling and root planing alone [26–29].

Recently, a novel concept consisting of enhancing biofilm removal during nonsurgical therapy by means of a sodium hypochlorite/amino acids followed by application of a cross-linked hyaluronic acid gel (xHyA) gel was suggested as a novel strategy to improve the outcomes of nonsurgical periodontal therapy [30, 31]. Results from two case series have shown statistically significant clinical improvements compared to baseline following scaling and root planing in conjunction with sodium hypochlorite/amino acid and xHyA, thus suggesting that this strategy may represent a valuable novel strategy in non-surgical periodontal treatment.

However, to the best of our knowledge, at present no randomized controlled clinical trials have evaluated the potential clinical relevance of this novel concept as compared to mechanical debridement alone.

Therefore, the aim of this randomized controlled clinical study was to compare the clinical outcomes obtained with either mechanical subgingival debridement in conjunction with sodium hypochlorite/amino acid gel followed by subsequent application of xHyA, or with mechanical debridement alone.

Material and methods

Study design

This study was conducted as a 6-months prospective, examiner-blind, randomized controlled clinical trial with a parallel design. The study was performed according to CONSORT guidelines for randomized controlled clinical trials (http://www.consort-statement.org/) [32]. Ethical permission was issued by the Regional Biomedical Research Ethics Committee (No. BE-2–87). Prior to participation, all patients signed a written informed consent form. After signing the informed consent form, the patients were randomly assigned to the control or test groups (allocation ratio 1:1). The study was conducted between September 2019 and January 2022. In addition, the study protocol was registered at ClinicalTrials.gov, NCT04662216.

Study population

All patients included in the study were enrolled and treated at the Department of Dental and Oral Pathology at the Lithuanian University of Health Sciences in Kaunas, Lithuania.

Inclusion criteria:

- Males and females ≥ 18 years old.
- Periodontitis stages II–III, grades A/B, generalised [5].
- Good general health (i.e., absence of systemic diseases and no intake of medication which may affect periodontal health).
- Presence of at least 20 teeth (wisdom teeth excluded).
- Absence of removable dentures.
- Patients willing to provide written informed consent and willing to complete the 6-month study follow-up.

Exclusion criteria:

- Patients already included in other clinical trials.
- Smokers.
- Periodontal treatment during the last 12 months.
- Antibiotic treatment 3 months prior to the start of the trial.
- Antibiotic prophylaxis required for dental treatment.
- Ongoing medication that may affect the clinical features of periodontitis.
- Pregnant/lactating.
- Allergies to sodium hypochlorite

Sample size calculation

At the start of the study, a significance level of $\alpha = 0.05$, a relevant average difference in PD of 1 mm between study groups with a standard deviation of 1 mm and a power (1—α) of at least 0.8 were set to calculate the minimum number of necessary cases (at least 20 per group). Assuming any possible dropouts during the study period, the number of patients was increased to 24 in each group. A power calculation at the end of the study with the given number of cases yielded a power of 99.6%.

Periodontal treatment

Baseline periodontal measurements were obtained 2 weeks prior to the treatment, which was followed by professional supragingival tooth cleaning and individual oral hygiene instructions for all included patients. These treatments included manual toothbrushes and interdental brushes. All patients were provided the same type of toothpaste (Elmex Enamel Protection, Gaba GmbH, Germany) and tooth (CS 5460, Curaprox, Curaden, Switzerland) and interdental (TePe, Tepe Mundhygienprodukten, Sweden) brushes. Oral hygiene instructions were reinforced at each follow-up visit, but no further treatment was rendered.

Two weeks later, under local anaesthesia, subjects in the control group underwent full-mouth SRP performed with ultrasonic (Satelec/Acteon suprasson newtron ultrasonic scaler) and hand instruments (LM SharpDiamond 1/2, 7/8, 11/12, 13/14 SD mini Gracey and Gracey curettes, LM Dental™, Finland). Subsequently, all teeth were polished using a low-abrasive paste (Lunos Super Soft, RDA < 5, Dürr Dental, Germany). Mechanical debridement took on average 3.5 h per patient.

In the test group, full-mouth SRP was performed as follows: in all pockets with PD ≥ 4 mm a sodium hypochlorite/amino acid gel (Perisolv®, Regedent AG, Zürich, Switzerland) was instilled into the pockets and kept there for 60 s before subgingival instrumentation. Subgingival instrumentation was carried out with the same ultrasonic and hand instruments and the application of sodium hypochlorite/amino acid gel was repeated until the instrumentation was considered sufficient (i.e., for a total of 2–3 times) (Fig. 1). All treatments were performed with magnifying glasses (4.5X – Ergo Advanced, Univet, Rezzato BS, Italy) and sufficient instrumentation was attained when root surfaces exhibited smooth surfaces upon probing with an explorer probe (Explorer-Periodontal Probe 8-520B, LM Dental™, Finland). Following SRP, a mixture of natural and cross-linked hyaluronic acid (high molecular) gel (Hyadent® BG, Regedent AG, Zürich, Switzerland) was instilled in the pockets using a blunt needle (Fig. 2).

Clinical measurements

The following clinical parameters were assessed using a Williams periodontal probe to the nearest mm (LM 51 ES, LM-Dental™, Finland) on all teeth at 6 sites per tooth (i.e., mesio-buccal (mb), mid-buccal (b), disto-buccal (db), mesio-oral (mo), mid-oral (o) and disto-oral (do)) at baseline (T0), 3 months (T1) and 6 months (T2) following the treatment:

- Bleeding on probing (BOP), defined as the percentage of sites positive to bleeding within 10 s after probing (%). BOP was assessed for treated sites (PD ≥ 4 mm) and full mouth (FMBOP).
- Plaque index (PI), defined as the percentage of sites with visual plaque on the tooth surface (%). PI was assessed at treated sites (PD ≥ 4 mm) as well as the full mouth (FMPI).
- Probing depth (PD), measured in millimetres from the gingival margin to the bottom of the probed pocket.
- Recession (REC), measured in millimetres from the gingival margin to the cemento-enamel junction or to the margin of a cervical restoration.

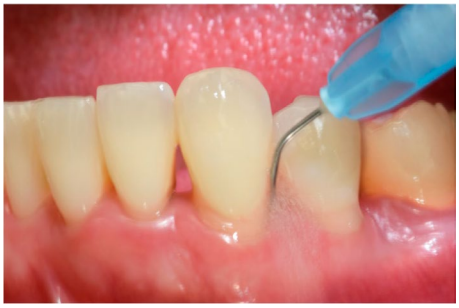

Fig. 1 Application of sodium hypochlorite/amino acid gel to the periodontal pocket

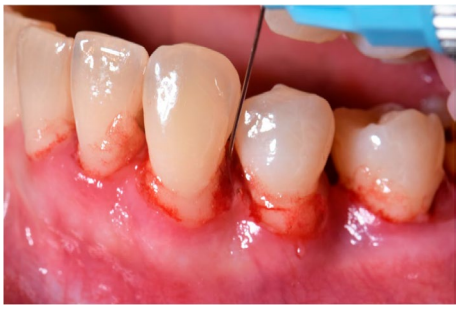

Fig. 2 Application of a mixture of natural and cross-linked hyaluronic acid (high molecular)to the periodontal pocket

- Clinical attachment level (CAL), calculated by adding PD and REC at each site.

At each visit, the clinical examiner had to record possible complications or adverse events related to the tested materials or study interventions, as well as those reported by study subjects.

Outcomes

For data analysis, PDs were subdivided into two categories: moderate (PD 4–6 mm) and deep (PD ≥ 7 mm). The primary outcome variable was the mean PD change from baseline to 6 months in moderate sites. Secondary outcome variables included PD change in deep pockets at 6 months, as well as CAL changes in moderate and deep sites. In addition, mean BOP and PI changes from baseline to 6 months in all treated sites (PD ≥ 4 mm) and the full mouth were evaluated.

Blinding

Clinical measurements and initial supragingival tooth cleaning were performed by a blinded calibrated examiner (U.M.D.), who was not aware in any of the cases of the type of treatment performed. All recordings were made without access to previous measurements to avoid bias.

To ensure blindness, the treatment procedures were performed by one experienced periodontist (E.R.).

The patients were not aware to which group they had been assigned. Periodontal treatment was performed in a sterile field (face drapes were used) to eliminate the possibility for patients to observe the procedure.

A third investigator (I.N.), unaware of the type of treatment performed, processed coded data for statistical analysis.

Randomization and allocation concealment

Forty-eight patients were randomized into two treatment groups. A computer-generated randomization table was created. Patients were assigned unique numbers from 1 to 48, and 2 sets of randomized numbers were generated (24 for control group subjects and 24 for test). Allocation concealment was performed using sealed envelopes to be opened before periodontal treatment. The generation of the random sequence allocation and the assignment of participants to interventions were performed by the investigator, distinct from the clinical examiner and the clinician who performed the treatment.

Calibration

Five patients, not related to the study, each diagnosed with periodontitis stages II–III [5], were used to calibrate the examiner (U.M.D.). The examiner was asked to evaluate PD, REC, CAL, BOP and PI at 6 sites per tooth on 2 separate appointments, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were equal to the millimetre at > 90% level. The examiner was not aware of the procedure to be performed.

Statistical analysis

Statistical analysis was performed with the IBM SPSS 27 software package (IBM Corp.). Data analysis was performed using the patient as the statistical unit. For all clinical parameters, mean values per subject and per visit were calculated. In particular, PD and CAL of moderate pockets at baseline and at 3- and 6-month follow-ups were obtained by averaging PDs and CALs in moderate sites for each patient at baseline, 3- and 6-month follow-ups. Similarly, per-patient PD and CAL of deep pockets at baseline (and at 3 and 6 months) were obtained by averaging PD and CAL values in deep sites for each patient at baseline, 3 and 6 months. Per-patient BOP and PI were obtained by calculating a percentage share of tooth sites with BOP and plaque for each patient by classifying pockets by baseline PD (all treated sites with PD ≥ 4 mm and the full mouth).

The Shapiro–Wilk test was performed to assess whether clinical periodontal measures followed a normal distribution. If data followed a normal distribution, a paired-samples *t* test was performed to evaluate before- and after-treatment comparisons within groups. If the data did not follow a normal distribution, the Wilcoxon signed rank test was performed on related samples to assess before- and after-treatment comparisons within the groups. The between-group comparisons of measures were obtained by either the independent-samples *t* test (if a parameter followed a normal distribution) or the Mann–Whitney test (if a specific measure followed a non-normal distribution). The significance level was set at 0.05.

Results

Participant flow

All 48 patients completed the study. Each treatment group (SRP or SRP + sodium hypochlorite/amino acid + xHyA) consisted of 24 randomly selected patients. A flowchart of the study is depicted in the CONSORT flow diagram (Fig. 3). In all subjects, healing was uneventful. No adverse effects of sodium hypochlorite/amino acid and xHyA were observed during the study period.

Baseline characteristics

Clinical and demographic baseline characteristics of the 48 participants are shown in Table 1. The baseline examination revealed that the two study groups showed similar characteristics for PD, CAL, bleeding (BOP and FMBOP) and plaque scores with no significant differences between the groups (except for PI and FMPI) (Table 1A). Furthermore, regarding the number of type of treated teeth, no statistically significant differences were observed between control and test groups (Table 1B).

Effect on clinical parameters

PD changes during the study period were analysed for different pocket categories: mean moderate (4–6 mm) and mean deep (PD ≥ 7 mm) pockets. Data is presented in Table 2.

In mean moderate pockets, the baseline values did not reveal a statistically significant difference between control and test groups (4.8 ± 0.2 and 4.7 ± 0.2, respectively, $p = 0.417$). Both groups showed statistically significant improvements at 3 and 6 months compared to baseline ($p < 0.001$); however, statistically significantly higher reductions were observed in favour for the test group at both points in time ($p < 0.001$) (Table 2). The change of PD between 3 and 6 months differed statistically significantly between groups in favour for the test group ($p = 0.002$) (Fig. 4).

Baseline PD values in mean deep pockets category were not statistically significantly different between control and test groups (8.0 ± 0.7 and 8.2 ± 0.9, respectively, $p = 0.443$). Both groups reached statistically significant improvements at 3 and 6 months compared to baseline ($p < 0.001$); however, PD reduction in the test group was statistically significantly higher compared to the control group at both follow-ups ($p < 0.001$) (Table 2). The change between 3 and 6 months did not differ between the groups ($p = 0.096$) (Fig. 5).

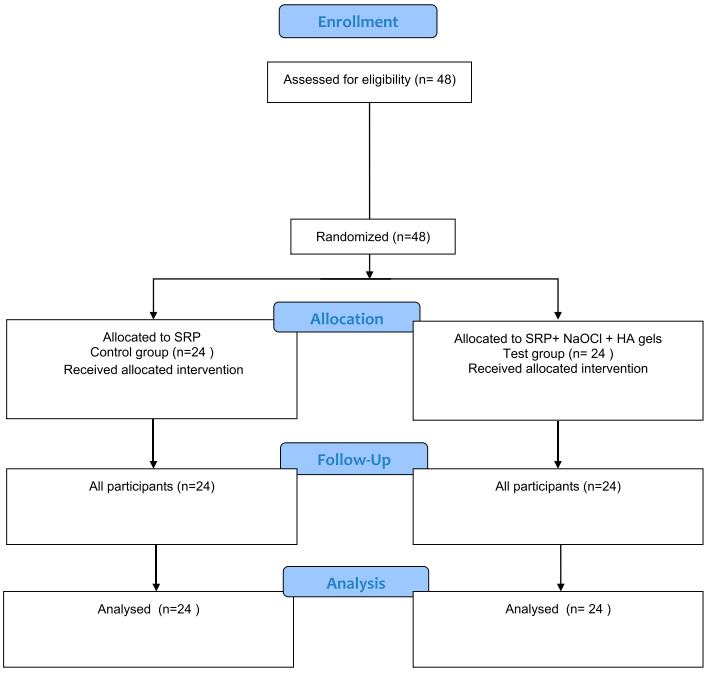

Fig. 3 CONSORT flow diagram of participant recruitment

Table 1 Clinical and demographic characteristics of sample population at the baseline

A. Characteristics of sample population at the baseline							
	SRP ($N=24$)		SRP + NaOCl + HA ($N=24$)		*P* value		
Age (years)	49.3 ± 11.2		47.3 ± 10.7		0.53[a], n.s		
Gender, *n* (%)							
Males	7 (29.2)		6 (25)		0.745[b],		
Females	17 (70.8)		18 (75)		n.s		
Periodontitis stage, *n* (%)							
Stage II	16 (66.7)		17 (70.8)		0.134[b],		
Stage III	8 (33.3)		7 (29.2)		n.s		
Grade A	13 (54.2)		12 (50.0)				
Grade B	11 (45.8)		12 (50.0)		0.242[b], n.s		
PD (mm)	5.3 ± 0.6		5.2 ± 0.4		0.592[c], n.s		
CAL (mm)	5.5 ± 0.5		5.6 ± 0.6		0.546[c], n.s		
PI (%)	38.8 ± 26		60.6 ± 10.9		**0.002[c]**		
BOP (%)	81.8 ± 16.2		83.2 ± 15.5		0.687[c], n.s		
FMPI (%)	35.7 ± 23.7		52.9 ± 11.4		**0.003[c]**		
FMBOP (%)	68.9 ± 20.3		76.5 ± 18.2		0.184[c], n.s		
B. Distribution of treated teeth							
Treatment	Second Molars	First Molars	Second Premolars	First Premolars	Canines	Lateral Incisors	Central incisors
Control group (*n*)	88	84	89	91	94	94	95
Test group (*n*)	86	90	86	89	96	96	96
p	0.549	0.187	0.505	0.682	0.153	0.153	0.317

BOP – bleeding on probing; CAL – clinical attachment level; FMBOP – full-mouth bleeding on probing; FMPI – full-mouth plaque index; PD – probing depth; PI – plaque index

n.s. not significant

[a] Independent-samples *t* test

[b] Fisher's exact test for the 2 × 2 table, sex by group (SRP, SRP + NaOCl + xHyA)

[c] Mann–Whitney U test for two independent groups

Mann–Whitney U test for two independent groups

Table 2 PD (mean (SD)) at sites with moderate (4–6 mm) and deep (≥ 7 mm) pockets

	Control group ($n=24$)	Test group ($n=24$)	*p* value
Moderate pockets (4–6 mm)			
Baseline	4.8(0.2)	4.7(0.2)	0.417[a]
After 3 months	2.9(0.7)	2.2(0.4)	< 0.001[a]
Baseline vs. 3 months	< 0.001[b]	< 0.001[b]	< 0.001[a]
After 6 months	3.0(0.6)	1.8(0.4)	
Baseline vs. 6 months	< 0.001[b]	< 0.001[b]	
Deep pockets (≥ 7 mm)			
Baseline	8.0(0.7)	8.2(0.9)	0.443[a]
After 3 months	4.4(1.4)	2.9(1.1)	< 0.001 [a]
Baseline vs. 3 months	< 0.001[b]	< 0.001[b]	< 0.001
After 6 months	4.3(1.0)	2.4(1.0)	
Baseline vs. 6 months	< 0.001[b]	< 0.001[b]	

[a] Statistical analysis by Student's t test for two independent groups

[b] Paired Samples T Test for two dependent groups

CAL changes in mean moderate and mean deep pockets are reported in Table 3.

At baseline, in mean moderate pockets group, the CAL values were slightly higher in the control group (4.8 ± 0.3 mm) compared to the test group (4.6 ± 0.2 mm; $p=0.026$). Both groups reached significant improvements at 3 and 6 months compared to baseline ($p<0.001$); however, a statistically significant difference between groups was observed in favour of the test group at both points in time ($p<0.001$) (Table 3). Mean CAL change between the 3- and 6-month follow-ups was statistically significantly different between the groups in favour for the test group ($p=0.004$) (Fig. 6).

In mean deep pockets baseline, CAL values were not statistically significantly different and measured 7.9 ± 0.6 mm in the control group and 8.1 ± 0.7 mm in the test group ($p=0.412$), respectively. Both groups reached statistically significant improvements at both follow-ups, compared to baseline ($p<0.001$); however, statistically significantly

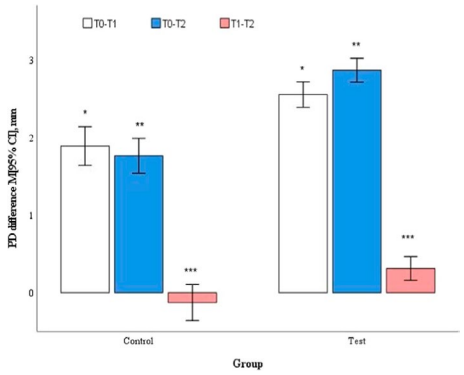

Fig. 4 Mean changes in PD in moderate pockets (4-6 mm) at different time points. $^{*,**}p<0.001$, $^{***}p=0.002$ by Student's t test for two independent groups. T0 – baseline; T1 – 3 months follow-up; T2 – 6 months follow-up

Table 3 CAL (mean ± SD) at sites with moderate (4–6 mm) and deep (≥ 7 mm) pockets

	Control group ($n=24$)	Test group ($n=24$)	p value
Moderate pockets (4–6 mm)			
Baseline	4.8(0.3)	4.6(0.2)	0.026[a]
After 3 months	3.1(0.8)	2.4(0.6)	< 0.001[a]
Base vs. 3 months	< 0.001[b]	< 0.001[b]	< 0.001[a]
After 6 months	3.1(0.7)	2.0(0.5)	
Base vs. 6 months	< 0.001[b]	< 0.001[b]	
Deep pockets (≥ 7 mm)			
Baseline	7.9(0.6)	8.1(0.7)	0.412[a]
After 3 months	4.5(1.2)	3.2(1.4)	0.002 [a]
Base vs. 3 months	< 0.001[b]	< 0.001[b]	< 0.001 [a]
After 6 months	4.6(1.0)	2.8(1.3)	
Base vs. 6 months	< 0.001[b]	< 0.001[b]	

[a] Statistical analysis by Student's t test for two independent groups

[b] Paired Samples T Test for two dependent groups

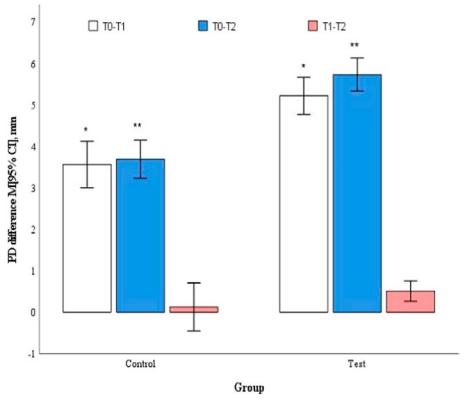

Fig. 5 Mean changes in PD in deep pockets (≥ 7 mm) at different study time points. $^{*,**}p<0.001$, by Student's t test for two independent groups. T0 – baseline; T1 – 3 months follow-up; T2 – 6 months follow-up

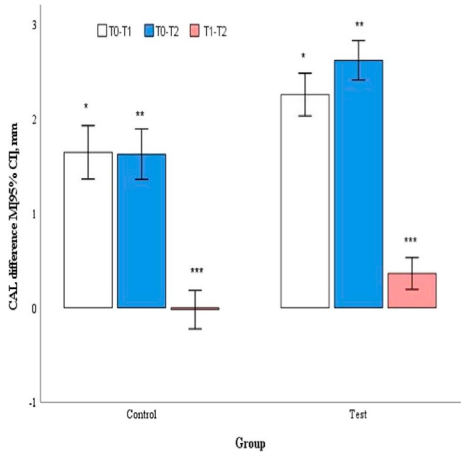

Fig. 6 Mean changes in CAL in moderate pockets (4-6 mm) at different study time points. $^{*,**}p<0.001$, $^{***}p=0.004$ by Student's t test for two independent groups. T0 – baseline; T1 – 3 months follow-up; T2 – 6 months follow-up

better improvements were achieved in favour for the test group ($p<0.001$) (Table 3). Mean CAL change between 3- and 6-month follow-up did not show a statistically significant difference between the groups ($p=0.077$) (Fig. 7).

BOP changes were evaluated for treated sites (PD ≥ 4 mm) and full mouth (FMBOP).

Regarding full-mouth measurements, baseline FMBOP values were similar in test (76.5 ± 18.2%) and control (68.9 ± 20.3%) groups ($p=0.184$). Both study groups reached significant improvements at 3 and 6 months compared to baseline ($p<0.001$). The difference between groups was not statistically significant at the 3-month follow-up ($p=0.06$) but reached a statistically significant difference in favour for the test group at the 6-month follow-up ($p<0.001$) (Table 4).

The analysis of treated pockets (PDs ≥ 4 mm) revealed no statistically significant difference in baseline BOP values between test and control groups ($p=0.687$). Although both

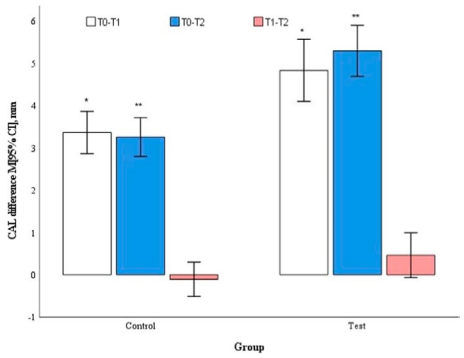

Fig. 7 Changes in CAL in deep pockets (≥7 mm) at different study time points. $^{*}p=0.002$, $^{**}p<0.001$, by Student's t test for two independent groups. T0 – baseline; T1 – 3 months follow-up; T2 – 6 months follow-up

groups showed statistically significant improvements at 3- and 6-month follow-ups compared to baseline ($p<0.001$), the reduction of BOP was statistically significantly better in the test group compared to the control group at both points in time ($p=0.018$ and $p<0.001$, respectively) (Table 4).

PI changes were evaluated for treated sites (PD ≥ 4 mm) and the full mouth (FMPI).

Baseline FMPI values were higher in the test group (52.9 ± 11.4%) than in the control one (35.7 ± 23.7%) ($p=0.003$). However, both groups showed significant improvements at 3- and 6-month follow-ups compared to baseline ($p<0.001$). The intergroup comparison revealed a statistically significant difference between groups in favour for the test group at 6 months ($p=0.006$) (Table 5).

A similar pattern was observed in the analysis for PI at treated pockets. In particular, higher PI (%) values were reported in the test group than the control group ($p=0.002$). Both study groups showed statistically significant improvements at 3- and 6-month evaluations, compared to baseline ($p<0.001$). No statistically significant difference was observed between groups at the 3-month evaluation ($p=0.714$), whereas at the 6-month examination, the reduction in PI was statistically significantly greater in the test group ($p=0.018$) (Table 5).

Analysis of frequency distributions of shallow, medium, and deep pockets

Additionally, the analysis of frequency distribution of shallow (1–3 mm), medium (4–6 mm) and deep (≥7 mm) sites at baseline, 3 and at 6 months was performed (Table 6). At baseline, subjects in the control group had 1518 (41.2%) sites with moderate pockets (4-6 mm) and test group 1803 (48.6%) sites, respectively. At 6 months this number reduced to 803 (22.6%) in control and 234 (7.7%) sites in the test

Table 4 BOP (%) at treated sites (PD ≥ 4 mm) and full mouth (mean ± SD)

	BOP		*P* value	FMBOP		*P* value
	Control ($n=24$)	Test ($n=24$)		Control ($n=24$)	Test ($n=24$)	
Baseline	81.8 ± 16.2	83.2 ± 15.5	0.687[a]	68.9 ± 20.3	76.5 ± 18.2	0.184[a]
After 3 months	39.1 ± 15.9	28.3 ± 14.6	**0.018**[a]	33.3 ± 13.7	25.9 ± 12.3	0.06[a]
Baseline vs 3 months	**< 0.001**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	
After 6 months	48.9 ± 14.5	17.6 ± 11.5	**< 0.001**[a]	40.8 ± 13.8	15.6 ± 9.9	**< 0.001**[a]
Baseline vs 6 months	**< 0.001**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	

[a] Statistical analysis by Student's t or Mann–Whitney test for two independent groups

[b] Wilcoxon Signed Ranks Test for two dependent groups

Table 5 PI (%) at treated sites (PD ≥ 4 mm) and full mouth (mean ± SD)

	PI		*P* value	FMPI		*P* value
	Control	Test		Control	Test	
Baseline	38.8 ± 26	60.6 ± 10.9	**0.002**[a]	35.7 ± 23.7	52.9 ± 11.4	**0.003**[a]
After 3 months	20.3 ± 16.7	18.8 ± 11.4	0.714[a]	19.3 ± 15.0	17.1 ± 9.7	0.893[a]
Baseline vs 3 months	**< 0.001**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	
After 6 months	26.5 ± 20.5	12.7 ± 8.9	**0.018**[a]	23.5 ± 16.6	11.2 ± 7.9	**0.006**[a]
Baseline vs 6 months	**0.039**[b]	**< 0.001**[b]		**< 0.001**[b]	**< 0.001**[b]	

[a] Statistical analysis by Student's t or Mann–Whitney test for two independent groups

[b] Wilcoxon Signed Ranks Test for two dependent groups

group with a statistically significant difference between the groups ($p < 0.001$). Similarly, the number of deep pockets (≥ 7 mm) changed from 277 (7.6%) to 35 (1.0%) in control and from 298 (8.7%) to 4 (0.1%) in test at 6 months evaluation with a statistically significant difference between the groups ($p = 0.003$) (Table 6).

No sub-analysis between different tooth types was performed since the results are presented only for moderate (PD 4–6 mm) and deep sites (PD ≥ 7 mm) without including furcation involved teeth.

Discussion

The present randomized clinical trial has investigated the clinical outcomes obtained with the subgingival application of a combination of sodium hypochlorite/amino acid and xHyA gels in conjunction with non-surgical periodontal therapy in untreated periodontitis patients. The results have shown that in patients diagnosed with stages II–III periodontitis, SRP combined with sodium hypochlorite/amino acid and xHyA gels resulted in statistically significantly higher clinical improvements evidenced through PD reduction, CAL gain, and decrease of BOP and PI values as compared to SRP alone.

An interesting observation of the study is related to PD and CAL changes between 3 and 6 months in moderate pockets. In particular, no statistically significant change was observed in the control group between the 3- and 6-month follow-ups, whereas in the test group, the change reached statistical significance. This observation appears to indicate that the test group demonstrated gradual improvements from month 3 to month 6, even though no additional treatment was performed. This finding may bear clinical relevance since it may suggest that the clinical improvements following the adjunctive sodium hypochlorite/amino acid and xHyA to SRP occur over a longer period of time (e.g., up to 6 months). Additionally, this observation may also suggest that a period of 3 months following nonsurgical periodontal therapy might be too early for making a final decision on the need for additional therapy (e.g., periodontal surgery). A similar pattern supporting the gradual improvement, was also observed for FMBOP and FMPI, where no statistically significant differences were observed between the groups at the 3-month follow-up, while it reached statistical significance at 6 months in favour of the test group.

This observation might be explained by the mode of action of xHA. In particular, the high molecular weight cross-linked HA that was used in this clinical trial can maintain its stability for 4 to 6 weeks which in turn, may serve as explanation for its prolonged activity [33].

When interpreting the clinical outcomes, it must be emphasized that the goal of non-surgical periodontal treatment is PD ≤ 4 mm with negative BOP [34]. The results of the current study have shown that the need for further treatment appears to be smaller in the test group, as demonstrated by the analysis of the change of number of moderate (4–6 mm) and deep pockets (≥ 7 mm) over time. In detail, in the control group, the total number of pockets with PD 4–6 mm decreased from 1518 to 803 with the corresponding values of 1803 and 234 in the test group. Similarly, the number of deep sites reduced from 277 to 35 in control and from 298 to 4 in test group.

As stated by Salvi et al., generally, a PD reduction of approximately 1–1.5 mm in moderate pockets (4–6 mm) and 2–2.5 mm in deep pockets (≥ 6 mm) can be expected [35] following mechanical debridement. This occurs concomitantly with CAL gain of approximately 0.5 mm in moderate pockets at baseline and 1.5 mm in deeper sites [35]. Any additional pocket reduction or CAL gain would, therefore, represent a true clinical benefit of the adjunctive materials used. This observation was also confirmed in the present study where in moderately deep sites, the mean PD change from baseline to 6 months measured 1.7 mm in the control group and 2.9 mm in test group, respectively, with the corresponding values of 3.7 mm and 5.8 mm, at deep sites (PD ≥ 7 mm). In moderately deep pockets, the mean CAL gain from baseline to 6 months measured 1.6 mm in the control group and 2.6 mm in test group, while in deep pockets, the corresponding values measured 3.2 mm and 5.3 mm, respectively.

When interpreting the results, one may ask the question to what extent each of the used adjunctive materials contributed to the additional improvements observed in

Table 6 Number of sites with shallow (1–3 mm), medium (4–6 mm) and deep (≥ 7 mm) pockets in test and control groups at different study timepoints

	1–3 mm			4–6 mm			≥ 7 mm		
	Control	Test	*P* value	Control	Test	*P* value	Control	Test	*P* value
Baseline	1916 (51.2%)	1603 (42.7%)	**0.05***	1518 (41.2%)	1803 (48.6%)	**0.041***	277 (7.6%)	298 (8.7%)	0.52
After 3 months	2938 (78.6%)	3284 (88.2%)	**0.013***	728 (20.3%)	402 (11.5%)	**0.018***	39 (1.1%)	12 (0.3%)	0.053
After 6 months	2859 (76.4%)	3398 (92.2%)	**0.006***	803 (22.6%)	234 (7.7%)	**< 0.001***	35 (1.0%)	4 (0.1%)	**0.003***

Data in bold represents statistically significant differences between test and control groups

the test group. In this respect, it is important to emphasize that the present study has used the combination of the two materials as a single concept, thus combining the effects of sodium hypochlorite/ amino acid gel to facilitate mechanical debridement and biofilm removal with the well-known wound-healing facilitating effects of xHyA. Based on previous findings from *in vitro* and animal experiments, it was hypothesized that the inherent effect of NaOCl to facilitate mechanical debridement and biofilm removal, may lend additional support to xHyA to express its wound healing improving properties [20, 23–25].

Despite the inherent positive effects of the used combination approach, it should be kept in mind that combining two materials and their use in conjunction with scaling and root planing also means a higher therapy effort in terms of time and costs. Additionally, it should be also emphasized that the present has only evaluated the outcomes in moderate (PD 4–6 mm) and deep sites (PD ≥ 7 mm) at teeth without furcation involvement. Obviously, further studies are warranted to evaluate the potential effect of this treatment approach in furcation involved teeth.

However, to the best of our knowledge, this is the first RCT evaluating the outcomes following the adjunctive application of sodium hypochlorite/amino acid gel and xHyA to scaling and root planing for untreated periodontal disease.

A recently published retrospective analysis of 29 clinical cases evaluated the adjunctive application of sodium hypochlorite/amino acid and a mixture of natural and cross-linked hyaluronic acid (high molecular) gels to SRP for treating residual periodontal pockets in patients diagnosed with periodontitis stages II–IV who were included into periodontal maintenance [30]. The authors reported an overall PD reduction exceeding 2 mm, associated with a similar CAL gain (2.02 mm). The results are comparable with the results obtained in this study. However, it must be emphasized that the study included compliant patients who already underwent nonsurgical periodontal treatment, as well as patients diagnosed with periodontitis stage IV, and therefore, direct comparisons are difficult. However, the same protocol has been evaluated in a very recent case series consisting of a total of twenty-one systemically healthy, non-smoking patients diagnosed with stage II-III periodontitis [31]. Compared to baseline, a statistically significant mean reduction of PD values was obtained after 3- and 6- months, amounting 2.6 ± 0.4 mm, and 2.9 ± 0.4 mm, respectively ($p < 0.001$), while mean CAL gain measured 2.3 ± 0.5 mm at 3- months, and 2.6 ± 0.5 mm at 6-months in comparison to baseline ($p < 0.001$). Mean reduction of BOP values amounted to 54.9 ± 16.9% at 3- months, and to 65.6 ± 16.4% at 6-months, respectively ($p < 0.001$). The number of moderate pockets (4–5 mm) reduced from 1808 at baseline to 274 at 6 months evaluation, and the number of deep (≥ 6 mm) pockets changed from 319 to 3, respectively [31]. These results compare well to those obtained in the present study, thus pointing to the potential clinical relevance of this novel clinical protocol.

Moreover, the adjunctive application of sodium hypochlorite/amino acid and hyaluronic acid gels to SRP has been tested separately in several clinical studies. On one hand, a recent clinical trial has evaluated the effect of the adjunctive application of sodium hypochlorite gel to SRP in residual periodontal pockets [9]. The findings revealed statistically significant PD reduction favouring the used of the sodium hypochlorite/amino acid gel, compared to a placebo ($p = 0.028$), as well as a statistically significant CAL gain at 6 months in the NaOCl-treated group, compared to the application of CHX gel ($p = 0.0026$).

One the other hand, the results of the studies on the adjunctive application of hyaluronic acid to non-surgical periodontal therapy are inconsistent. For instance, some of the studies found statistically significant improvements for the adjunctive application of hyaluronic acid to SRP in terms of PD and BOP reductions and CAL gain [27, 29], whereas in other studies adjunctive application of hyaluronic acid did not reach statistically significant differences in the investigated clinical parameters compared to SRP alone [36, 37].

Obviously, when interpreting the current results, certain the following limitations need to be mentioned: a) the study included a relatively small sample size and was of relatively short duration (i.e., 6 months), and b) only systemically healthy, non-smoking patients diagnosed with periodontitis stages II and III exhibiting adequate oral hygiene skills were included in the study.

Conclusion

Within their limits the present data indicate that: a) Both treatments resulted in statistically significant improvements in all evaluated clinical parameters, and b) The adjunctive subgingival application of sodium hypochlorite/amino acid gel and xHyA to SRP yielded statistically significantly higher improvements compared to SRP alone.

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Data availability All data underlying the results are available as part of the article and no additional source data is applicable.

Declarations

Competing interests The authors declare no competing interests.

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Microbiological Effects of Sodium Hypochlorite/-Amino Acids and Cross-linked Hyaluronic Acid Adjunctive to Non-surgical Periodontal Treatment

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Purpose: To investigate the microbiological outcomes obtained with either subgingival debridement (SD) in conjunction with a gel containing sodium hypochlorite and amino acids followed by subsequent application of a cross-linked hyaluronic acid gel (xHyA) gel, or with SD alone.

Materials and Methods: Forty-eight patients diagnosed with stages II-III (grades A/B) generalised periodontitis were randomly treated with either SD (control) or SD plus adjunctive sodium hypochlorite/amino acids and xHyA gel (test). Subgingival plaque samples were collected from the deepest site per quadrant in each patient at baseline and after 3 and 6 months. Pooled sample analysis was performed using a multiplex polymerase chain reaction (PCR)-based method for the identification of detection frequencies and changes in numbers of the following bacteria: *Aggregatibacter actinomycetemcomitans* (A.a), *Porphyromonas gingivalis* (P.g), *Tannerella forsythia* (T.f), *Treponema denticola* (T.d), and *Prevotella intermedia* (P.i).

Results: In terms of detection frequency, in the test group, statistically significant reductions were found for P.g, T.f, T.d and P.i ($p < 0.05$) after 6 months. In the control group, the detection frequencies of all investigated bacterial species at 6 months were comparable to the baseline values ($p > 0.05$). The comparison of the test and control groups revealed statistically significant differences in detection frequency for P.g ($p = 0.034$), T.d ($p < 0.01$) and P.i ($p = 0.02$) after 6 months, favouring the test group. Regarding reduction in detection frequency scores, at 6 months, statistically significant differences in favour of the test group were observed for all investigated bacterial species: A.a ($p = 0.028$), P.g ($p = 0.028$), T.f ($p = 0.004$), T.d ($p < 0.001$), and P.i ($p = 0.003$).

Conclusions: The present microbiological results, which are related to short-term outcomes up to 6 months post-treatment, support the adjunctive subgingival application of sodium hypochlorite/amino acids and xHyA to subgingival debridement in the treatment of periodontitis.

Keywords: cross-linked hyaluronic acid, microbiology, non-surgical periodontal therapy, periodontitis, periopathogenic bacteria, sodium hypochlorite/amino acids

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Periodontitis is a chronic, inflammatory disease characterised by microbial dysbiosis, resulting in the destruction of connective tissue attachment and alveolar bone.[1,5,6,16] Periodontal treatment aims to reduce or eliminate the periodontal-pathogenic biofilm from the periodontal pockets and the surrounding periodontal tissues.[21] Therefore, the thorough

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mechanical disruption and removal of subgingival biofilm and calculus are key components of cause-related periodontal therapy, aiming to reestablish clinical health as evidenced by shallow probing depths and the absence of bleeding on probing.[34]

However, the complete removal of plaque and calculus is often limited due to anatomical factors (e.g., furcation involvement, deep pockets, anatomical grooves, or concavities), the operator's manual skills, and various patient-related factors (e.g., smoking status or systemic diseases). It has been demonstrated that up to 30% of the total surface area of subgingivally debrided roots may still be covered with residual plaque and calculus.[21] In order to further enhance the elimination of subgingival bacterial biofilm, various adjunctive materials with antimicrobial activity have been utilised.[27]

Recently, a novel concept termed "Clean and Seal" in conjunction with subgingival instrumentation has been suggested to improve the outcomes of non-surgical periodontal therapy.[10,25,26] The two constituents of "Clean and Seal" are sodium hypochlorite/amino acids (Perisolv, Regedent; Zürich, Switzerland) and cross-linked hyaluronic acid (high molecular) (xHyA) gels (Hyadent BG, Regedent).

Preclinical studies have shown that sodium hypochlorite is able to alter biofilm matrices and act in particular against Gram-negative species associated to periodontitis.[17] Moreover, favourable cell survival and spreading of periodontal ligament cells has been observed after the application of sodium hypochlorite/amino acids gel to root surfaces.[29] Clinically, the additive value of sodium hypochlorite/amino acids gel has been reported in treating deep periodontal pockets in untreated periodontitis,[15] residual periodontal pockets,[18,24] peri-implant-mucositis[14] and peri-implantitis.[28]

Preclinical evidence on cross-linked hyaluronic acid has demonstrated that this formulation is not only biocompatible with periodontal tissues but also enhances the proliferative, migratory, and wound healing properties of cells involved in soft-tissue wound healing.[3] Furthermore, cross-linked hyaluronic acid strongly induces the growth of osteoprogenitors and is able to maintain their stemness, thus potentially regulating the balance between self-renewal and differentiation during bone regeneration.[2] Importantly, histological evidence from animal studies revealed that the adjunctive application of cross-linked hyaluronic acid resulted in significant regeneration of periodontal tissues in treating intrabony defects, gingival recessions, or furcation defects as compared to surgical controls.[30-32] Findings from a systematic review have shown that the adjunctive application of hyaluronic acid to non-surgical periodontal treatment resulted in statistically significant improvements in probing depth reduction and gain in clinical attachment compared to controls.[11]

Recent findings from clinical studies have provided evidence indicating that the adjunctive application of sodium hypochlorite/amino acid and cross-linked hyaluronic acid gels to SD may result in significant clinical improvements, as evidenced by the reduction of probing pocket depths (PD), bleeding on probing (BOP), and gain in clinical attachment (CAL). This applies both to patients with untreated periodontitis and patients enrolled in maintenance but still exhibiting residual pockets.[10,25,26]

To the best of our knowledge, no clinical studies to date have reported on the microbiological outcomes following the treatment using this novel concept for non-surgical periodontal therapy. Therefore, the aim of this study was to investigate the potential microbiological advantages of this strategy in the treatment of periodontitis.

MATERIALS AND METHODS

Experimental Design

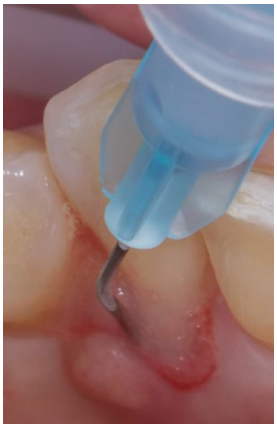
This randomised, controlled, parallel study included 48 non-smoking patients, diagnosed with stages II-III (grades A, B) generalised periodontitis, aged between 30 to 72 years (mean ± SD), who attended the Department of Dental and Oral Pathology at the Lithuanian University of Health Sciences in Kaunas, Lithuania, for periodontal treatment. The study's inclusion criteria were the absence of systemic diseases and no intake of medication which may affect periodontal health, the presence of at least 20 teeth, and absence of removable dentures. The study's exclusion criteria were: smokers, periodontal treatment during last 12 months, antibiotic treatment 3 months prior to the start of the trial, antibiotic prophylaxis required for dental treatment, pregnant/lactating women, and known allergies to sodium hypochlorite. The study protocol was registered at ClinicalTrials.gov, NCT04662216. All patients were enrolled between September 2019 and January 2022. Each patient was given detailed information of the study protocol and was required to sign an informed consent form.

Treatment Procedures

After an initial screening visit for recruitment and supragingival cleaning, patients were assigned randomly to the control or test groups (control group: 24 patients; test group: 24 patients). Demographic details, randomisation, allocation concealment and study design are described in detail in a related paper reporting clinical outcomes.[26] In brief, subjects in the control group underwent full-mouth SD performed with ultrasonic (Satelec/Acteon suprasson newtron ultrasonic scaler, Acteon; Norwich, UK) and hand instruments (LM SharpDiamond 1/2, 7/8, 11/12, 13/14 SD mini Gracey and Gracey curettes, LM; Parainen, Finland). Subsequently, all teeth were polished using a low-abrasive paste (Lunos Super Soft, RDA < 5, Dürr Dental; Bietigheim-Bissingen, Germany). In the test group, full-mouth SD was performed as follows: in all pockets with PD ≥ 4 mm, a sodium hypochlorite/amino acid gel (Perisolv, Regedent) was inserted into the pockets and left there for 60 s before subgingival instrumentation (Fig 1). Subgingival instrumentation was carried out with the same ultrasonic and hand instruments, and the application of sodium hypochlorite/amino acid gel was repeated until instrumentation was considered sufficient (i.e., a total of 2–3 times). Following SD and polishing, a mixture of natural and cross-linked hyaluronic acid (high molecular) gel (Hyadent BG, Regedent) was inserted in the pockets using a blunt needle (Fig 2).

Outcomes

The primary outcome variable was the change in detection frequency of *Aggregatibacter actinomycetemcomitans* (A.a), *Por-*



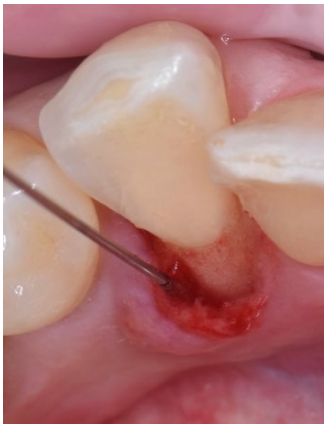


Fig 1 The application of sodium hypochlorite/amino acid gel to the periodontal pocket prior to subgingival debridement.

Fig 2 Application of a mixture of natural and cross-linked hyaluronic acid (high molecular) to the periodontal pocket after subgingival debridement.

phyromonas gingivalis (P.g), *Tannerella forsythia* (T.f), *Treponema denticola* (T.d), and *Prevotella intermedia* (P.i) from baseline to 6 months. Secondary outcome variables included the change of detection scores (0-4, which correspond to the number CFUs, see Table 1) of the respective bacteria as well as changes in PD, CAL, BOP and plaque index (PI) at sampled sites from baseline to 6 months.

Microbial Sampling

Subgingival plaque samples were collected at baseline (prior to SD) and at 3 and 6 months from the deepest pocket per quadrant by the same investigator (U.M.D). Following a thorough removal of supragingival plaque and calculus using periodontal curettes and sterile cotton pellets, each site was dried and isolated with cotton rolls. A sterile endodontic paper point ISO #30 (Dentsply Sirona; Bensheim, Germany) was inserted and left in place for 20 s. Four samples per patient were collected in a coded sterile-sealed Eppendorf tube and sent to the laboratory (Department of Laboratory Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania) for analysis. There, these samples were kept frozen at -20°C until further processing (for one day), and then at -80°C until the microbiological analysis was performed (not more than 30 days later). Molecular analysis of the subgingival plaque samples was performed manually in three steps:

- deoxyribonucleic acid (DNA) extraction;
- multiplex amplification with biotinylated primers;
- reverse hybridisation.

DNA Extraction

DNA extraction was performed using DNA purification from swab samples kit (Swab, version 0517, A&A Biotechnology; Gdynia, Poland). 700 µl of lysis solution and 20 µl of proteinase K were added to the original Eppendorf tubes containing the paper points with subgingival plaque samples. The tube contents were thoroughly mixed, briefly centrifuged, and incubated for 20 min at 37°C with mixing at 500 rpm. After incubation, the samples were mixed, centrifuged, and the resulting liquid was applied to the spin columns. The columns were centrifuged for 1 min at 12,000 rpm. Two washing cycles were performed using new 2-ml tubes and 500 µl of washing solution each time. The washing solution was centrifuged at 12,000 rpm for 1 min the first time and for 2 min the second time. The washed and spun columns were transferred to new 1.5-ml tubes, and 150 µl of elution buffer heated to 75°C was added, incubated for 3 min at room temperature, and centrifuged for 1 min at 12,000 rpm. The resulting DNA samples were stored at -80°C until further analysis.

Multiplex DNA Amplification

DNA samples were analysed using molecular genetic assay for combined identification of five periodontopathogenic bacterial species (micro-IDent VER 2.0, Hain Lifescience; Nehren, Germany) including *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia,* and *Treponema denticola*. Master mix of the amplification enzymes was freshly prepared before testing each batch of the DNA samples. 45 µl of master mix and 5 µl of DNA samples or the negative control (molecular-biology–grade water) were prepared and mixed in separately designated laboratory spaces. The negative control was used along with each 24-sample batch. Polymerase chain reaction (PCR) for DNA amplification was performed in the thermal cycler according to the protocol provided by the diagnostic kit's manufacturer. Amplification products were stored at 2-4°C until further processing.

Reverse Hybridisation

Before starting the test procedure, as stated in the manufacturer's instructions, reagents were brought to room tempera-

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Table 1 Semi-quantitative interpretation of the test results

Bacterial species	Color intensity of the test strip bands representing detection scores 0-4				
	0	1	2	3	4
Aggregatibacter actinomycetemcomitans	$<10^3$ CFU/ml	10^3 CFU/ml	$<10^4$ CFU/ml	$<10^5$ CFU/ml	$>10^6$ CFU/ml
Porphyromonas gingivalis	$<10^4$ CFU/ml	10^4 CFU/ml	$<10^5$ CFU/ml	$<10^6$ CFU/ml	$>10^7$ CFU/ml
Prevotella intermedia					
Tannerella forsythia					
Treponema denticola					

Table 2 Distribution of sampled sites

Treatment	Second molars	First molars	Second premolars	First premolars	Canines	Lateral incisors	Central incisors
Control group (n)	10	5	9	10	16	25	21
Test group (n)	9	9	14	11	21	13	19
p	0.621	0.244	0.503	0.152	0.327	0.021	0.504

Mann-Whitney U-test for two independent groups.

ture (20-25°C) or heated to 45°C, and necessary dilutions were prepared. First, 20 µl of denaturation solution and 20 µl of amplified DNA sample were mixed and incubated at room temperature for 5 min. 1 ml of pre-warmed hybridisation buffer was added, and test strips were placed into each well containing denatured DNA samples. The prepared wells were incubated for 30 min at 45°C in a shaking water bath. After incubation, the hybridisation buffer was aspirated, and 1 ml of stringent wash solution was added to each well. The wells were incubated for 15 min at 45°C in the shaking water bath. The stringent wash solution was removed, and each strip was washed with 1 ml of rinse solution for 1 min on a shaking platform. 1 ml of diluted conjugate solution was added to each well and incubated for 30 min at room temperature on the shaking platform. Conjugate was removed, and each strip was washed for 1 min three times on a shaking platform: twice using rinse solution and once using distilled water. 1 ml of diluted substrate solution was added to each well and incubated protected from light and without shaking for 15 min. After test strip bands became clearly visible, they were briefly washed twice using distilled water, dried between two layers of absorbent paper, pasted on the provided evaluation sheet and stored protected from light.

Evaluation and Interpretation of Microbiological Results

First, developed test strips were inspected for effective and correct testing procedure by observing three control bands (conjugate control, hybridisation control, and amplification control). After making sure all three control bands were correctly developed, five bacterial species-specific bands were analysed by a semi-quantitative approach. According to the developed color intensity, 0, 1, 2, 3, or 4 points were assigned to each band. The color intensity of the bands is expressed semi-quantitatively as detection scores 0-4, which represent the number of CFUs/ml (Table 1).

Clinical Measurements

The following clinical parameters were measured to the nearest mm using a Williams periodontal probe (LM 51 ES, LM-Dental; Parainen, Finland) from the deepest site per quadrant at baseline, 3 and 6 months following the treatment:

- Bleeding on probing (BOP), defined as the percentage of sites positive for bleeding within 10 s after probing (%).
- Plaque index (PI), defined as the percentage of sites with visual plaque on the tooth surface (%).
- Probing depth (PD), measured in mm from the gingival margin to the bottom of the probed pocket.
- Recession (REC), measured in mm from the gingival margin to the cementoenamel junction or to the margin of a cervical restoration.
- Clinical attachment level (CAL), calculated by adding PD and REC at each site.

Blinding

Clinical measurements and microbial sampling were performed by a blinded calibrated examiner (U.M.D.), who was not aware in any of the cases of the type of treatment performed. To ensure blindness, the treatment procedures were performed by one experienced periodontist (E.R.). A third investigator

Table 3 Detection frequencies sorted by periodontopathogen (%)

Periodontopopathogen	Treatment strategy	Baseline	3 months	6 months
Aa	Control group	42.5	54.2	58.3
	Test group	45.8	29.2	33.3
Pg	Control group	75.0	58.3	75.0*
	Test group	87.5[ab]	41.7[a]	41.7*[b]
Tf	Control group	91.7[a]	62.5[a]	79.2
	Test group	83.3[ab]	54.2[a]	58.3[b]
Td	Control group	87.5[a]	58.3[a]	79.2*
	Test group	95.8[ab]	41.7[a]	33.3*[b]
Pi	Control group	58.3	29.2	45.8*
	Test group	45.8[ab]	20.8[a]	8.3*[b]

*$p < 0.05$, between-group differences (X^2 test). [a]$p < 0.05$, baseline and after 3 months (McNemar test); [b]$p < 0.05$, baseline and after 6 months (McNemar test).

(L.P.) performed microbiological analysis and was unaware of neither treatment procedures nor clinical measurements. I.N. processed coded data for statistical analysis.

Statistical Analysis

Statistical analysis was performed with the IBM SPSS 27 software package (IBM; Armonk, NY, USA). Data analysis was performed using the patient as the statistical unit. The difference in the distribution of sampled sites in terms of tooth group was examined using the Mann-Whitney U-test for two independent groups. For clinical changes at sampled sites, mean values per subject and per visit were calculated for each clinical parameter. The Shapiro-Wilk test was performed to assess whether clinical periodontal measures followed a normal distribution. If data followed a normal distribution, a paired-samples t-test was performed to evaluate before- and after-treatment comparisons within groups. If the data did not follow a normal distribution, the Wilcoxon signed-rank test was performed on related samples to assess before- and after-treatment comparisons within the groups. The between-group comparisons of measures were obtained by either the independent-samples t-test (if a parameter followed a normal distribution) or the Mann-Whitney test (if a specific measure followed a non-normal distribution).

Differences in detection frequency (0 = undetected and 1 = detected) between the control group and the test group at baseline and at 3 and 6 months were analysed using the X^2 test. The within-group changes were evaluated by McNemar test.

The changes of the detection frequency scores were recorded and classified into one of the following categories: 0: not detectable; or detectable with a score of 1, 2, 3 or 4 (Table1). Intragroup comparisons of detection scores of periopathogen species between the baseline and 3- and 6-month evaluation were performed using the Wilcoxon signed-rank test. The Mann-Whitney test was used for intergroup comparisons of detection scores for each timepoint. The significance level was set at 0.05.

RESULTS

All 48 patients completed the study. The distribution of sampled sites was equal in both groups in terms of tooth group, except for lateral incisors (Table 2).

Detection Frequency of Periodontopathogens

Table 3 displays the detection frequencies for each periodontopathogen at different time points in test and control groups. The results were expressed as the proportion of patients (%) positive for a given pathogen.

In the control group, after 3 months, statistically significant reductions were detected for T.f and T.d ($p < 0.05$), whereas after 6 months, the detected frequencies of the respective bacteria recovered to pretreatment levels and were comparable to the baseline values ($p > 0.05$). In the test group, statistically significant reductions were found for for P.g, T.f, T.d and P.i after 3 and 6 months ($p < 0.05$). The comparison of the test and control groups pointed to statistically significant differences in detection frequency of P.g ($p = 0.034$), T.d ($p < 0.01$) and P.i ($p = 0.02$) after 6 months, favouring the test group.

Changes of the Detection Scores of Periodontopathogens

Table 4 shows detection scores for A.a, P.g, T.f, T.d, and P.i at baseline, 3- and 6-month follow-ups.

At baseline, no statistically significant differences were observed between control and test groups in terms of detection scores of the investigated periodontal pathogenic species ($p > 0.05$). In the control group at 3 months, a statistically significant decrease in detection scores from baseline was found for P.g ($p = 0.013$), T.f ($p = 0.007$), T.d ($p = 0.003$) and P.i ($p = 0.012$). At 6 months, statistically significant reductions from baseline remained for P.g ($p = 0.039$) and T.f ($p = 0.048$). The test group at 3 months demonstrated a statistically significant decrease in detection scores from baseline for all investigated periopatho-

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Table 4 Detection frequency scores for A.a, P.g, P.i, T.f, T.d at baseline, 3- and 6-month follow-up visits

Species	Timepoint	Detection score	Total, n (%)	Control group, n (%)	Test group, n (%)	p–value**
A.a	Baseline	0	22 (45.8)	9 (37.5)	13 (54.2)	0.174
		1	2 (4.2)	1 (4.2)	1 (4.2)	
		2	4 (8.3)	2 (8.3)	2 (8.3)	
		3	8 (16.7)	4 (16.7)	4 (16.7)	
		4	12 (25.0)	8 (33.3)	4 (16.7)	
	3 months	0	28 (58.3)	11 (45.8)	17 (70.8)	0.044
		1	2 (4.2)	1 (4.2)	1 (4.2)	
		2	6 (12.5)	4 (16.7)	2 (8.3)	
		3	6 (12.5)	2 (8.3)	4 (16.7)	
		4	6 (12.5)	6 (25.0)	0	
	*p–value		0.013	0.231	0.011	
	6 months	0	26 (54.2)	10 (41.7)	16 (66.7)	0.028
		1	4 (8.3)	1 (4.2)	3 (12.5)	
		2	4 (8.3)	3 (12.5)	1 (4.2)	
		3	6 (12.5)	3 (12.5)	3 (12.5)	
		4	8 (16.7)	7 (29.2)	1 (4.2)	
	*p–value		0.085	0.064	0.016	
P.g	Baseline	0	9 (18.8)	6 (25.0)	3 (12.5)	0.884
		1	1 (2.1)	–	1 (4.2)	
		2	2 (4.2)	1 (4.2)	1 (4.2)	
		3	11 (22.9)	4 (16.7)	7 (29.2)	
		4	25 (52.1)	13 (54.2)	12 (50.0)	
	3 months	0	24 (50.0)	10 (41.7)	14 (58.3)	0.099
		1	3 (6.3)	1 (4.2)	2 (8.3)	
		2	6 (12.5)	3 (12.5)	3 (12.5)	
		3	8 (16.7)	4 (16.7)	4 (16.7)	
		4	7 (14.6)	6 (25.0)	1 (4.2)	
	*p–value		<0.001	0.013	<0.001	
	6 months	0	20 (41.7)	6 (25.0)	14 (58.3)	0.006
		1	7 (14.6)	4 (16.7)	3 (12.5)	
		2	8 (16.7)	4 (16.7)	4 (16.7)	
		3	6 (12.5)	3 (12.5)	3 (12.5)	
		4	7 (14.6)	7 (29.2)	–	
	*p–value		<0.001	0.039	<0.001	
T.f	Baseline	0	6 (12.5)	2 (8.3)	4 (16.7)	0.846
		1	3 (6.3)	3 (12.5)	–	
		2	5 (10.4)	3 (12.5)	2 (8.3)	
		3	18 (37.5)	8 (33.3)	10 (41.7)	
		4	16 (33.3)	8 (33.3)	8 (33.3)	
	3 months	0	20 (41.7)	9 (37.5)	11 (45.8)	0.088
		1	8 (16.7)	1 (4.2)	7 (29.2)	
		2	8 (16.7)	5 (20.8)	3 (12.5)	
		3	10 (20.8)	7 (29.2)	3 (12.5)	
		4	2 (4.2)	2 (8.3)	–	
	*p–value		<0.001	0.007	<0.001	
	6 months	0	15 (31.3)	5 (20.8)	10 (41.7)	0.004
		1	10 (20.8)	3 (12.5)	7 (29.2)	
		2	9 (18.8)	4 (16.7)	5 (20.8)	
		3	12 (25.0)	10 (41.7)	2 (4.2)	
		4	2 (4.2)	2 (8.3)	–	
	*p–value		<0.001	0.048	<0.001	
T.d	Baseline	0	4 (8.3)	3 (12.5)	1 (4.2)	0.878
		1	10 (20.8)	4 (16.7)	6 (25.0)	
		2	22 (45.8)	11 (45.8)	11 (45.8)	
		3	12 (25.0)	6 (25.0)	6 (25.0)	
		4	–	–	–	
	3 months	0	24 (50.0)	10 (41.7)	14 (58.2)	0.125
		1	13 (27.1)	6 (25.0)	7 (29.2)	
		2	10 (20.8)	7 (29.2)	3 (12.5)	
		3	1 (2.1)	1 (4.2)	–	
		4	–	–	–	
	*p–value		<0.001	0.003	<0.001	
	6 months	0	21 (43.8)	5 (20.8)	16 (66.7)	<0.001
		1	13 (27.1)	6 (25.0)	7 (29.2)	
		2	12 (25.0)	11 (45.8)	1 (4.2)	
		3	2 (4.2)	2 (8.3)	–	
		4	–	–	–	
	*p–value		<0.001	0.083	<0.001	
P.i	Baseline	0	23 (47.9)	10 (41.7)	13 (54.2)	0.413
		1	5 (10.4)	4 (16.7)	1 (4.2)	
		2	4 (8.3)	–	4 (16.7)	
		3	12 (25.0)	8 (33.3)	4 (16.7)	
		4	4 (8.3)	2 (8.3)	2 (8.3)	
	3 months	0	36 (75.0)	17 (70.8)	19 (79.2)	0.399
		1	2 (4.2)	1 (4.2)	1 (4.2)	
		2	7 (14.6)	3 (12.5)	4 (16.7)	
		3	3 (6.3)	3 (12.5)	–	
		4	–	–	–	
	*p–value		<0.001	0.012	0.014	
	6 months	0	35 (72.9)	13 (54.2)	22 (91.7)	0.003
		1	4 (8.3)	3 (12.5)	1 (4.2)	
		2	5 (10.4)	4 (16.7)	1 (4.2)	
		3	4 (8.3)	4 (16.7)	–	
		4	–	–	–	
	*p–value		<0.001	0.091	0.003	

n: frequencies; *according to Wilcoxon tests for intragroup comparison of pathogen detection scores between successive timepoints; **according to Mann–Whitney tests for intergroup comparisons of pathogen detection scores for each timepoint.

Table 5 Clinical data of sampled sites (mean ± SD) at different time points

	Control group	Test group	p-value
PD (mm)			
Baseline	6.4 (1.0)	6.6 (1.2)	0.569[a]
After 3 months	3.3 (1.0)	2.5 (0.9)	0.02[a]
Baseline vs 3 months	<0.001[b]	<0.001[b]	
After 6 months	3.6 (0.8)	2.0 (0.8)	<0.001[a]
Baseline vs 6 months	<0.001[b]	<0.001[b]	
3 months vs 6 months	0.096[b]	0.003[b]	
CAL (mm)			
Baseline	6.4 (1.2)	6.4 (1.4)	0.844[a]
After 3 months	3.5 (1.0)	2.8 (1.2)	0.017[a]
Baseline vs 3 months	<0.001[b]	<0.001[b]	
After 6 months	3.9 (1.0)	2.3 (1.1)	<0.001[a]
Baseline vs 6 months	<0.001[b]	<0.001[b]	
3 months vs 6 months	0.084[b]	0.003[b]	
BOP (%)			
Baseline	92.1 (5.9)	94.2 (4.4)	0.429[a]
After 3 months	52.1 (14.2)	32.2 (14.6)	0.003[a]
Baseline vs 3 months	<0.001[b]	<0.001[b]	
After 6 months	59.4 (16.2)	19.2 (11.2)	<0.001[a]
Baseline vs 6 months	<0.001[b]	<0.001[b]	
3 months vs 6 months	0.072[b]	0.002[b]	
PI (%)			
Baseline	66.2 (22.1)	68.2 (11.2)	0.622[a]
After 3 months	21.2 (17.1)	19.2 (11.2)	0.002[a]
Baseline vs 3 months	<0.001[b]	<0.001b	
After 6 months	26.2 (21.3)	13.3 (6.8)	0.006[a]
Baseline vs 6 months	0.041[b]	<0.001[b]	
3 months vs 6 months	0.062[b]	0.003[b]	

[a] Statistical analysis using the Mann-Whitney test for two independent groups. [b] Paired-samples t-test for two dependent groups.

genic species: A.a ($p = 0.011$), P.g ($p < 0.001$), T.f ($p < 0.001$), T.d ($p < 0.001$) and P.i ($p = 0.014$). These results were maintained after 6 months: A.a ($p = 0.016$), P.g ($p < 0.001$), T.f ($p < 0.001$), T.d ($p < 0.001$) and P.i ($p = 0.003$). The intergroup analysis exhibited statistically significant differences in detection scores between control and test groups for A.a ($p = 0.044$) at the 3-month evaluation and for A.a ($p = 0.028$), P.g ($p = 0.006$), T.f ($p = 0.004$), T.d ($p < 0.001$) and P.i ($p = 0.003$) at the 6-month evaluation, favouring the test group.

Clinical Changes at Sampled Sites

Clinical changes at sampled sites are depicted in Table 5.

At baseline, no statistically significant differences were observed between test and control groups in any of the investigated clinical parameters ($p > 0.05$).

Regarding PD changes, both groups demonstrated statistically significant reductions in PD after 3 and 6 months; however, the difference between groups was statistically significant in favour of the test group at both timepoints ($p = 0.02$ and $p < 0.001$, respectively). Importantly, the PD change between 3- and 6- month follow-ups was statistically significant in the test group ($p = 0.003$), but did not demonstrate a statistically significant reduction in the control group ($p = 0.096$).

The intragroup comparisons pointed to a statistically significant gain in CAL in both groups at 3- and 6- month evaluations ($p < 0.05$), and intergroup analysis revealed statistically significant differences between groups at the respective timepoints ($p = 0.017$ and $p < 0.001$, respectively) in favour of the test group. The change in CAL between 3- and 6- months was statistically significant in the test group ($p = 0.003$), but did not demonstrate statistically significant improvements in the control group ($p = 0.084$).

Regarding changes in BOP, both study groups statistically significantly improved at 3 and 6 months compared to baseline ($p < 0.001$). The difference between groups was statistically significant at both the 3-month ($p = 0.003$) and the 6-month follow-up ($p < 0.001$). The change between 3- and 6-month evaluation was statistically significant in the test group ($p = 0.002$) but not ($p = 0.072$) in the control group.

In terms of PI, both groups showed statistically significant improvements at 3- and 6-month follow-ups compared to baseline ($p < 0.05$). The intergroup comparison revealed a statistically significant difference between groups in favour of the test group at 3 ($p = 0.002$) and 6 months ($p = 0.006$). The change between 3- and 6-month evaluation was statistically significant in the test group ($p = 0.003$) but not ($p = 0.062$) in the control group.

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DISCUSSION

Recent studies indicated that clinical outcomes of non-surgical periodontal therapy can be improved by the adjunctive subgingival application of sodium hypochlorite/amino acids and cross-linked hyaluronic acid gels.[10,25,26] The present study investigated the microbiological impact of subgingivally delivered sodium hypochlorite/amino acids and cross-linked hyaluronic acid gels as adjuncts to same-day full-mouth subgingival debridement. To the authors' best knowledge, this is the first study to clinically evaluate the microbiological outcomes of this novel concept (i.e., "Clean and Seal") for non-surgical periodontal therapy.

Based on the present data, both treatment approaches (i.e., subgingival debridement and subgingival debridement in conjunction with sodium hypochlorite/amino acids and cross-linked hyaluronic acid gels) led to statistically significant microbiological shifts. However, these shifts exhibited different patterns between the test and control groups. In particular, after 3 months, both groups demonstrated statistically significant reductions in the detection frequency of T.f and T.d ($p < 0.05$), with the test group additionally showing a statistically significant reduction for P.i and P.g ($p < 0.05$). After 6 months, the detection frequency of T.f and T.d was comparable to baseline in the control group ($p > 0.05$), whereas statistically significant reductions ($p < 0.05$) compared to baseline were sustained in the test group for the respective bacterial species (T.f, T.d, P.i, and P.g). At this point, it is important to mention that the frequency of detecting A.a was unaffected by both treatment approaches ($p > 0.05$).

Similar findings have been reported in previous clinical studies on the effects of subgingival debridement on periodontal pathogens using molecular techniques, such as DNA probes and PCR amplification.[9,12,33] More specifically, only the levels of P.g, T.f, T.d and P.i statistically significantly decreased after non-surgical periodontal therapy,[9,12,33] while such changes were found to be statistically insignificant in terms of decreasing A.a.[9,33] These findings once again support the results from previous reports which failed to demonstrate the effectiveness of subgingival debridement alone in reducing A.a. levels.[35] Moreover, several studies have shown that statistically significant reductions in detection frequency of P.g, T.f, and T.d may be a characteristic feature of successful periodontal therapy.[7] Our observations align well with this statement, since at 3 months, a statistically significant reduction in detection frequency was found for P.g, T.f and T.d in the test group ($p < 0.05$) and for T.f and T.d in the control group ($p < 0.05$), whereas after 6 months, the reductions remained stable for P.g, T.f and T.d only in the test group ($p < 0.05$).

Regarding the changes of detection scores, after 3 months, both study groups demonstrated statistically significant reductions of T.f, T.d, P.g, and P.i ($p < 0.05$) compared to baseline, while a statistically significant reduction of A.a was only observed in the test group ($p = 0.001$). At 6 months, a statistically significant reduction compared to baseline persisted for P.g and T.f in the control group ($p < 0.001$). However, in the test group, the reduction remained statistically significant for all investigated periodontal pathogenic species compared with baseline ($p < 0.05$). These findings corroborate those obtained in a recent 12-month randomised controlled clinical trial[24] that evaluated changes in detection scores for five periodontal pathogenic species and pointed towards statistically significant benefits of the adjunctive application of sodium hypochlorite/amino acids gel to subgingival debridement in reducing the detection scores of P.g ($p = 0.015$) and T.f ($p = 0.004$). However, the levels of A.a remained unchanged compared with baseline ($p = 0.098$).[24] Moreover, another clinical trial, which investigating the presence or absence of six target microorganisms in pockets treated with either ultrasonic instrumentation (control) or ultrasonic instrumentation supplemented with sodium hypochlorite/amino acid gel (test), found statistically significant reductions in T.f from baseline to day 7 ($p < 0.05$) and in T.d from baseline to month 4 ($p < 0.05$) in the test group.[19]

The differences observed in the present analysis between the test and control groups regarding detection frequencies and changes in detection scores may be attributed to the additive antimicrobial effects of sodium hypochlorite/amino acid and cross-linked hyaluronic acid gels.[17,23] Based on previous findings from in-vitro and animal experiments, it may be hypothesised that the ability of sodium hypochlorite/amino acids to facilitate mechanical debridement and biofilm removal may lend additional support to xHyA in expressing its bacteriostatic and wound healing properties.[17,23,30-31] In fact, as pointed out by the numerous clinical studies,[4,8,13,20] mechanical debridement alone has only limited efficacy in eradicating all bacteria, particularly keeping in mind that bacteria may reside in soft tissues, root surface irregularities and dentinal tubules.[22]

The present work also analysed microbial samples taken from treated patients from our previous randomised clinical trial;[26] the results are reported here. We therefore show that previously reported clinical data[26] align well with the microbiological outcomes reported in this paper. When interpreting the data, it is important to point out that the obtained microbiological findings correspond well with the clinical outcomes assessed after 3 and 6 months after treatment. In particular, after 3 months, both study groups demonstrated statistically significant improvements in PD, BOP, PI reductions and CAL gain with a statistically significant difference in favour of the test group. An interesting finding was that after 6 months, the test group exhibited gradual and significant clinical improvements in PD, CAL, BOP, and PI compared to the 3-month evaluation. In contrast, the results in the control group remained unchanged or showed signs of relapse.

Taken together, these findings demonstrate that the microbiological benefits of sodium hypochlorite/amino acids and cross-linked hyaluronic acid gels were sustained over a 6-month period, indicating a long-term microbiological effect. Furthermore, a connection between clinical and microbiological status can be confirmed; however, it remains unclear whether a decrease in subgingival microbiota led to an improvement in clinical conditions or vice versa. When interpreting the results, the question arises as to what extent each of the adjunctive substances used (i.e., sodium hypochlorite/amino acids and cross-linked hyaluronic acid) contributed to the additional microbiological improvements observed in the test group. In this respect, it is important to emphasise that the present study used the combination of the two materials as a

single concept. Therefore, further studies are needed to better understand the separate and combined effects of the two components on the clinical and microbiological outcomes.

CONCLUSION

The microbiological results of the present study support the adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA to subgingival debridement in the treatment of periodontitis.

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RESEARCH

Histological evaluation of nonsurgical periodontal treatment with and without the use of sodium hypochlorite / amino acids and cross-linked hyaluronic acid gels in dogs

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Abstract

Objectives To evaluate periodontal wound healing following scaling and root planing (SRP) in conjunction with the application of sodium hypochlorite/amino acids and cross-linked hyaluronic acid (xHyA) gels in dogs.

Materials and Methods In four beagle dogs, 2-wall intrabony defects were created and metal strips were placed around the teeth. Clinical parameters were measured 4 weeks after plaque accumulation. The experimental root surfaces were subjected to SRP with either the subgingival application of a sodium hypochlorite/amino acid gel and a xHyA gel (test group) or SRP alone (control group) using a split-mouth design. Clinical parameters were re-evaluated at 6 weeks. The animals were sacrificed at 8 weeks for histological analysis.

Results The test group showed significant improvements in all clinical parameters compared to the control group. Histologically, the test group exhibited statistically significantly greater new bone formation [i.e., length of newly formed bone, new bone area] compared with the control group ($p < 0.05$). Furthermore, statistically significantly greater formation of new attachment [i.e., linear length of new cementum adjacently to newly formed bone with inserting collagen fibers] and new cementum was detected in the test group compared with the control group at 8 weeks ($p < 0.05$ and $p < 0.01$, respectively).

Conclusion The adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA gels to SRP offers an innovative novel approach to enhance periodontal wound healing/regeneration.

Clinical relevance The present findings have for the first-time shown histologic evidence for periodontal regeneration in support of this novel treatment modality.

Keywords Periodontitis · Periodontal wound healing/regeneration · Non-surgical periodontal therapy · Cross-linked hyaluronic acid · Sodium hypochlorite/amino acids

Introduction

Periodontitis is a highly prevalent chronic inflammatory disease caused by dysbiotic dental plaque, leading to the destruction of connective tissue attachment and loss of alveolar bone, ultimately resulting in tooth loss [1–3]. The treatment of periodontitis is multi-staged and first steps of non-surgical periodontal therapy (NSPT) include supragingival plaque control, followed by subgingival scaling and root planing (SRP), aimed at eliminating biofilms, endotoxins, and calculus. This treatment reduces inflammation and reestablishes a favorable environment for oral hygiene measures [4–6]. The efficacy of SRP has been well reported by gains in clinical attachment level (CAL), reductions in periodontal pocket depth (PPD) and in the frequency of

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bleeding on probing (BOP) [4, 7, 8]. However, SRP does not always result in closure of periodontal pockets and the outcomes may be influenced by several patient related factors (e.g., smoking level, oral hygiene), anatomical factors (e.g., tooth type and surface, furcation involvement) and operator's experience [9–11]. The available evidence from histological studies indicates that SRP typically leads to a reparative type of healing characterized by formation of a long junctional epithelium and limited or no regeneration of cementum, periodontal ligament and bone [12–16]. Consequently, various strategies including the use of antibiotics, antiseptics and different biological agents adjunctive to SRP have been used to effectively control the bacterial biofilm caused inflammation and enhance wound healing [15, 17–25].

Recently, a novel formulation of sodium hypochlorite (NaOCL) gel buffered with leucine, lysine, and glutamine acid (Perisolv®, Regedent AG, Zürich, Switzerland) has been suggested as an adjunct to SRP. Since it has been shown that the active ingredients in the gel create chloramines, which have a strong antimicrobial effect and can penetrate the biofilm [26], it has been suggested that its use may aid for both the mechanical removal of hard and soft subgingival bacterial deposits and the detoxification of the root surface [26, 27]. In this respect, positive clinical effects of a sodium hypochlorite gel were reported in studies treating deep pockets at teeth [28, 29] and dental implants [27].

Hyaluronic acid (HyA) is a major natural glycosaminoglycan component of the extracellular matrix in many tissues such as skin, joints, eyes, and periodontium and has several unique physiochemical and biological properties including hygroscopic, viscoelastic, bacteriostatic, anti-inflammatory, anti-oedematous, pro-angiogenetic and osteoinductive nature [30–35]. HyA is currently also available in cross-linked form (cross-linked HyA: xHyA) for various applications in tissue engineering, serving as biologics/scaffolds to further improve the overall mechanical properties and provide a longer degradation period compared with non-cross-linked HyA [36, 37]. Results from clinical studies, indicate positive outcomes evidenced by significant gain of CAL, PPD reduction and improved BOP values have been reported following the adjunctive application of xHyA to nonsurgical and surgical periodontal therapy [17, 31, 38]. Furthermore, a recent series of preclinical studies has demonstrated periodontal regeneration, evidenced by formation of cementum, periodontal ligament, and bone, following the application of xHyA in conjunction with reconstructive periodontal surgery for recessions, intrabony, and furcation defects [39–41].

Very recently, a novel two-step approach consisting of enhanced biofilm removal during nonsurgical therapy by means of a sodium hypochlorite/amino acid followed by application of a xHyA gel was suggested to improve the outcomes of nonsurgical periodontal therapy [42–44]. Results from two case series have shown statistically significant clinical improvements compared to baseline following SRP in conjunction with sodium hypochlorite/amino acid and xHyA [42, 43], thus suggesting that this strategy may represent a valuable novel strategy in non-surgical periodontal treatment. Additionally, a randomized controlled clinical study has assessed the clinical outcomes achieved through either mechanical subgingival debridement in conjunction with a sodium hypochlorite/amino acids-containing gel followed by subsequent application of a xHyA gel, or mechanical debridement alone [44]. The results have shown that both treatments led to statistically significant improvements in all evaluated clinical parameters, but the adjunctive subgingival application of sodium hypochlorite/amino acids and xHyA to SRP resulted in statistically significantly greater improvements compared to SRP alone [44].

However, to the best of our knowledge, at present no histological data are available evaluating the healing following the use of this novel approach for non-surgical therapy. Therefore, the aim of this study was to histologically evaluate in dogs, the healing following nonsurgical periodontal therapy and in conjunction with sodium hypochlorite/amino acid gel and xHyA application.

Methods and materials

Animals

Four healthy male beagle dogs, 26 to 38 months of age and weighing 9 to 15 kg, were used in this study. The animals were housed and monitored daily for the duration of the study in the Animal Experimentation Facility Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. They were kept in individual cages at 20–26 ℃, relative humidity of 30–70%, and a 12-h light/dark cycle. Approximately 300 g of solid food (NVE-10, Nippon Pet Food, Co., Ltd. Tokyo, Japan) was provided to each animal daily and water was available ad libitum. All procedures during the in-life phase were approved by the ethical committee of the Animal Research Center of Kagoshima University, Japan (Project Approval No. D22017; Date of approval: 23 January 2023). This study conformed to the ARRIVE guidelines for preclinical animal studies.

Induction of experimental periodontitis

All surgical procedures were performed under general and local anesthesia using aseptic routines by one experienced surgeon (Yo.S.). Before surgical procedures, antibiotics (dihydrostreptomycin sulfate aqueous suspension for injection, 0.05 ml/kg; Mycillin Sol Meiji for veterinary use, Meiji Seika Pharma Co. Ltd, Tokyo, Japan) were administered intramuscularly. General anesthesia was induced with

intramuscular injection using medetomidine hydrochloride (Domitor®, 0.08 ml/kg IM; Orion Corporation, Espoo, Finland), 0.08 ml/kg of midazolam (Dormicum®, IM; Maruichi Pharmaceutical, Osaka, Japan) and 0.02 ml/kg of butorphanol tartrate (Vetorphale® 5 mg, Meiji Seika Pharma, Tokyo, Japan). After sedation, the anesthesia was maintained by inhalation of sevoflurane (0.5%–5.0%, Mylan Pharma Co., Ltd. Osaka, Japan) and a nitrogen/oxygen (2:1) mixture using an intracircuit vaporizer for spontaneous breathing. Local anesthesia was achieved with lidocaine HCl/epinephrine (2%, 1:80,000; Xylocaine; Fujisawa Inc., Osaka, Japan). The bilateral mandibular first and third premolars were carefully extracted to provide enough space for creation of intrabony defects. After a 8-week healing interval, two-wall intrabony defects (5 mm wide and 5 mm deep) were prepared bilaterally at the mesial aspect of the mandibular fourth premolars (P4) and at the distal aspect of the mandibular second premolars (P2) (four defects per dog). Following elevation of the mucoperiosteal flap, defects were created by using fissure burs with a sterile saline coolant (Fig. 1a). Cementum was removed using Gracey curettes and a chisel. Reference notches were made with a #1 round bur on the root surface at the cementoenamel junction (CEJ), and on the crown surface, to indicate the precise center plane of the intrabony defects and to allow an optimal histomorphometric analysis. To prevent spontaneous healing and induce plaque accumulation, metal strips were fixed to the tooth surface in the intrabony defects with a self-cure dental adhesive resin cement (Super Bond C&B, Sun Medical Co., Ltd., Moriyama, Japan) (Fig. 1b). The flaps were repositioned and stabilized with 4–0 silk sutures. Ketoprofen for analgesia (Capisten IM 50 mg, 2 mg/kg, 0.1 ml/kg; Kissei Pharmaceutical Co. Ltd, Matsumoto, Japan) and an antibiotic (Mycillin Sol) were administered daily for 2 days following the surgeries. Intraoral periapical radiographs at selected sites including the teeth (P2 & P4) were taken immediately after the treatment. The sutures were removed after 14 days of healing. To promote plaque formation, the animals were fed a soft diet during the induction period (Fig. 1c). After 4 weeks, bone loss progression was confirmed by the radiographs and the metal strips were removed without flap reflection. Acrylic stents with a groove on the mid-proximal root surfaces where the deepest pockets were detected were then fabricated to standardize the location of periodontal probe for clinical measurements (Fig. 1d) during this study.

Non-surgical periodontal therapy

Plaque control was performed with routine (3 times a week) flushing of the oral cavity with 2% chlorhexidine gluconate solution for 2 weeks prior to the treatment. To prevent mixing of gel type agents to the other site in the same side of the mandible, split-mouth design was employed in this study. Experimental 16 teeth (i.e., bilateral P2 & P4) were designated test and control side by coin flipping. Immediately before SRP, professional supragingival mechanical tooth cleaning was performed for the teeth. On one side, teeth were treated by SRP with sodium hypochlorite/amino acid gel followed by a cross-linked hyaluronic acid (xHyA) gel application (test group), whereas teeth of the contralateral side were treated by SRP only (control group) by the same experienced operator (T.N.). In the test group, SRP was performed as follows: in the teeth a sodium hypochlorite/amino acid gel (Perisolv®, an alkaline 0.95% sodium hypochlorite solution and a slightly viscous alkaline gel containing glutamic acid, leucine, lysine, carboxymethyl cellulose and titanium dioxide, Regedent AG, Zürich, Switzerland) was instilled into the periodontal pockets using a blunt needle for

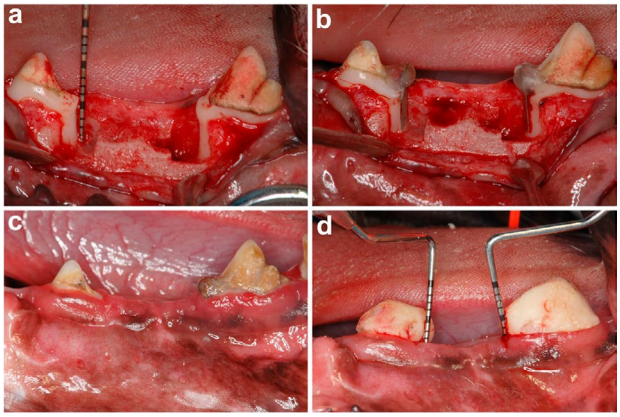

Fig. 1 **a** Surgically created two-wall intrabony defects in mandible. **b** Placement of the metal strips on the denuded root surfaces. **c** Plaque accumulation after 4 weeks. **d** Standardized measurement of clinical parameters by using customized acrylic stents with guiding grooves at baseline

30 s (Fig. 2a) prior to saline irrigation and instrumentation using an ultrasonic device (ENAC 10WA, Osada, Tokyo, Japan) and an ultrasonic tip (ST35, Osada, Tokyo, Japan) for 15 s (Fig. 2b), followed by hand instrumentation with manual curettes (LM Sharp Diamond Mini Gracey 11/12, 13/14 SD curettes, LM Dental™, Finland) through 5 traction movements in buccal and interproximal area (Fig. 2c), and the same process was repeated again. Following the final saline irrigation, the xHyA (hyadent BG®, a gel formulation containing butanediol diglycidyl ether-cross-linked HA (1000 kDA HA monomers) and non-cross-linked HA (2500 kDA) in a ratio 8:1, made from biotechnologically produced synthetic HA, REGEDENT AG, Zurich, Switzerland) gel (0.1 ml/tooth) was instilled in the pockets using a blunt needle (Fig. 2d). Teeth in the control group underwent the identical procedure except the sodium hypochlorite/amino acid and xHyA application. After the treatments, no antibiotics or analgesics were administrated, and the animals were fed a hard diet and the aforementioned oral hygiene regimen was performed daily for 8 weeks to reduce plaque formation.

Clinical evaluation

The following clinical parameters were assessed using a periodontal probe (UNC 15 Hu-Friedy, Chicago, IL., USA) to the nearest mm on all teeth at one site per tooth by one experienced and blinded examiner (F.S.) at baseline (Fig. 1d) and 6 weeks following the treatment: (a) probing pocket depth (PPD), (b) clinical attachment level (CAL) measured from the acrylic stent margin to the bottom of the probed pocket and (c) bleeding on probing (BOP). BOP was evaluated simultaneously with PPD by recording the presence (+) or absence (-) of bleeding up to 15 s after probing.

Histologic preparation

Eight weeks after the non-surgical therapy, intraoral radiographs were taken, and the animals were euthanized with an overdose of sodium thiopental. The teeth were removed together with the surrounding soft and hard tissues. The tissue blocks were fixed in 10% buffered formalin, trimmed according to intraoral radiographs and the reference notch on the crown, and rinsed in phosphate-buffered saline. The samples were decalcified in Kalkitox™ (Wako Pure Chemical Industries, Ltd., Osaka, Japan), dehydrated, and embedded in paraffin. Serial 6-µm-thick sections were then prepared along the mesiodistal plane and were stained with hematoxylin and eosin or with azan.

Histomorphometric analysis

All specimens were analyzed under a light microscope (BX51; Olympus Corp., Tokyo, Japan) equipped with a computerized image system (WinROOF2015; Mitani Corporation, Tokyo, Japan). For histomorphometric analysis, three sections approximately 90 µm apart were selected from the most central area of each two-wall defect, identified by the length of the root canal and the reference notches. The mean value of each histomorphometric parameter was then calculated for each site. To evaluate intra-examiner reproducibility, sixteen sections from all sites were read by a single blinded examiner at two different moments (48 h apart), and inter-calibration of the examiner was accepted at 90% level. The following parameters were measured by the examiner (T.I.). 1. Defect height (DH): distance between the apical extent of root planing and the CEJ. 2. Junctional epithelium length (JE): distance between the apical extension of the junctional epithelium and the CEJ. 3. Connective

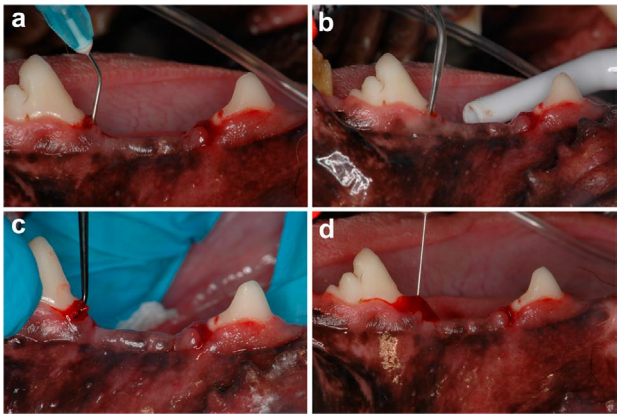

Fig. 2 **a** Application of sodium hypochlorite/amino acid gel to the periodontal pocket. SRP performed using an ultrasonic device with an ultrasonic tip (**b**) and hand instruments (**c**). **d** Application of a xHyA gel to the periodontal pocket

tissue adhesion (without cementum) (CT): distance between apical extent of the junctional epithelium and the coronal extent of the newly formed cementum. 4. New bone length (NB): distance between the apical extent of root planing and the coronal extent of newly formed alveolar bone along the root surface. 5. New bone area (NBA): newly formed trabecular bone within a template (5 × 5 mm) that served as a standardized proxy for the defect site. The template was aligned parallel to the root surface interfacing the apical extension of the root planing [45]. 6. New cementum length (NC): distance between apical extent of root planing and coronal extent of newly formed cementum on the denuded root surface.

7. New attachment length (NA): linear length of the root surface covered by NC adjacent to newly formed bone, with functionally oriented collagen fibers.

8. Periodontal ligament score (PDL score): which was obtained by grading the periodontal ligament with the reported scoring system outlined by Wikesjö et al. [46].

Statistical analysis

The primary outcome of this study was the histomorphometric outcome in terms of NA, measured for the treatment groups at 8 weeks. Clinical parameters were evaluated as secondary outcomes. However, due to the limited number of pre-clinical studies in dogs with a comparative design and primary outcome, no power analysis for sample size calculation could be performed. For obvious ethical reasons, sample size was set to an absolute minimum (4 animals) and the animal was chosen as the unit for the statistical analysis. The means and standard deviations for each parameter were calculated for each of the treatment groups. Wilcoxon signed rank test was used to compare the clinical parameters between baseline and at the 6 week follow up. Mann–Whitney U test was used to compare the clinical and histological values between the control and test groups.

For the comparison of the proportions of sites showing bleeding on probing (BOP), Fisher's exact test was used. A P value of < 0.05 was considered statistically significant. All calculations were performed with statistical software (BellCurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

Clinical observations

Postoperative clinical healing was uneventful at all 16 (8 sites/group) sites in the control and test groups. No visible adverse reactions, including suppuration, abscess formation, or increased tooth mobility, were observed throughout the entire experimental period. Visual gingival redness seemed to remain longer or recur in the control group after the treatment (Fig. 3).

Clinical measurements

The values for clinical parameters at the baseline and 6-week examinations in both treatment groups are shown in Table 1. The baseline examination revealed that the two study groups demonstrated similar characteristics for PPD, CAL, and BOP score with no significant differences between the groups. Both treatment groups showed clinical improvements at 6 weeks compared to baseline. Mean PPD reduction between the baseline and 6 weeks follow-up was statistically significantly different between the groups in favor for the test group ($p < 0.05$). The test group showed better results in terms of mean CAL gain compared to control group, however, no statistically significant difference was detected between the groups. The number of bleeding (BOP +) sites was markedly reduced in the test group at 6 weeks compared to baseline. Similarly, there was statistically significant difference in the score between the groups.

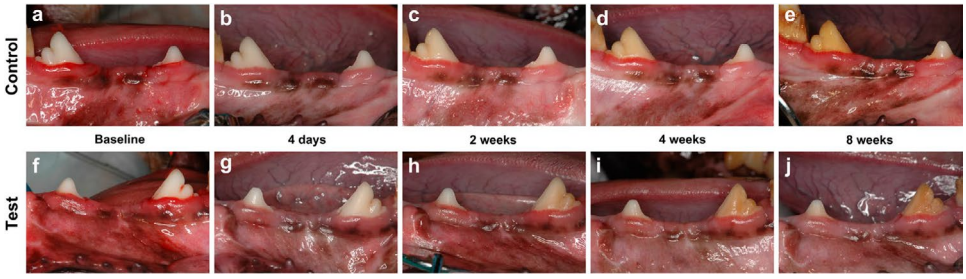

Fig. 3 Clinical overview at baseline, 4 days, 2, 4 and 8 weeks in the control (**a**-**e**) and the test group (**f**-**j**)

Table 1 Clinical parameters for each treatment at baseline and 6 weeks (means ± SD)

Parameters	Control	*N* = 4 animals Test
PPD (mm)		
Baseline	5.46 ± 0.82	5.50 ± 0.57
6 weeks	2.12 ± 0.32	1.25 ± 0.50
PPDreduction	3.34 ± 0.54	4.25 ± 0.50[†]
CAL (mm)		
Baseline	5.71 ± 0.84	5.59 ± 0.47
6 weeks	2.93 ± 0.23	1.68 ± 0.89
CAL gain	2.78 ± 0.79	3.90 ± 0.82
BOP (+) n (%)		
Baseline	8 (100)	8 (100)
6 weeks	6 (75.0)	1 (12.5)[*] [†]

PPD probing pocket depth, *CAL* clinical attachment level, *BOP* bleeding on probing

[*] Significantly different from baseline within each group ($p<0.01$)

[†]Significantly different from control group ($p<0.05$)

Descriptive histology

In the control group, a collapse of the soft tissue could be observed in three teeth and the oral gingival epithelium was partially thickened with deeper rete ridges than normal one (Fig. 4a and b) in four teeth. Also, most sites (seven out of eight teeth) showed the slight to moderate widespread inflammatory cell infiltrate mostly at the tips of gingiva (Fig. 4a and b). Downgrowth of junctional epithelium was detected slightly below the CEJ (Fig. 4a and e). Periodontal defect was mostly occupied by fibrous connective tissue (Fig. 4a and c) and slight superficial (two teeth) and inflammatory (one tooth) root resorption areas were seen on the root surfaces without cementum formation (Fig. 4e and f) in three teeth. Extensive proximal host bone resorption and varying degrees of spontaneous bone formation occurred along the root surfaces (Fig. 4a and d) in all sites. Two teeth in the control group presented no cementum formation at all and predominantly acellular cementum formation was restricted at the apical extension of instrumentation (Fig. 4a, f and g) in five teeth. Most specimens (5/8, 62.5%) in the control group showed non-functional disordered periodontal ligament like tissue or collagen fibers detached from the root surfaces (Figs. 4f and g and Fig. 6a).

In the test group, residual xHyA with reticular appearance was well integrated with gingival connective tissue at the coronal portion of the defects in all sites (Fig. 5a and b). Some remnants of xHyA were observed around/in the newly formed bone and occasionally between new cementum and new bone (Fig. 5a, c, d and f). Marked soft tissue atrophy or

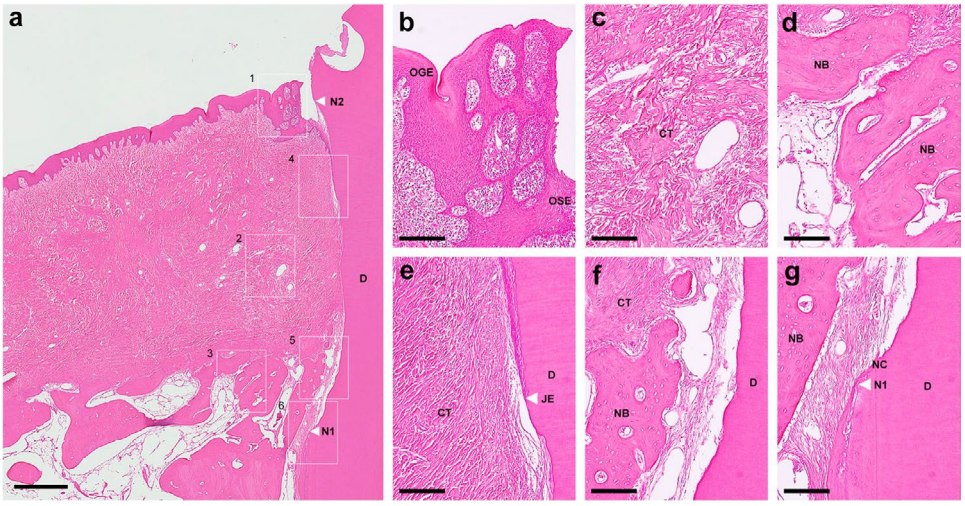

Fig. 4 **a** Histologic overview of defect treated with SRP alone (control group). (scale bar, 1 mm; hematoxylin and eosin stain). **b** Higher magnification of the box 1 area. **c** Higher magnification of the box 2 area. **d** Higher magnification of the box 3 area. **e** Higher magnification of the box 4 area. **f** Higher magnification of the box 5 area. **g** Higher magnification of the box 6 area. (scale bar, 200 µm; hematoxylin and eosin stain). D, root dentin; N_1, apical end of root planing; N_2, cementoenamel junction; OGE, oral gingival epithelium; OSE, oral sulcular epithelium; JE, apical end of junctional epithelium; CT, gingival connective tissue; NB, new bone; NC, new cementum

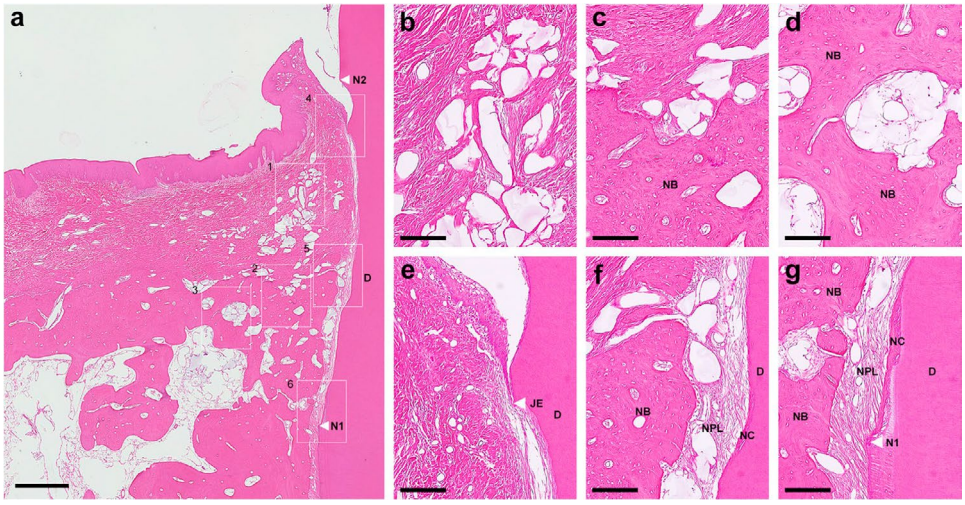

Fig. 5 **a** Histologic overview of defect treated with SRP with a sodium hypochlorite and amino acids gel and a cross-linked hyaluronic acid gel (xHyA) gel (test group). (scale bar, 1 mm; hematoxylin and eosin stain). **b** Higher magnification of the box 1 area. **c** Higher magnification of the box 2 area. **d** Higher magnification of the box 3 area. **e** Higher magnification of the box 4 area. **f** Higher magnification of the box 5 area. **g** Higher magnification of the box 6 area. (scale bar, 200 µm; hematoxylin and eosin stain). D, root dentin; N_1, apical end of root planing; N_2, cementoenamel junction; JE, apical end of junctional epithelium; CT, gingival connective tissue; NB, new bone; NC, new cementum; NPL, new periodontal ligament

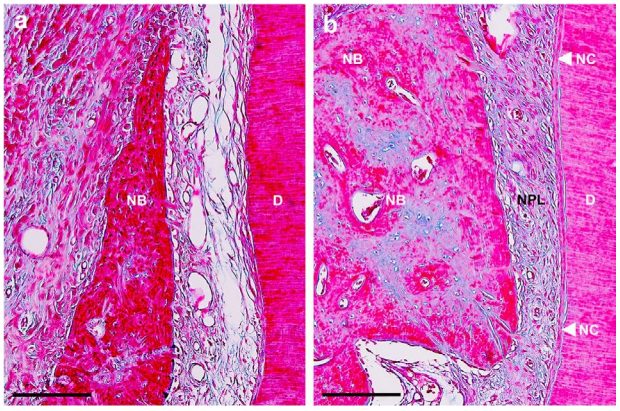

Fig. 6 **a** Higher magnification view of the middle portion of the defect treated with SRP alone (control group). Loosely arranged collagen fibers were seen near the root dentin without new cementum. (scale bar, 200 µm; azan stain). **b** Higher magnification view of the middle portion of the defect treated with SRP with a sodium hypochlorite and amino acids gel and a cross-linked hyaluronic acid gel (xHyA) gel (test group). Dense obliquely oriented collagen fibers were observed between the new bone and cementum. (scale bar, 200 µm; azan stain). D, root dentin; NB, new bone; NC, new cementum; NPL, new periodontal ligament

pathological change of gingival epithelium was not noted (Fig. 5a). A limited inflammatory response was observed in the tips of gingiva around three teeth (Fig. 5e). Apical extension of junctional epithelium was mostly restrained at the CEJ (Fig. 5a and e). Superficial root resorption was detected on the root surface of one tooth. New bone formation extended from the host bone toward the coronal region of the defects (6/8, 75%) (Fig. 5a). Newly formed bone was well integrated with the original bone and characterized by cancellous bone, which consists of a network of bony trabeculae containing bone marrow, blood vessels, osteoblasts, and osteocytes (Fig. 5a, c and d). A continuous layer of new cellular/acellular cementum was seen, with or without inserting collagen fibers running perpendicular to the root surfaces, was observed covering half of the defect area (Fig. 5a, f and g) in 5 teeth. The highly vascularized and dense new periodontal ligament-like tissue, which was formed between the new cementum and new bone (Figs. 5f and gandFig. 6b), maintained its width up to the coronal portion in the test group. No ankylosis was observed in any of the teeth.

Histomorphometric analysis

The results of histomorphometric analysis are shown in Table 2. No statistically significant differences were detected between the groups in regard to the following measurements (DH, JE, and CT). However, the length of CT (without cementum formation) in the test group was smaller than that observed in the control group. The length of new cementum was statistically significantly ($P < 0.01$) greater in the test (2.46 ± 0.77 mm) group than in the control (0.85 ± 0.84 mm) group. The test (1.75 ± 0.65 mm) group yielded statistically significantly ($P < 0.05$) greater formation of new attachment (i.e., linear length of NC adjacent to newly formed bone, with functionally oriented collagen fibers) compared with control (0.48 ± 0.79 mm) group. Moreover, the PDL scores in the test (2.87 ± 1.59) group was statistically significantly ($P < 0.05$) higher than that in the control (1.00 ± 0.94) group. The amount of newly formed bone (e.g., the length of NB and the area of NB) in the test (3.01 ± 0.64 mm and 5.75 ± 2.21 mm^2, respectively) group was statistically significantly ($P < 0.05$) greater than that in the control (2.26 ± 0.64 mm and 3.14 ± 1.94 mm^2, respectively) group.

Table 2 Histomorphometric comparisons between test and control groups 8 weeks after treatment. (means ± SD)

Parameters	Control	$N = 4$ animals Test
DH (mm)	5.60 ± 0.42	5.77 ± 0.52
JE (mm)	1.22 ± 0.45	1.03 ± 0.31
CT (mm)	2.84 ± 1.33	1.86 ± 1.00
NC (mm)	0.85 ± 0.84	2.46 ± 0.77**
NA (mm)	0.48 ± 0.79	1.75 ± 0.65*
PDL score (1–5)	1.00 ± 0.94	2.87 ± 1.59*
NB (mm)	2.26 ± 0.64	3.01 ± 0.64*
NBA (mm^2)	3.14 ± 1.94	5.75 ± 2.21*

DH defect height, *JE* junctional epithelium length, *CT* connective tissue adhesion (without cementum), *NB* new bone length, *NBA* new bone area, *NC* new cementum length, *NA* new attachment length, *PDL score* periodontal ligament score

* Significantly different from control group ($p < 0.05$)

** Significantly different from control group ($p < 0.01$)

Discussion

The present study has, for the first time, provided histological evidence of periodontal regeneration following the adjunctive subgingival application of sodium hypochlorite/amino acids and a xHyA gels to SRP. The histological results were consistent with the greater clinical improvements observed in terms of PPD reduction, CAL gain, and reduction of inflammation in the test group compared to the control group, which in tern, underscores the potential clinical significance of these findings.

The clinical findings obtained in this animal study are in line with the results from clinical studies reporting that SRP combined with sodium hypochlorite/amino acid and xHyA gels resulted in statistically significantly higher clinical improvements evidenced through PPD reduction, CAL gain, and decrease of BOP score (values) as compared to baseline [42, 43] or SRP alone [44]. In line with the clinical findings, the histologic analysis revealed that the test treatment yielded statistically significantly greater amounts of new connective attachment and new cementum formation than the control one. In the defects treated by SRP with a sodium hypochlorite and amino acids containing gel and a xHyA gel, dense functionally oriented collagen fibers with numerous blood vessels were predominantly observed between the newly formed cementum and the newly formed bone showing high PDL scores compared to the teeth treated by SRP alone. In addition, statistically significantly greater new bone was measured in the test group compared with the control one.

When interpreting these positive results, it must be emphasized that the present study has used the combination of the two different materials as a single treatment adjunctive to SRP. The cleaning effect was expected by the sodium hypochlorite and amino acids gel, which create chloramines. Chloramines have a strong antimicrobial effect and minimize the effects of hypochlorite on sound dentin/root cementum and healthy soft tissue [26, 28, 47]. Additionally, in vitro, and clinical studies have demonstrated that the sodium hypochlorite/amino acid gel can facilitate SRP to disrupt the biofilm, by dissolving necrotic tissue, and by softening calculus and thus reducing

friction during instrumentation [26–29]. The positive effect on the healing was expected by subsequent application of a xHyA gel since several in vitro studies have demonstrated that HyA significantly stimulates blood clot formation [30, 48], induces angiogenesis [30, 33] and increases osteogenesis [30, 34] as a biological modulator for promoting periodontal wound healing/regeneration. Additionally, recent studies have shown that the surgical application of the same high molecular xHyA yielded statistically significant improvements characterized by PPD reduction and CAL gain in human intrabony defects [38] and effectively promoted periodontal tissue regeneration in canine 2-wall intrabony, gingival recession and class III furcation defects [39–41].

When interpreting the results it is important to point out that the study did not include treatment groups treated with sodium hypochlorite/amino acids gel or xHyA. Therefore, it is unclear to what extent each of the used adjunctive materials may have contributed to the favorable outcomes obtained in the test group. A very recent animal study has shown that treatment of experimental periodontitis in rats using the combination of sodium hypochlorite/amino acids gel and SRP yielded to better outcomes in terms of gingival bleeding index, tooth mobility and the overall aspect of the gingival structures than treatment with SRP alone. However, the histologic evaluation demonstrated a predominantly reparative type of healing characterized by non-functionally oriented collagen fibers and unrestored alveolar ridges following the treatment with sodium hypochlorite/amino acids gel and SRP [49]. Megally et al. reported that subgingival ultrasonic debridement with sodium hypochlorite/amino acid gel resulted in a clinically relevant PPD reduction and CAL gain in residual pockets in subjects in maintenance care. However, these improvements were comparable to that obtained in the ultrasonic debridement alone without statistically significant differences [28]. Also, Pilloni et al. demonstrated that subgingival instrumentation with the local adjunctive use of xHyA yielded statistically significant clinical and microbiological improvements compared to baseline in residual periodontal pockets, although there was a lack of statistically significant differences in the outcomes following the subgingival instrumentation with placebo control [50]. These results may justify the rationale for the novel approach including the combined use of the two materials adjunctive to SRP as a single treatment procedure. Additionally, it is important to emphasize that the present animal study was designed to assess the biologic potential of the very recently introduced clinical protocol for nonsurgical periodontal treatment [42–44].

An interesting observation in this study is related to the changes in bleeding scores; i.e., while gingival inflammation decreased within a week in both control and test groups, gingival swelling and redness gradually increased during the 8 weeks following treatment in the control group. On the contrary, no such increase occurred in the test group. This clinical observation was consistent with the histologically observed inflammatory cell infiltrate at the coronal part of gingiva in the control group. In the test group, varying degrees of xHyA remnants were consistently observed, but they did not appear to interfere with tissue integration in all periodontal defects around teeth. These findings are also in agreement with those from previous preclinical studies that have demonstrated the presence of residual xHyA in two-wall intabony and class III furcation defects in dogs [40, 41].

An important finding that warrants further attention is the fact that this novel approach for non-surgical periodontal therapy (NSPT) resulted in chronic two-wall intrabony defects in comparable amounts of NC (2.46 ± 0.77 mm) and NA (1.75 ± 0.65 mm) to those (3.20 ± 1.29 mm, 2.43 ± 1.29 mm respectively) obtained in the same initial size of acute two-wall intrabony defects treated with a surgical approach and the application of xHyA in dogs [40]. These findings indicate that the application of xHyA in NSPT is clinically beneficial, and the high molecular weight xHyA can maintain its stability for 4 to 8 weeks [40, 41, 51]. From a clinical perspective, the results of the present study suggest that this novel approach offer additional benefits in NSPT and decrease the need for surgical periodontal therapy.

In contemporary periodontology, the adjunctive use of EMD [20, 21], antibiotics [22, 23], lasers and antimicrobial photodynamic therapy [24, 25], has been repeatedly investigated as adjunctive approaches to NSPT. However, additional benefits compared to SRP alone were not consistently observed while the relatively high cost, some risks of allergy and the necessity of specific equipment for these treatment modalities need also to be considered. On the other hand, the novel approach evaluated in the present study is based on the use of a highly biocompatible and non-animal origin materials adjunctive to SRP. The sodium hypochlorite cleaning gel may offer further advantages to NSPT by facilitating the mechanical removal of the biofilm, thus enhancing the effects of the xHyA gel [42]. Moreover, the xHyA gel enhances blood clot stability and attracts several growth factors [38, 52, 53] which play a key role in periodontal wound healing/regeneration [54]. However, it needs to be emphasized that despite the fact that these results are encouraging, they were obtained in experimentally created defects including a small number of animals. Therefore, further studies are required to confirm the clinical relevance and the predictability of this novel treatment approach in NSPT.

Conclusion

In conclusion, the present data offer histological evidence supporting the use of adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA gels in NSPT to enhance periodontal wound healing and regeneration.

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Author contribution Conceptualization: Yoshinori Shirakata, and Anton Sculean. Methodology: Yoshinori Shirakata, and Anton Sculean. Validation: Eglė Ramanauskaite, and Kazuyuki Noguchi. Investigation: Yoshinori Shirakata, Toshiaki Nakamura, Fumiaki Setoguchi, Shohei Matsumura, Takatomo Imafuji, Yukiya Shinohara, and Masayuki Iwata. Data curation: Toshiaki Nakamura. Writing-original draft preparation: Yoshinori Shirakata, Writing-review and editing: Eglė Ramanauskaite, and Anton Sculean. Visualization: Yukiya Shinohara, Supervision: Anton Sculean. Project administration: Yoshinori Shirakata, and Anton Sculean. Funding acquisition: Yoshinori Shirakata. All authors gave their final approval and agreed to be accountable for all aspects of the work.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and all procedures performed in studies involving animals were in accordance with the ethical standards of the ethical committee of the Animal Research Center of Kagoshima University, Japan (Project Approval No. D22017; Date of approval: 23 January 2023). This study conformed to the ARRIVE guidelines for preclinical animal studies. For this type of study, informed consent is not required.

Competing interests The authors declare no competing interests.

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PADĖKA

Pirmiausia, norėčiau išreikšti nuoširdžią padėką savo disertacijos vadovei Profesorei Vitai Mačiulskienei už nuolatinę paramą, palaikymą, padrąsinimą ir tikėjimą mano idėjomis.

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Atskiras padėkos žodis keliauja statistikei Irenai Nedzelskienei, kuri visada besąlygiškai padėjo ir kurios dėka daugybė matematinių skaičiavimų įgavo prasmę.

Šis darbas nebūtų įmanomas be didžiulės visuomet padėti pasiruošusių kolegų pagalbos. Noriu atskirai padėkoti Fam Yuming, Urtei Marijai Dvylienei, Aušrai Rusilienei – be Jūsų besąlyginės pagalbos ir supratimo nebūčiau čia, kur esu dabar.

Galiausiai, tačiau svarbiausiai, noriu padėkoti savo šeimai – sesei ir tėvams – Jūs esate svarbiausia mano pasiekimų dalis.