Análise química e topográfica da neoformação óssea por distração osteogênica em mandíbula de ovelhas com uso de laserterapia: estudo piloto

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Doutorado em Odontologia
Concentração CTBMF

Porto Alegre, 2012

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIRURGIA E TRAUMATOLOGIA BUCOMAXILOFACIAL

Doutorado

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Artigo apresentado como parte dos requisitos obrigatórios para obtenção do título de Doutor em Odontologia, pela Pontifícia Universidade Católica do Rio Grande do Sul, na área de concentração em Cirurgia e Traumatologia Bucomaxilofacial.

Orientadora: Prof. Dra. Marília Gerhardt de Oliveira

Co-Orientador: Prof. Dr. Angelo Luiz Freddo

Linha de Pesquisa: Laser em Odontologia

Porto Alegre, Abril 2012.

Dedico este trabalho:

À Deus pela ajuda e iluminação em todos os momentos. Foi Contigo que falei em pensamentos durante todo esse tempo.

A minha esposa, Micéli:

A tua presença ao meu lado me dá força para seguir adiante. Te agradeço pelos conselhos e principalmente pelo carinho. Que esta criança que tu carregas seja merecedora de todo o nosso amor...Te amo muito!!!

Ao meu Pai, Walter:

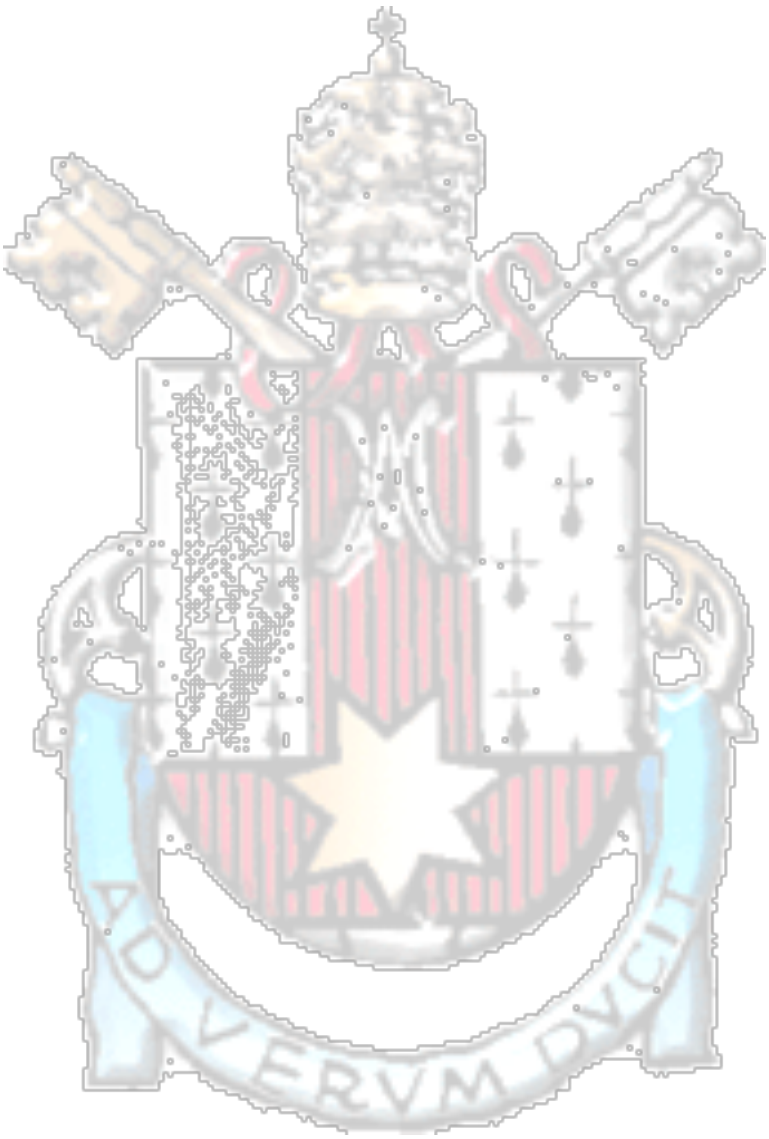
Pela ajuda e apoio em todas as horas mesmo quando deverias dedicar-se a sua própria saúde.

A minha mãe Zeni:

Por estarem sempre me ouvindo, me dando apoio e carinho, incondicionalmente.

A minha filha Nicolle:

Tu és o meu bem mais precioso, obrigado pela graça do teu sorriso.



Agradecimentos

AGRADECIMENTOS:

À professora Marília Gerhardt de Oliveira... Sou muito grato pela sua disponibilidade, pela dedicação e principalmente, por sua amizade. Tenho certeza de que além de uma ótima orientadora, tenho uma amiga muito especial. Obrigada por tudo, Doutora!!!

Ao professor Angelo Freddo...Foi um privilégio ter te conhecido. Vi em você uma pessoa muito dedicada, determinada e minuciosa. És exemplar! Muito obrigado!

Ao CNPq e a CAPES, por viabilizar recursos para a realização deste Curso de Doutorado em Odontologia, área de concentração em Cirurgia e Traumatologia Bucomaxilofacial

À Pontifícia Universidade Católica do Rio Grande do Sul, na pessoa do Diretor, Marcos Túlio Mazzini Carvalho, por nos proporcionar a oportunidade de aprendizado, com excelente infra-estrutura física e qualidade elevada de ensino superior.

Ao professor José Antônio Poli de Figueiredo, pelo esforço e dedicação prestados frente ao Programa de Pós-Graduação em Odontologia... és um exemplo de professor e coordenador!

Aos demais professores do Programa de Pós-Graduação, pelos ensinamentos grandiosos, pelas demonstrações de amor pelo ensino, e pela qualidade das aulas e discussões que presenciei.

Aos funcionários da Secretaria de Pós-Graduação... muito obrigada por sempre me ajudar em tudo que fosse necessário. Ana, Marcos, Davenir e Carlos,

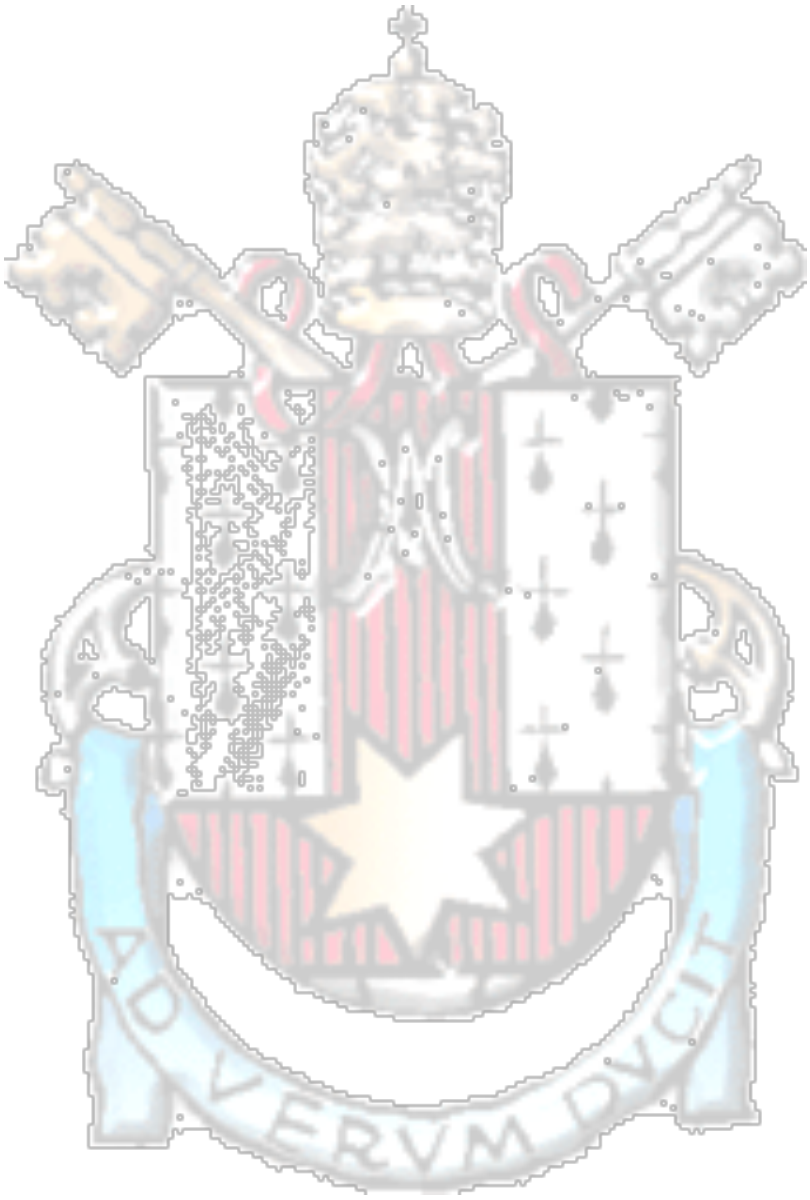
vocês são exemplos de dedicação numa sala de onde muitos passam e requisitam a ajuda de vocês.

Ao colega e amigo Lêonilson Gaião, uma das excelentes amizades que o Curso me proporcionou, amizade esta que iniciou no mestrado e perdurará por toda a vida.

Aos meus colegas de doutorado... minha gratidão pelos encontros, conversas, disponibilidade. Foi um prazer ter estado com vocês!

Aos meus irmãos Carla e Juliano, agradeço a amizade e os muitos momentos de felicidade.

Aos Professores de Cirugia e Traumatologia da UFSM, pois foi lá que minha trajetória iniciou, e vocês foram meus grandes incentivadores. Aprecio a dedicação, o companheirismo e a vontade de ensinar. Obrigado Profª. Marcia, Prof. Gustavo, Profª. Alexsandra, Prof. Jorge, e Prof. Carlos.



Sumário

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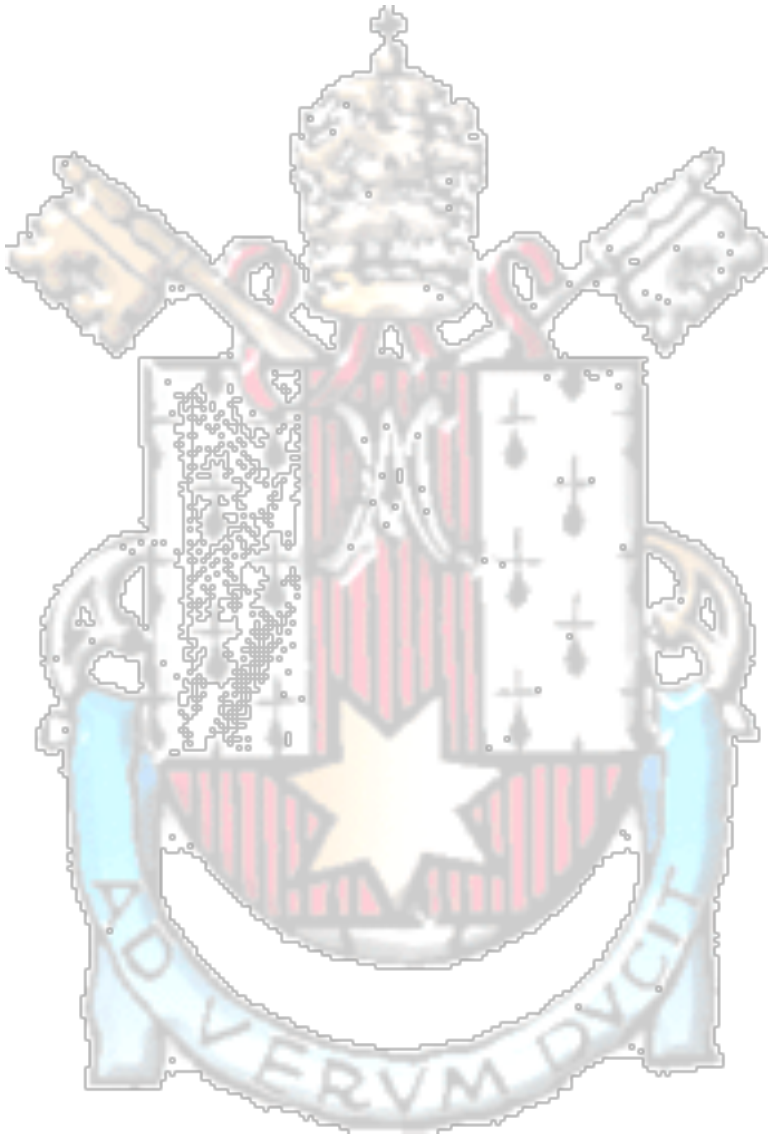


Resumo

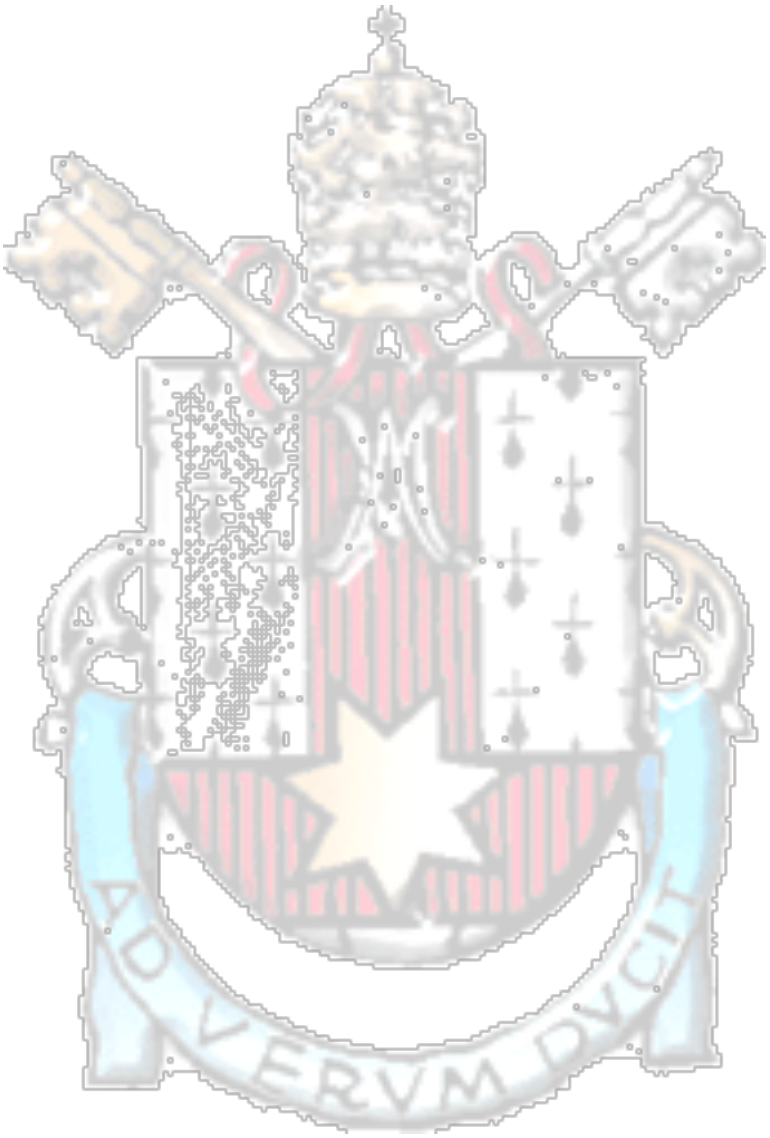
Resumo

Objetivos: Verificar, em estudo preliminar, a importância da utilização terapêutica da laserterapia (830nm) no que diz respeito à topografia óssea (Microscopia Eletrônica de Varredura - MEV) e a composição química (Espectrômetro por Dispersão de Energia - EDS) do osso neoformado mandibular por distração osteogênica (DO) associada à laserterapia (LLLT) em modelo experimental animal ovelha.

Metodologia: A amostra deste estudo foi composta por cinco ovelhas fêmeas da raça Corriedale, com peso entre 40 e 45 quilos, divididas em cinco unidades experimentais (grupos A, B, C, D e E), com utilização do laser em diferentes etapas da distração osteogênica. Nos grupos A, B e C, a irradiação inicial foi aplicada logo após o término da sutura e a cada 48 horas (total de oito aplicações). No grupo D, a primeira irradiação ocorreu no último dia da ativação do distrator, seguindo com mais sete aplicações a cada 48 horas (totalizando de oito aplicações). O grupo E (controle) não recebeu nenhuma irradiação. Os pontos foram analisados no MEV com magnificação de 80x, 300x e 1000x e os espectros por EDS realizados sobre a imagem em um aumento de 300X.

Resultados: De acordo com o teste t-Student pareado, na medular das ovelhas que sofreram distração óssea, os valores médios de Carbono e Cálcio são estatisticamente maiores que as respectivas médias. Nas corticais interna e externa não ocorreram diferenças estatisticamente significativas nas médias dos elementos comparados aos valores de referência ($p > 0,05$). A topografia óssea mostrou-se muito semelhante em todas as amostras.

Conclusão: A laserterapia com comprimento de onda infravermelha (GaAlAs, 830nm), 50mW de potência, irradiação cutânea com três pontos de densidade de energia de 5J/cm^2 por sessão, totalizando 120J/cm^2 ao final do tratamento, é benéfica quando aplicada no período de maturação do osso, pois sugere um aumento na dureza do tecido ósseo neoformado.



Abstract

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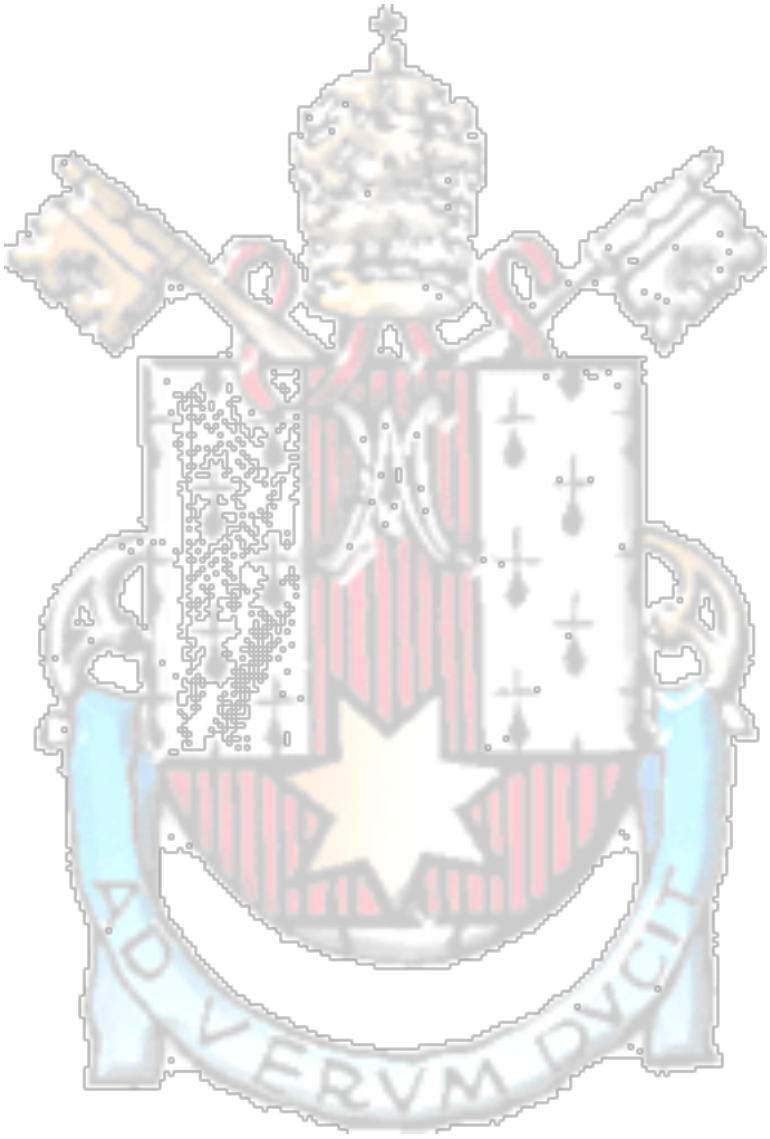
Objectives: To conduct a preliminary analysis of the therapeutic effects of laser therapy (830nm) with regard to surface topography (scanning electron microscopy - SEM) and chemical composition (energy dispersive spectrometry - EDS) of the newly formed bone during distraction osteogenesis (DO) combined with low-level laser therapy (LLLT) in an experimental sheep model.

Methods: Five female Corriedale sheep weighing 40 to 45kg (experimental units A, B, C, D, and E) were treated with LLLT at different stages of OD, as follows: sheep A, B, and C received the first irradiation session immediately after closure of the surgical wound and new sessions then every 48 hours (at a total of eight sessions); sheep D received the first irradiation session on the last day of device activation, followed by seven additional applications every 48 hours (at a total of eight sessions); sheep E (control) was not irradiated. Bone samples were analyzed via SEM at 80x, 300x, and 1000x magnification, and EDS spectra, at 300x magnification.

Results: According to the Student t test for paired samples, the medullary bone of sheep mandibles treated with DO presented statistically higher mean values of carbon and calcium when compared with untreated mandibles (controls). The internal and external cortical layers did not show statistically significant differences in relation to reference values ($p > 0.05$). Bone surface topography was very similar in all specimens.

Conclusion: Infrared laser therapy as used in our study (GaAlAs, 830nm, 50mW power, cutaneous irradiation at three sites, and energy density of 5J/cm^2 per session at a total of 120J/cm^2), has benefits when applied at the beginning of the bone consolidation period, suggesting an increased hardness and resistance of the newly formed bone tissue.

Keywords: Distraction osteogenesis, laser therapy, electron microscopy.



Artigo em Português

Análise química e topográfica da neoformação óssea por distração osteogênica em mandíbula de ovelhas com uso de laserterapia: estudo piloto

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Resumo

Objetivos: Verificar, em estudo preliminar, a importância da utilização terapêutica da laserterapia (830nm) no que diz respeito à topografia óssea (Microscopia Eletrônica de Varredura - MEV) e a composição química (Espectrômetro por Dispersão de Energia - EDS) do osso neoformado mandibular por distração osteogênica (DO) associada à laserterapia (LLLT) em modelo experimental animal ovelha.

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Descritores: Distração osteogênica, laserterapia, microscopia eletrônica.

Introdução

A distração osteogênica (DO) é uma técnica que envolve a formação de um osso novo, entre superfícies ósseas vascularizadas, após osteotomia ou corticotomia, por meio de aparelhos funcionais. Por meio da osteotomia, seguida por movimentos lentos, promovidos por aparelhos específicos para esse fim, o *gap* é, inicialmente, preenchido por um calo ósseo, o qual é substituído por tecido ósseo (1).

Algumas pesquisas também têm avaliado a influência da laserterapia no processo de reparação óssea com consequente efeito na DO. Nesse sentido, Dörtbudak *et al*.(2) avaliou o efeito do laser diodo (690nm) em culturas de osteoblastos de ratos, concluindo que a irradiação com laser diodo não ablativo tem um efeito bioestimulador nos osteoblastos *in vitro.* Já Weber *et al*.(4) avaliaram histologicamente a influência da radiação laser, utilizando, para tanto, um laser diodo infravermelho (GaAlAs, 830nm, 50mW, CW) no processo de reparo de enxertos ósseos autógenos, concluiu que nos grupos em que o laser foi aplicado na loja cirúrgica, no transoperatório, a atividade de remodelação óssea mostrou-se qualitativa e quantitativamente mais exuberante, tendo um efeito de biomodulação positiva sobre o processo de reparo ósseo em enxertos ósseos quando aplicado no transoperatório. Lopes *et al*.(5) comprovaram também o efeito positivo da laserterapia no processo de reparo ósseo.

Miloro *et al.* (6) utilizou o laser de arseneto de gálio-alumínio (GaAlAs) em seu estudo e concluiram que o laser acelera o processo de regeneração óssea durante a fase de consolidação, permitindo a remoção mais precoce do aparelho distrator, reduzindo, assim, a morbidade cirúrgica.

Diversas pesquisas clínicas e científicas têm sido realizadas com o objetivo de aperfeiçoar as técnicas da DO, dos aparelhos distratores, verificar os vetores de distração, assim como fatores que podem influenciar na reparação óssea existente após a distração osteogênica. Entretanto alguns estudos tem focado no efeito do LLLT em termos de topografia e composição clinica do osso neoformado na DO.

Nesse contexto, o objetivo da presente pesquisa foi verificar, em estudo piloto, a importância da utilização terapêutica do laserterapia (830nm) no que se refere à topografia óssea (MEV) e à composição química (EDS) do osso neoformado mandibular por distração osteogênica em modelo experimental animal ovelha, assim como, também, verificar se o tempo de uso do distrator, durante a fase de maturação, irá alterar as propriedades ósseas nesse modelo animal.

Metodologia

Esta pesquisa está de acordo com as normas de pesquisa nacional e internacional (número de registro no Comitê de Ética e Pesquisa e no Comitê de Ética para Animais da PUC/RS - 0219/07; 075/08).

Foram selecionadas cinco ovelhas fêmeas da raça *Corriedale*, com idade de 2 anos e peso entre 40 e 45 quilos. Todos os animais foram inspecionados e submetidos a exames laboratoriais básicos, sendo que nenhum apresentava qualquer doença ou alteração que pudessem interferir nos resultados deste estudo.

Os cinco animais selecionados foram divididos, aleatoriamente, em cinco unidades experimentais (grupos A, B, C, D e E). Cada grupo permaneceu com o distrator osteogênico externo de acordo com a Tabela 1. A laserterapia foi realizada no período de latência/ativação do distrator (grupos A, B e C) ou no período de maturação óssea (grupo D). O grupo E não recebeu nenhuma irradiação por LLLT. Todos os animais permaneceram em observação por 60 dias.

Durante todo o período do experimento os animais permaneceram no Hospital de Clínicas Veterinárias (HCV) da Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. Cada animal foi alojado em baias apropriadas, sendo que cada um foi identificado por uma placa.

Tabela 1- Organização dos grupos experimentais

Grupos	Tempo com distrator	Utilização do laserterapia	Morte	Dose total em joules
Grupo A	50 dias	Latência/ativação	60 dias	120 J
Grupo B	40 dias	Latência/ativação	60 dias	120 J
Grupo C	33 dias	Latência/ativação	60 dias	120 J
Grupo D	33 dias	Maturação	60 dias	120 J
Grupo E	33 dias	Sem laserterapia	60 dias	0J

Fonte: dados da pequisa CEP PUCRS-0219/07

Os animais submeteram-se à anestesia geral que foi obtida através da indução com o uso de acepromazina 0,05mg/kg, meperidina 2mg/kg, quetamina 4mg/kg e máscara de halotano. Para a intubação utilizou-se propofol 8ml. A manutenção da anestesia foi realizada com halotano em O_2 100%.

A profilaxia antibiótica foi realizada com a administração endovenosa de ampicilina sódica, 10mg/kg. Após a perda de consciência, os espécimes foram posicionados em decúbito lateral direito.

A cirurgia seguiu os seguintes passos: tricotomia da região submandibular esquerda, antissepsia, infiltração local com 3ml de 1% de lidocaína e epinefrina 1:100.000, e incisão (tipo Risdon) 1cm abaixo da região basilar esquerda da mandíbula, medindo aproximadamente 3cm. Os tecidos foram divulsionados utilizando-se uma abordagem por planos. Os afastadores do tipo Farabeuf foram posicionados expondo a superfície lateral da mandíbula. As osteotomias foram realizadas com serra reciprocicante pela face lateral e medial da mandíbula, na região do ângulo mandibular Figura 1.

O aparelho distrator foi fixado através de quatro parafusos transcutâneos sob irrigação abundante de soro fisiológico, conforme mostra a Figura 2. O aparelho distrator foi, então, ativado até a obtenção de uma resistência à ativação do aparelho distrator, para que a fratura pudesse ser concluída com o uso de cinzéis retos. Foi

realizada sutura da ferida operatória, por planos, com fio monofilamentar de náilon nº.. 4-0 em pontos isolados. A sutura de pele foi removida depois de sete dias. Após o ato cirúrgico, os espécimes receberam morfina 0,4mg/kg de 8/8h durante 72h e pentabiótico (associação de penicilina benzatina, procaína e estreptomicina) a cada 24h durante sete dias.

Utilizou-se como Protocolo da Distração Osteogênica:

Período de Latência – cinco dias (1º. ao 5º.)

Durante os cinco primeiros dias pós-operatórios, o distrator osteogênico não foi ativado, apenas inspecionado e higienizado com iodofor alcoólico a 1%.

Período de Ativação – 15 dias (6º. ao 20º.)

A partir do sexto dia pós-operatório, em todos os grupos, iniciaram-se as ativações do aparelho distrator, avançando 1mm por dia, até que se completassem 15 mm de extensão ao final do experimento.

Período de Maturação Óssea – 13 - 30 dias (21º. ao 33º., 40º. ou 50º.)

Após o período de alongamento, o aparelho distrator foi mantido inativo por um período de 30 dias no grupo A, 20 dias no grupo B e 13 dias nos grupos C, D e E para que ocorresse a consolidação óssea. Quando decorrido o tempo de maturação óssea para cada grupo, o dispositivo de distração foi removido com anestesia local.

O aparelho utilizado para irradiação foi o Thera Laser®5 (DMC, São Carlos, SP, Brasil), devidamente calibrado, com meio ativo GaAlAs (arseneto de gálio e alumínio), com comprimento de onda de 830ηm. Nas ovelhas 1, 2 e 3, a irradiação inicial foi aplicada logo após o término da sutura e a cada 48 horas, totalizando oito aplicações, ou seja, as irradiações iniciavam no período de latência e se estendiam pelo período de ativação. No animal nº. 4, a primeira irradiação aconteceu no último dia da ativação do distrator, seguindo com mais sete aplicações a cada 48 horas, totalizando oito aplicações durante o período de maturação óssea. Por ser indolor, esse procedimento não necessitou do uso de sedação ou anestésicos. Os espécimes 1, 2, 3 e 4 receberam irradiações pontuais sobre a região distraída. Demarcaram-se três pontos de irradiação de 5J/cm2, totalizando 15J/cm2 por sessão, na potência de 50mW, em modo contínuo, com um tempo de 1,41 minutos. A energia total aplicada, ao final do experimento, correspondeu a 120J/cm2. A

ovelha nº. 5 ou grupo E, controle, não recebeu nenhuma irradiação, porém o animal passou pelo mesmo protocolo de irradiação ativação com a ponteira desligada.

Quando atingiram 60 dias do procedimento cirúrgico, os espécimes foram mortos por meio de anestesia profunda com os mesmos anestésicos utilizados para o procedimento cirúrgico, ocasionando morte por parada cardiorrespiratória, respeitando-se, dessa forma, a Resolução nº. 714, de 20 de junho de 2002, do Conselho Federal de Medicina Veterinária, que dispõe sobre procedimentos e métodos de eutanásia em animais e dá outras providências. Constatada a morte dos animais, pelos sinais vitais, as mandíbulas foram dissecadas e retiradas com descoladores e cinzéis e conservadas em glutaraldeído.

Na Faculdade de Física da PUCRS, as peças foram cortadas ao meio, com auxílio de um minimotor de corte (Proxxon®-50HZ; 40W), dividindo-se a mandíbula em duas hemiarcadas. Em seguida, a região do osso neoformado e uma porção do osso antigo foram osteotomizada no sentido vertical, separando-as das demais áreas.. A seguir, as peças foram levadas a um dessecador pelo período de uma semana e depois a um forno vácuo a uma pressão atmosférica de 5 Pascal, por mais uma semana, com o objetivo de eliminar remanescentes de glutaraldeído no interior do tecido ósseo, evitando-se reações químicas no momento da inclusão da resina.

As porções de interesse da mandíbula, agora incluídas em formas quadradas pré-moldadas de PVC e preenchidas com resina de fibra de vidro (Fiberglass® incolor), foram cortadas axialmente com auxílio de uma serra-fita (St1101, Starrett®) em três blocos após tomarem presa. Ou seja, cada mandíbula foi separada em três blocos, todos devidamente identificados; amostra superior, medial (lado superior ou inferior) e inferior.

As peças foram preparadas no laboratório e, após, os blocos ósseos foram analisados em um microscópio eletrônico de varredura, Philips XL 30. Os pontos foram analisados no MEV com magnificação de 80x, 300x e 1000x, no modo SE (detector de elétrons secundários) e BSE (detector de elétrons retroespalhados).

Os espectros por EDS foram realizados sobre a imagem em um aumento de 300X com o objetivo de analisar a composição química da amostra, conforme mostra a Tabela 2, sendo que para cada uma das análises (corticais e medular), foram

tomados três pontos aleatórios para a obtenção de uma média das quantidades dos níveis de oxigênio (O), carbono (C), cálcio (Ca) e fósforo (P), onde se obteve como grupo controle o tecido ósseo sadio do lado oposto da mandíbula do grupo E.

Tabela 2- Mensurações dos testes com MEV

Análises	Cortical interna	Cortical externa	Medular
SE	80X; 300X e 1000X	80X; 300X e 1000X	80X; 300X e 1000X
BSE	80X; 300X e 1000X	80X; 300X e 1000X	80X; 300X e 1000X
EDS	300X	300X	300X

Fonte: dados da pequisa CEP PUCRS-0219/07

Para a análise dos resultados foram descritas a quantidade de cada elemento em cada região com uso de médias e o desvio padrão, assim como comparadas as médias de cada elemento em cada região com o valor de referência (controle) com uso de testes t-Student pareado(6).

Os testes foram realizados com nível de significância de 5%.

Resultados

Análise EDS

A Tabela 3 mostra que na medular das ovelhas que sofreram distração óssea e submetidos à laserterapia, os valores médios de fósforo e oxigênio são menores que os respectivos controles (grupo E), assim como os elementos carbono e cálcio são em média maiores que os respectivos controles (grupo E). Na cortical interna e

na externa não há diferenças significativas nas médias dos elementos comparados aos valores do grupo controle (E).

GRUPOS		A		B		C		D		E	
REGIÃO	**ELEMENTO**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cortical Interna	Carbono	26,24	12,05	18,88	11,98	31,43	15,50	19,99	10,93	24,55	12,79
	Oxigênio	31,23	15,12	35,71	15,89	32,76	15,83	36,06	17,23	21,98	9,87
	Fósforo	16,06	7,30	19,96	12,05	18,84	11,64	21,35	10,18	24,55	11,63
	Cálcio	17,81	10,05	35,44	15,71	16,95	7,43	32,09	16,55	28,57	13,47
Medular	Carbono	11,91	3,25	28,73	13,85	33,74	16,25	10,24	3,87	13,21	4,99
	Oxigênio	35,42	17,15	38,24	16,75	28,10	14,81	37,09	17,79	24,99	11,75
	Fósforo	20,86	10,83	14,19	6,90	16,78	7,35	21,60	13,23	24,99	11,75
	Cálcio	30,45	14,12	28,25	18,65	21,25	13,75	31,11	16,79	16,52	6,88
Cortical Externa	Carbono	26,06	12,49	20,95	12,49	19,62	12,14	10,58	2,87	22,55	10,73
	Oxigênio	26,78	13,25	41,38	18,15	21,69	13,52	29,96	15,99	30,46	15,18
	Fósforo	19,73	11,60	12,55	4,49	23,42	14,99	24,17	15,64	19,20	11,69
	Cálcio	27,42	14,25	25,67	12,98	35,26	17,25	35,28	17,05	27,72	12,88

Tabela 3. Descrição das médias dos pontos de cada elemento por região nas ovelhas que sofreram distração óssea e resultado dos testes comparativos com os respectivos valores de referência

Fonte: dados da pequisa CEP PUCRS-0219/07

Análise descritiva da topografia óssea (MEV)

Todos os espécimes deste estudo foram avaliados no dia 60 de pós-operatório. As peças foram analisadas com aumentos de 80X, 300X e 1000X. Em todos os grupos em que foi utilizada a laserterapia, encontraram-se resultados semelhantes, com um tecido ósseo maduro e com poucas lacunas preenchidas por tecido fibrosado, porém localizou-se uma acentuada deposição de matriz óssea quando comparado com o grupo controle (Figura 3).

Discussão

Nesta pesquisa, optou-se usar uma técnica de distração osteogênica já bastante utilizada na literatura (7,8,9,10), com um período de latência de cinco dias, frequência de ativação de uma vez ao dia e ritmo de 1mm diário.

A caracterização morfológica dos materiais é de fundamental importância para se definir seu comportamento. Sendo assim, a técnica de microscopia

eletrônica de varredura (MEV) tem se demonstrado essencial na observação e análise da microestrutura de superfícies (11). O MEV é um dos mais versáteis instrumentos disponíveis para a observação e análise de características microestruturais de objetos sólidos. A principal razão de sua utilidade é a alta resolução que pode ser obtida quando as amostras são observadas; valores da ordem de 2 a 5 nanômetros são geralmente apresentados por instrumentos comerciais, enquanto instrumentos de pesquisa avançada são capazes de alcançar uma resolução melhor que 1nm (12).

Após o término da distração, o período de contenção é utilizado para que o tecido regenerado adquira a resistência bioelástica necessária para resistir às forças de recidiva. Períodos com amplitude entre 2,4 a 10 semanas já foram utilizados após a DO mandibular tanto em humanos quanto em modelos animais (13).

A redução deste período de contenção com remoção precoce do distrator é que se baseia, fundamentalmente, o aprimoramento da técnica da distração osteogênica. O tempo de contenção, que é objetivo deste estudo, variou para cada animal. Nas ovelhas 1 e 2, utilizou-se tempo de consolidação já consagrados na literatura, sendo 30 dias e 20 dias, respectivamente (17). Nas outras três, o tempo de consolidação foi reduzido ao máximo, isto é, por um período de 13 dias, de forma não encontrada na literatura para este modelo biológico.

Nesta pesquisa, foi utilizado o laser GaAlAs arseneto de gálio e alumínio) com comprimento de onda de 830ηm (infravermelho), devido à propriedade de penetração tecidual maior que o laser vermelho. Os laseres infravermelhos possuem uma maior penetração nos tecidos subcutâneos em decorrência da sua baixa absorção na água ou nos pigmentos da pele (14).

O protocolo de irradiações utilizado baseou-se em trabalhos anteriormente desenvolvidos no grupo de pesquisa Deformidades Faciais, com financiamento do CNPq, nos quais a laserterapia (LLLT) foi aplicada, nas amostras experimentais, a cada 48 horas, totalizando oito irradiações. Tal protocolo foi semelhante ao utilizado por Weber *et al.* (3) e por Rodrigo *et al*. (15).

A análise descritiva da microscopia eletrônica mostrou que a topografia óssea submetida à laserterapia possuía menor volume de lacunas e uma maior presença de matriz óssea (17), o osso distraído de todos os animais já estava com características de tecido ósseo maduro. Constatou-se que as características do

tecido ósseo nesses grupos foi muito semelhante, porém ocorreu contração (recidiva) pós-operatória nos grupos em que foi removido o distrator mais precocemente.

Na análise por EDS, todas as ovelhas obtiveram valores estatisticamente semelhantes quanto aos elementos carbono, oxigênio, fósforo e cálcio, mas nas medidas do osso medular distraído foi encontrado um aumento, principalmente nos níveis de cálcio, o que sugere um osso mais resistente nas ovelhas que utilizaram a laserterapia tanto na fase de ativação como na de maturação, uma vez que a ossifição consolida-se da região cortical para a medular (16), tendendo a mineralizar-se em um momento mais tardio, quando fica demonstrado que a laserterapia atuou de forma positiva em tal porção óssea.

Conclusão

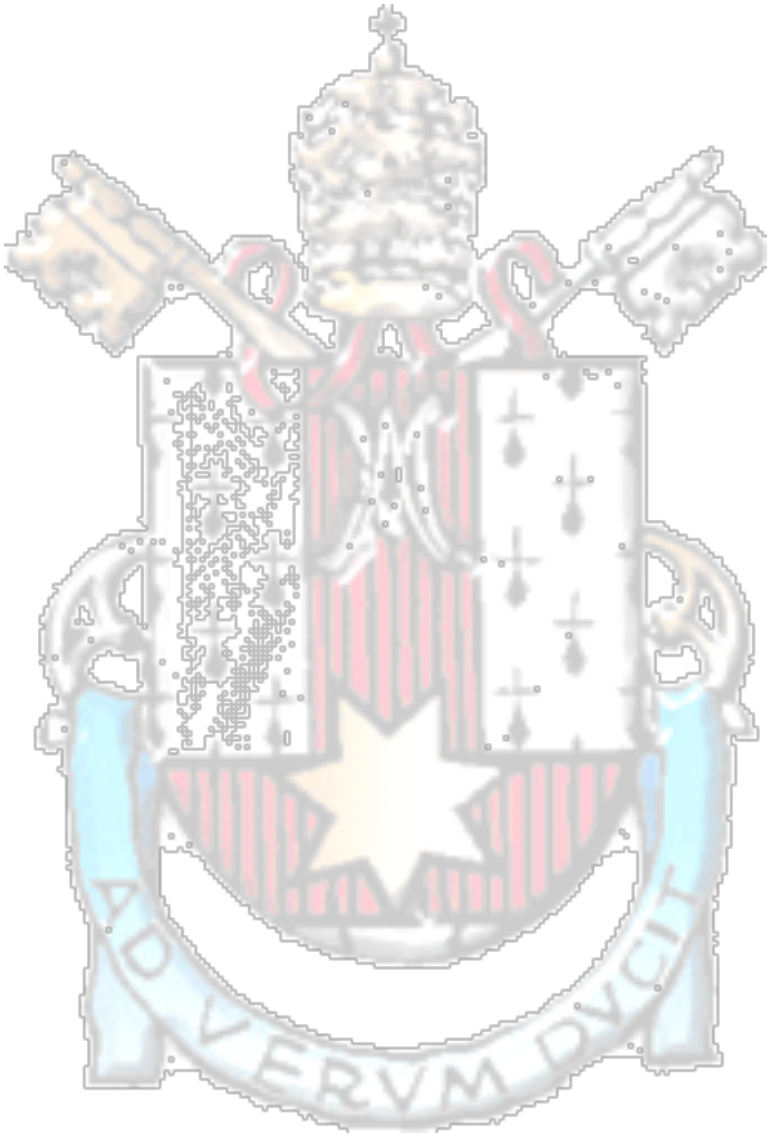
A laserterapia com comprimento de onda infravermelho (GaAlAs, 830nm), 50mW de potência, irradiação cutânea com três pontos de densidade de energia de 5J/cm$_2$ por sessão, totalizando 120J/cm$_2$ ao final do tratamento, demostrou ser benéfica quando aplicada no período de maturação do osso, o que sugere um aumento na dureza do tecido ósseo neoformado, principalmente na porção medular com um aumento nos níveis de cálcio e, por conseguinte, resistência à contração.

Mesmo quando o distrator osteogênico é removido com 33,40 ou 50 dias, a composição química e a topografia óssea neoformada são muito semelhantes, o que permite concluir que, mesmo tendo gerado alguma recidiva após a distração, o osso dos 33, 40, 50 e 60 dias tem sua estrutura biológica fundamental plenamente constituída.

Referências

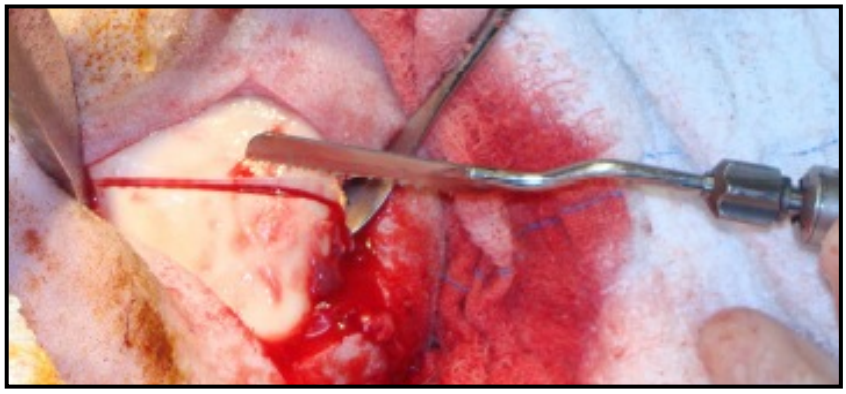
1. Stewart KJ, Weyand B, Van`t Hof RJ, White SA, Lvoff GO, Maffulli N, et al. A quantitative analysis of the effect of insulin-like growth factor-1 infusion during mandibular distraction osteogenesis in rabbits. Br J Plast Surg.1999 jul; 52(5):343-50.
2. Dortbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implants sites. Clin Oral Implants Res. 2002 ;13(3):288-92.
3. Weber J.B et al. Laser therapy improves healing of bone defects submitted to autologous bone graft. Photomed Laser Surg. 2006 Feb;24(1):38-44.
4. Kirkwood BR, Sterne JAC (2006). Essential medical statistics. 2nd ed. Blackwell Science: Massachusetts, USA. p.502
5. Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Cristinamartins M. Infrared laser light reduces loading time of dental implants: a Raman spectroscopic study. Photomed. Laser Surg. 2005 Feb;23(1):27-31
6. Miloro M, Miller JJ, Stoner JA.Low-Level laser effect on mandibular distraction osteogenesis. J Oral Maxillofac Surg. 2007 Feb;65(2):168-76.
7. Diner PA, Kollar EM, Martinez H, Vazquez MP. Intra-oral distraction for mandibular lengthening: a technical innovation. J Craniomaxillofac Surg,1996. Apr;24(2):92-5.
8. McTavish J, Marucci DD, Bonar SF, Walsh WR, Poole MD. Does the sheep mandible relapse following lengthening by distraction osteogenesis? J Craniomaxillofac Surgery.2000 Oct;28(5): 251-7.
9. Mattick CR, Chadwick SW, Morton ME. Mandibular advancement using an intra-oral osteogenic distraction technique: a report of three clinical cases. J Orthod.2001 Jun;28(2):105-14.
10. Glowacki J, Shusterman EM, Troulin M, Holmes r, Perrott D, Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plast Recontr Surg.2004 Feb;113(2):566-73.
11. Nagatani T, Saito S, Sato M, Yamada M.Development of an ultra high resolution scanning electron microscope by means of a field emission source and in-lens system. Scanning Microscopy. v.11, 901-909, 1987
12. Sena, L. A.; Produção e comparação de compósitos hidroxiapatita-colágeno para aplicações biomédicas , Rio de Janeiro UFRJ, 2004, 95 p.

13. Faber, J.; Azevedo, R.B.; Bao, S.N. Aplicações da distração osteogênica na região dentofacial: o estado da arte. R Dental Press Ortodon Ortop Facial, v.10, n.4, p.25-33, jul/ago. 2005
14. Pinheiro, A.L.B.; Brugnera junior, A.; Zanin F.A.A. Aplicação do laser na odontologia. São Paulo: Santos, 2010, 436p.
15. Rodrigo SM, Cunha A, Pozza DH, Blaya DS, Moraes JF, Weber JB, et al. Analysis of the systemic effect of red and infrared laser therapy on wound repair. Photomed Laser Surg. 2009;27:929-35.
16. Freddo AL, Hübler R, de Castro-Beck CA, Heitz C, de Oliveira MG. A preliminary study of hardness and modulus of elasticity in sheep mandibles submitted to distraction osteogenesis and low-level laser therapy Med Oral Patol Oral Cir Bucal. 2012 Jan 1;17 (1):e102-7.
17. Aronson, J.R, Preliminary studied of mineralization during distraction osteogenesis. Clinical Orthopaedics and Related Research, Philadelphia, n. 250, p. 43-49, jan. 1990



Figuras do Artigo em Português

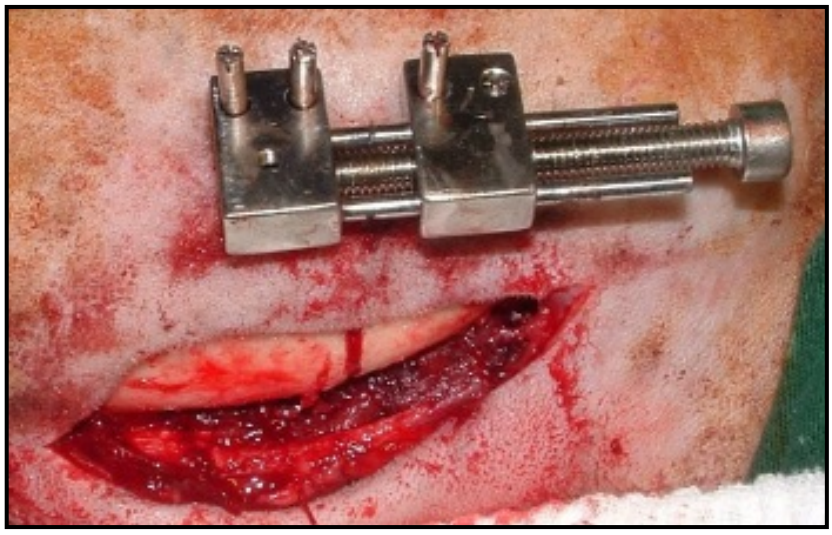
Figura 1



Fonte: dados da pequisa CEP PUCRS-0219/07

Figura 1 - Osteotomia da cortical lateral da mandíbula com a utilização de serra reciprocicante sob irrigação constante.

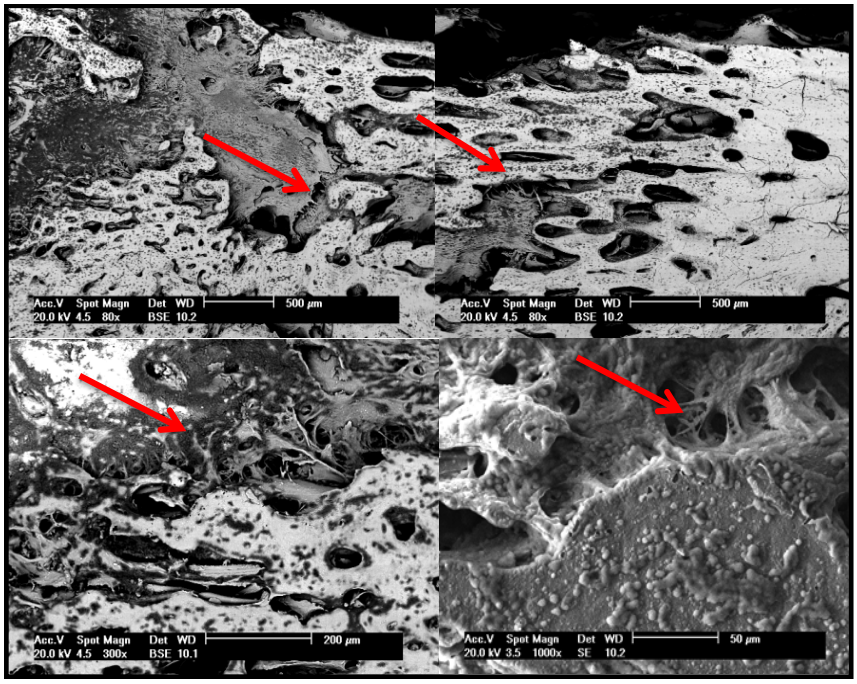
Figura 2



Fonte: dados da pequisa CEP PUCRS-0219/07

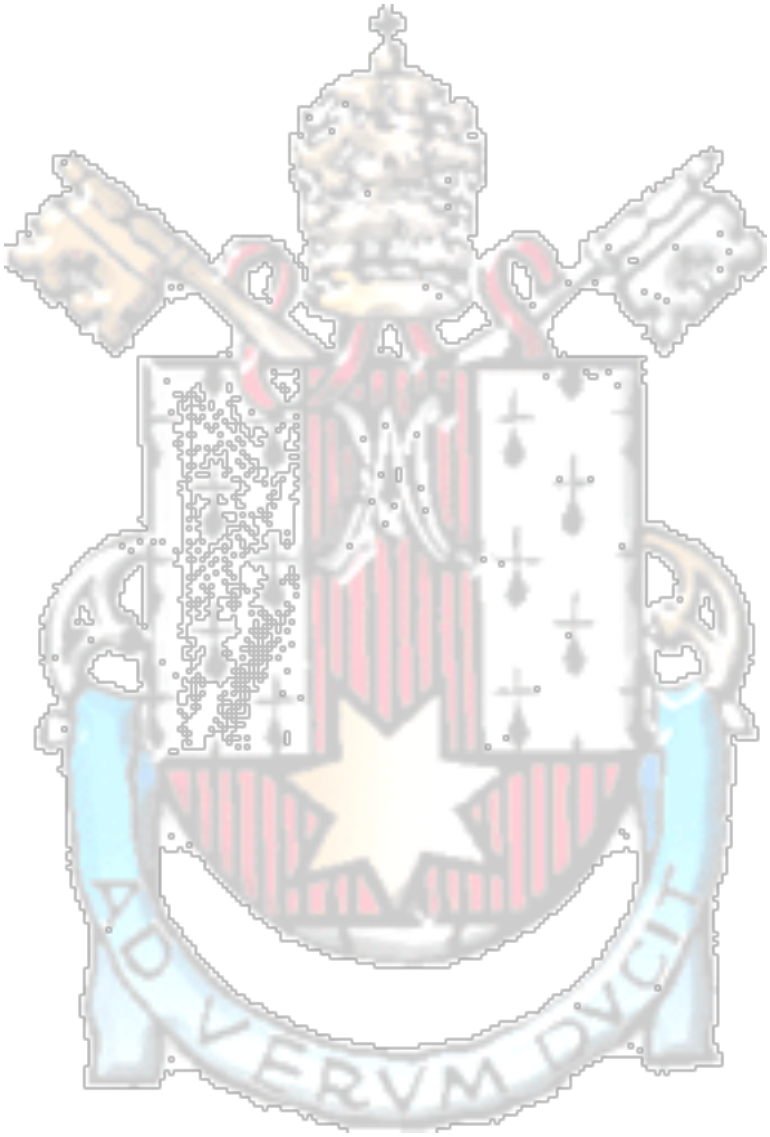
Figura 2 - Instalação do distrator mandibular externo.

Figura 3



Fonte: dados da pequisa CEP PUCRS-0219/07

Figura 3 – Tecido ósseo neoformado na região da cortical externa da ovelha A (aumentos de 80X, 300X e 1000X), onde é evidenciada deposição de matriz óssea.



Artigo em Inglês

Chemical and topographic analysis of sheep mandibles treated with distraction osteogenesis and laser therapy: pilot study

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Abstract

Objectives: To conduct a preliminary analysis of the therapeutic effects of laser therapy (830nm) with regard to surface topography (scanning electron microscopy - SEM) and chemical composition (energy dispersive spectrometry - EDS) of the newly formed bone during distraction osteogenesis (DO) combined with low-level laser therapy (LLLT) in an experimental sheep model.

Methods: Five female Corriedale sheep weighing 40 to 45kg (experimental units A, B, C, D, and E) were treated with LLLT at different stages of OD, as follows: sheep A, B, and C received the first irradiation session immediately after closure of the surgical wound and new sessions then every 48 hours (at a total of eight sessions); sheep D received the first irradiation session on the last day of device activation, followed by seven additional applications every 48 hours (at a total of eight sessions); sheep E (control) was not irradiated. Bone samples were analyzed via SEM at 80x, 300x, and 1000x magnification, and EDS spectra, at 300x magnification.

Results: According to the Student t test for paired samples, the medullary bone of sheep mandibles treated with DO presented statistically higher mean values of carbon and calcium when compared with untreated mandibles (controls). The internal and external cortical layers did not show statistically significant differences in relation to reference values ($p > 0.05$). Bone surface topography was very similar in all specimens.

Conclusion: Infrared laser therapy as used in our study (GaAlAs, 830nm, 50mW power, cutaneous irradiation at three sites, and energy density of 5J/cm^2 per session at a total of 120J/cm^2), has benefits when applied at the beginning of the bone consolidation period, suggesting an increased hardness and resistance of the newly formed bone tissue.

Keywords: Distraction osteogenesis, laser therapy, electron microscopy.

Introduction

Distraction osteogenesis (DO) is a bone lengthening technique that involves the formation of new bone tissue to fill defects or spaces found in vascularized bone surfaces following osteotomy or corticotomy procedures, via the use of functional devices. In DO, the bone lengthens gradually in response to adjustments made to the distraction device; the space is initially filled with a bone callus and subsequently replaced with bone tissue (1).

Some studies have assessed the influence of low-level laser therapy (LLLT) on bone healing and consequently on DO results. Dörtbudak *et al.* (2), for example, assessed the effect of diode laser (690nm) on rat osteoblast and concluded that irradiation with non-ablative diode laser has a biostimulatory effect on osteoblasts *in vitro.* Weber *et al.* (3,4) conducted a histological analysis to assess the influence of irradiation with an infrared diode laser (gallium-aluminum-arsenide, GaAlAs, 830nm, 50mW, CW) on the healing of autologous bone grafts. Those authors found that specimens receiving laser therapy trans-operatively on the surgical bed (G2 and G4) presented more evident bone remodeling activity both quantitatively and qualitatively, suggesting a positive biomodulative effect of trans-operative LLLT on the healing of bone defects associated with autologous bone grafts. Lopes *et al.* (5) also observed positive effects of laser therapy on the bone healing process.

Miloro *et al.* (6) used (GaAlAs) and concluded that LLL applied during the bone consolidation period accelerates bone regeneration, allowing for earlier device removal and thus reducing surgical morbidity.

Several clinical and experimental studies have been conducted with the aims of improving DO techniques and distraction devices, investigating distraction vectors, and assessing factors that may potentially influence bone healing after DO. However, few studies so far have focused on the effects of LLLT in terms of topography and chemical composition of the new bone formed in DO.

Therefore, the objective of the present study was to conduct a pilot study, or a preliminary analysis, of the therapeutic effects of LLLT (830nm) with regard to bone surface topography (assessed by scanning electron microscopy, SEM) and chemical composition (assessed by energy dispersive spectrometry, EDS) of the new mandibular bone formed in DO in experimental sheep models. Moreover, we

investigated whether different times of use of the distraction device during the bone consolidation period had any influence on the properties of newly formed bone.

Methods

The present study was conducted in accordance with applicable national and international research guidelines and was approved by the Research Ethics Committee of the institution where it was developed (protocol nos. 0219/07 and 075/08, PUCRS).

Five female Corriedale sheep aged 2 years and weighing 40-45kg were included in the investigation. All animals were inspected and submitted to standard tests. None of the animals presented any conditions or abnormalities that could interfere with the results of the present study.

The five sheep were considered as five experimental units (sheep A, B, C, D, and E) and randomly assigned to different treatment regimens (Table 1). LLLT was carried out either during activation of the distraction device (sheep A, B, and C) or in the bone consolidation period (sheep D). Sheep E was not treated with LLLT. All animals were observed for 60 days.

Throughout the study period, the animals remained in the animal hospital of Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. Animals were housed in appropriate isolated caging and identified using a ear tag.

Tabela 1- experimental organizaion

Group	Distractor	LLLT	Death	joules
A	50 days	Latency/activation	60 days	120 J
B	40 days	Latency/activation	60 days	120 J
C	33 days	Latency/activation	60 days	120 J
D	33 days	Maturation	60 days	120 J
E	33 days	LLLT off	60 days	0J

Font: CEP PUCRS-0219/07

General anesthesia was induced with acepromazine 0.05mg/kg, meperidine 2mg/kg, ketamine 4mg/kg, plus halothane delivered by mask. Subsequently, sheep were intubated after administration of 8ml of propofol. Anesthesia was maintained with halothane in 100% oxygen.

Antibiotic prophylaxis was performed with intravenous ampicillin sodium at 10mg/kg. After the animals became unconscious, they were positioned in right lateral decubitus.

Surgery was performed as follows: trichotomy of the left submandibular region, antisepsis, local infiltration of 3mL of 1% lidocaine and epinephrine at a 1:100,000 ratio, and a Risdon type incision measuring approximately 3cm, performed 1cm below the left submandibular region. Tissues were cut in planes. Farabeuf retractors were placed so as to expose the lateral surface of the mandible. Osteotomies were performed with a reciprocating saw through the medial and lateral faces of the mandible, in the mandibular angle region (Figure 1).

The distraction device was fixed with four transcutaneous screws under copious irrigation (Figure 2). Then, it was activated until resistance was encountered, so that the produced fracture could be completed using straight chisels. The surgical wound was closed in layers with single interrupted sutures using 4.0 monofilament nylon. Skin sutures were removed after seven days. Following surgery, sheep received morphine at 0.4mg/kg every 8 hours for 72h, and pentabiotics (benzathine, procaine, and streptomycin penicillin combined) every 24h for seven days.

The DO protocol adopted is described in detail elsewhere (16).Briefly, there was 1) a five-day latency period, during which the distraction device was only inspected and cleaned with 1% iodophor alcohol (not activated); 2) a 15-day activation period, with device activation starting on the sixth postoperative day, at 1mm per day, to a total of 15mm; and 3) a 13- to 30-day bone consolidation period (30 days in sheep A, 20 days in sheep B, and 13 days in sheep C, D, and E), during which the distractor remained in the animals working as a rigid fixation device, to allow bone maturation. Distractor removal was carried out under local anesthesia.

Laser irradiation was performed using Thera Laser® system (DMC, São Carlos, Brazil), properly calibrated, with gallium-aluminum-arsenide (GaAlAs) as the

active medium, and wavelength of 830ηm. In sheep A, B, and C, irradiation was first applied immediately after closure of the surgical wound (i.e. in the latency period) and then every 48 hours (into the activation period), at a total of eight sessions. In sheep D, the first irradiation session took place on the last day of device activation, and then every 48 hours, also at a total of eight sessions. Because laser therapy is painless, no sedation or anesthetics were necessary. Specimens obtained from sheep A, B, C, and D received laser doses directly on the distraction site. Three sites received 5 J/cm^2 each, totaling 15 J/cm^2, with a power of 50mW, in a continuous wave mode, for 1.41 minutes. Total energy applied to each sheep was 120J/cm^2. Although sheep E (control) was not irradiated, the animal underwent the same protocol, however with the equipment turned off.

Sixty days after surgery, animals were killed under deep anesthesia using the same anesthetic agents employed before surgery. Death was caused by cardiorespiratory arrest, as recommended by Brazilian Resolution no. 714/2002 issued by the Brazilian Federal Council of Veterinary Medicine, which provides guidelines for euthanasia of animals used in research. Once death was confirmed by the absence of vital signs, mandibles were dissected and removed with a chisel and a gouge, and subsequently stored in glutaraldehyde.

At the Physics Laboratory of the institution where the study was carried out, mandibles were sectioned in two halves with the aid of a mini-lathe (Proxxon®-50HZ; 40W). Then, vertical osteotomies were performed to generate specimens containing both newly formed bone and old bone for subsequent analysis. Subsequently, specimens were placed in a desiccator cabinet and kept there for one week, and then transferred to a vacuum chamber, at 5 Pascal atmospheric pressure, for another week, to eliminate any glutaraldehyde remnants that might be present inside the bone tissue. These measures were taken to avoid chemical reactions at subsequent stages of the experiment.

The specimens of interest were inserted in pre-molded square polyvinyl chloride (PVC) pipes filled with clear fiberglass resin (Fiberglass®). After fixation, blocks were sectioned in axial direction in three blocks using a band saw (St1101, Starrett®). All blocks were properly identified as upper, middle (upper or lower), or lower portion.

Following specimen preparation, each bone block was analyzed in a scanning electron microscope (Philips XL 30) at 80x, 300x, and 1000x magnification, in secondary electron (SE) and backscatter electron (BSE) modes.

EDS spectrum images were acquired at 300x magnification to allow analysis of the chemical composition of specimens (Table 2). Three sites were randomly selected for each of the analyses (cortical and medullary bone), in order to assess mean levels of oxygen, carbon, calcium, and phosphorus. The control specimen used in EDS analysis was comprised of healthy mandibular bone tissue collected from the untreated side of sheep E.

Table 2- Parameters used in scanning electron microscopy

Analyses	Internal cortical layer	External cortical layer	Medullary bone
SE	80X; 300X e 1000X	80X; 300X e 1000X	80X; 300X e 1000X
BSE	80X; 300X e 1000X	80X; 300X e 1000X	80X; 300X e 1000X
EDS	300X	300X	300X

Font: CEP PUCRS-0219/07

Results were expressed as means and standard deviation for each of the elements and regions assessed. The means of each element were compared with reference (control) values using the Student t test for paired samples (6).

Significance was set at 5%.

Results

EDS analysis

Table 3 shows that the medullary bone of sheep mandibles treated with DO and LLLT presented statistically lower mean values of phosphorus and oxygen when compared with the untreated mandible (control, sheep E). Carbon and calcium, in turn, showed higher means when compared with the control (sheep E). The internal and external cortical layers did not show statistically significant differences in relation to the values obtained for sheep E.

Table 3. Means obtained for each element and region assessed in sheep treated with distraction osteogenesis and in the untreated sheep (control)

	A		B		C		D		E	
Region/element	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Internal cortical layer										
Carbon	26.24	12.05	18.88	11.98	31.43	15.50	19.99	10.93	24.55	12.79
Oxygen	31.23	15.12	35.71	15.89	32.76	15.83	36.06	17.23	21.98	9.87
Phosphorus	16.06	7.30	19.96	12.05	18.84	11.64	21.35	10.18	24.55	11.63
Calcium	17.81	10.05	35.44	15.71	16.95	7.43	32.09	16.55	28.57	13.47
Medullary bone										
Carbon	11.91	3.25	28.73	13.85	33.74	16.25	10.24	3.87	13.21	4.99
Oxygen	35.42	17.15	38.24	16.75	28.10	14.81	37.09	17.79	24.99	11.75
Phosphorus	20.86	10.83	14.19	6.90	16.78	7.35	21.60	13.23	24.99	11.75
Calcium	30.45	14.12	28.25	18.65	21.25	13.75	31.11	16.79	16.52	6.88
External cortical layer										
Carbon	26.06	12.49	20.95	12.49	19.62	12.14	10.58	2.87	22.55	10.73
Oxygen	26.78	13.25	41.38	18.15	21.69	13.52	29.96	15.99	30.46	15.18
Phosphorus	19.73	11.60	12.55	4.49	23.42	14.99	24.17	15.64	19.20	11.69
Calcium	27.42	14.25	25.67	12.98	35.26	17.25	35.28	17.05	27.72	12.88

Font: CEP PUCRS-0219/07

Descriptive analysis of bone topography (SEM)

All specimens were assessed at the 60th postoperative day. The specimens of the four sheep treated with LLLT showed similar topographic properties, with mature bone tissue and few spaces filled with fibrous tissue. However, treated specimens showed higher amounts of bone matrix deposition when compared with the control sheep (Figure 3).

Discussion

The DO technique used in the present study, with a latency period of five days and device activation at 1mm per day, has been extensively described in the literature (7-10).

The morphological characterization of materials is extremely important to assess their behavior. In this sense, SEM has become an essential tool in the observation and analysis of surface microstructures (11). SEM is one of the most flexible techniques currently available for the assessment of microstructural characteristics of solid materials. The main reason for this widespread use of SEM is the high resolution that can be obtained: while commercially available instruments

usually offer a resolution ranging from 2 to 5 nanometers, advanced research instruments can reach a resolution of 1nm or better (12).

At the end of the activation period, the bone consolidation (or retention) period is important to ensure that the newly regenerated tissue will acquire the necessary bioelasticity to resist relapse. Consolidation periods ranging from 2.4 to 10 weeks have been described after mandibular DO both in humans and in animal models (13).

Current developments in the field of DO lie precisely in reducing the bone consolidation period and thus allowing the distraction device to be removed as early as possible. Therefore, we chose to use different consolidation times for each animal. In sheep A and B, consolidation periods already consolidated in the literature were adopted, namely 30 and 20 days, respectively (17). In the other three animals, consolidation time was reduced to 13 days, a time frame not previously adopted in sheep.

We used GaAlAs laser at a wavelength of 830ηm (infrared) because infrared light spectra have a greater ability to penetrate tissues when compared with red lasers. The greater penetration associated with infrared lasers in subcutaneous tissues is due to their low absorption in water or skin pigments (14).

The irradiation protocol employed in the present study was based on previous studies conducted by the Facial Deformities Group, financed by CNPq, in which LLLT was applied every 48 hours, at a total of eight irradiation sessions (3,15).

The descriptive analysis of SEM findings showed that the topography of specimens treated with LLLT presented fewer spaces and higher amounts of bone matrix (17), i.e., the distracted bone of all animals presented characteristics compatible with mature bone. We also observed that the bone tissue of all specimens treated with LLLT was very similar, however postoperative relapse (contraction) was observed in the sheep that had the distraction device removed earlier.

In the EDS analysis, all sheep presented statistically similar values for carbon, oxygen, phosphorus, and calcium. However, distracted medullary bone samples showed increased values for these elements, especially calcium, suggesting the formation of more resistant bone tissue in sheep treated with LLLT in both the device activation and the bone consolidation periods. Because bone consolidation takes place from the cortical layers toward the medullary cavity (16), the presence of higher

rates of calcium in this region confirms the positive effects of LLLT on new bone formed by distraction osteogenesis.

Conclusion

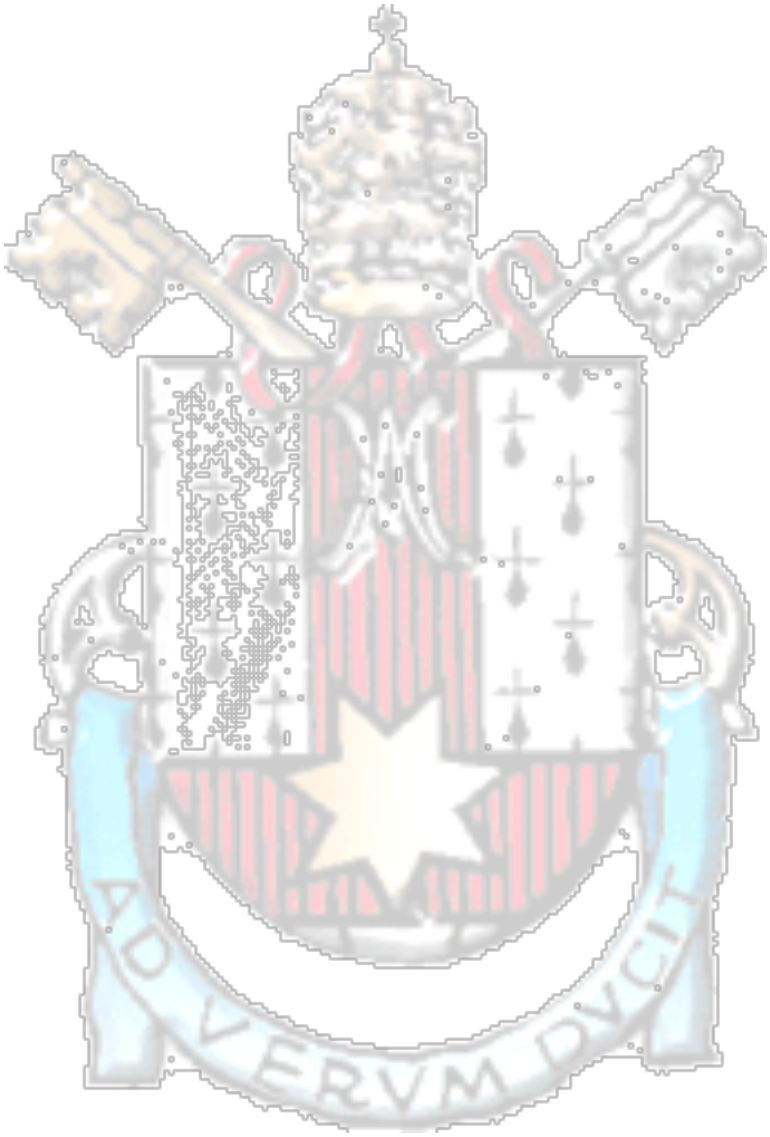
Laser therapy with infrared laser therapy (GaAlAs, 830nm), 50mW of power, cutaneous irradiation at three sites, energy density of 5J/cm^2 per session, at a total of 120J/cm^2 at the end of the study, showed benefits when applied at the bone consolidation period, suggesting an increased hardness of the newly formed bone tissue, especially in the medullary cavity, with increased calcium levels and consequently increased resistance to contraction.

Device removal at 33, 40, or 50 days did not alter the chemical composition and topographic properties of the newly formed bone, which were similar in all specimens. This finding suggests that, in spite of the possibility of relapse after DO, use of a distraction device for 33, 40, 50, or 60 days results in a fully developed biological bone structure.

References

1. Stewart KJ, Weyand B, Van`t Hof RJ, White SA, Lvoff GO, Maffulli N, et al. A quantitative analysis of the effect of insulin-like growth factor-1 infusion during mandibular distraction osteogenesis in rabbits. Br J Plast Surg.1999 jul; 52(5):343-50.
2. Dortbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implants sites. Clin Oral Implants Res. 2002 ;13(3):288-92.
3. Weber J.B et al. Laser therapy improves healing of bone defects submitted to autologous bone graft. Photomed Laser Surg. 2006 Feb;24(1):38-44.
4. Kirkwood BR, Sterne JAC (2006). Essential medical statistics. 2nd ed. Blackwell Science: Massachusetts, USA. p.502.
5. Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Cristinamartins M. Infrared laser light reduces loading time of dental implants: a Raman spectroscopic study. Photomed. Laser Surg. 2005 Feb;23(1):27-31
6. Miloro M, Miller JJ, Stoner JA. Low-level laser effect on mandibular distraction osteogenesis. J Oral Maxillofac Surg. 2007 Feb;65(2):168-76.
7. Diner PA, Kollar EM, Martinez H, Vazquez MP. Intra-oral distraction for mandibular lengthening: a technical innovation. J Craniomaxillofac Surg,1996. Apr;24(2):92-5.
8. McTavish J, Marucci DD, Bonar SF, Walsh WR, Poole MD. Does the sheep mandible relapse following lengthening by distraction osteogenesis? J Craniomaxillofac Surgery.2000 Oct;28(5): 251-7.
9. Mattick CR, Chadwick SW, Morton ME. Mandibular advancement using an intra-oral osteogenic distraction technique: a report of three clinical cases. J Orthod.2001 Jun;28(2):105-14.
10. Glowacki J, Shusterman EM, Troulin M, Holmes R, Perrott D, Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plast Reconstr Surg. 2004 Feb;113(2):566-73.
11. Nagatani T, Saito S, Sato M, Yamada M. Development of an ultra high resolution scanning electron microscope by means of a field emission source and in-lens system. Scanning Microscopy. v.11, 901-909, 1987
12. Sena, L. A.; Produção e comparação de compósitos hidroxiapatita-colágeno para aplicações biomédicas , Rio de Janeiro UFRJ, 2004, 95 p.

13. Faber, J.; Azevedo, R.B.; Bao, S.N. Aplicações da distração osteogênica na região dentofacial: o estado da arte. R Dental Press Ortodon Ortop Facial, v.10, n.4, p.25-33, jul/ago. 2005
14. Pinheiro, A.L.B.; Brugnera Junior, A.; Zanin F.A.A. Aplicação do laser na odontologia. São Paulo: Santos, 2010, 436p.
15. Rodrigo SM, Cunha A, Pozza DH, Blaya DS, Moraes JF, Weber JB, et al. Analysis of the systemic effect of red and infrared laser therapy on wound repair. Photomed Laser Surg. 2009;27:929-35.
16. Freddo AL, Hübler R, de Castro-Beck CA, Heitz C, de Oliveira MG. A preliminary study of hardness and modulus of elasticity in sheep mandibles submitted to distraction osteogenesis and low-level laser therapy. Med Oral Patol Oral Cir Bucal. 2012 Jan 1;17 (1):e102-7.
17. Aronson, J.R, Preliminary studied of mineralization during distraction osteogenesis. Clinical Orthopaedics and Related Research, Philadelphia, n. 250, p. 43-49, jan. 1990



Figuras do artigo em Inglês

Figure 1

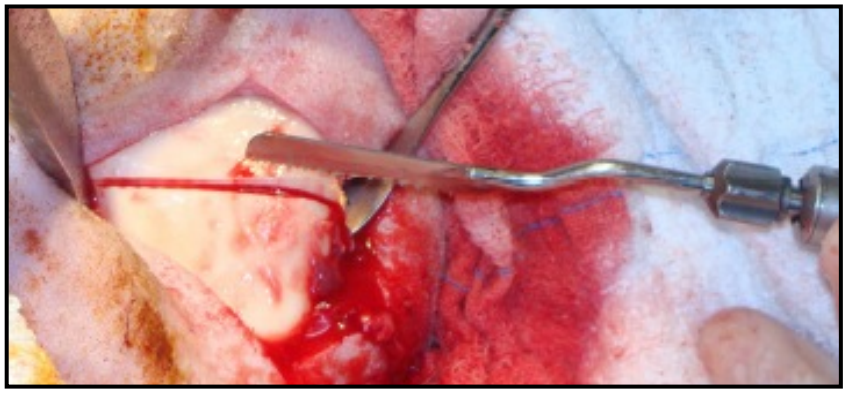


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Figure 1 - Osteotomy of the lateral cortical layer of the mandible using a reciprocating saw.

Figure 2

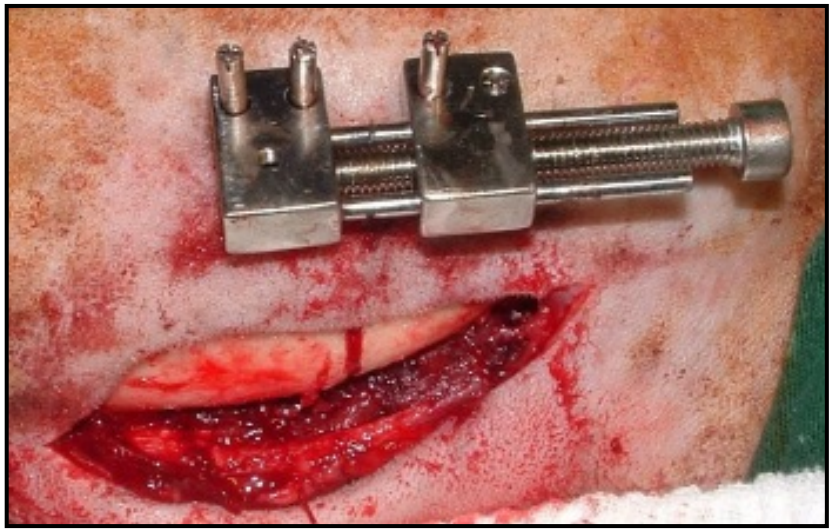

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Figure 2 - Placement of the external mandibular distraction device.

Figure 3

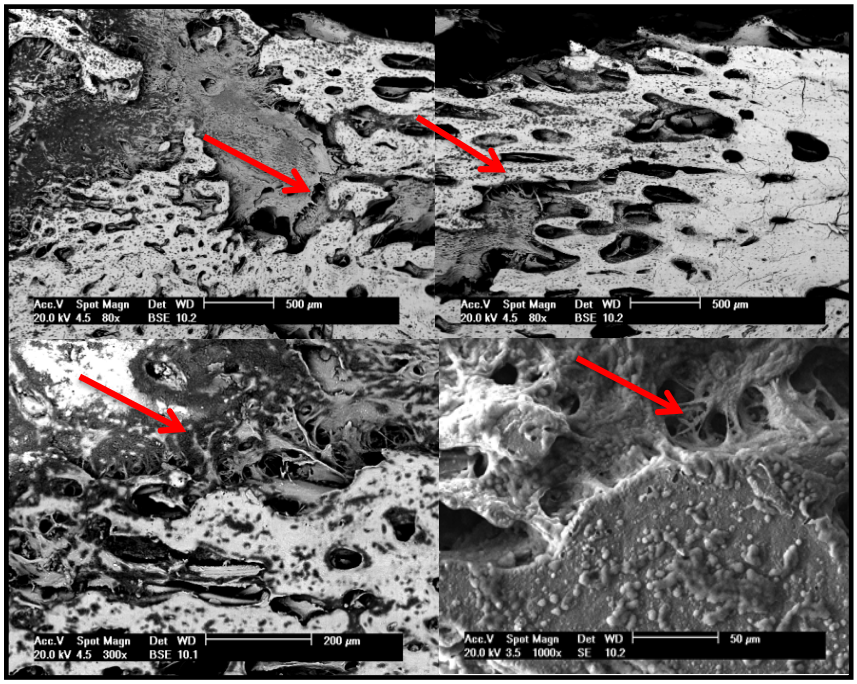

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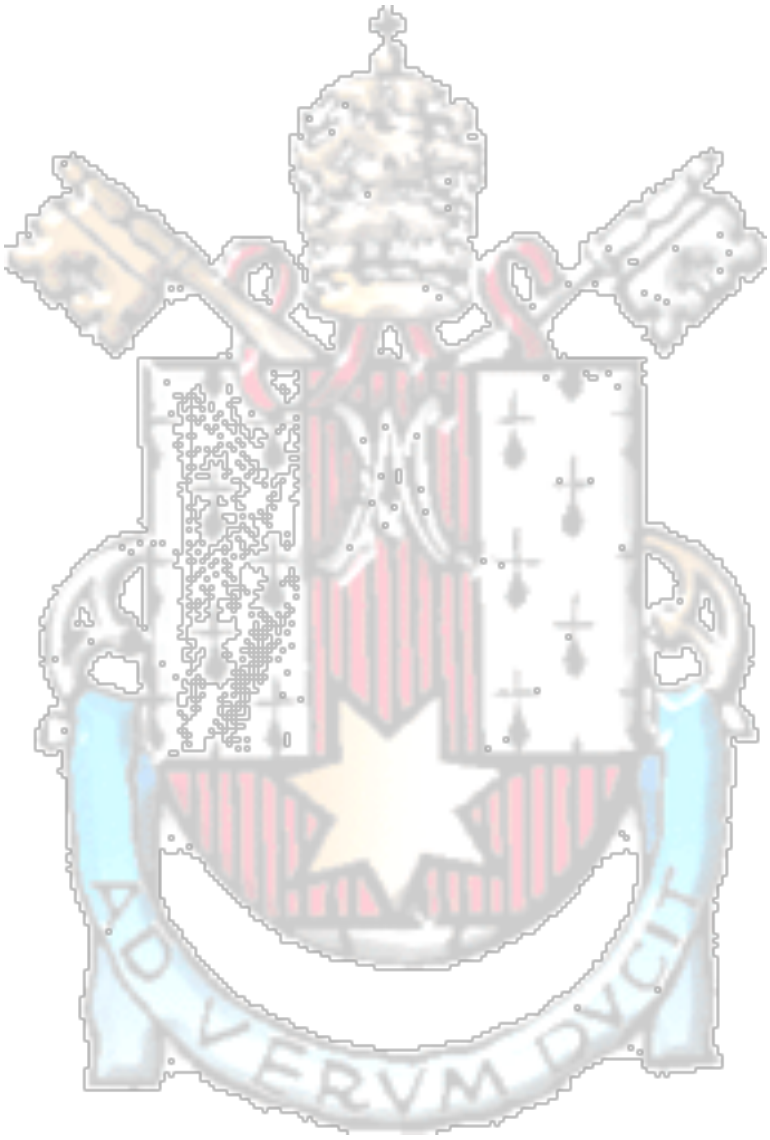
Figure 3 - Newly formed bone tissue at the external cortical layer of sheep A (80x, 300x, and 1000x magnification) showing bone matrix deposition.



Anexos

Anexo 1

Liberação do comitê de Etica e Pesquisa

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

PUCRS

Ofício 075/08-CEUA Porto Alegre, 23 de outubro de 2008.

Senhor Pesquisador:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 08/00040, intitulado: **"Avaliação por nanodureza, xrf e xrd do osso mandibular de ovelhas submetidas à distração osteogênica".**

Sua investigação está autorizada a partir da presente data.

Relatórios do andamento do projeto devem ser entregues a este Comitê.

Atenciosamente,

Profa. Dr. Anamaria Feijó
Coordenadora do CEUA – PUCRS

Ilmo. Sr.
Prof. Dr. Roberto Hübler
Laboratório GEPSI - TECNOPUC
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Anexo II
Relatório dos artigos realizados no programa de pós graduação, Doutorado em CTBMF período 2009-2012

Artigo 1

Med Oral Patol Oral Cir Bucal. 2010 Jul 1;15 (4):e616-8. Evaluation of the effect of LLLT in osteogenic distraction

Journal section: Oral Surgery doi:10.4317/medoral.15.e616
Publication Types: Research

Histological evaluation of the effect of low-level laser on distraction osteogenesis in rabbit mandibles

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Received: 01/07/2009
Accepted: 28/11/2009

Abstract

Objectives: This study evaluated the action of low level laser therapy (LLLT) on the percentage of newly forme bone in rabbit mandibles that underwent distraction osteogenesis (DO).

Study design: Ten rabbits underwent bone lengthening according to the following protocol: Latency – 3 days; Activation – 7 days 0.7 mm/d; and Consolidation – 10 days. The control group was composed of 4 rabbits. The experimental group, composed of 6 rabbits, received infrared GaAlAs LLLT (λ=830 nm, P=40 mW) according to the following protocol: point dose of 10 J/cm2 applied directly on the bone site that underwent DO during bone consolidation at 48-hour intervals.

Results: The percentage of newly formed bone was greater in the LLLT group (57.89%) than in the control group (46.75%) (p=0.006).

Conclusion: The results suggest that LLLT had a positive effect on the percentage of newly formed bone. Betterquality
bone sites may allow early removal of the osteogenic distractors, thus shortening total treatment time.

Key words: Distraction osteogenesis, lasers, physical process, basic research.
Kreisner PE, Blaya DS, Gaião L, Maciel-Santos MES, Etges A, Santana-Filho M, Oliveira MG. Histological evaluation of the effect of low-level laser on distraction osteogenesis in rabbit mandibles. Med Oral Patol Oral

Introduction

Distraction osteogenesis (DO) has been shown to be an alternative treatment for facial bone reconstruction. A
distraction devices is used to induce bone growth in the site of deformity (1). Long-term stability of DO has not been well documented, and some studies have found cases of instability and recurrence (2-5). Therefore, some authors have attempted to accelerate bone maturation and to improve the physical properties of lengthened bone (6-8).

Bone regeneration creates a response involving blood vessels, cells, and extracellular matrix. Vascular supply, protein synthesis, and mineralization are all fundamental processes to guarantee tissue regeneration after trauma (9). The use of low level laser therapy (LLLT) seems to have a positive effect on the repair of soft tissues and bone (10). DO involves metabolic events that can be modulated by using LLLT, which may reduce total treatment time and ensure, therefore, greater patient comfort (11).

Material and Methods Experimental procedures in this study were approved by the Science and Ethics Committee of the School of Dentistry and the Ethics in Research Committee of the Pontifícia Universidade Católica do Rio Grande do Sul. Ten adult male New Zealand rabbits (Oryctolagus cuniculus) were admitted to the Animal Laboratory of the State Foundation of Health Research and Production (Fundação Estadual de Produção e Pesquisa em Saúde - FEPPS) and underwent DO of the right side of the mandible. The animals were anesthetized with 0.1 mg/kg 2% xylazine hydrochloride (Anasedan®, Agribands do Brasil, Paulínia, SP, Brazil) and 3 mg/kg zolazepam and tiletamine (Zoletil®, Virbac do Brasil Ltda., São Paulo, SP, Brazil). The right submandibular area was shaved and cleaned with 4% chlorhexidine. Sterile surgical drapes were used to isolate the operating field. Enrofloxacin (50 mg) was administered as antibiotic prophylaxis one hour before the procedure and in the next three days. After infiltration of 0.9 ml lidocaine and 2% epine phrine, a 3 cm incision was made on the skin along the lower edge of the right side of the mandible. The mandible was exposed by carefully elevating the subperiosteal plane. Burs and osteotomes were used to produce a corticotomy. The inferior alveolar nerve was preserved. The distractor (PROMM®, Porto Alegre, Brazil) was fixed to the mandible with four 1.5 x 5 mm screws per pendicular to the corticotomy. The wound was irrigated with saline solution and closed in layers. The DO was applied using a 3-day latency period, a single 0.7 mm daily activation of the distractor for 7 days and a 10-day consolidation period. The animals were randomly divided into 2 groups. The control group comprised 4 rabbits, and the experimental group, 6 rabbits that received doses of 10 J/cm2 per point at each 48 hours during the consolidation period,which totaled 50 J/cm2. Gallium-aluminum arsenide (GaAlAs) laser was used at 830 nm and 40 mW. At the end of consolidation, the animals were killed in a carbon dioxide chamber according to the recommendation of the Brazilian Committee for Animal Experiments. The specimens were decalcified with 5% citric acid and routinely prepared to be embedded in paraffin and stained with HE (hematoxylin and eosin). Lateromedial 4-µm-thick sections were obtained, and 3 sections of each animal were selected.

To measure the areas of new bone formation, each slide was subdivided into experimental units (EU) under light microscopy and 100x magnifica tion. images were subsequently captured with a digital camera coupled to the microscope. Images of EU were organized in files to ensure that examiners, who were previously calibrated, were blinded to study groups. The free software ImageTool® for Windows 3.0 (University of Texas Health Science Center, San Antonio, (USA) was used to measure areas of newly formed bone (NFB) in square pixels. After the measurement of the area of newly formed bone (AN) and the total EU area (AT), the percentages of newly formed bone were calculated as AN/AT*100. A t test was used for the statistical
comparison of group results.

Results

The percentage of bone neoformation was used for histological evaluation. Table 1 shows the mean values of NFB by animal. These means were calculated according to the measurements of experimental units on each slide. A t parametric test for independent samples was used to analyze measures, and results showed differences in NFB between the control and experimental groups ($p<0.05$).

The percentage of NFB was greater in the LLLT group (57.89%) than in the control group (46.75%) ($p=0.006$) (Table 1). A statistically significant difference was found when variables were compared between groups taking into consideration non-homogeneity (Table 1), which suggests that the effect of LLLT was positive in the experimental group).

Discussion

The percentage of NFB is the standard criterion to evaluate bone tissue or substitutes, and was used by Cerqueira et al. (11), Saito and Shimizu (12), and Miloro et al. (13) to evaluate DO.

The adoption of a quantitative measure, such as the percentage of NFB, reduces examiner interference found in qualitative analyses, in which the examiner describes specific items on the slides, or in semi-quantitative analysis, in which scores are assigned by the examiner o a group of items that are characteristic of a certain process.

As this description or score assignment is subjectively made by the examiner, there is a greater possibility of interference on the results. The use of an already consolidated and widely used test (HE) makes it possible to compare results, to generate innovative protocols for the use of LLLT in DO, and to reduce risks and complications inherent to this technique.

The methods used in this study and the results obtained showed that LLLT had a positive effect on the percentage of NFB in the mandible of rabbits that underwent DO.

References

1. Pereira MA, Luiz de Freitas PH, da Rosa TF, Xavier CB. Understanding distraction osteogenesis on the maxillofacial complex: a literature review. J Oral Maxillofac Surg. 2007;65:2518-23.
2. McCarthy JG, Schreiber J, Karp N, Thorne CH, Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg. 1992;89:1-8.
3. Douglas LR, Douglass JB, Smith PJ. Intraoral mandibular distraction osteogenesis in a patient with severe micrognathia secondary to TMJ ankylosis using a tooth and bone-anchored device (PIT device): a case report. J Oral Maxillofac Surg. 2000;58:1429-33.
4. Marquez IM, Fish LC, Stella JP. Two-year follow-up of distraction osteogenesis: its effect on mandibular ramus height in hemifacial microsomia. Am J Orthod Dentofacial Orthop. 2000;117:130-9.
5. Mofid MM, Manson PN, Robertson BC, Tufaro AP, Elias JJ, Vander Kolk CA. Craniofacial distraction osteogenesis: a review of 3278 cases. Plast Reconstr Surg. 2001;108:1103-14.
6. Farhadieh RD, Gianoutsos MP, Dickinson R, Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg. 2000;105:889-95.
7. Hagiwara T, Bell WH. Effect of electrical stimulation on mandibular distraction osteogenesis. J Craniomaxillofac Surg. 2000;28:12-9.
8. Shimazaki A, Inui K, Azuma Y, Nishimura N, Yamano Y. Low-intensity pulsed ultrasound accelerates bone maturation in distraction osteogenesis in rabbits. J Bone Joint Surg Br. 2000;82:1077-82.
9. Fernández-Tresguerres-Hernández-Gil I, Alobera-Gracia MA, del-Canto-Pingarrón M, Blanco-Jerez L. Physiological bases of bone regeneration I. Histology and physiology of bone tissue. Med Oral Patol Oral Cir Bucal. 2006;11:E47-51.
10. Weber JB, Pinheiro AL, de Oliveira MG, Oliveira FA, Ramalho LM. Laser therapy improves healing of bone defects submitted to autologous bone graft. Photomed Laser Surg. 2006;24:38-44.
11. Cerqueira A, Silveira RL, Oliveira MG, Sant'ana Filho M, Heitz C. Bone tissue microscopic findings related to the use of diode laser (830 nm) in ovine mandible submitted to distraction osteogenesis. Acta Cir Bras. 2007;22:92-7.
12. Saito S, Shimizu N. Stimulatory effects of low-power laser irradiation on bone regeneration in midpalatal suture during expansion in the rat. Am J Orthod Dentofacial Orthop. 1997;111:525-32.
13. Miloro M, Miller JJ, Stoner JA. Low-level laser effect on mandibular distraction osteogenesis. J Oral Maxillofac Surg. 2007;65:168-76.

Artigo 2

Rev. odonto ciênc. (Online) vol.26 no.3 Porto Alegre 2011

http://dx.doi.org/10.1590/S1980-65232011000300003

ORIGINAL ARTICLE

Titanium alloy miniscrews for orthodontic anchorage: an in vivo study of metal ion release

ABSTRACT

PURPOSE: To examine and compare the levels of several metal ions released in the saliva of patients with orthodontic appliances, at different time points before and after insertion of a miniscrew. **METHODS:** Saliva of patients (n=20) was collected at four time points: before miniscrew placement (T1), 10 minutes (T2), 7 days (T3) and 30 days after miniscrew placement (T4). The salivary samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). The release of nine different metal ions was observed: titanium (Ti), zinc (Zn), chromium (Cr), nickel (Ni), iron (Fe), copper (Cu), aluminum (Al), Vanadium (V) and cobalt (Co). Data were analyzed by descriptive statistics. Salivary metal concentrations from different time points of miniscrew treatment were compared using Wilcoxon paired tests (a=5%). **RESULTS:** At time point T4, there was a quantitative increase in the salivary concentration of Cu, Ti, V, Zn, as well as a quantitative decrease in the salivary concentration of Al, Co, Cr, Fe, Ni, when compared with T1. **CONCLUSION:** It can be concluded that the placement of fixed orthodontic appliances associated with miniscrews does not lead to an increase of salivary metal ion concentrations.

Key words: Metal ions; miniscrews; ICP-MS; ICP-OES; orthodontic appliances

Introduction

Ti -6A- 4V is the most frequently used titanium alloy for medical implants and orthodontic miniscrews because of its excellent properties (1,2). However, several studies suggested the cytotoxicity and dissolution of this alloy (2-4) and its corrosion products (5-8). In addition, the concentrations of these metals have been measured in the blood, urine and tissues (9).

In the alloy Ti-6Al-4V, superficial oxide is composed of TiO_2, with small amounts of Al_2O_3, hydroxylic groups and water (10). Its superficial oxide layer is less stable than that of commercially pureTi because the Al and V, which are added to stabilize the α and β phases, respectively, desestabilize the alloy, making it more vulnerable to corrosion (11). As V is not present in the superficial oxide layer of Ti-6Al-4V (10), Ti and Al are the metal ions most likely to be released from the Ti-6Al-4V surface(12).

According to Hanawa (10), the most harmful components of metallic implants are Co from Cr-Co alloy, Ni from stainless steel and V from Ti-6Al-4V alloy. However, both V and Al in Ti-6Al-4V are potentially toxic (13,14). The Al ions affect the proliferation, metabolic activity and differentiation of osteoblasts (12). Some toxic effects attributed to Al accumulation in the human body have been described in the literature (encephalopathia and senile dementia of the Alzheimer's type) (15). The element may also be associated with osteomalacia and pulmonary granulomatosis (14).

Vanadium is an essential element for the functioning of our organism (16). However, toxic vanadium may elicit local or especially systemic reactions or inhibit cellular proliferation. Vanadium may be cytotoxic for alveolar macrophages and synovial fibroblasts, interferes with mitosis and chromosome distribution and therefore presents a real risk of carcinogenicity (16,17). Titanium ions may induce a decrease in the number and activity of osteoblasts, macrophages and leukocytes (12), hampering osteogenesis.

The oral environment is ideal for the biodegradation of metals due to its thermal, microbiological and enzymatic properties (18). Thus, it is uncertain whether these alloys, which are used in miniscrews, produce corrosion debris as a result of wear and whether the debris is cytotoxic to bone (9). It is well known that metals released from orthodontic appliances, metal restorations and metal prostheses can cause potentially toxic effects on tissues, but it is unknown whether metals released from miniscrews used as orthodontic anchorage are potentially toxic.

Therefore, the aim of the present study was to examine and compare the levels of several metal ions released in the saliva of patients with orthodontic appliances and with miniscrews as orthodontic anchorage.

Methods

This study was approved by The Committee of Ethics and Research of the Pontifical Catholic University of Rio Grande do Sul (PUCRS/ Brazil), in accordance with national and international norms of research in human beings (registration number = 09/04788).

Sample

A total of 20 patients (12 females and 8 males) were included in the study. The mean age of the sample was 21.4 years old (range: 16 to 32 years old) and these patients were selected from a private clinic. None of the patients were smokers, had pre-existing systemic diseases or were under any pharmacological treatment.

All patients were treated by the same orthodontist (M.B.) and were within the 6^{th} and 8^{th} month of treatment. The fixed orthodontic appliance consisted of: 8 bands and 20 bonded brackets. None of the patients had palatal or lingual appliances weIded to the bands or any extraoral orthodontic appliances. None of the patients had any amalgam fillings or metallic restorations.

A preadjusted straight-wire appliance was used in all cases (10.10.971 reference, special roth brackets set with hook on the canines and premolars 0,56 x 0,76 mm – Morelli, Sorocaba, SP, Brazil). Both the permanent maxillary and mandibular molars were banded (Morelli, Sorocaba, SP, Brazil). First maxillary molar bands had triple buccal tubes with hooks, whereas first mandibular molar bands had double bucal tubes with hooks. Second maxillary and mandibular molar bands had single buccal tubes. Metallic brackets were directly bonded on incisors, canines, and premolars. A 0.018-inch or 0.020-inch stainless steel archwire was placed on both arches (Morelli, Sorocaba/SP, Brazil) and tied with elastics (Morelli, Sorocaba, SP, Brazil).

All patients were classified as class II malocclusion with left or right subdivision, that is, they possessed malocclusion Class I on one side and class II on the counter-lateral side. These patients, therefore, needed correction for superior arch asymmetry, which was achieved through molar distalization with the use of a miniscrew as orthodontic anchorage in the superior arch, placed interdentally between the roots of the superior first and second pre-molars. The miniscrews were placed by the same oral and maxillofacial surgeon (D.B.) with the same surgical technique.

All miniscrews remained stable as an anchorage unit for the appliance during the orthodontic treatment. Before the treatment, all patients gave signed informed consent (in accordance with bioethics norms) to the treatment plan, which consisted of implantation of one miniscrew in the superior arch. Those patients that chose not to accept the treatment with miniscrew were excluded from the study without any disruption to their treatment.

Mini-screw installation

A surgical guide made of orthodontic wire was used in all patients to verify the receptor site. Periapical radiographs were made before miniscrew implantation, in order to verify the miniscrew site without damaging

the teeth or anatomic structures.

Miniscrews were installed under local anesthesia of the soft tissues at the implant receptor site. The entire procedure was carried out under sterile conditions. The miniscrew was inserted with a manual handpiece screwdriver (Sin Implant Systems, São Paulo, SP, Brazil) and considered immobile and stable at the moment of placement. Self-tapping miniscrews with a total length of 10 mm, screw head of 3 mm and 1.2 mm of diameter were obtained from Sin Implant Systems (Sin Implant Systems, São Paulo, SP, Brazil).

After installation, a periapical radiograph was taken to evaluate the position of the miniscrew. After the surgical procedure, oral hygiene with an extra-soft toothbrush and the use of a 0.12% chlorhexidine mouth rinse were prescribed. No other medications were prescribed. The miniscrews were used in the maxilla and were loaded two weeks after placement.

Collection and processing of saliva

Samples of stimulated saliva were collected by the following method: the patient thoroughly rinsed the mouth with deionized water for 1 minute (Dermapelle, Santa Maria, RS, Brazil). Next, the patient spit non-stimulated salivary secretion at different time points. Approximately 10 mL of saliva were collected into a sterile glass tube. After collection, the samples were stored at -20°C in a freezer.

For metal determinations, saliva samples (1 mL) were digested in a hot water bath (80°C, 1 hour) with 1 mL of 14 mol L^{-1} nitric acid (Merck, Darmstadt, Germany) and 0.5 mL of 30% (v/v) hydrogen peroxide (Synth, Diadema, SP, Brazil) in polypropylene tubes (Sarstedt, Nümbrecht, Germany). After digestion, samples were diluted to 5 mL with ultra pure water and centrifuged (Nova Técnica, Piracicaba, SP, Brazil) at 3,000 rpm for 4 minutes prior to analysis.

Estimation of metal ions released

Metal content in digested saliva samples was determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). Co, Cr, Ni, and V were determined by ICP-MS using an inductively coupled plasma mass spectrometer (PerkinElmer-SCIEX, model Elan DRC II, Thornhill, Canada), equipped with a concentric nebulizer, a cyclonic spray chamber and a quartz torch with a quartz injector tube (2 mm i.d.). Instrumental performance optimization, including nebulizer gas flow rate, ion lens voltage, and torch alignment, was carried out according to manufacturer instructions (Perkin-Elmer-Sciex, Elan version 3.0, Software guide, 1006920 A, 2003, Thornhill, Canada). An inductively coupled plasma optical emission spectrometer (ICP-OES – Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany with an axial view configuration) was used for Al, Zn, Cu, Fe and Ti determinations. Nebulization was performed through a crossflow nebulizer coupled to a Scott double pass type nebulization chamber. Plasma operating conditions and selected wavelengths used, and they were used as recommended by the instrument manufacturer (Spectro Ciros CCD, Software version 01/March 2003, Spectro Analytical Instruments, Kleve, Germany). For ICP-MS and ICP-OES determinations, argon 99.996% (White Martins-Praxair, Sao Paulo, Brazil) was used for plasma generation, for nebulization, and as the auxiliary gas.

Statistical analysis

Data were analyzed with descriptive statistics and normality test (Shapiro-Wilk). Comparisons of salivary element concentrations among different times of mini-implant treatment were analyzed by Wilcoxon paired tests (α=5%). The software *Statistical Package for Social Sciences* (SPSS, Chicago, Illinois, USA) was used to perform the statistical analysis.

Results

Results from the analysis of the concentration of Al, Ti, Cr, Ni, Fe, Cu, Co, Zn and V ions released at different times of miniscrew .

After 10 minutes of exposure to the treatment, it can be observed that there was an increase in the salivary concentration of the ions: Fe, Ti, V, Cr and Zn and a decrease in: Al, Co, Cu, and Ni.

At 7 days after miniscrew insertion, there was a quantitative increase of Al, Co, Cr, Cu, Ni, Ti and V.

At 30 days after insertion, there was a quantitative increase in the salivary concentration (μg/L) of: Cu, Ti, V, Zn and a quantitative decrease in the salivary concentration of: Al, Co, Cr, Fe, Ni, when compared with concentrations found before insertion. However, there were no statistically significant differences in the salivary ion concentrations at any of the times studied

Discussion

This study investigates the release of metal ions from fixed orthodontic appliances, particularly with the use of miniscrews as orthodontic anchorage.

The main advantage of the present in vivo study is that the concentrations of salivary metals ions were recorded in the natural oral environment of the patient where actual adverse effects of increased metal concentrations take place. So, this study was carried out to investigate the metal ion concentrations in saliva of patients with fixed orthodontic appliances (20 brackets, 8 bands and wires) and one miniscrew.

The average number of brackets used in a study depends upon whether the patients are treated using an extraction or nonextraction approach and whether the brackets are only placed on a single arch or on both arches. In this study the patients were treated without extraction of pre-molars, brackets are placed on both arches, and the fixed orthodontic appliances were produced by the same manufacturer, in order to avoid additional variables in the study.

Thus, a systemic toxic effect from orthodontic appliances is highly unlikely. However, even such small quantities of metal ions can cause allergic reactions, especially because fixed orthodontic appliances remain in the oral cavity for a long period of time (2 to 3 years approximately). For an allergic reaction to occur in the oral mucous membrane, the antigenic potential has to be 5 to 12 times stronger than that on the skin surface. However, various clinical manifestations of hypersensitive reactions to fixed orthodontic appliances have been reported (19,20). Moreover, it was reported that nickel ions released from dental alloys can accumulate in the cells over time, and this may have multiple harmful effects on cells (21). A number of studies have been carried out on the biocompatibility of orthodontic materials, with the aim of determining a limit of biological tolerance and assessing whether the ions released from such materials are within these limits.

In relation to the Ti-6Al-4V alloy components from miniscrew (Ti, Al and V), it was found that Ti was increased at all time points (T1/T2/T3/T4); Al was decreased from T1 to T2, increased from T2 to T3 and decreased from T3 to T4; V increased from T1 to T2 and from T2 to T3, while from T3 to T4 it decreased, though these differences were not statistically significant. While there were no statistically significant differences, it is important to note that the quantitative increase for Ti was observed at all time points, which may be explained by the fact that it is the element with the greatest concentration in the alloy and because of the formation of a superficial titanium oxide layer.

Al and V presented a slight increase at 7 days after miniscrew insertion, which may be due to the fact that V is not present in the superficial oxide layer of Ti-6Al-4V (10) and Ti and Al are the metal ions most likely to be released from the Ti-6Al-4V surface (12). There was a higher Ni value in T1 than T2, T3 and T4. However it cannot be affirmed that this value was due to the release of Ni from the orthodontic appliance, as it had been inside the mouth for a period of approximately 6 to 8 months. Nor could it be attributed to the orthodontic wire, as all patients had stainless steel wires.

There was a minimal difference in the V concentration at the different time points measured, which would not incur in an alarming situation, especially because the remain in the intraoral environment for a limited time. Our results are in accordance with those found by Morais et al., 2007 (9), who detected varying quantities of Ti, Al and V, proving that metal ions were released by Ti-6Al-4V orthodontic miniscrews. However, the authors observed extremely low quantities of these metal ions. Based on the findings of this study, it can by asserted that the quantity of metal ions release is not proportional to the metal content in the alloy, which corroborates with the findings of other studies (19,20).

The method of saliva sampling, processing, and analysis also adds to the variability of the results. Several methods have been suggested to collect resting and stimulated whole saliva (22,23). Most commonly, saliva is collected by draining or spitting into a tube (23). This was done in our study as in previous studies (24). Standardization of saliva collections is important when saliva is used as research material, since saliva composition varies greatly both intra- and inter-individually (18). Although the ICP-MS and ICP-OES used in this study is a time-consuming and expensive technique, it is highly sensitive, accurate and capable of the determination of a range of metals and several non-metals at concentrations below one part in 10 (12).

Saliva contains acids arising from the degradation and decomposition of food, which increases the corrosion potential of stainless steel and Ti-6Al-4V. In the oral cavity, the bracket-archwire ligation induces "fretting corrosion" of metal surfaces of both the bracket and archwire, as both are moving elements. The presence of complex intraoral flora and accumulation of plaque and its byproducts also add to this variation (18). Moreover, according to Edgar and O' Mullane (25), hormones, drugs, and various diseases also influence saliva composition. In our study, we aimed to exclude patients who presented any systemic disturbance in order to reduce the number of variables that could alter the results.

In summary, metals are present in the saliva of patients with metal brackets, bands and miniscrews depending on a number of variables. The metal contents differed, though not significantly, between consecutive samples per individual. No data are available for a safe limit to metal exposure in saliva. More studies are necessary, especially to investigate the amount of metal ions release when two or more miniscrews are utilized as orthodontic anchorage

Conclusions

Based on the findings of this study, it can be concluded that:

1. Orthodontic appliances and miniscrews released metal ions, but in a quantity not proportional to the metal concentration in these materials.

2. There was no significant difference in the metal ion concentration among the different time points after miniscrew placement.

3. The placement of miniscrew leads to an increase of salivary titanium ion concentrations that is not statistically significant at all periods of saliva collection.

References

1. Morais LS, Serra GS, Muller CA, Palermo EFA, Andrade LR, Meyers MA, Elias CN. In vivo metal ion release from Ti-6Al-4V orthodontic mini-implants. Rev Mater 2007;12:290-7. [Links]

2. Ichinose S, Muneta, T, Sekiya I, Itoh S, Aoki H, Tagami M. The study of metal ion release and citotoxicity in Co-Cr-Mo and Ti-Al-V alloy in total knee prosthesis-scanning electron microscopic observation. J Mat Science: Materials in Medicine 2003;14:79-86. [Links]

3. Metikos-Hukovic M, Kwokal A, Piljac J. The influence of niobium and vanadium on passivity of titanium-based implants in physiological solution. Biomaterials 2003;24:3765-75. [Links]

4. Evans EJ. Cell damage in vitro following direct contact with fine particles of titanium, titanium alloy and cobalt-chrome-molybdenum alloy. Biomaterials 1994;15:713-7. [Links]

5. Haynes DR, Rogers SD, Hay S, Pearcy MJ, Howie DW. The differences in toxicity and release of bone-resorbing mediators induced by titanium and cobalt-chromium-alloy wear particles. J Bone Joint Surgery 1993;75:825-34. [Links]

6. Jacobs JJ, Skipor AK, Black J, Urban RM, Galante JO. Release and excretion of metal in patients who have a total hip-replacement component made of titanium-base alloy. J Bone Joint Surg 1991;73A:1475-86. [Links]

7. Dobbs HS, Minski MJ. Metal ion release after total hip replacement. Biomaterials 1980;1:193-8. [Links]

8. Urban RM, Jacobs JJ, Gilbert JL, Galante JO. Migration of corrosion products from modular hip prostheses. Particle microanalysis and histopathological findings. Ibid 1994; 74:1345-59. [Links]

9. Morais LS, Serra GG, Muller CA, Andrade LR, Palermo EF, Elias CN, Meyers M. Titanium alloy mini-implants for orthodontic anchorage: immediate loading and metal ion release. Acta Biomater 2007;3:331-9. [Links]

10. Hanawa T. Metal ion release from metal implants. Mat Sci Eng C 2004;24:745-52. [Links]

11. Goldberg JR, Gilbert JL. The electrochemical and mechanical behavior of passivated and TiN/AlN-coated CoCrMo and Ti6Al4V alloys. Biomat 2004;25:851-64. [Links]

12. Saldaña L, Barranco V, Garcia-Alonso MC, Vallés G, Escudero ML, Munuera L et al. Concentration-dependet effects of titanium and aluminum ions released from termally oxidized Ti6Al4V alloy on human osteoblasts. J Biom Mat Res 2006;77:220-9. [Links]

13. Latysh V, Krallics G, Alexandrov I, Fodor A. Application of bulk nanostructured materials in medicine. Current Appl Phys 2006;6:262-6. [Links]

14. Sedarat C, Harmand MF, Naji A, Nowzari H. In vivo kinetic evaluation of titanium alloy biodegradation. J Period Res 2001;36:269-74. [Links]

15. Liu P, Yao YN, Wu SD, Dong HJ, Feng GC, Yuan XY. The efficacy of deferiprone on tissues aluminum removal and copper, zinc, manganese level in rabbits. J Inorg Bioch 2005;99:1733-7. [Links]

16. Heinemann G, Fichtl B, Vogt W. Pharmacokinetics of vanadium in humans after intravenous administration of a vanadium containing albumin solution. Brit J Clin Pharmacol 2003;55:241-45. [Links]

17. Barceloux DG. Vanadium. J Toxicol Clin Toxicol 1999;37:265-78. [Links]

18. Schipper RG, Silletti E, Vingerhoeds MH. Saliva as research material: biochemical, physicochemical and practical aspects. Arch Oral Biology 2007;52:113-115. [Links]

19. Hwang CJ, Shin JS, Cha JY. Metal release from simulated fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2001;120:383-91. [Links]

20. Grimsdottir MR, Gjerdet NR, Hensten-Pettersen A. Composition and in vitro corrosion of orthodontic appliances. Am J Orthod Dentofacial Orthop 1992;101:525-32. [Links]

21. Hamano H. Fundamental studies on biological effects of dental metals: nickel dissolution, toxicity and distributions in cultured cells. J Stomatol Soc 1991;59:456-78. [Links]

22. Kocadereli l, Atac PA, Kale PS, Ozer D. Salivary nickel and chromiurn in patients with fixed orthodontic appliances. Angle Orthod 2000;70:431-4. [Links]

23. Eliades T, Athanasiou AE. In vivo aging of orthodontic alloys:Implications for corrosion potential, nickel release, and biocompatibility. Angle Orthod 2002;72:222-37. [Links]

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Artigo 3

Rev. odonto ciênc. (Online) vol.25 no.3 Porto Alegre July/Sept. 2010

ORIGINAL ARTICLE

Patient's perception on mini-screws used for molar distalization

ABSTRACT

PURPOSE: The objective of this study was to evaluate and compare the perceived pain intensity, side effects and discomfort related to the moment of placement, during mechanics and removal of a mini-screw for molar distalization in orthodontic treatment. **METHODS:** The sample consisted of 30 adult patients with a mean age of 30 years old, with class II malocclusion subdivision right or left. A mini-screw was installed in each patient, in the maxillary arch to provide a molar distalization. The patients answered a questionnaire to assess their opinions on the treatment. **RESULTS:** Ninety percent of the patients chose mini-screws over pre-molar extraction (orthodontic camouflage), or the use of an extra-oral appliance (Kloehn cervical traction) or another non-compliance treatment to class II. Aphthous ulcer was the side effect most frequent after placement of the mini-screw (30%). The greatest discomfort was felt during infiltration anesthesia (27%), though 23% reported no discomfort during placement. Eighty-three percent of the patients reported no pain during placement, which may be associated with the degree of satisfaction with the treatment (100%) and recommendation of the procedure to other patients (100%). **CONCLUSION:** Mini-screws were well accepted by the patients and were efficient for molar distalization when utilized in association with orthodontic treatment for Class II malocclusion.

Key words: Orthodontics; Angle Class II malocclusion; orthodontic anchorage procedure

Introduction

The use of mini-screws to obtain absolute anchorage has recently become very popular in clinical orthodontic approaches (1). Mini-screws can be used either as direct anchorage units, when clinical forces are applied to the mini-screw, or as indirect anchorage units, when the forces are applied to dental units that are stabilized by the mini-screw (2).

During the past few years, the application of mini-screws include a wide array of cases, including: the correction of deep overbites (3-5), closure of extraction spaces (6,7) correction of canted occlusal plane (4), extrusion and uprighting of impacted molars (8,9), molar mesialization (10,11), alignment of dental midlines (4), extrusion of impacted canines (12,13), molar intrusion (14,15), intermaxillary anchorage for the correction of sagittal discrepancies (12,16,17), en-masse retraction of anterior teeth (17), maxillary molar distalization (9,16), and correction of vertical skeletal discrepancies that would otherwise require orthognathic surgical procedure (18,19).

For class II malocclusion assimetries, the mini-screw is an ideal dispositive to assist the orthodontic treatment because the professional thus does not depend on patient collaboration in the use of orthodontic rubber bands, or of an appliance of Kloehn cervical traction, for example, in order to achieve successful treatment (1). In addition, these implements make it possible to avoid pre-molar extractions in the case of Class II camouflage and

laboratory procedures in the case of non-compliance alternatives treatment (*e.g.*, distal jet).

These temporary accessories for orthodontic anchorage offer a number of advantages, including: easy placement and removal which does not require any particular surgical procedure, low cost, small size, variety of locations that can be inserted, patient collaboration is limited to maintaining good oral hygiene, and immediate orthodontic force/pressure reducing the total treatment time (2,6,20,21). The literature on mini-screw implants is diverse, however, the majority of studies is limited to the clinical application, success rate, properties, osseointegration and loading. Nonetheless, their use is still questioned in terms of apprehension, intolerance and psychological factors involved with this procedure (22).

This study aimed to verify the patient's perception on mini-screws for molar distalization during orthodontic treatment as well as the pain felt by patients during placement and the occurrence of unforeseen events during their utilization as orthodontic anchorage. In addition, this study assessed the patient's opinion on the treatment procedure.

Methodology

This study was approved by the Committee of Ethics and Research of the Pontifical Catholic University of Rio Grande do Sul (PUCRS/ Brazil), in accordance with the national and international standards for research in human beings.

Thirty consecutive patients who were undergoing ortho- dontic treatment between 6 to 8 months were included in the sample. All of them had class II malocclusion with left or right subdivision, that is, they had malocclusion Class I on one side and Class II on the counter-lateral side. These patients, therefore, needed correction for maxillary arch asymmetry, which was achieved through molar distalization with the use of mini-screw as orthodontic anchorage in the maxillary arch, interdentally between the roots of the first molar and second pre-molars.

Patients were undergoing orthodontic treatment in a private clinic. All patients were treated by the same orthodontist (M.B.) and mini-screws were placed by the same oral and maxillofacial surgeon (D.B.) with the same surgical technique. Before the treatment, all patients signed an informed consent form regarding the research protocol and the treatment plan, which consisted of implantation of one mini-screw in the maxillary arch. Those patients that chose not to accept the treatment with mini-screw and chose another treatment to correct the malocclusion were excluded from the study without any disruption to their treatment.

A surgical guide made of orthodontic wire was used in all patients to verify the receptor site (Fig. 1). Periapical radiographs were made before the mini-screw implantation in order to verify the mini-screw site without damage to the teeth or anatomic structures.

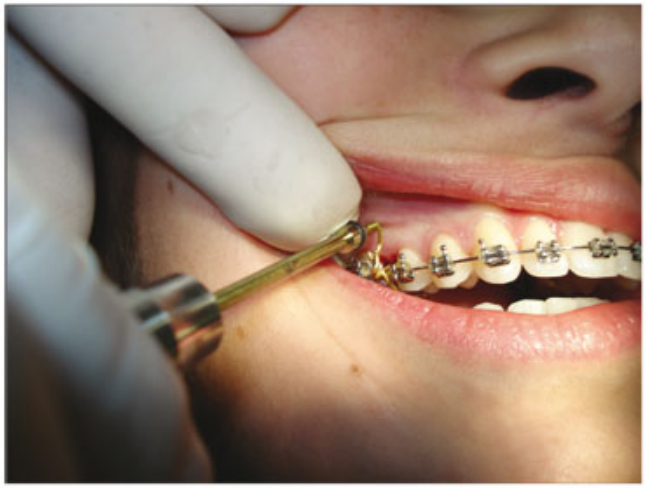

Fig. 1. Location of the self-tapping mini-screw implantation using a surgical guide.

Mini-screws were installed under local anesthesia of the soft tissues at the implant receptor site. The entire procedure was carried out under sterile conditions. The mini-screw was inserted with a manual handpiece screwdriver (Sin Implant Systems, São Paulo,SP, Brazil) and considered immobile and stable at the moment of placement. Self-tapping mini-screws with total length of 10 mm, screw head of 3 mm and 1.2 mm of diameter, were obtained from Sin Implant Systems (Sin Implant Systems, São Paulo, SP, Brazil). After installation, a periapical radiograph was taken to evaluate the position of the mini-screw. After the surgical procedure, oral hygiene with an extra-soft toothbrush and the use of a 0.12% chlorhexidine mouth rinse were prescribed. No medications were prescribed.

The mini-screws were used in the maxilla and were loaded two weeks after placement. The force applied to the mini-screw with the sliding jig mechanics was on average 300 g (Fig. 2). No medication or anesthesia was used for removal of the mini-screws. The mini-screw was grasped using a handpiece screwdriver and removed by anticlockwise rotation, with no lateral movement.

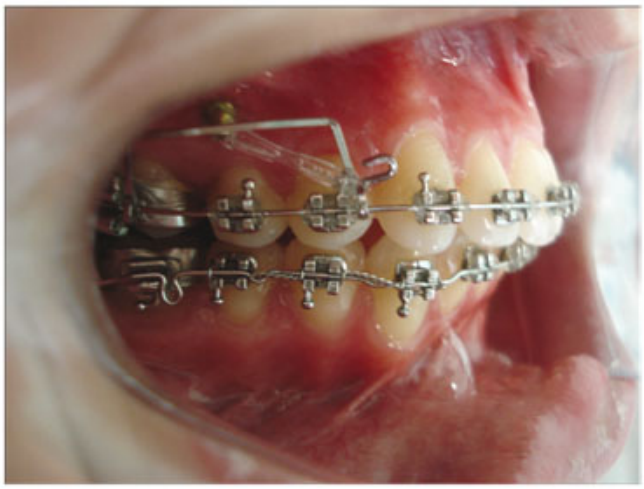

Fig. 2. Mini-screw with the sliding jig mechanics for molar distalization.

All patients responded to a questionnaire (Tables 1, 2 and 3) at three different time points: immediately after the mini-screw placement (T1), 30 days after placement (T2) and immediately after the mini-screw removal (T3). The questionnaire contained questions about patient acceptance including psychological aspects, degree of acceptance, pain, discomfort, tolerance and side effects.

Table 1. Results of the first questionnaire given to patients immediately after the placement of mini-screw.

Immediately after placement of the mini-screw:
1. Which treatment option would you choose for your treatment plan if you had bilateral class II malocclusion? a) An extra-oral appliance – 3% b) 2 mini-screws – 90% c) 2 extraction sites (pre-molars) – 3% d) distal jet, pendulum, jasper jumper or another non-compliance treatment – 4%
2. Have you ever experienced any type of extraction procedure? a) Yes – 83% b) No – 17%
3. Have you ever experienced any type of restoration procedure? a) Yes – 67% b) No – 33%
4. To what would you compare the pain felt during placement of the mini-screw? a) To a restoration – 6% b) An extraction – 17% c) Other dental procedure – 77%
5. What was the degree of pain felt during the placement of the mini-screw? a) Slight pain – 10% b) Moderate pain – 7% c) Severe pain – 0% d) No pain – 83%

Table 2. Results of the second questionnaire given to patients 30 days after the mini-screw placement.

At 30 days after the mini-screw placement:
6. Which side effects did you experience after mini-screw placement? a) Aphthous ulcer – 30% b) Restricted mouth opening – 0% c) Irritation inside mouth –10% d) Gingival inflammation – 10% e) Difficulty chewing – 3% f) No discomfort – 47%
7. What made you feel the most uncomfortable after the mini-screw placement? a) The mini-screw guide jig –10% b) The initial orthodontic pressure – 17% c) The anesthesia for placement – 27% d) The pressure of the mini-screw placement – 23% e) No discomfort – 23%
8. Was cleaning the mini-screw difficult? Yes – 10% No – 90%
9. How much time do you think is acceptable for the mini-screws to remain in the mouth? a) 1-3 months – 10% b) 3-6 months – 10% c) 6-9 months – 20% d) Other – 60%
10. Would you recommend this procedure to other patients? a) Yes – 100% b) No – 0 %
11. Are you satisfied with the treatment so far? a) Yes – 100% b) No – 0%

Table 3. Results of the third questionnaire given to patients immediately after the removal of the mini-screw.

Immediately after the removal of the mini-screw:
12. What was the degree of pain felt during the removal of the mini-screw? a) Slight pain – 17% b) Moderate pain – 0% c) Severe pain – 0% d) No pain – 83%

Results

Thirty patients between 21 and 39 years of age (mean age = 30 years), 19 females and 11 males, were treated. A total of 30 mini-screws was installed. The mini-screws were removed after molar distalization (Table 1, 2 and 3) after 6 to 9 months (mean time = 7.5 months).

There were no failures, *i.e.*, all mini-screws remained stable as an anchorage unit for the appliance during the orthodontic treatment.

Discussion

The questionnaire used in this study was useful to verify some aspects related to patient adaptation, side effects and degree of patient sensitivity at different times of the proposed treatment. The results can be used as a reference for future interventions, planning and questionnaires about experiences with these temporary accessories for orthodontic anchorage, especially in the treatment of Class II malocclusion.

As for the choice of orthodontic treatment for the correction of malocclusion (bilateral class II malocclusion), 90% of the patients selected the placement of 2 mini-screws rather than extraction of 2 pre-molars (3%), the use of an extra-oral appliance (3%) or another non-compliance treatment as distal jet, pendulum or jasper jumper (4%). This corroborates that the mini-screw is a practical appliance as patient cooperation is not necessary for its utilization, and in most cases it allows for treatment without extractions and results in successful treatment of the patient's type of malocclusion (22).

The patients were questioned about previous extractions and restorations in order to determine the number of patients that had undergone dental procedures for which they retained a memory of pain. Of the total sample, 83% had undergone some type of extraction, and 67% had had some type of dental restoration. These dental procedures are more common to happen in an dental office.

Pain affects quality of life and treatment cooperation (23), and the feeling of pain is a subjective parameter (24). When asked about pain felt during placement of the mini-screw, 77% of the patients were unable to compare it to pain from extraction or restoration, and 25% of those reported pain similar to that felt during anesthesia, 12.5% reported fear, 12.5% reported dental pressure on the first day after placement and 50% reported "nothing to compare". Although these alternatives were not included in the questionnaire, these patient reports are important as many expressed the same feeling about mini-screw placement. The remaining 17% reported pain similar to that felt during restoration, and 7% reported pain similar to that of extraction.

Despite the attempt to compare the pain felt to some previous dental procedure, 83% of the patients classified mini-screw placement as "without pain". This confirms the previous findings by Cornelis et al. (24), who reported that 82% of their patients said that the surgical experience was better than expected, with little or no pain. However, the perception of pain intensity is subjective and influenced by many other factors such as anxiety levels and motivational attitude (25).

Thirty days after the procedure, the patients were questioned about side effects and 30% reported aphthous ulcers, 20% gingival inflammation, 3% difficulty chewing and 47% reported no side effects. This is in accordance with the study by Tseng et al. (1), who reported that 40% of patients had no side effects (inflammation or ulcers) after placement of mini-screws.

The greatest discomfort related to the technique was the application of anesthesia during placement of the mini-screw (27%), followed by discomfort during mini-screw placement (pressure) (23%), the initial orthodontic force applied by the mini-screw (17%), and the mini-screw guide jig (10%). Nevertheless, 23% reported no discomfort whatsoever associated with the treatment alternative. However, all patients reported that while they attempted to choose the most suitable question alternative, they did not feel discomfort that would lead them to remove the mini-screw.

Forces of 300 g were reported for distalization maxillary molars with a lever-arm (4). In our study, the forces of 300 g (measured with a Richmond gauge) were used for molar distalization and did not cause much discomfort to the patient (17%). In general, the mini-screws were well tolerated by the patients (1,25). No patient requested to have the mini-screw removed and after 30 days, they did not mind having the mini-screw in place (60%). Twenty percent expressed the desire to have the mini-screw removed after 6-9 months, 10% after 3-6 months and 10% after 1-3 months. These patients expressed mildly negative comments such as "no one likes to have a foreign body in their mouth".

Almost all of the individuals had no difficulty in cleaning the mini-screw (90%), would recommend the procedure to a friend (100%) and were satisfied with the treatment (100%). No medication or anesthesia was used for the mini-screw removal. However, 83% of the patients felt no pain during removal, indicating no need for medication or anesthesia, as the application of anesthesia would cause a greater sensation of pain than the mini-screw removal itself.

Conclusions

Based on the present results, it can be concluded that:

2. 90% of the patients prefer the utilization of mini-screws rather than premolar extraction (orthodontic camouflage), the use of an extra-oral appliance (Kloehn cervical traction) or another non-compliance treatment (distal jet, jasper jumper or pendulum);

3. For the side effects felt after mini-screw placement, aphthous ulcer was the most frequent followed by gingival inflammation. More than 40% of the patients reported no side effects.

4. The greatest discomfort felt during placement was that of infiltration anesthesia followed by the pressure during mini-screw placement.

5. The majority of the patients reported no pain during mini-screw placement or removal, which may be associated with the degree of satisfaction with the treatment and the willingness to recommend the procedure to a friend.

6. Mini-screws were well accepted by the patients.

References

1. Papadoulos MA, Tarawneh BDS. The use of mini-screw implants for temporary skeletal anchorage in orthodontics: A comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:e6-e15. [Links]

2. Kanomi R. Mini-implant for orthodontic anchorage. J Clin Orthod 1997:31;763-7. [Links]

3. Creekmore TD, Eklund MK. The possibility of skeletal anchorage. J Clin Orthod 1983;17:266-9. [Links]

4. Carano A, Velo S, Leone P, Siciliani G. Clinical applications of the mini-screw anchorage system. J Clin Orthod 2005;39:9-24. [Links]

5. Ohnishi H, Yagi T, Yasuda Y, Takada K. A mini-implant for orthodontic anchorage in a deep overbite case. Angle Orthod 2005;75:444-52. [Links]

6. Park H, Bae S, Kyung H, Sung J. Micro-implant anchorage for treatment of skeletal Class I bialveolar protrusion. J Clin Orthod 2001;35:417-28. [Links]

7. Yun S, Lim W, Chun Y. Molar control using indirect mini-screw anchorage. J Clin Orthod 2005;39:661-4. [Links]

8. Park H, Kyung H, Sung J. A simple method of molar uprighting with micro-implant anchorage. J Clin Orthod 2002;36:592-6. [Links]

9. Bae S, Park H, Kyung H. Clinical application of micro-implant anchorage. J Clin Orthod 2002;36:298-302. [Links]

10. Giancotti A, Greco M, Mampieri G, Arcuri C. The use of titanium mini-screws for molar protraction in extraction sites. J Clin Orthod 2003;37:575-9. [Links]

11. Kyung HM, Park HS, Bae SM, Sung JH, Kim IB. Development of orthodontic microimplants of intraoral anchorage. J Clin Orthod 2003;37:321-8. [Links]

12. Papadoulos MA. Overview of the intra-maxillary non-compliance appliances with absolute anchorage. In: Papadoulos MA (ed.). Orthodontic treatment for the Class II non-compliant patient: current principles and techniques. Edinburgh: Elsevier, Mowby; 2006. p.341-4. [Links]

13. Park HS, Kwon OW, Sung JH. Micro-implant anchorage for forced eruption of impacted canines. J Clin Orthod 2004;38:297-302. [Links]

14. Bae SM, Kyung HM. Mandibular molar intrusion with mini-screw anchorage. J Clin Orthod 2006;40:107-8. [Links]

15. Yao CC, Lee JJ, Chen HY, Chang ZC, Chan Hf, Chen YJ. Maxillary molar intrusion with fixed appliances and mini-implant anchorage studied in three dimensions. Angle Orthod 2005; 75:754-60. [Links]

16. Chung K, Kim SH, Kook YC. Orthodontic microimplant for distalization of mandibular dentition in class III correction. Angle Orthod 2005;75:119-28. [Links]

17. Kyung SH, Choi JH Park YC. Mini-screw anchorage used to protract lower second molars into first molar extraction sites. J Clin Orthod 2003;37:575-9. [Links]

18. Kuroda S, Katayama A, Takano-Yamamoto T. Severe anterior open- bite case treated using titanium screw anchorage. Angle Orthod 2004;74:558-67. [Links]

19. Park HS, Kwon TG, Kwon OW. Treatment of open bite with microscrew implant anchorage. Am J Orthod Dentofacial Orthop 2004;126:627-36. [Links]

20. Kuroda S, Sugawara Y, Deguchi T, Kyung HM, Takano-Yamamoto T. Clinical use of mini-screw implants as orthodontic anchorage: Success rates and postoperative discomfort. Am J Orthod Dentofacial Orthop 2007:131; 9-15. [Links]

21. Liou EJ, Pai BC, Lin JC. Do mini-screws remain stationary under orthodontic forces? Am J Orthod Dentofacial Orthop 2004:126;42-7. [Links]

22. Feldmann I, List T, Feldmann H, Bondemark L. Pain intensity and discomfort following surgical placement of orthodontic anchoring units and premolar extraction. Angle Orthodont 2007:77; 578-85. [Links]

23. Krukemeyer AM, Arruda AO, Inglehart MR. Pain and orthodontic treatment. Angle Orthod 2009;79:1175-81. [Links]

24. Cornelis MA, Scheffler NR, Nyssen-Behets C, De Clerk Hj, Tulloch JFC. Patient's and orthodontist's perceptions of miniplates used for temporary skeletal anchorage: A prospective study. Am J Orthod Dentofacial Orthop 2008;133:18-24. [Links]

25. Justens E, De Bruyn H. Clinical outcome of mini-screws used as orthodontic anchorage. Clin Impl Dent Relat Res 2008;10: 174-80. [Links]

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Received: Jan 20, 2010 Accepted: April 4, 2010

Anexo III
Carta de Submisão do Artigo :

Elsevier Editorial System(tm) for International Journal of Oral & Maxillofacial Surgery
Manuscript Draft

Manuscript Number: ***IJOMS-D-12-00067***

Title: ***Effects of low-level laser therapy on distraction osteogenesis: a histological analysis***

Article Type: Research Paper

Keywords: Osteogenesis, Distraction, Lasers, Histology.

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