UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS ODONTOLÓGICAS

Patrícia Cabral Passos

BIOCOMPATIBILIDADE DE SCAFFOLDS NANOFIBROSOS CONTENDO METRONIDAZOL OU CIPROFLOXACINA EM MODELO DE IMPLANTAÇÃO SUBCUTÂNEA EM RATOS

Santa Maria, RS
2016

Patrícia Cabral Passos

BIOCOMPATIBILIDADE DE SCAFFOLDS NANOFIBROSOS CONTENDO METRONIDAZOL OU CIPROFLOXACINA EM MODELO DE IMPLANTAÇÃO SUBCUTÂNEA EM RATOS

Projeto de Dissertação apresentado ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**.

Orientadora: Profa. Dra. Karla Zanini Kantorski

Santa Maria, RS

2016

Patrícia Cabral Passos

BIOCOMPATIBILIDADE DE SCAFFOLDS NANOFIBROSOS CONTENDO METRONIDAZOL OU CIPROFLOXACINA EM MODELO DE IMPLANTAÇÃO SUBCUTÂNEA EM RATOS

Projeto de Dissertação apresentado ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Periodontia da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**.

Aprovado em 29 de julho de 2016:

_____

Karla Zanini Kantorski, Dra. (UFSM)
(Presidente da Banca/Orientadora)

_____

Patrícia D. Melchiors Angst, Dra. (UNIFRA)

_____

Fabrício Batistin Zanatta, Dr. (UFSM)

Santa Maria, RS
2016

DEDICATÓRIA

*Aos meus pais, **José Solano** e **Iraci**, e aos meus irmãos, **Diorgnis** e **Patrique**, os quais são aqueles que estiveram ao meu lado incentivando e apoiando minha vida acadêmica. São minha inspiração, meu orgulho e o meu maior valor. A quem amo incondicionalmente.*

AGRADECIMENTOS

*Aos meus pais, **Iraci** e **José Solano**, por todo esforço, empenho, dedicação a mim e aos meus irmãos, Patrique e Diorgnis. Vocês são meus exemplos de amor, de coragem, de força. São aqueles que caminham ao meu lado, ensinam valores, incentivam minhas metas. Algum dia espero ser 1/3 do que são e retribuir as abdicações de sonhos que tiveram para pudéssemos alcançar o que almejamos. Muito obrigada por tornar tudo possível. Tenho orgulho de pertencer a essa família e ter vocês como pais.*

*Aos meus irmãos, **Diorgnis** e **Patrique**, pelos conselhos, pela proteção, pelo carinho, pelo companheirismo.*

*A todos os **demais familiares**, que são fundamentais para tornar minha vida mais alegre, me proporcionando momentos inigualáveis de completa ternura e afeto. Nossa família tem como sobrenomes risadas, amor e muitas histórias.*

*Á minha orientadora, **Karla Zanini Kantorski**, agradeço pelas oportunidades proporcionadas e ensinamentos que foram determinantes para meu crescimento profissional e intelectual. Professora, tu és exemplo de profissional e repetirei aqui o que já te falei pessoalmente, tenho certeza da escolha de orientadora que fiz e da tua competência. A ti devo minha escolha pela periodontia. Obrigada por ter confiado no meu trabalho, ter entregue a mim uma pesquisa desta grandeza e ter dedicado inúmeras horas para que este trabalho fosse possível. Agradeço também aos momentos de amizade, conversa e descontração. Espero ter correspondido tuas expectativas.*

*Ao professor **Marco Cícero Bottino**, a colaboração e parceria com esta pesquisa.*

*Ao professor **Fabricio Zanatta**, pelos ensinamentos, discussões e incentivo. É motivante ver o entusiasmo com que o senhor busca o seu aprimoramento como profissional, dos seus alunos e da disciplina. Assim como, a forma com o que o senhor levanta hipóteses, questiona e sugere mudanças. Levarei comigo as palavras de carinho e de apoio para que eu melhorasse meu empenho em apresentações em público.*

*Ao professor **Carlos Heitor,** pelos ensinamentos e discussões junto ao grupo de periodontia, sendo um dos responsáveis pelo meu crescimento intelectual. Sou admiradora do seu conhecimento na literatura clássica e contemporânea de diferentes linhas de pesquisa na periodontia.*

*À professora **Cristiane Danesi**, pela disponibilidade, conhecimentos e contribuição neste trabalho, bem como a sua dedicação para a concretização do LAPBIO que sem dúvidas contribuirá e facilitará pesquisas no Programa de Pós-graduação em Ciências Odontológicas (PPGCO).*

*Ao funcionário do LAPBIO**, Everton Pittaluga**, e ao veterinário, **Roberto Marinho**, pelo envolvimento e disponibilidade para com a realização deste trabalho.*

*Ao professor **João Marcelo Oliveira,** pela receptividade e disponibilidade no laboratório de Botânica Estrutural (LABOTE). Obrigado pelo conhecimento compartilhado e pela oportunidade de trabalhar em um ambiente completamente novo e enriquecedor. Sua dedicação com seus alunos e laboratórios é admirável.*

*Aos professores **Patrícia Angst** e **José Mariano da Rocha**, pelas considerações pontuais realizadas na qualificação desta dissertação. Suas contribuições foram de grande valia para a conclusão desta pesquisa.*

*À futura colega de profissão e doutoranda **Raquel Barcellos**, pela colaboração, ajuda e paciência para desvendar os mistérios oxidativos.*

*À mestranda **Juliana Moro,** pela parceria nesta pesquisa. Obrigada por compartilhar comigo momentos de descontração, risadas, angústias e dúvidas. Foi bom trabalhar com uma pessoa com o astral como o teu. Lembrarei com carinho das aventuras no LAPBIO e biotério.*

*Ao **grupo de epidemiologia**, em especial ao amigo **Bernardo Agostini** pela troca de conhecimentos.*

*À colega e amiga **Silvana Martins Rodrigues**, pelos momentos indescritíveis em que passamos juntas nessa etapa das nossas vidas. Sil levarei comigo todas nossas conversas, madrugadas em claro, compartilhamento de experiências, mas, principalmente o teu apoio*

incondicional. Tu és uma pessoa incrível e tenho certeza que esta parceria se estenderá por anos.

*Ao amigo **Danilo Dutra**, pela paciência, apoio, conversas, troca de conhecimento e discussões. Sabes do tamanho da minha admiração por ti, como profissional e ser humano. Tu foste um dos incentivadores para escolha da periodontia e da pesquisa. Tua amizade foi um dos presentes que a odontologia me proporcionou e espero seguir com as discussões, críticas e carinho por muito tempo ainda. Muito obrigada.*

*Aos irmãos de coração, **Lauren Bortoli**, **Lucas Barão**, **Nayana Rovedder**, **Piekalas** e "**verdadeiros**" pelo apoio, paciência, carinho, dedicação, parceria em diversos momentos, mas principalmente naqueles em que tudo parecia desmoronar. Mais uma vez repito, sorte a minha ter pessoas como vocês ao meu lado.*

*Aos coordenadores do PPGCO, professores **Thiago Ardenghi e Luis Felipe Valandro**, pela competência e dedicação ensinamentos e competência em prol do crescimento do nosso programa de pós-graduação.*

*Ao **curso de Odontologia da UFSM, bem como todos os professores, funcionários e servidores,** que direta ou indiretamente participaram de minha formação.*

*Á **CAPES**, pela bolsa de estudos concedida.*

Á todas as pessoas que de alguma forma ajudaram e participaram da realização dessa dissertação.

RESUMO

BIOCOMPATIBILIDADE DE SCAFFOLDS NANOFIBROSOS CONTENDO METRONIDAZOL OU CIPROFLOXACINA EM MODELO DE IMPLANTAÇÃO SUBCUTÂNEA EM RATOS

AUTORA: Patrícia Cabral Passos
ORIENTADORA: Karla Zanini Kantorski

Evidências mostram que não há membranas ideais, ou seja, biocompatíveis, biodegradáveis, com propriedades mecânicas e físicas adequadas que permitam a reestruturação dos tecidos periodontais. A técnica do eletrospinning tem demonstrado bons resultados no processamento de scaffolds confeccionados a partir de polímeros. As nanofibras formadas por esta técnica possuem características que se assemelham a matriz extracelular. O objetivo do presente estudo foi avaliar a biocompatibilidade de scaffolds nanofibrosos de polidioxonona (PDS II®) contendo metronidazol ou ciprofloxacina em modelo de implantação subcutânea em ratos Wistar. A PDS II® é um poliéster biocompatível com diversas aplicações na área médica. Nossa hipótese conceitual considerou que scaffolds com antimicrobianos teriam semelhante comportamento inflamatório quando comparados ao controle positivo PDS. Sessenta ratos machos adultos foram randomizados em seis grupos: controle negativo (SHAM) - animais com incisão e loja cirúrgica, ausentes de scaffolds; controle positivo (PDS) - animais com scaffolds de PDS, 1 scaffold PDS com 25%wt de metronidazol (1MET), 2 scaffolds PDS com 25%wt de metronidazol (2MET), 1 scaffold PDS com 25%wt de ciprofloxacina (1CIP), 2 scaffolds com 25%wt de ciprofloxacina (2CIP). Os animais foram eutanasiados em 3 (n=30) e 30 dias (n=30), correspondendo a resposta inflamatória inicial e tardia, respectivamente. Os desfechos avaliados foram degradação de fibras colágenas (Picrosírius Red e Tricrômico de Masson), atividade de enzimáticas celulares (mieloperoxidase e N-Acetil β-D-Glicosaminidase) e perfil oxidativo local [espécies reativas de oxigênio (ROS), peroxidação lipídica (LP), proteína carbolinada (PC), catalase (CAT), vitamina C (VIT.C) e glutationa reduzida (GSH)]. Os dados foram analisados estatisticamente ($p<0,05$) através do teste Two-way ANOVA (tratamento e tempo). Coletivamente, os resultados mostram que scaffolds com antibióticos possuem menor resposta inflamatória se comparado ao grupo PDS. Entre as nanofibras com antibióticos, o grupo que apresentou melhor resposta inflamatória inicial e tardia foi 2 CIP. Os achados desta pesquisa sugerem o potencial estudo destes scaffolds com metronidazol e ciprofloxacina em modelos regenerativos, capazes de confirmar a efetividade dessas matrizes artificiais para a regeneração periodontal.

Palavras-chave: Antibióticos. Biomateriais. Eletrospinning. Eletrospun. Engenharia tecidual Polidioxonona. Regeneração.

ABSTRACT

BIOCOMPATIBILITY OF NANOFIBERS SCAFFOLDS CONTAINING METRONIDAZOLE OR CIPROFLOXACIN IN SUBCUTANEOUS IMPLANTATION MODEL

AUTHOR: Patrícia Cabral Passos
ADVISOR: Karla Zanini Kantorski

Evidence shows there is no ideal membrane, i.e., biocompatible, biodegradable, with adequate mechanical and physical properties that enable reorganization of periodontal tissues. Eletrospinning technique has demonstrated good processing results on scaffolds made from polymers. Nanofibrous formed by this technique have characteristics that resemble the extracellular matrix. The aim of this study was to evaluate the biocompatibility of nanofibrous polydioxanone scaffolds (PDS II®) containing metronidazole or ciprofloxacin in subcutaneous implantation model in rats Wistar. PDS II® is biocompatible polyester having various applications in the medical field. Our conceptual hypothesis considers that scaffolds with antimicrobials have similar inflammatory behavior when compared to PDS positive control. Sixty adult male rats were randomized into 6 groups: negative control (SHAM) – animals with incision and surgical pocket, without scaffold; positive control (PDS) – animals with PDS scaffolds; 1MET – animals with one PDS scaffold containing 25%wt metronidazole; 2MET – animals with two PDS scaffolds containing 25%wt metronidazole, 1CIP – animals with PDS scaffolds containing 25%wt ciprofloxacin; 2CIP – animals with two PDS scaffolds containing 25%wt ciprofloxacin. Animals were euthanized at 3 days (n=30) and 30 days (n=30), corresponding to initial and late inflammatory responses, respectively. Outcomes measures were the degradation of collagen fibers (Picrosirius Red and Masson´s trichrome), activity of cellular enzyme (Myeloperoxidase activity and N-acetyl-β-D-glucosaminidase activity) and local oxidative profile (reactive oxygen species, lipid peroxidation, protein carbonyl, vitamin C, catalase and reduced glutathione). Dara were analyzed statistically ($p<0.05$) by two-way ANOVA (treatment and time). Collectively, results show that antibiotics scaffolds have a lower inflammatory response compared to PDS group. Among the nanofibers with antibiotics, the group showed better early and late inflammatory response was 2CIP. The findings of this research suggest the potential study of these scaffolds with metronidazole and ciprofloxacin in regenerative models, able to confirm the effectiveness of these artificial matrices for periodontal regeneration.

Keywords: Antibiotics. Biomaterials. Electrospinning. Electrospun. Polydioxanone. Regeneration. Tissue enginnering.

LISTA DE ILUSTRAÇÕES

MANUSCRITO

LISTA DE TABELAS

MANUSCRITO

LISTA DE ABREVIATURAS E SIGLAS

Ad libitum	À vontade
ADA	American Dental Association
CAT	Catalase
CIP	Ciprofloxacina
DP	Doença periodontal
DTNB	Ácido nitrobenzóico
DNPH	Dinitrofenilidrazina
DTNB	Ácido nitrobenzóico
DO\ Ml	Densidade ótica por mL da amostra
EO	Estresse oxidativo
FDI	Federação dentária internacional
g	Grama
GSH	Glutationa reduzida
GSSH	Glutationa peroxidase
HO_2^-	Hidroperoxila
H_2O_2	Peróxido de hidrogênio
IC	Intervalo de confiança
IP	Intraperitoneal
IV	Intravenoso
IM	Intramuscular
J/cm²	Joule por centímetro quadrado
Kv	Quilo volt
Kda	Quilo Dalton
MDA	Malondialdeído
Ml	Mililitro
MET	Metronidazol
MPO	Mieloperoxidase
mW	Miliwatts
NAG	N-acetil-β-D-glicosaminidase
nm	Nanômetros
mm	Milímetros
µm	Micrômetros
µs	Micro-segundos
mg/Kg	Miligrama por quilograma
O_2^-	Superóxido
OH^-	Hidroxila
PBS	Solução de tampão fosfato
PDS	Polidioxanona
PCL	Poli(ε-Caprolactone)
PLA	Poli(ácido lático)
PGA	Poli(ácido glicolico)
ROG	Regeneração óssea guiada
ROS	Espécies reativas de oxigênio
RTG	Regeneração tecidual guiada
SOD	Superóxido dismutase
TBA	Ácido tiobarbitúrico
Wt	Unidade de concentração de sólidos em líquidos

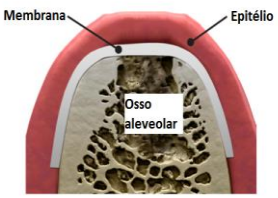
SUMÁRIO

1 INTRODUÇÃO

A periodontite é uma doença infecto-inflamatória que afeta os tecidos periodontais de suporte, podendo resultar, caso não tratada, na perda do elemento dentário (PAGE et al., 1997). A resposta imuno inflamatória resultante da interação, hospedeiro susceptível e biofilme bacteriano, promove a destruição dos tecidos periodontais. A alta prevalência da periodontite (ALBANDAR et al., 1999; KASSEBAUM et al., 2014; PETERSEN et al., 2005; SUSIN et al., 2004) e suas sequelas, como defeitos ósseos, recessão gengival e bolsas periodontais, tem levado ao estudo de técnicas que permitam o restabelecimento dos tecidos periodontais específicos perdidos (BOTTINO et al., 2011; KARRING et al.,1993; WANG, 2005).

Modalidades terapêuticas utilizando uma variedade de procedimentos, tais como cirúrgicos (WALTER et al., 2009), enxertos ósseos combinados ou não com proteínas (JUNG et al., 2003; TAHERI et al., 2009), materiais osteocondutores\osteoindutores (STAVROPOULOS; WIKESJO, 2012; TROMBELLI et al., 2010), fatores exógenos de crescimento (CHEN et al., 2010; HOSOKAWA et al., 2000; KNOW et al., 2010), tecnologia baseada em células e genes (CHEN et al., 2010; YANG et al., 2013), estão sendo estudadas. Neste contexto, a regeneração tecidual guiada (RTG) é considerada uma técnica promissora para reestruturar os tecidos periodontais perdidos. Esta técnica consiste na utilização de uma membrana como interface entre o tecido conjuntivo ou epitelial e ligamento/osso alveolar (Figura 1). Estas barreiras são essenciais uma vez que o tipo de célula que repovoa a superfície radicular após a cirurgia determina a natureza da inserção que se formará (MELCHER, 1976). Assim, a membrana funciona como uma barreira mecânica que impede o tecido conjuntivo e epitelial de migrar para o interior do defeito, favorecendo a recolonização do local pelas células progenitoras localizadas do ligamento remanescente e ou células sanguíneas, para diferenciação em novo aparato periodontal (BOTTINO et al., 2012).

Figura 1 - Esquema do posicionamento ideal da membrana durante a regeneração tecidual guiada.



FONTE: Bottino et al., 2012

Porém, o sucesso desta técnica tem sido limitado pela ausência de membranas ideais, ou seja, biocompatíves, biodegradáveis, com propriedades mecânicas e físicas adequadas, tais como estabilidade, resistência à força de rasgamento, flexibilidade, capacidade de manter a função de barreira protetora 4-6 semanas que permita a reorganização dos tecidos periodontais, e com porosidade suficiente que possibilite a troca de nutrientes e células (BECKER et al., 1987; BEHRING et al., 2008; BOTTINO; THORMAS; JANOWSKI, 2011; GENTILE et al., 2011; KARRING et al., 1993; SUSIN & WIKESJO, 2013). Assim, pesquisadores têm avançado no conhecimento de biomateriais combinados à alta tecnologia para o desenvolvimento de materiais que consigam suprir todas estas características.

Neste sentido, a técnica do eletrospinning descrita por Bottino e colaboradores (2011; 2013) tem demonstrado auspiciosos resultados para o processamento de scaffolds que são matrizes tridimensionais artificiais as quais mimetizam a matriz extracelular natural. Esta técnica consiste na utilização de uma seringa carregada com uma solução de polímero sob alta tensão e uma placa posicionada a uma distância pré-estabelecida a partir da ponta da agulha. A diferença de potencial supera a tensão superficial da gotícula do fluído que resulta na formação do chamado cone de Taylor, onde os jatos de fluído instáveis são arremessados até a placa (BOTTINO et al., 2012). Os jatos de fluído tendem a secar e formar fibras com uma média de diâmetro variando em dezenas de microns até nanômetros (AGARWAL; GREINER; WENDORFF, 2009). Os scaffolds formados por essa técnica possuem poros interconectados e fibras nanoparticuladas os quais são características mais favoráveis se comparado a microfibras ou qualquer outra forma morfológica (BOTTINO et al., 2012). As nanofibras podem estimular a interação entre as células, aumentar a taxa de proliferação, manter o fenótipo celular, suportar a diferenciação celular (LI et al., 2002). Assim, scaffolds sintetizados a partir de polímeros naturais (colágeno, quitosana) e sintéticos (coprolactone, polidioxonona) estão sendo estudados (BOTTINO et al., 2013; LIN et al., 2011).

Modelos experimentais de implantação subcutânea em ratos têm sido utilizados para avaliação da biocompatibilidade de diferentes scaffolds, considerado pela Associação Dentária Americana (ADA) (1972; 1982) e Fundação Dentária Internacional (FDI) (ISO, 1997) um teste válido nas etapas iniciais na pesquisa da histocompatibilidade para aceitação em futuras pesquisas clínicas. Biocompatibilidade é definida como a avaliação da resposta produzida pelo contato de um material com os tecidos do hospedeiro (ADTM, 2006). Existe uma ampla variedade de técnicas e tempos de avaliação as quais possibilitam a verificação da reação do hospedeiro aos materiais implantados para determinação da sua biocompatibilidade.

O tipo de célula predominante no infiltrado inflamatório em períodos distintos de pós-implantação, o aspecto da matriz extracelular e as fibras colágenas são importantes fatores a serem avaliados (BAVARIYA et al., 2013; MULDER et al., 2013) visto que o predomínio dos eventos vasculares, celulares, reparativos e enzimáticos ocorrem em tempos distintos durante a inflamação.

Durante a resposta imediata a um agente lesivo as células predominantes são na maioria neutrófilos e monócitos, posteriormente com a participação de linfócitos e plasmócitos. A mieloperoxidase (MPO) e N-acetil-β-D-glucosamidase (NAG) são biomarcadores inflamatórios indiretos que sinalizam a atividade de neutrófilos, monócitos e macrófagos (BAILEY, 1988). Entre suas funções, a MPO contribui para a defesa do organismo contra agentes patogênicos através da liberação de espécies reativas de oxigênio (ROS) (KLEBANOFF, 2005; VAN DER VEEN; WINTHER; HEERINGA, 2009). O excesso destes produtos oxidativo, que excede a capacidade de defesa antioxidante enzimática [superóxido dismutase (SOD), catalase (CAT), glutationa redutase e glutationa peroxidase] e não enzimática [vitamina C (VIT. C), vitamina E, glutationa reduzida (GSH)] do organismo, pode causar danos aos lipídios, proteínas e outros componentes celulares dos tecidos, caracterizando o estresse oxidativo (EO) (AKALIN et al., 2008; CHAPPLE, 1997; CHAPPLE; MATTHEW, 2007; HALLIWELL; GUTTERIDGE, 1984; SCHAFER; BUETTNER, 2001). Já a NAG é uma enzima lisossomial que é produzida por monócitos ativados. Uma vez ativados, os macrófagos sofrem degranulação promovendo a liberação de mediadores inflamatórios que promoverão o recrutamento e ativação de outras células inflamatórias (LAWRENCE; GILROY, 2007).

Além das características celulares e vasculares na resposta mediata, a fibrose é responsável pelo processo de reparo tecidual (ROBBINS et al., 2008). Reparação é um estado dinâmico, que compreende diferentes processos simultâneos, entre os quais, a inflamação, a proliferação celular e a síntese ou remodelagem dos elementos que constituem a matriz extracelular, tais como o colágeno tipo I e III (THOMAS; O`NEILL; HARDING, 1995). Na fase proliferativa ocorre a deposição de colágeno tipo III e na sua remodelagem há maturação colagenosa, na qual o colágeno tipo III é substituído por colágeno tipo I (EYDEN; TZAPHLIDOU, 2001; JUNQUEIRA; COSSERMELLI; BRENTANI, 1978; LIU et al., 1997).

Na busca por scaffolds ideais para a RTG, diferentes tipos de scaffolds estão sendo desenvolvidos e testados em modelo subcutâneo. Scaffolds de borato de vidro bioativos com diferentes porosidades (DELIORMANLI; LIU; RAHAMAN; 2012), scaffolds nanofibrosos

de quitosana reticuladas ou não com genipina (BAVARIYA et al., 2013), scaffolds de hidrogéis de polietilenoglicol (PEGDA) (BROWNING et al., 2014), scaffolds estruturados em multicamadas funcionais (BOTTINO; THOMAS; JANOWSKI, 2011; ERISKEN; KALYON; WANG, 2008) são alguns exemplos. Contudo, degradação, exposição prematura, alta densidade que dificulta o manuseio, taxa de degradação não uniforme, e falhas devido à infecção (BOTTINO et al., 2012), sugerem a necessidade de novas pesquisas para o desenvolvimento de scaffolds alternativos (BAVARIYA et al., 2013; BOTTINO et al., 2012).

Bottino e colaboradores (2013; 2014) testaram, *in vitro*, scaffolds nanoparticulados de PDS contendo os antimicrobianos MET ou CIP. Scaffolds contendo CIP inibiram o crescimento de ambos os biofilmes testados (*P. gingivalis e E. faecalis)* enquanto que o de MET inibiu somente crescimento de *P. gingivalis.* Contudo, scaffolds de CIP à 25wt% mostraram-se citotóxicos para células embrionárias da polpa dentária. No aspecto mecânico, o scafflold de MET à 25wt% apresentou maior resistência às tensões quando comparado ao de CIP. Ambos os scaffolds contendo antibióticos a 25wt% obtiveram redução significativa do diâmetro das fibras quando comparado ao scaffold sem a incorporação dos antimicrobianos. A presença de antimicrobianos nos scaffolds para o controle e redução da infecção tem sido comprovada por estudos anteriores (KIM et al., 2004; RUCKH et al., 2012). A terapêutica adjuvante proporcionada pelos antibióticos em dosagens controladas e uniformes sobre os danos periodontais pode ter implicações positivas na redução do percentual de falhas dos scaffolds devido à infecção (BOTTINO et al., 2012). Essa estratégia pode favorecer um ambiente livre de biofilmes e propício para a RTG, ao mesmo tempo minimizando os efeitos adversos associados ao uso sistêmico destes fármacos (BOTTINO et al., 2013). Os achados prévios promissores *in vitro* de scaffolds de PDS, quanto a parâmetros mecânicos, microbiológicos e estruturais, podem indicar um potencial uso destes scaffolds nanofibrosos com antibióticos em estudos em animais.

Logo, o objetivo deste estudo foi avaliar a biocompatibilidade inicial e tardia de scaffolds nanofibrosos de PDS contendo MET ou CIP a 25%wt, em modelo de implantação subcutânea em ratos. A biocompatibilidade será mensurada pelo perfil oxidativo tecidual, perfil enzimático celular (atividade das enzimas mieloperoxidase e N-acetil-β-D-glucosaminidade) e degradação de fibras colágenas. A hipótese deste estudo considerou que a incorporação de antibióticos ao scaffold de PDS resultaria em comportamento inflamatório semelhante à matriz de PDS.

2 REVISÃO DE LITERATURA

2.1 PERIODONTIA X REGENERAÇÃO

Periodontite é uma inflamação crônica que afeta a integridade do aparato de inserção causando a destruição dos tecidos periodontais e podendo culminar na perda dos dentes (HAFFAJEE; SOCRANSKY,1994; PIHLSTROM; MICHALOWICZ; JOHNSON, 2005). A utilização de procedimentos regenerativos visando à restauração dos tecidos periodontais de suporte vem se tornando comum, devido às demandas estéticas (recessão gengival, defeitos ósseos), dolorosas (sensibilidade radicular), anatômicas e funcionais (sítios com envolvimento de furca, implantes), visando melhorar o prognóstico dos dentes tratados periodontalmente em longo prazo (CORTELLINE & TONETTI, 2015; GOTTLOW, 1986; LINDIE; LANG; KARRING, 2008). Estes tratamentos englobam uma variedade de abordagens cirúrgicas, enxertos ósseos, materiais osteocondutores\osteoindutores, tecnologia baseada em células e genes e engenharia tecidual (BOTTINO, 2012).

O termo regeneração é definido como a reprodução ou reconstituição de uma parte perdida ou lesada, de tal forma que a arquitetura e função destes tecidos sejam completamente recuperadas (GLOSSARY OF PERIODONTAL TERMS, 1992). Lager e Vacanti (1993) propuseram a engenharia tecidual como uma terapia restauradora de tecidos perdidos e suas funções, onde os princípios para a regeneração tecidual envolviam a combinação de três elementos: membranas, células regenerativas, e moléculas sinalizadoras de células ou fatores de crescimento.

Há dois tipos de membranas utilizadas na terapia regenerativa, reabsorvíveis e não reabsorvíveis. O padrão ouro das membranas não reabsorvíveis são as confeccionadas com politetrafluoroetileno de alta densidade (PTFE) e PTFE reforçadas com titânio. São membranas inertes e biocompatíveis. Estudos sugerem que o reforço de titânio pode levar a uma maior capacidade regenerativa devido ao suporte mecânico adicional proporcionado pelo titânio contra as forças compressivas exercidas pelos tecidos (JOVANOVIC; NEVINS, 1995). Uma das desvantagens das membranas não reabsorvíveis é a necessidade de uma cirurgia adicional para sua remoção, o que implica em dor e desconforto ao paciente, além do custo adicional. Para reduzir estes prejuízos foram desenvolvidas as membranas reabsorvíveis (BOTTINO et al., 2012).

Técnicas de processamento com base na fusão de materiais sólidos ou evaporação de solventes foram utilizadas para fabricar membranas à base de polímeros biodegradáveis

(LIAU et al., 2007). A maioria das membranas reabsorvíveis para regeneração periodontal podem ser confeccionadas a partir dos polímeros: ácido poliglicólico (PGA), ácido polilático (PLA), policaprolactone (PCL) e seus copolímeros (BOTTINO et al., 2012). As membranas à base de poliésteres sintéticos são biocompatíveis, fáceis de manipular e biodegradáveis. Embora essas membranas tenham demonstrado inicialmente alta resistência, as mesmas perderam suas propriedades estruturais e mecânicas dentro de 4 semanas (MILELLA et al., 2001). Membranas à base de colágeno foram sugeridas como alternativa aos polímeros sintéticos, porém o alto custo, pouca definição das fontes comerciais e pobre desempenho *in vivo* (BEHRING et al., 2008; BOTTINO et al., 2012) sugerem o estudo de novas alternativas.

Na busca de membranas periodontais biocompatíveis e biodegradáveis com características e propriedades adequadas, aprimorou-se as técnicas de síntese de polímeros naturais e sintéticos como fundição (LIAO et al., 2007; MILELLA et al., 2001), filtração dinâmica (TENG et al., 2009) e eletrospinning (BOTTINO et al., 2014; BOTTINO; THOMAS; JANOWSKI, 2011; YANG et al., 2009). A técnica do eletrospinning possui vantagens por ser de fácil execução, versátil e relativamente de baixo custo (LIM; MAO, 2009). Essa técnica proporciona a síntese de scaffolds que funcionam como matrizes artificiais com arquitetura tridimensional (3D) que impedem a migração de tecidos para o interior do defeito, proporcionando um ambiente adequado para a RTG (IKADA, 2006).

Neste contexto, os scaffolds de PDS foram testados. A Poli (p-dioxanona) é um poliéster biocompatível que apresenta excelentes propriedades mecânicas, com poder de degradação (VAN DER VEEN; WINTHER; HEERINGA, 2009). Este polímero degrada-se por hidrólise química no organismo, geralmente resultando em moléculas de baixo peso molecular, podendo ser metabolizado ou reabsorvido pelo corpo (VAN DER VEEN; WINTHER; HEERINGA, 2009). Entre as características do PDS estão a flexibilidade, a memória elástica e a resistência (BOLAND et al., 2005; GONOO et al., 2015; VAN DER VEEN; WINTHER; HEERINGA, 2009). Além da aplicação do PDS como material de sutura, este polímero é utilizado como substituto da válvula mitral e reparo da válvula tricúspide (CHRISTENSON; KALANGOS, 2009; KALANGOS et al., 2006), confecção de stents de órgãos vasculares e não vasculares (LI et al., 2014; LISCHKE et al., 2011; REPICI et al., 2010; VONDRYS et al., 2011; ZAMIRI et al., 2010). Na forma de scaffolds, suas propriedades mecânicas são comparáveis com os principais componentes da matriz extracelular nativa, ou seja, colágeno e elastina (GONOO et al., 2015) , e os dados *in vivo* (GOLLAPUDI et al., 2014) sugerem que a exposição a longo prazo aos scaffolds de PDS não alteram as funções das células do sistema de defesa imune inata e adaptativa. Neste contexto,

os scaffolds de polidioxonona foram testados (BOTTINO et al., 2013; 2014; KAMOCKI; NOR; BOTTINO, 2015)

Bottino e colaboradores (2014) verificaram a média do diâmetro das fibras de scaffolds de PDS comparados aos scaffolds de PDS sintetizados com antimicrobianos. Os scaffolds de PDS puro possuíam maior média de diâmetro da fibra (1158±402 nm), seguido em ordem decrescente do scaffold com MET 5%wt (1108±383 nm), scaffold 25%wt MET (944±392 nm), scaffold 5%wt CIP (871± 309 nm) e scaffold 25% CIP (765±288 nm). As características mecânicas destes scaffolds foram observadas anteriormente (BOTTINO et al., 2013), como teste de resistência à tração. Houve uma pequena redução dessas propriedades nos scaffolds com MET e CIP se comparado ao scaffold controle (PDS). Entretanto, estes scaffolds com antimicrobianos foram eficazes no controle bacteriano (BOTTINO et al., 2013; 2014), e não apresentaram redução das células viáveis, exceto o scaffold com CIP a 25%wt.

2.2 ANTIMICROBIANOS

A presença de periodontopatógenos afeta negativamente o sucesso da regeneração periodontal e o controle desses agentes lesivos é de extrema importância para que a terapia alcance os resultados almejados (BOTTINO et al., 2012). A utilização inadequada de agentes microbianos pode causar resistência bacteriana (AMINOV, 2009), gerar reações de hipersensibilidade e infecções oportunistas (CASSEL; MEKALANOS, 2001). Diante deste cenário, pesquisadores buscam projetar materiais capazes de liberar estas drogas de maneira controlada e localizada por um período de tempo específico, como os scaffolds de PDS confeccionados com metronidazol e ciprofloxacina (BOTTINO et al., 2012; 2014). Estes scaffolds de PDS com antibióticos (25 wt%) tiveram liberação de 12% de metronidazol e 40% de ciprofloxacina durante um período de 7 dias, 1 e em quantidade acima da concentração inibitória mínima dos periodontopatógenos testados (BOTTINO et al., 2014).

Metronidazol é um antibiótico de amplo espectro utilizado no tratamento das infecções periodontais (FREEMAN; KLUTMAN; LAMB, 1997), atua contra bacilos anaeróbios Gram-negativos e bacilos e cocos Gram-positivos (EL-KAMEL; ASHRI; ALSARRA, 2007), com positivos resultados contra *Porphyromonas gingivalis*, em scaffolds de PDS (BOTTINO et al., 2012). Esse antimicrobiano apresenta boa penetração e distribuição nos tecidos, liga-se fracamente às proteínas plasmáticas, atingindo uma concentração plasmática máxima em 1 ou 2 horas (DRUG INFORMATION HANDBOOK, 2005),

Já a ciprofloxacina é uma fluoroquinolona utilizada isolada ou em conjunto com o metronidazol no tratamento das DP, devido sua ação contra o aneróbio facultativo *A. actinomycetemcomintans*. No estudo de Bottino e colaboradores (2014), scaffolds de PDS contendo ciprofloxacina inibiram o crescimento de *Fusubacterium nucleatum* e foram mais eficazes contra *Aggregatibacter actinomycetemcomitans* se comparado aos scaffolds de PDS com metronidazol. A utilização de um scaffold confeccionado por um polímero biodegradável aprovado pela FDI, comumente usado em aplicações médicas e odontológicas como material de sutura, capaz de liberar de forma direta e controlada antimicrobianos durante a terapia regenerativa periodontal é altamente desejável (BOTTINO et al., 2014).

2.3. BIOCOMPATIBILIDADE

O termo biocompatibilidade refere-se ao conjunto de propriedades desejáveis de um material que será inserido em um organismo vivo. A compatibilidade biológica abrange o potencial citotóxico, alergênico e mutagênico dos materiais aos tecidos vivos (COSTA; HEBLING; SOUZA, 2001). Os materiais que preenchem os requisitos biológicos são denominados biomateriais, termo que se refere a qualquer substância que possa ser utilizada em período de tempo indeterminado como parte de um sistema e que objetive o tratamento ou reposição de qualquer tecido (ANUSAVICE, 2005). Pesquisas envolvendo avaliação da toxicidade de materiais devem obedecer a critérios específicos e aceitos internacionalmente (SCHMALZ, 2002). Vários testes podem ser efetuados para verificar a compatibilidade biológica de um material. A Federação Dentária Internacional – FDI (ISO 7405) e a American National Standards Institute\ American Dental Association –ANSI\ ADA recomendam três níveis de avaliação para materiais dentários, baseados em uma sequência de protocolos de pesquisa, os quais possuem como objetivo final determinar através de testes *in vitro* e *in vivo*, a segurança da aplicação clínica dos materiais odontológicos em seres humanos (COSTA; HEBLING; SOUZA, 2001).

Os testes iniciais ou preliminares *in vitro* determinam de maneira preliminar o comportamento biológico de materiais e de seus componentes de modo a simular as reações biológicas desencadeadas quando colocados em contato com os tecidos do organismo (HANKS et al., 1996). Modelos *in vitro*, como cultura de células, são restritos, pois não podem prever a atividade biológica ou tóxica possível da interação de diferentes materiais com células, fatores moleculares e o meio ambiente. Um tipo de scaffold pode comportar-se diferente *in vitro, in vivo*, e em diferentes partes de um mesmo animal (LIU; CAO, 2007).

Essa variabilidade interna ocorre devido à resposta tecidual do hospedeiro frente ao biomaterial implantado. Segundo Spangberg & Langeland (1973), quando um material for considerado tóxico *in vitro*, sempre causará irritação tissular, entretanto, uma baixa toxicidade *in vitro* não implica em uma baixa irritação tecidual *in vivo*, pois há muitos fatores envolvidos como a reabsorbilidade, solubilidade nos tecidos, fragmentação pelos fagócitos ou até uma reação inflamatória induzida (MIYAGAKI, 2011).

Os testes secundários são aqueles que avaliam a toxicidade local realizados em animais de pequeno porte. Estes testes *in vivo* verificam a sensibilidade e o padrão de resposta tecidual de materiais implantados em tecido conjuntivo subcutâneo e ósseo de animais (COSTA; HEBLING; SOUZA, 2005; SOUSA et al., 2006). O terceiro nível são os testes que envolvem animais como cães e macacos, entretanto são considerados de alto custo, demorados e difíceis de serem controlados (HAUMAN; LOVE, 2003).

2.4. MODELO SUBCUTÂNEO

O modelo de implantação subcutânea em pequenos animais é ideal para examinar a resposta tecidual devido às propriedades físicas de um determinado material, como forma, densidade, dureza as quais podem influenciar nas reações teciduais apresentadas (ANUSAVICE, 1998). Este modelo pré-clínico é amplamente utilizado pela praticidade, baixo custo (GHANAATI et al., 2010), sendo considerado válido nas etapas preliminares da pesquisa de histocompatibilidade de diversos materiais (ADA, 1972).

A taxa de absorção subcutânea em animais parece ser mais rápida do que em seres humanos. A fisiologia da hipoderme e sua diferença entre as espécies precisam ser considerados. O panículo carnoso localizado na derme de ratos facilita a absorção de fármacos devido à presença de capilares e linfa ausente (WELLS et al., 2010). Mais pesquisas são necessárias para melhorar a compreensão dos eventos durante a absorção subcutânea, porém sabe-se que compósitos moleculares com peso inferior ou igual a 16 kDa atingem a circulação através de capilares sanguíneos, já moléculas de peso superior entram na circulação indiretamente através de vasos linfáticos (RICHTER; BHANSALI; MORRIS, 2012).

2.5. INFLAMAÇÃO

O uso de materiais biocompatíveis e o grau de inflamação causado são de grande importância para a recuperação dos tecidos e do paciente (ZAFALON, 2004). A inflamação é uma reação dos tecidos vascularizados a um agente agressor caracterizada pela saída de líquidos e de células do sangue para o interstício. Vários modelos experimentais foram utilizados para compreender os eventos vasculares que ocorrem durante a inflamação, antes restritas aos sinais cardinais calor, eritema, edema, dor- descrita por Cornelius Celsus - e alterações funcionais por Virchow (CONSOLARO, 2009).

A resposta inflamatória ocorre devido à liberação de mediadores que induzem modificações na microcirculação e migração de fagócitos para o interstício. Os mediadores (citocinas) ficam armazenados em células (mastócitos), em terminais nervosos ou são produzidos por outras células (monócitos, macrófagos, neutrófilos, linfócitos e células parenquimatosas ou do estroma). Esses mediadores são liberados em tempos diferentes durante o processo inflamatório. Os mediadores imediatos são responsáveis pelo início dos fenômenos vasculares e exsudativos, enquanto os de liberação mediata atuam na manutenção desses fenômenos e início dos fenômenos reparativos. Assim, são reconhecidos dois tipos de inflamação: aguda e crônica (ROBBINS et al., 2008).

2.5.1 Inflamação aguda

A resposta imediata a um agente lesivo possui dois principais componentes: alterações vasculares e eventos celulares. Segundo Kumar e colaboradores (1995), os fenômenos vasculares são caracterizados por aumento do fluxo sanguíneo para a área lesada, resultante da dilatação das artérias e abertura dos capilares. O aumento da permeabilidade vascular resulta em acúmulo de líquido extracelular rico em proteínas, que forma o exsudato. Posteriormente, ocorre a exsudação de líquido e proteínas plasmáticas e acúmulo de leucócitos. O tipo de leucócito varia com o tempo da resposta inflamatória. Na maioria das inflamações agudas, os neutrófilos predominam o infiltrado inflamatório durante as primeiras 4 a 24 horas, sendo substituídos por monócitos em 24 a 48 horas (COOK-MILLS; DREEM; 2005; MAYADAS; CULLERE; LOWELL, 2014).

A ativação leucocitária resulta em fagocitose de substâncias nocivas, produção de mediadores que amplificam a reação inflamatória e produção de substâncias que incluem enzimas lisossômicas e espécies reativas de oxigênio (ROS) (MAYADAS; CULLERE;

LOWELL, 2014). Quando secretadas em níveis baixos ou moderadas, as ROS podem aumentar a expressão das moléculas de adesão, citocinas e quimiocinas, amplificando a cascata de mediadores inflamatórios, apresentando efeitos benéficos nas funções fisiológicas (GUTTERIDGE; HALLIWELL, 2010). Entretanto, o acúmulo dessas ROS podem causar danos a lipídios, carboidratos, proteínas e outros componentes celulares, caracterizando o estresse oxidativo (CHAPPLE, 1997; CHAPPLE; MATTHEW, 2007; HALLIWELL; GUTTERIDGE, 1984).

2.5.2 Inflamação crônica e reparo

Ao contrário da inflamação aguda, que é caracterizada pelas alterações vasculares e celulares, a inflamação crônica caracteriza-se pela infiltração de células mononucleadas (macrófagos, linfócitos e plasmócitos), destruição tecidual induzida pelos produtos das células inflamatórias e reparo envolvendo angiogênese e fibrose (SHERWOOD; TOLIVER-KINSKY, 2004). Os monócitos ao alcançar o tecido extravascular sofrem transformação em macrófagos maiores, que quando ativados, resultam em um aumento do seu metabolismo e maior capacidade de destruir o agente lesivo. Após a ativação, os macrófagos secretam uma variedade de produtos que, se desimpedidos, resulta em lesão tecidual e fibrose. Estes produtos são proteases, ROS, citocinas e fatores de crescimento (LAWRENCE; GILROY, 2007). As citocinas e fatores de crescimento regulam a quimiotaxia e proliferação de fibroblastos, a síntese de colágeno e angiogênese necessários para o processo de reparação tecidual (GRANSTEIN et al., 1987).

2.6. BIOMARCADORES ENZIMÁTICOS INFLAMATÓRIOS: MIELOPEROXIDASE (MPO) E N-ACETIL-β-D-GLICOSAMINIDASE (NAG)

A detecção da atividade das enzimas MPO e NAG pode ser utilizada como marcador da atividade de leucócitos PMN, monócitos e macrófagos. A MPO é uma enzima chave na imunidade inata na defesa contra agentes patogênicos (KLEBANOFF, 2005). Esta enzima é uma hemeproteína catiônica, considerada como a principal constituinte dos grânulos azurófilos dos neutrófilos e encontrada em menor quantidade em monócitos. Liga-se facilmente a superfícies bacterianas, componentes da matriz extracelular e membranas celulares (VEEN; WINTHER; HEERINGA, 2009). A MPO é prontamente liberada dentro do fagossomo ou para o espaço extracelular após sua ativação por diferentes agonistas (LAU;

BALDUS, 2006). A MPO catalisa reações através do peróxido de hidrogênio, formado pelo sistema NADPH oxidase, produzindo o ácido hipocloroso (BORREGAARD; COWLAND, 1997; FAURSCHOU; BORREGAARD, 2003). O conjunto MPO, peróxido de hidrogênio e ácido hipocloroso provou ser um poderoso sistema antimicrobiano. Além da sua função de gerar ROS, a MPO tem sido descrita como um potente mediador inflamatório, envolvida na homeostase celular, induzindo tanto a iniciação como a manutenção de numerosos eventos inflamatórios (ROMAN; WENDLAND; POLANCZYK, 2008).

A NAG é uma enzima hidrolítica lisossomial presente em níveis elevados em macrófagos ativados, considerada um indicador indireto da presença de células mononucleadas no foco inflamatório crônico (BAILEY, 1988; OLIVEIRA et al., 2014). Estas bioenzimas inflamatórias quando ativadas, sofrerão degranulação promovendo a liberação de mediadores inflamatórios, incluindo aminas, citocinas, quimiocinas e mediadores lipídicos (LAWRENCE; GILROY, 2007).

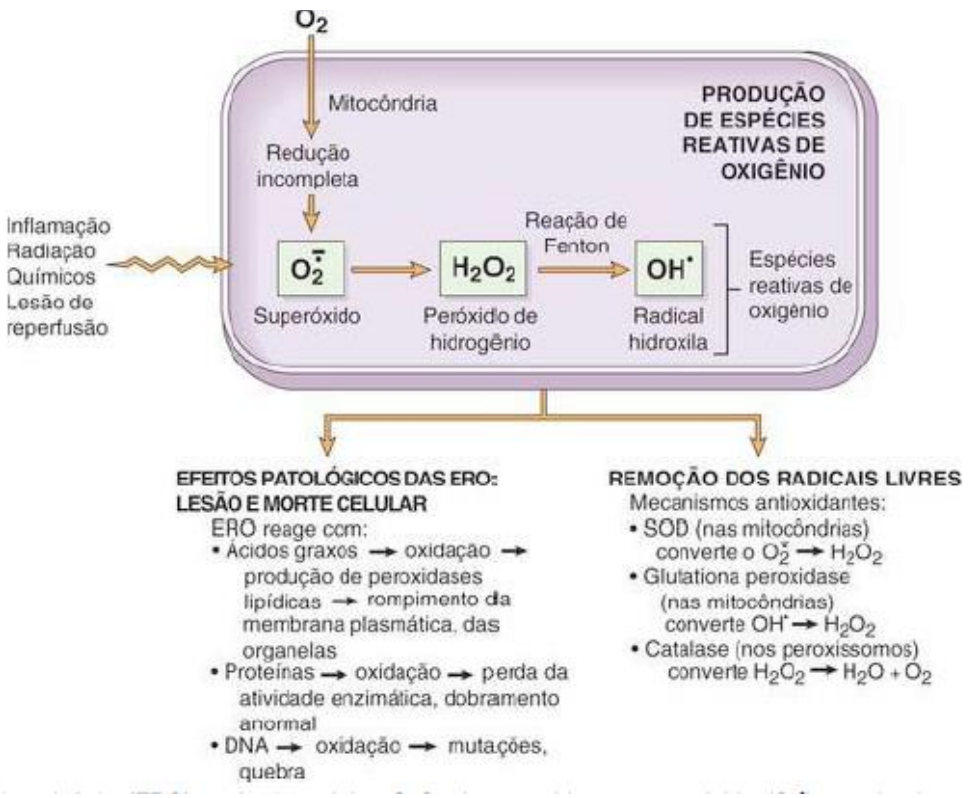
2.7. RADICAIS LIVRES E ESTRESSE OXIDATIVO

Radicais livres são espécies químicas (átomos ou moléculas) os quais têm um número ímpar de elétrons em sua órbita externa. Os elétrons não possuem um pareamento na última camada o que confere uma alta reatividade e instabilidade desses estados químicos (HALLIWELL, 1992; HALLIWELL; GUTTERIDGE, 1984). As ROS são produzidas durante o metabolismo mitocondrial fisiológico através das reações de redução-oxidação. Os principais radicais gerados na produção parcial de oxigênio são os radicais superóxido (O_2^-), peróxido de hidrogênio (H_2O_2), hidroxila (OH^-) e hidroperoxila (HO_2^-) (HALLIWELL; GUTTERIDGE, 1984).

As células desenvolveram múltiplos mecanismos para a proteção contra ROS e abrangem a proteção enzimática ou não enzimática. No sistema enzimático estão incluídos superóxido dismutase (SOD), a catalase (CAT), a glutationa redutase e glutationa peroxidase (SCHAFER; BUETTNER, 2001). Este sistema atua diminuindo a produção dos radicais livres. Já no sistema não enzimático estão incluídos a glutationa reduzida (GSH), vitamina C (VIT. C) e vitamina E. A GSH está presente na maioria das células e sua capacidade redutora é determinada pelo grupamento –SH, presente na cisteína. Após a exposição da GSH ao agente oxidante, ocorre sua oxidação a GSSG, onde a glutationa peroxidase (GSSH) protege contra a lesão catalisando a degradação de radicais (HEBBEL, 1986). As vitaminas C e E podem bloquear a formação de radicais livres ou removê-los.

Quando a produção de ROS aumenta ou quando os sistemas de remoção desses radicais são ineficientes, o resultado é o excesso de radicais livres que se denomina estresse oxidativo (EO) (HALLIWELL, 1993). O EO é um processo deletério que pode ser um importante mediador de danos a estruturas celulares, incluindo os lipídios de membranas, proteínas e DNA. A lesão celular mediada por radicais livres ocorre através de três reações: peroxidação lipídica das membranas onde as ligações duplas de lipídios das membranas são atacadas pelos radicais livres, que geram interações lipídio-radical, gerando peróxidos instáveis e reativos os quais alteram a estrutura e permeabilidade das membranas celulares (MELLO; HOFFMAN; MENEGHINI, 1983, TSAI et al., 2005); ligação cruzada das proteínas onde os radicais livres promovem essa ligação por sulfidrila, resultando na degradação ou perda da atividade enzimática, podendo causar também fragmentação dos polipeptídios (YAN; TRABER; PACKER, 1995); fragmentação do DNA onde os radicais livres reagem com a timina do DNA mitocondrial e nuclear produzindo a quebra do filamento único no DNA, resultando na morte celular (BRIGANTI; PICARDO, 2003).

Figura 2 - Esquema do papel das espécies reativas de oxigênio (EROs).



FONTE: Robins e colaboradores (2008).

2.8 COLÁGENO

O colágeno é uma macromolécula composta de três cadeias polipeptídicas de aminoácidos, entrelaçadas umas com as outras em uma configuração helicoidal sendo o componente mais importante na reparação de tecidos (THORNTON; BARBUL, 1997). À medida que as moléculas de colágeno se agrupam, sua composição passa a receber denominações diferentes como colágeno, procolágeno, microfibrilas, fibrilas e fibras colágenas (GARTNER; HIATT, 2003; JUNQUEIRA; CARNEIRO, 2004; MINOR, 1980; ZAGRIS, 2000).

As fibras colágenas apresentam-se estruturalmente distintas, variando sua forma, tamanho, espessura, orientação e posição das cadeias helicoidais. Devido a essa variabilidade, localização e função nos tecidos, as fibras colágenas foram classificadas em diferentes tipos. Atualmente, a família de colágenos é composta por mais de 20 tipos (EYDEN; TZAPHLIDOU, 2001; KESLER et al., 2000; OTTANI et al., 2001). 80 a 90% do colágeno encontrado no organismo humano é classificado em tipos I,II e III (LODISH et al., 2000). As fibras colágenas tipo I produzidas por fibroblastos, conferem a resistência tensora dos tecidos. Apresenta estrutura fibrilar, com fibras espessas e densas, altamente birrefringentes à luz polarizada. São encontradas em todos os tecidos conjuntivos, principalmente na pele, ossos, tendões e parede dos vasos sanguíneos (BARTOLD et al., 2000; JUNQUEIRA; CARNEIRO, 2004; KESLER et al., 2000; MONTES; JUNQUEIRA, 1991; SCHROEDER; LISTGARTEN, 1997).

O colágeno tipo III ou fibrilas de reticulina tende sempre estar associado com o tipo I. Ao contrário do tipo I, estas fibras tem pouca resistência à tensão, porém confere a integridade estrutural. Apresenta estrutura fibrilar, são finas e alongadas com baixa birrefringência à luz polarizada, exibindo padrão reticular e encontradas de maneira difusa nos tecidos (BARTOLD et al., 2000; JUNQUEIRA et al., 1982; SCHROEDER; LISTGARTEN, 1997; OTTANI et al., 2001). Durante o processo de reparo, o remodelamento do colágeno modifica a proporção entre o colágeno tipo I e tipo III. A resistência de um tecido poderia ser mais bem definida pela determinação da proporção entre fibras colágenas tipo I e tipo III, ou seja, pela presença de colágeno maduro e imaturo (KORUDA; ROLANDELLI, 1990).

Mulder e colaboradores (2012) avaliaram o efeito de scaffolds isotrópicos e anisotrópicos de poliuretano na orientação do colágeno em modelo subcutâneo. Eles verificaram que scaffolds isotrópicos continham fibras colágenas de 4 semanas em diante com

colágeno alinhado aleatoriamente. Já os scaffolds anisotrópicos continham fibras colágenas a partir de 8 semanas alinhados ao longo dos poros. O estudo mostrou que a arquitetura dos poros presentes nos scaffolds guiam a deposição e orientação do colágeno. Porém nesse estudo, não houve a diferenciação entre os tipos de fibras colágenas formadas.

3 ARTIGO – BIOCOMPATIBILITY OF POLYDIOXANONE E-SPUN NANOFIBROUS SCAFFOLD CONTAINING METRONIDAZOLE OR CIPROFLOXACIN USING THE SUBCUTANEOUS IMPLANTATION MODEL IN WISTAR RATS

Este artigo será submetido ao periódico *Dental Materials*, *Elsevier*, ISSN: 0109-5641, Fator de impacto = 3.769; Qualis A1. As normas para publicação estão descritas no Anexo A.

Biocompatilbility of polydioxanone e-spun nanofibrous scaffold containing metronidazole or ciprofloxacin using the subcutaneous implantation model in Wistar rats

Patrícia C. Passos[a], Juliana Moro[b], Roberto Maciel[b], Raquel C.S. Barcelos[c], Sara Marchesan de Oliveira[d], Cristiane C. Danesi[b], Marco Cícero Bottino[e], Karla Z. Kantorski[a].

[a] School of Dentistry, Federal University of Santa Maria, Periodontology Unit, Santa Maria, Rio Grande do Sul State, Brazil.

[b] School of Dentistry, Federal University of Santa Maria, Department of Stomatology, Pathology Unit, Santa Maria, Rio Grande do Sul State, Brazil.

[c] Federal University of Santa Maria, Department of Biochemistry, Santa Maria, Rio Grande do Sul State, Brazil.

[d] Federal University of Santa Maria, Department of Toxicology Biochemical, Santa Maria, Rio Grande do Sul State, Brazil.

[e] Indiana University School of Dentistry, Department of Restorative Dentistry ,Indianapolis, USA.

Autor correspondente:

Karla Zanini Kantorski

Rua Marechal Floriano Peixoto, 1184, CEP 97015-372, Centro, Santa Maria, Brasil.

Telefone: +55-55-3220-9284

E-mail: kzkantorski@gmail.com (Dr. Kantorski KZ)

ABSTRACT

Objectives: To evaluate the biocompatibility of polydioxanone (PDS II®) scaffolds for periodontal regeneration containing metronidazole (MET) or ciprofloxacin (CIP) to 25% wt in a model of subcutaneous implantation in rats Wistar.

Methods: Sixty adult male rats were randomized into six groups: negative control (SHAM) – subcutaneous pocket without scaffold; positive control (PDS) - one PDS II® scaffold; 1MET – one PDS II® containing MET, 2MET – two PDS II® scaffolds, 1 CIP – one PDS II® scaffold, 2 CIP – two PDS II® scaffolds. The animals were sacrificed 3 and 30 days, to evaluate initial and late inflammatory response, respectively. Outcomes were: profile oxidative, myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase and collagen fibers. The evaluations were performed by a blinded examiner for the experimental groups. The date were statistically analyzed ($p<0.05$).

Results: Antibiotic incorporation to the PDS matrices improved the oxidative profile when compared to PDS scaffold, particularly in 2CIP group. There were no significant ($p>0.05$) changes in quantify and qualify of collagen fibers in all groups.

Significance: Findings of this research suggest a potential study of these scaffolds PDS with MET and CIP in regenerative models, able to confirm the effectiveness of these artificial matrices for periodontal regeneration.

Key words: Antibiotics. Biomaterials. Drug delivery. Electrospinning. Periodontitis. Polydioxanone. Regeneration. Tissue enginnering.

1. INTRODUCTION

Periodontitis is an inflammatory disorder that leads to the destruction of tooth support tissues and, ultimately, resulting in tooth loss. This destruction is caused by an imbalance between a wide range of microorganisms and host response, which is modified by various genetic, epigenetic, and environmental factors [1]. Periodontitis is very common in developed and developing countries [2–4]. Data from World Health Organization showed that severe periodontitis exists in 5-20% of most adult populations worldwide [5], and moreover, severe periodontitis is the sixth-most prevalent condition in the world [6].

The periodontitis treatment consist mechanical removal of biofilm and mineralized deposits on root surface by scaling and root planing (SRP) [7]. Even though, some clinical gain in tissue support may be attained, SRP do not support regeneration of the lost alveolar bone. Many patients have to live with the undesirables' consequences of the advanced periodontitis, such as exposed root surfaces, loss of keratinized attached gingiva, alveolar craters, tooth mobility, and anatomical sequelae that difficult the adequate control plaque.

Periodontal-regenerative technologies can be applied: (i) to increase the bone support of severely compromised teeth, reducing the degree of residual tooth mobility; (ii) to decrease the pocket depth, which has been associated with an increased probability of tooth loss in patients attending supportive periodontal therapy [8]; (iii) to reach adequate aesthetic features such as no, or minimal gingival recession; (iiii) to obtain tissue anatomy that facilitates the plaque control, particularly in furcation lesions; (iiiii) and to improve function and prognosis of periodontally compromised teeth. "The possibility of changing the prognosis of a tooth from "questionable" into "favorable" would help clinicians and patients to maintain teeth over time with appropriate function" [9].

Periodontal regenerative medicine has focused on regenerative materials on one side, and on novel surgical approaches on the other side [10]. Evidences from animals studies

showed that under optimal condition for wound healing, relevant periodontal regeneration occur, and, moreover, space provision positively influences the extent of periodontal regeneration [11–13].

Barrier membranes have been employed in so-called guide tissue/bone regeneration (GTR/GBR) [14,15]. Historically, GTR devices have been used to mechanically select the cells able to repopulate the blood clot, preventing connective and epithelial tissue migration into the defect. Then, progenitor cells located in the remaining periodontal ligament, adjacent alveolar bone, and/or blood are able to repopulate the root area and differentiate into a new periodontal support apparatus [16]. RTG devices should be designed with sufficient strength to avoid the membrane collapse into defect assuring the space-provision necessary for regenerative process [11–13]. Simultaneously, they should present biocompatibility to allow integration with the host tissues without eliciting inflammatory event, porous to allow nutrient exchange while preventing soft tissue infiltration, and to have adequate degradation rate, since these membranes must function during 4 weeks [17,18] to allow successful regeneration of the periodontal system.

Current membrane, with few exceptions, does not possess structural integrity to resist compressive forces to provide or maintain space provision. They commonly collapse or are compresses into the periodontal defects [19,20], resulting in limited or none regeneration [16]. Conventional non-resorbable membranes have as disadvantage the necessity of a second surgery, which implicates in additional discomfort and economic burden. Resorbable membranes do not require a secondary surgical procedure. The polyester-based membranes are biodegradable, allow tissue integration and are easier to handle when compared to non-resorbable membranes. However, their poor cell response is a great drawback [19,21,22]. Collagen membranes have excellent cell affinity and biocompatibility [21,22], but they show relatively poor mechanical and dimensional stability due to their rapid degradation and

precipitate collapse [21,22]. The disadvantages presented by both non-resorbable and resorbable membranes make clear the necessity of the "ideal" membrane development. The limitations of current membranes are evidenced by clinical studies. GTR-based procedures are generally considered unpredictable given the great variability found in clinical outcomes [23,24].

Numerous authors have been explored electrospinning technique to generate nanofibrous scaffolds for tissue regeneration [19,25,26]. The advantage of e-spun nanofibrous scaffolds is that their pore size are too small to allow cellular infiltration, but they allow fluid and nutrients exchange [27]. E-spun nanofibrous scaffolds resemble the morphologies of the native extracellular matrix, and their three-dimensional structure (3D) shows a high surface area of improved hydrophilicity and wettability [28–30]. These characteristics stimulate the cell-matrix extracellular interactions, increase the proliferation rate, maintain cell phenotype, support differentiation of stem cells, and support in vivo-like-three-dimensional matrix adhesion [31,32]. Researchers also have explored e-spun nanofibrous scaffolds incorporating antimicrobial agents [33–36] with promising outcomes in the infection control [33–35].

The synthesis and characterization of a mechanically strong biodegradable polydioxanone polymer-based e-spun antibiotics-containing nanofibrous scaffold was reported by Bottino and colleagues [37–39]

These scaffolds presented: (i) adequate mechanical properties to sustain handling, and to maintain its structure during regenerative procedures; (ii) inhibitory effect on biofilm of *Porphyromonas gingivalis*, *Aggragatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* with no inhibitory effect on commensal bacteria biofilm, such as *Lactobacillus casei* and *Streptococus salivarius*, (iii) and controlled release of drug. Biodegradable nanofibrous drug delivery systems could promote a bacterial free environment favorable to

GTR, and could help in the failures percentage reduction due to the infection, while minimizing the adverse effects associated with the use of the systemic antibiotics.

Discovery and characterization of biological mechanisms and materials involves the use of *in vivo* animals' models. Thus, our proposal was to evaluate the biocompatibility of polydioxanone e-spun antibiotics-containing nanofibrous scaffold in subcutaneous implantation model in rats, which is commonly used for this aim [40–43]. We evaluated initial (3 days) and late (30 days) inflammatory response on the following outcomes: tissue oxidative profile, myeloperoxidase, N-acetyl-β-D-glucosaminidase and collagen fiber degradation, and systemic inflammatory response from white blood counts.

2. MATERIAL AND METHODS

2.1 Synthesis of electrospun drug delivery systems

Polydioxanone monofilament surgical sutures (PDS II®, Ethicon, Somerville, NJ, USA) were cut into pieces and placed into glass vials containing dichloromethane (Sigma Aldrich, St Louis, MO, USA) to remove the violet dye [38]. Clear PDS was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma Aldrich) at a 10wt% concentration with stirring. Metronidazole (Sigma Aldrich) with molecular weight (M_w) of 171.15 g/mol, or ciprofloxacin (Sigma Aldrich) with M_w of 331.346 g/mol, were directly added in 25wt% concentration (respective to the total polydioxanone/PDS weight) and mixed together under vigorous stirring. A custom-made electrospinning apparatus was used to synthesize the different drug delivery systems following standard electrospinning practices [37,38].

Pure PDS or antibiotic-containing PDS solutions were individually loaded into plastic syringes fitted with a 27-gauge stainless steel needle and electrospun directly over glass slides under optimized parameters (i.e., 15 kV power voltage to the needle tip, 20 cm collection distance and a flow rate of 1 ml h^{-1}). The fibers were collected at room temperature on the aluminum foil covered rotating mandrel containing the glass slides. The electrospun scaffolds

were dried at room temperature in vacuum desiccators for at least 48 h to remove any residual solvent [37,38].

2.2 Experimental design

The experimental protocols followed ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and was approved by Committe on Animal Research of the Federal University of Santa Maria, Brazil (4141250315).

Sixty rats (male, *Rattus norvegicus albinus*, Wistar) with 90 days old (250-300 g) were housed (5 animals per cage). They were fed with regular rat pellets and distilled water *ad libitum*. The room presented controlled temperature (23±1^{o}C), relative humidity of the air close to 60% and exhaust air, noise control (maximum 85 decibels), and standard light-dark cycle (12 hours each). Before the experimental procedures, the animals were allowed to acclimatize to the laboratory environment for a period of 2 weeks.

The animals were allocated into two groups (30 rats per group) to evaluate the initial (3 days) and late (30 days) inflammatory response to the scaffolds implantation. A computer program (Random Allocation Software, version 1.0, May 2004) was used to randomly allocate the animals into the experimental groups according Table 1. One study team member (RM) performed the randomization process.

Table 1. Experimental groups description.

Experimental groups		Animal number to evaluate initial inflammatory response: 3 days	Animal number to evaluate late inflammatory response: 30 days
SHAM	One subcutaneous pocket, no scaffold.	5	5
1PDS	One polydioxanone scaffold.	5	5
1MET	One polydioxanone scaffold containing metronidazole 25wt% per rat.	5	5
2MET	Two polydioxanone scaffolds containing metronidazole 25wt% per rat.	5	5
1CIP	One polydioxanone scaffold containing ciprofloxacin 25wt% per rat.	5	5
2CIP	Two polydioxanone scaffolds containing ciprofloxacin 25wt% per rat.	5	5

The surgical procedures were performed for one operator (PCP) and in the order of the randomization sequence. The operator preformed subcutaneous pockets and after was informed if the animals received the scaffold or not. The scaffolds presented the same size, coloration and flexibility. Thus, the operator was masked to the experimental groups.

Animals were anesthetized with ketamine (70 mg/kg) and xylazine (6 mg/kg), via intramuscular. The back of each rat shaved, and scrubbed with chlorhexidine (2%) to disinfect and draped for sterile surgery. One incision (3 cm) was performed through the skin on right side of the midline. For animals of the 2MET and 2CIP groups, one incision (3 cm) on each side of the midline with at least 2 cm distance from each other was performed. In each incision, a pouch was created subcutaneously (3 - 4 cm^2) using blunt dissection. Scaffold (2 x 2 cm) was implanted in each subcutaneous pouch, with exception of the SHAM animals. The skin incisions were subsequently stitched with non-resorbable 6.0 sutures (Ethicon) and adhesives strips (Leukostrip, Smith & Nephe Ltda, SP, Brazil).

After 3 and 30 days, the animals were anesthetized with an inhalation of isoflurane (3%) (Isoflurano, BioChimico, Itatiaia, RJ, Brazil) in an oxygen carrier. Blood was collected by cardiocentese (22G1 needle and 5 ml syringe) in EDTA anticoagulant tubes. Euthanasia was performed by total exsanguinations at the corresponding time point (3 and 30 days), and implants retrieved for evaluations.

Immediately after death, the biomaterials were explanted together with the surrounding peri-implantation tissue. The biopsy was sectioned in two equal segments. One segment was used to analyze the tissue oxidative status and cellular enzymatic profile, and the other segment was fixed in 4% buffered formalin for 72 h prior to histological analysis (Figure 1).

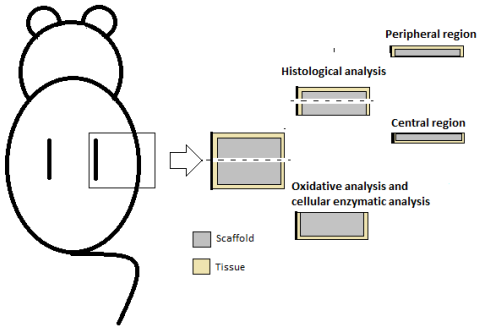


Figure 1. Samples diagram for oxidative, cellular enzymatic activity and histological analysis

2.3 Evaluation methods

2.3.1 Oxidative status

Oxygen reactive species (ROS) are produced in mitochondria during respiratory chain electron transport and they should be rapidly transformed into more inactive species by the antioxidant defenses. However, the increased production of ROS during the inflammatory process cannot be counteracted by a concomitant increase in enzymatic (catalase, glutathione, superoxide dismutase) and nonenzimatic (vitamin C) natural antioxidant defenses producing cellular damage, which can be measured from protein carbonylation and lipidic peroxidation. Oxidative stress occurs when there is an imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis, promoting tissue damage.

2.3.1.1 ROS generation with DCH

ROS levels were quantified using the oxidant-sensing fluorescent probe, 2,7-dichlorofluorescein diacetate (DCHF-DA). The oxidation (DCHF-DA) to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. Tissue samples were added to a medium containing TrisHCl buffer (0.01 nM, pH 7.4) and centrifuged (15 minutes, 3040 rpm). After homogenization, DCHF-DA was added to the solution for 1 hour until fluorescence measurements procedure. DCF-RS levels were corrected by the protein content [44].

2.3.1.2 Protein carbonyl (PC) determination

PC is a type of protein oxidation that can be promoted by ROS. It usually refers to a process that forms reactive ketones or aldehydes that can be reacted by 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones. PC was measured according to Yan et al. (1995) with some alterations. Aliquots of tissue sample homogenized in TrisHCl buffer (10 mM, pH 7.4) were mixed with 0.2 ml of DNPH (10 mM). After 1 hour of incubation at room temperature in the dark, 0.5 ml of denaturing buffer (150 mM sodium phophate buffer, pH 6.8, 3% SDS), 2 ml of heptane (99.5%) and 2 ml of ethanol (99.8%) were added sequentially, mixed for 40 seconds and centrifuged for 15 minutes. Thereafter, the protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each sample was measured at 370 nm against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22 000 M^{-1} cm^{-1}. The results were expressed as the nmol carbonyl/g $tissue^{-1}$[45].

2.3.1.3 Lipidic peroxidation (LP) estimation

LP or reaction of oxygen with unsaturated lipids occurs when reactive oxygen species attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells. One product of lipid peroxidation is the malondialdehyde (MDA), which present facile reaction with thiobarbituric acid (TBA) yields an intensely colored chromogen fluorescent red. LP was measured by reaction of MDA with TBA, as described by Ohkawa et al. [46]. A pink chromogen was generated and spectrophotometrically measured at 532 nm. The TBARS levels (substances that react with TBA) were expressed as nmol of MDA g $tissue^{-1}$.

2.3.1.4 Catalase (CAT) activity

CAT is an enzyme that catalyses the decomposition of hydrogen peroxide to water and oxygen, protecting the cells from oxidative damage by ROS**.** CAT activity was spectrophotometrically quantified in tissue sample, which includes monitoring the disappearance of H_2O_2 in the presence of cell homogenate (pH 7 at 25^{o}C) at 240 nm. The disappearance of H_2O_2 is directly proportional to the CAT activity. The enzymatic activity was expresses as µmol H_2O_2 g tissue^{-1} min^{-1}.

2.3.1.5 Glutathione (GSH)

GSH is one the most important cellular antioxidants. GSH react with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) generating the 5-mercapto-2-nitro-banzoic acid (TNB) which is determined by its absorption at 412 nm. GSH levels were determined after the reaction of tissue sample homogenates DTNB, which is reduced by GSH, generating the yellow color solution measured at 412 nm (TNB). GSH levels were express as µmol GSH per g tissue sample [47].

2.3.1.6 Vitamin C (VIT C)

Ascorbic acid or VIT C is a redox catalystic, which can reduce, and thereby neutralize, ROS such as hydrogen peroxide. VIT C was estimated as described by Galley et al. [48], with some changes [49]. This method produces an orange chromogen by the reaction with dinitrophenylhydrazine at 37°C, measured spectrophotometrically at 520 nm. A standard curve using ascorbic acid was used to calculate the content of VIT C and expressed as mg VIT C g tissue^{-1}.

2.3.2 Histological analysis

The segment used to histological analysis was cut into two identical segments [41]. These tissue segments contained the peripheral and center region (Figure 1) of the implantation bed. Further processing involved dehydration of the biopsies and paraffin

embedding. Accordingly, from each animal two consecutive 5µm serial sections were cut from each segment (peripheral region- and center- of the biopsies). Masson's trichrome (MT) staining was used for the first section (Sigma Aldrich) and Picrosirius Red (PRed) (Picrosirius Red Staining, Easy Path®, São Paulo, SP, Brazil) for second section.

The sections dyed with MT were digitalized at x 200 magnification using optical microscopy (Axiovision; Carl Zeiss MicroImaging, Jena, Germany). From left margin of tissue sample, fields of view were numbered consecutively up to right margin. On average 14 fields were displayed. Of these, the fields 7, 8, and 9 were selected to determine the percentage of area occupied by collagen fibers from peripheral and central region. The image of the fields was obtained using image-processing software (Zen lite 2012 blue edition, Carl Zeiss MicroImagnig, Jena, Germany). The images were transferred to another images software (ImageJ v1.50i, National Institutes of Health, USA), in which they were binarized and the percentage of area filled by collagen fibers was calculated [50]. Descriptive analysis of collagen fiber was performed in the same fields (7, 8 and 9) using sections dyed with PRed at x 400 magnification using polarization microscopy (Leica, model DM2000, v. 4.0, Germany) (Figure 2).

All the histometric analyses were performed by one examiner (PCP), who was blinded to the experimental groups.

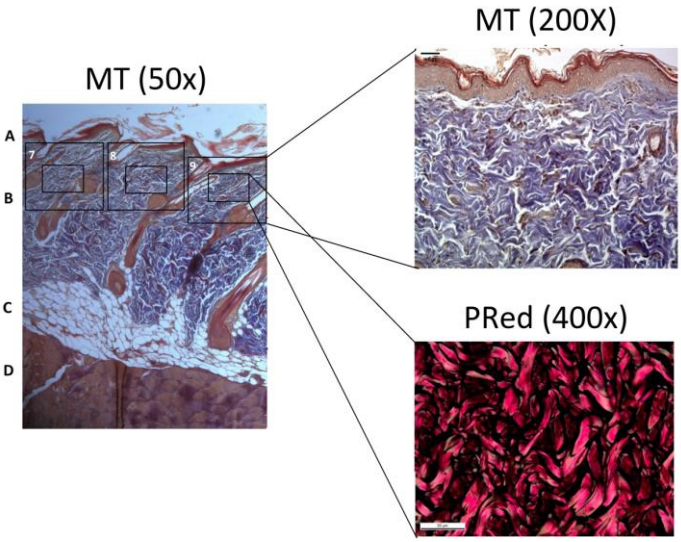


Figure 2. Diagram of the evaluation methods of collagen fibers from MT and PRed dye. A. Epidermis. B. Dermis. C. Subcutaneous tissue. D. Muscle tissue.

2.3.3 Measurement of myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities

MPO and NAG enzyme activities were evaluated as previously described by Oliveira et al. [51]. Tissue samples were homogenized in acetate buffer (80 mM, pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide and kept at 4°C. Immediately before the assay, the tissue homogenate was centrifuged at 11,000 x g for 20 min and the supernatant was then collected for assay.

To determine the MPO enzyme activity, the supernatant was incubated with 3,3′,5,5′-tetramethylbenzidine solution (18.4 mM) for 3 minutes at 37°C. The reaction was stopped on ice by the addiction of acetic acid. The color formed was analyzed by a spectrophotometer at 630 nm. Otherwise, in order to determine NAG activity, the supernatant was mixed with p-nitrofenil-2-acetamide-β-D-glucopyranoside (NAG 2.25 nM) and incubated for 60 minutes at 37°C. After this time, the reaction was stopped on ice by the addition of glycine buffer (0.2 µM, pH 10.4). The color formed was analyzed by a spectrophotometer at 405 nm. Both reactions were read on a Fisher Biotech Microkinetics BT 2000 microplate reader. Values were expressed as optical densities and corrected by ml sample.

2.4 Statistical Analysis

Mean and standard deviation (SD) were calculated for data of oxidative profile, MPO and NAG activities and percentage of collagen fiber.

Normality of the data was analyzed using the Shapiro-Wilk test. The intergroup analyses were performed with Two-way ANOVA and post hoc Duncan' test. Differences were considered significant when $p<0.05$. The program *Statistics 8.0* was used for all analyses.

3. RESULTS

3.1 Oxidative status

Oxidative status profile of the animals in 3 and 30 days were presented in Figure 3.

PDS scaffold statistically increases ROS production when compared to the SHAM for both evaluation periods. However, only in 3 days, PDS promote higher cellular damage from LP and PC when compared to the SHAM only in 3 days.

In 3 days, antibiotics scaffolds showed a significant statistically reduction of ROS when comparing to the PDS scaffold, with exception 1CIP group. 2CIP was the only group that simultaneously reduced the cellular damage from LP and PC when comparing to the PDS, presenting, thus, the best results in this evaluation time. In 30 days, the best results were verified from 1MET, 2MET and 2CIP that showed protective effect for cellular damage, yield lower statistically LP levels when compared to the PDS and SHAM, and, at the same time, they were statistically similar to the PDS and SHAM regard PC levels.

In 3 days, SHAM animals consumed highest levels of antioxidant defenses, especially VIT C, explaining its lower cellular damage. Despite PDS shows similar consume of antioxidant defense when compared to the antibiotics scaffolds, and similar CAT consume when compared to the SHAM, PDS presented highest cellular damage *via* LP. The imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis, promoting tissue

damage associated with oxidative stress in animals that received PDS scaffolds. In 30 days, 1CIP showed the highest PC levels and the lowest consume of CAT, GSH and VIT C. Thus, 1CIP do not obtained enough antioxidant defenses to stop cellular damage.

The oxidative results of the animals that received the implantation of two scaffolds were different from those that received only one scaffold. This finding was interesting because the oxidative analyses were performed from local tissue of only one scaffold.

3.2 MPO and NAG activities

MPO and NAG activity increased between 3 and 30 days for all groups (Table 2).

In 3 days, PDS group showed higher statistically MPO activity when compared to the other groups. With antibiotics, the MPO activity did not present statistical alterations when compared to SHAM. The incorporation antibiotics prevented the MPO activity increase caused by PDS. Only in 30 days statistical differences were observed for NAG activity. The PDS, 2MET and 2CIP groups showed the highest mean values. 2MET presented higher significantly NAG activity when compared to the PDS and SHAM.

Table 2. Mean (SD) of the levels of myeloperoxidase and N-acetyl-β-D-glucosaminidase.

		SHAM	**PDS**	**1MET**	**2MET**	**1CIP**	**2CIP**
MPO	3d	0.75^{b} (0.2)	1.43^{a} (0.4)	0.93^{b} (0.3)	0.8^{b} (0.1)	0.83^{b} (0.04)	0.79^{b} (0.07)
	30d	1.49^{A} (0.2)	1.53^{A} (0.2)	1.42^{A} (0.3)	1.73^{A} (0.4)	1.67^{A} (0.5)	1.76^{A} (0.6)
	P	0.00	0.66	0.03	0.00	0.00	0.00
NAG	3d	0.62^{a} (0.1)	0.68^{a} (0.1)	0.55^{a} (0.1)	0.73^{a} (0.02)	0.56^{a} (0.07)	0.7^{a} (0.1)
	30d	$0.86^{B.D}$ (0.1)	$1.09^{B.C}$ (0.1)	$0.96^{B.C}$ (0.1)	1.31^{A} (0.4)	0.69^{D} (0.1)	$1.12^{A.C}$ (0.2)
	P	0.05	0.00	0.00	0.00	0.27	0.00

MPO: myeloperoxidase; NAG: N-acetyl-β-D-glucosaminidase.
Two way ANOVA and Duncan´s test.
Line: Different lower case shows statistical difference between groups for MPO at 3 days ($p<0.05$). Different capital letters shows statistical difference between groups for NAGase at30 days ($p<0.05$).
Collumn: $p<0.05$ shows statistical difference intergroup.

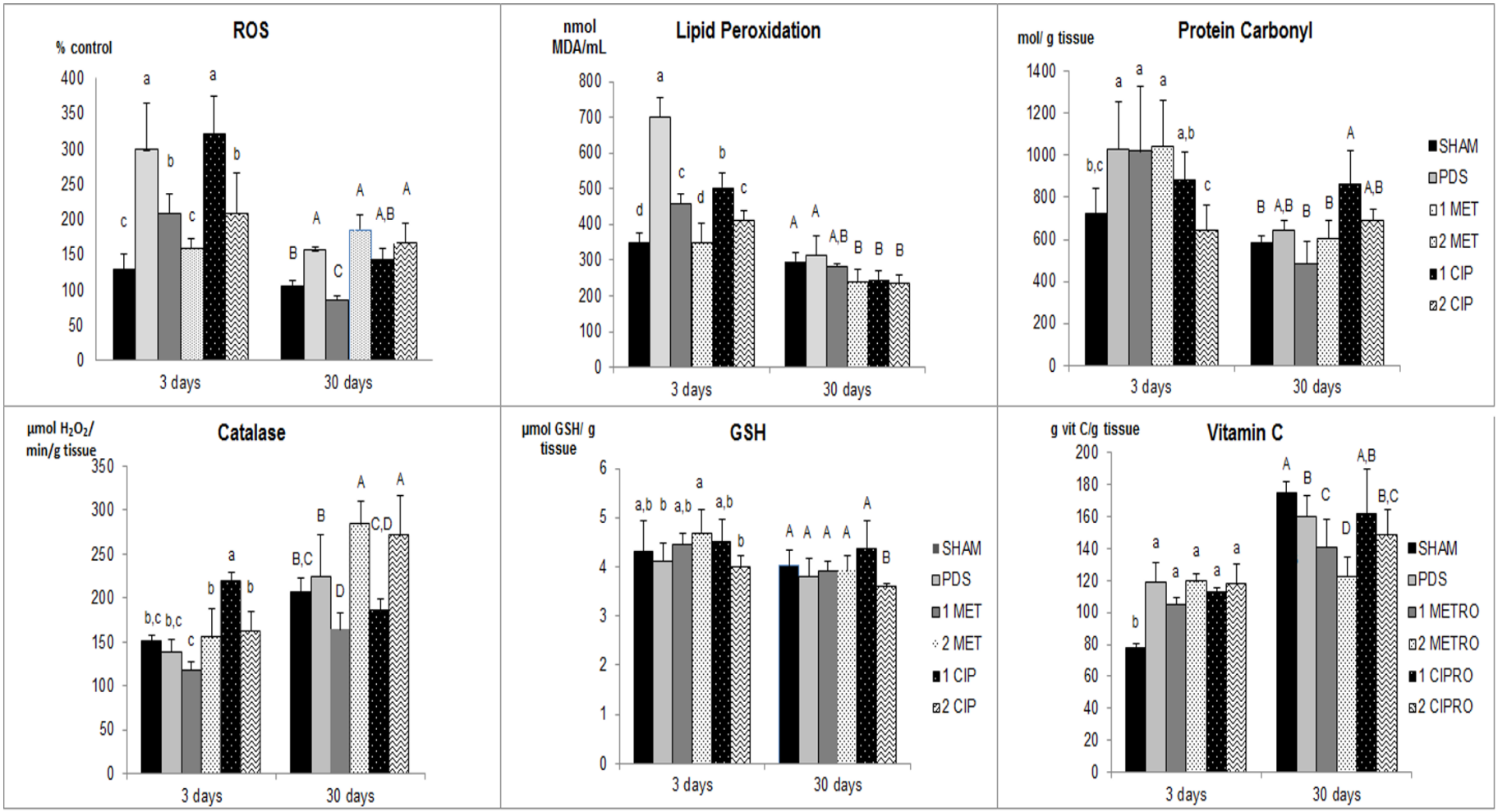

ROS
% control
3 days
30 days
Lipid Peroxidation
nmol MDA/mL
3 days
30 days
Protein Carbonyl
mol/ g tissue
3 days
30 days
SHAM
PDS
1 MET
2 MET
1 CIP
2 CIP
Catalase
µmol H_2O_2/ min/g tissue
3 days
30 days
GSH
µmol GSH/ g tissue
3 days
30 days
SHAM
PDS
1 MET
2 MET
1 CIP
2 CIP
Vitamin C
g vit C/g tissue
3 days
30 days
SHAM
PDS
1 METRO
2 METRO
1 CIPRO
2 CIPRO

Figure 3. Oxidative status profile in 3 and 30 days after scaffolds subcutaneous implantation. Data expressed as means and SD (n=5).
ROS: reactive oxygen species, LP: lipid peroxidation, PC: protein carbonyl, CAT: catalase, GSH: glutathione, VIT C: vitamin C.
Two way Anova, and Duncan's test.
Different lower case letters represent statistical differences between groups at 3 days ($p<0.05$).
Different capital letters represent statistical differences between groups at 30 days ($p<0.05$).

3.3 Collagen fibers

In 3 days and 30 days, no deleterious effect was observed with the scaffold implantation on collagen fibers. The exception was 2MET that showed lower significantly collagen fiber percentage than SHAM group (Figure 5).

All groups showed the same pattern of collagen fibers (PRed). Most of the fibers were type I, dense and thick. Few fibers type III were observed and were interlaced with fibers type I. These results demonstrated the collagen fibers quality was not altered by presence of scaffolds.

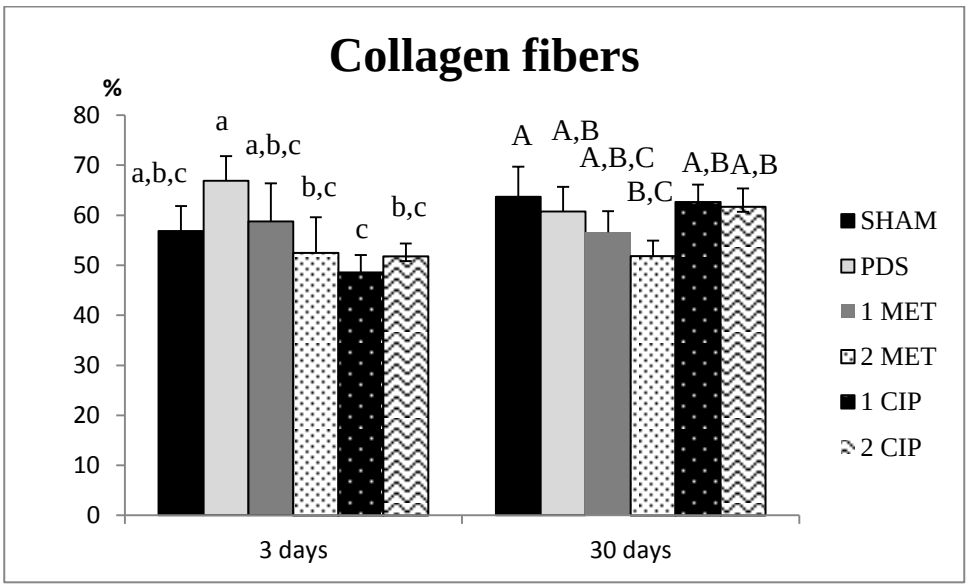


Figure 4. Mean percentage of collagen fibers at 3 and 30 days after scaffolds subcutaneous implantation. Data expressed as mean ± SD (n=5).
Two way Anova, and Duncan's test.
Different lower case letters represent statistical differences between groups at 3 days ($p<0.05$). Different capital letters represent statistical differences between groups at 30 days ($p<0.05$).

DISCUSSION

The proposal of the present study was to evaluate the biocompatibility of

polydioxanone e-spun antibiotics-containing nanofibrous scaffold in subcutaneous implantation model in rats. Polydioxanone is a bioresorble synthetic polymer, FDA-approved, and commonly used in medical and dental applications as suture material. No long-term cytotoxicity has been verified with PDS implanted for up to 6 month in mice [52]. Therefore, we presumed PDS scaffold should promote minimal inflammatory response when compared to SHAM animals. Collectively, our findings showed PDS scaffold presented the worse initial inflammatory response (3 days) demonstrating the higher ROS production, the higher MPO activity, and the higher cell damage from LP and PC when compared to SHAM animals; and, in general, the antibiotics inclusion was beneficial, since they promote reduction of inflammatory parameters when compared to the PDS scaffold. In late inflammatory response (30 days), usually, PDS scaffold presented the similar comportment to the SHAM animals, and scaffolds containing antibiotics, showed protective effect for cellular damage, yield lower LP levels when compared to the PDS and SHAM, and similar PC levels to the PDS and SHAM.

At sites of inflammation, there is considerable over production of various oxygen reactive species, such as superoxide radical (O2-), hydrogen peroxide (H2O2), and the reactive chlorine species hypochlorous acid (HOCl). These oxygen reactive species are produced in large quantities by activated neutrophils. Phagocytes (neutrophils and macrophages) respond to stimulation with a burst of oxygen consumption, and practically all-extra oxygen is converted to the superoxide anion and hydrogen peroxide. MPO, which is released from cytoplasmic granules of phagocytes by degranulation process, reacts with hydrogen peroxide, formed by the respiratory burst, to form a complex that can oxidize a large variety of substances generating chlorine and chloramines. The products of the MPO/hydrogen peroxide/chloride system are powerful oxidants, and in high concentrations can cause cellular damage due its high instability and reactivity [53–57]. Therefore, in the

initial inflammatory response, PDS caused greater cellular damage due the higher production of ROS consequence of the respiratory explosion of activated neutrophil confirmed by higher levels of MPO in this group.

In 3 days, generally, the antibiotics incorporation to the PDS scaffolds reduce ROS production, MPO activity, and cellular damage. All antibiotics scaffolds reduced the cellular damage originated from LP. But, only 2CIP simultaneously reduced the cellular damage from LP and PC when comparing to the PDS, presenting the best results in this period. The difference observed between the antibiotics types can be explained by differences of drugs release profile. Bottino et al. [37] showed data of the cumulative drug release as a percentage of the total weight recorded prior to the study. After 3 days, the PDS scaffold containing 25wt% ciprofloxacin showed a cumulative release of 28%, and its metronidazole counterpart release approximately 8%. In the third day, while PDS scaffold containing 25wt% ciprofloxacin was realizing 250 μg of the drug, its metronidazole counterpart released approximately 100 μg.

Tissue diffusion and bioavailability of a drug depend pH, local vascularization, presence of bacterial enzyme, drug physical and chemical properties, such as size and weight molecular, charge, dissociation constants (pKa), liposolubility, and partition coefficient [58,59]. Drugs administered by the subcutaneous route can reach the systemic circulation either by uptake by blood capillaries or indirectly by lymphatic vessels [60], and also can perform diffusion and advection into matrix extracellular before uptake by the lymphatic capillaries draining the site [59]. Small peptides and proteins leave the subcutaneous site by diffusion into blood capillaries. Transport of larger proteins from subcutaneous site involves travel through the interstitium and into the lymphatic system. Larger molecules have been found to be trapped at the site of administration for longer durations resulting in a reduced extent uptake [61]. The ciprofloxacin molecule (Mw 331.346 g/mol) is more weight and

larger than metronidazole molecule (Mw 171.15 g/mol). Therefore, it's possible that metronidazole molecules were uptake by blood capillaries or lymphatic vessels more rapidly, while ciprofloxacin molecules were kept at the site for longer durations permitting its diffusion and convection through the interstitium promoting higher concentrations of the drug on contralateral site where tissue sample was collected for analysis. Also, it's plausible that the ciprofloxacin bioavailability has been higher than metronidazole due the release of higher percentage and dosage of the drug from ciprofloxacin scaffold when compared to the metronidazole scaffold [38]. These aspects could explain the difference of results between the same antibiotics, but with different number of scaffolds, and also why 2CIP was only group that simultaneously reduced the cellular damage from LP and PC when comparing to the PDS in 3 days.

Previous studies demonstrated PDS scaffold containing 25wt% ciprofloxacin was cytotoxic on human dental pulp stem cells *in vitro* [38,39]. Our results demonstrated no deleterious effect of PDS scaffold containing 25wt% ciprofloxacin when compared to the other groups. On the other hand, we verified protective effects this scaffold on cellular damage from oxidative process and no impair on collagen fiber. According Liu & Cao [62], there are differences in the scaffold comportment *in vitro* and *in vivo*, and differences into of the same species accord sites of implantation.

All scaffold showed similar comportment on collagen fibers when compared to the SHAM, in 3 and 30 days. These findings demonstrated no dermis phenotypic alteration, probably due the scaffolds morphologies to allow the nutrients exchange [27,31,32,40]. Although, Bottino et al. [38] verified significant reduction of fiber diameter of the PDS matrices after antibiotics incorporation (25wt% ciprofloxacin 765±288 nm, and its metronidazole counterpart 944±392 nm) resulting in higher porosity when compared to the PDS matrix (1158±402 nm), these changes don't affected the exchange nutrients.

In all groups, an activity increase of MPO and NAG was verified between 3 and 30 days, without any increase of ROS or cellular damage, probably because there was simultaneous increase of the anti-oxidant defenses, particularly catalase e vitamin C. The increase of the MPO activity, between 3 and 30 days, can be explained from environment pH differences. In the initial inflammatory response, the environment presents a pH acid inactivating, partially, this enzyme [57]. In the late inflammatory response, there is higher macrophages activity acting in the tissue repair [63]. Interestingly, in 3 and 30 days, the higher degradation of collagen fiber occurred in the animals that received two metronidazole scaffolds. These animals also presented the higher mean values of NAG activity, indicating the necessity of higher tissue repair.

Collectively, the results of this study showed that PDS scaffold have no intense adverse effect on outcomes evaluated. But, PDS scaffold showed higher cellular damage via oxidative process when compared to SHAM. The antibiotic incorporation to the PDS matrices improved the oxidative profile when compared to PDS scaffold, particularly in animals that received two ciprofloxacin scaffolds. Then, we consider that the promising results of antimicrobial and mechanical properties [38,39] associated with the biocompatibility findings of the present study, become polydioxanone polymer-based e-spun antibiotics-containing nanofibrous scaffolds attractive for RTG. Further studies with other outcomes should be performed to confirm the biocompatibility these scaffolds, and to analyze degradation rate and structural integrity, which are decisive factors in periodontal regenerative context.

Acknowledgements

The researcher and collaborator, Marco Cicero Bottino, Indiana University School of Dentistry (IUPUI). This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES). The authors declare no conflict of interest.

REFERENCES

[1] Kornman KS. Mapping the Pathogenesis of Periodontitis: A New Look. J Periodontol 2008;79:1560–8. doi:10.1902/jop.2008.080213.

[2] Albandar JM, Brunelle JA, Kingman A. Destructive Periodontal Disease in Adults 30 Years of Age and Older in the United States, 1988-1994. J Periodontol 1999;70:13–29. doi:10.1902/jop.1999.70.1.13.

[3] Susin C, Vecchia CFD, Oppermann R V., Haugejorden O, Albandar JM. Periodontal Attachment Loss in an Urban Population of Brazilian Adults: Effect of Demographic, Behavioral, and Environmental Risk Indicators. J Periodontol 2004;75:1033–41. doi:10.1902/jop.2004.75.7.1033.

[4] Eke PI, Wei L, Thornton-Evans GO, Borrell LN, Borgnakke WS, Dye B, et al. Risk Indicators for Periodontitis in US Adults: National Health and Nutrition Examination Survey (NHANES) 2009 – 2012. J Periodontol 2016;2020:1–18. doi:10.1902/jop.2016.160013.

[5] Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005;83:661–9. doi:/S0042-96862005000900011.

[6] Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJL, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res 2014;93:1045–53. doi:10.1177/0022034514552491.

[7] Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J Clin Periodontol 2002;29 Suppl 2:6–16. doi:10.1034/j.1600-051X.29.s2.4.x.

[8] Zwahlen M, Np L. Influence of residual pockets on progression of periodontitis and tooth loss : Results after 11 years of maintenance 2008:685–95. doi:10.1111/j.1600-051X.2008.01245.x.

[9] Cortellini P, Tonetti MS. Clinical concepts for regenerative therapy in intrabony defects. Periodontol 2000 2015;68:282–307. doi:10.1111/prd.12048.

[10] Peridontol C. Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials : a randomized-controlled trial in intra-bony defects 2011:365–73. doi:10.1111/j.1600-051X.2011.01705.x.

[11] Polimeni G, Koo KT, Qahash M, Xiropaidis A V., Albandar JM, Wikesj?? UME. Prognostic factors for alveolar regeneration: Effect of tissue occlusion on alveolar bone regeneration with guided tissue regeneration. J Clin Periodontol 2004;31:730–5.

doi:10.1111/j.1600-051X.2004.00543.x.

[12] Polimeni G, Koo K, Xiropaidis V, Albandar JM. Prognostic factors for alveolar regeneration : bone formation at teeth and titanium implants 2004:927–32. doi:10.1111/j.1600-051X.2004.00590.x.

[13] Polimeni G, Susin C, Wikesj?? UME. Regenerative potential and healing dynamics of the periodontium: A critical-size supra-alveolar periodontal defect study. J Clin Periodontol 2009;36:258–64. doi:10.1111/j.1600-051X.2008.01369.x.

[14] Gottlow J, Nyman S, Lindhe J, Karring T, Wennstrom J. New attachment formation in the human periodontium by guided tissue regeneration Case reports. J Clin Periodontol 1986;13:604–16. doi:10.1111/j.1600-051X.1986.tb00854.x.

[15] Gottlow J, Karring T, Nyman S. Guided Tissue Regeneration following treatment of recession-type defects in the monkey. J Periodontol 1990;61:680–5.

[16] Haney JM, Nilvéus RE, Mcmillan PJ, Wikesjò UE. Repair in Dogs : Expanded Polytetrafluoroethylene Barrier Regeneration. J Periodontol 1993:883–90.

[17] Dickinson DP, Coleman BG, Batrice N, Lee J, Koli K, Pennington C, et al. Events of wound healing/regeneration in the canine supraalveolar periodontal defect model. J Clin Periodontol 2013;40:527–41. doi:10.1111/jcpe.12055.

[18] Susin C, Wikesjö UME. Regenerative periodontal therapy: 30 years of lessons learned and unlearned. Periodontol 2000 2013;62:232–42. doi:10.1111/prd.12003.

[19] Bottino MC, Thomas V, Janowski GM. A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. Acta Biomater 2011;7:216–24. doi:10.1016/j.actbio.2010.08.019.

[20] Wound healing following surgical and regenerative periodontal therapy 2015;68:83–98.

[21] Kikuchi M, Koyama Y, Yamada T, Imamura Y, Okada T, Shirahama N, et al. Development of guided bone regeneration membrane composed of ??-tricalcium phosphate and poly (L-lactide-co-glycolide-co-??- caprolactone) composites. Biomaterials 2004;25:5979–86. doi:10.1016/j.biomaterials.2004.02.001.

[22] Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: Morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96:1–11. doi:10.1007/s10266-008-0087-y.

[23] Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. Ann Periodontol 2003;8:266–

302. doi:10.1902/annals.2003.8.1.266.

[24] Needleman IG, Worthington H V, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev 2006;37:CD001724. doi:10.1002/14651858.CD001724.pub2.

[25] Zamani F, Amani-Tehran M, Latifi M, Shokrgozar MA, Zaminy A. Promotion of spinal cord axon regeneration by 3D nanofibrous core-sheath scaffolds. J Biomed Mater Res - Part A 2014;102:506–13. doi:10.1002/jbm.a.34703.

[26] Münchow EA, Pankajakshan D, Albuquerque MTP, Kamocki K, Piva E, Gregory RL, et al. Synthesis and characterization of CaO-loaded electrospun matrices for bone tissue engineering. Clin Oral Investig 2015. doi:10.1007/s00784-015-1671-5.

[27] Komath M, Rajesh J, Nandakumar K, Varma H. Calcium phosphate cement as a "barrier-graft" for the treatment of human periodontal intraosseous defects. Indian J Dent Res 2009;20:471. doi:10.4103/0970-9290.59459.

[28] Fu Y-C, Nie H, Ho M-L, Wang C-K, Wang C-H. Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/HAp composite scaffolds loaded with BMP-2. Biotechnol Bioeng 2008;99:996–1006. doi:10.1002/bit.21648.

[29] Srouji S, Ben-David D, Lotan R, Livne E, Avrahami R, Zussman E. Slow-release human recombinant bone morphogenetic protein-2 embedded within electrospun scaffolds for regeneration of bone defect: in vitro and in vivo evaluation. Tissue Eng Part A 2011;17:269–77. doi:10.1089/ten.TEA.2010.0250.

[30] Phipps MC, Clem WC, Catledge SA, Xu Y, Hennessy KM, Thomas V, et al. Mesenchymal stem cell responses to bone-mimetic electrospun matrices composed of polycaprolactone, collagen I and nanoparticulate hydroxyapatite. PLoS One 2011;6:1–8. doi:10.1371/journal.pone.0016813.

[31] Li M, Guo Y, Wei Y, MacDiarmid AG, Lelkes PI. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. Biomaterials 2006;27:2705–15. doi:10.1016/j.biomaterials.2005.11.037.

[32] Venugopal J, Prabhakaran M, Low S, Choon A, Zhang Y, Deepika G, et al. Nanotechnology for Nanomedicine and Delivery of Drugs. Curr Pharm Des 2008;14:2184–200. doi:10.2174/138161208785740180.

[33] Kim K, Luu YK, Chang C, Fang D, Hsiao BS, Chu B, et al. Incorporation and controlled release of a hydrophilic antibiotic using poly(lactide-co-glycolide)-based electrospun nanofibrous scaffolds. J Control Release 2004;98:47–56. doi:10.1016/j.jconrel.2004.04.009.

[34] Ruckh TT, Carroll DA. NIH Public Access 2012;23:1411–20. doi:10.1007/s10856-012-4609-3.Antimicrobial.

[35] Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS. Electrospun antibacterial polyurethane-cellulose acetate-zein composite mats for wound dressing. Carbohydr Polym 2014;102:884–92. doi:10.1016/j.carbpol.2013.10.070.

[36] Hu N, Wu H, Xue C, Gong Y, Wu J, Xiao Z, et al. Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. Biomaterials 2013;34:100–11. doi:10.1016/j.biomaterials.2012.09.020.

[37] Bottino MC, Arthur RA, Waeiss RA, Kamocki K, Gregson KS, Gregory RL. Biodegradable nanofibrous drug delivery systems: effects of metronidazole and ciprofloxacin on periodontopathogens and commensal oral bacteria. Clin Oral Investig 2014;18:2151–8. doi:10.1007/s00784-014-1201-x.

[38] Bottino MC, Kamocki K, Yassen GH, Platt J a, Vail MM, Ehrlich Y, et al. Bioactive nanofibrous scaffolds for regenerative endodontics. J Dent Res 2013;92:963–9. doi:10.1177/0022034513505770.

[39] Kamocki K, Nör JE, Bottino MC. Effects of ciprofloxacin-containing antimicrobial scaffolds on dental pulp stem cell viability - In vitro studies. Arch Oral Biol 2015;60:1131–7. doi:10.1016/j.archoralbio.2015.05.002.

[40] Deliormanli AM, Liu X, Rahaman MN. Evaluation of borate bioactive glass scaffolds with different pore sizes in a rat subcutaneous implantation model. J Biomater Appl 2014;28:643–53. doi:10.1177/0885328212470013.

[41] Ghanaati S, Barbeck M, Detsch R, Deisinger U, Hilbig U, Rausch V, et al. The chemical composition of synthetic bone substitutes influences tissue reactions in vivo: histological and histomorphometrical analysis of the cellular inflammatory response to hydroxyapatite, beta-tricalcium phosphate and biphasic calcium phosphate cer. Biomed Mater 2012;7:015005. doi:10.1088/1748-6041/7/1/015005.

[42] Sabri F, Boughter JD, Gerth D, Skalli O, Phung TCN, Tamula GRM, et al. Histological Evaluation of the Biocompatibility of Polyurea Crosslinked Silica Aerogel Implants in a Rat Model: A Pilot Study. PLoS One 2012;7. doi:10.1371/journal.pone.0050686.

[43] Bavariya AJ, Andrew Norowski P, Mark Anderson K, Adatrow PC, Garcia-Godoy F, Stein SH, et al. Evaluation of biocompatibility and degradation of chitosan nanofiber membrane crosslinked with genipin. J Biomed Mater Res - Part B Appl Biomater 2014;102:1084–92. doi:10.1002/jbm.b.33090.

[44] Randall RJ, Lewis a. The folin by oliver. Readings 1951;193:265–75. doi:10.1016/0304-3894(92)87011-4.

[45] Yan LJ, Traber MG, Packer L. Spectrophotometric Method for Determination of Carbonyls in Oxidatively Modified Apolipoprotein B of Human Low-Density Lipoproteins. Anal Biochem 1995;228:349–51. doi:10.1006/abio.1995.1362.

[46] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8. doi:10.1016/0003-2697(79)90738-3.

[47] OWENS CW, BELCHER R V. a Colorimetric Micro-Method for the Determination of Glutathione. Biochem J 1965;94:705–11. doi:10.1042/bj0940705.

[48] Galley HF, Davies MJ, Webster NR. Ascorbyl radical formation in patients with sepsis: Effect of ascorbate loading. Free Radic Biol Med 1996;20:139–43. doi:10.1016/0891-5849(95)02022-5.

[49] Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JB. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacol Toxicol 2001;88:119–25.

[50] Castro ML, Franco GCN, Branco-de-Almeida LS, Anbinder AL, Cogo-Müller K, Cortelli SC, et al. Down-Regulation of Protease Activated Receptor 2, Interleukin-17 and Other Pro-Inflammatory Genes by Subantimicrobial Doxycycline Dose in a Rat Periodontitis Model. J Periodontol 2015:1–11. doi:10.1902/jop.2015.150385.

[51] Oliveira SM, Silva CR, Wentz AP, Paim GR, Correa MS, Bonacorso HG, et al. Antinociceptive effect of 3-(4-fluorophenyl)-5-trifluoromethyl-1H-1- tosylpyrazole. A Celecoxib structural analog in models of pathological pain. Pharmacol Biochem Behav 2014;124:396–404. doi:10.1016/j.pbb.2014.07.005.

[52] Gollapudi S. Safety and Efficacy of Polydioxanone Nano-Fibers as Anti-Inflammatory Agents. J Nanomedine Biotherapeutic Discov 2014;04:2–7. doi:10.4172/2155-983X.1000127.

[53] Bailey PJ. [29] Sponge implants as models. Methods Enzymol 1988;162:327–34. doi:10.1016/0076-6879(88)62087-8.

[54] Malle. E., Waeg,G., Schreiber, R., Grone, E.F., Sattler, W., Grone H. Immunohistochemical evidence for the myeloperoxidadse/H2O2/halide system in human atherosclerotic lesions: colocalization of myeloperoxidase and hypochlorite-modified protiens. Eur J Biochem 2000;267:4495–503.

[55] Klebanoff SJ. Myeloperoxidase : friend and foe. J Leukoc Biol 2005;77:598–625.

doi:10.1189/jlb.1204697.1.

[56] Delporte C, Van Antwerpen P, Vanhamme L, Roumegu??re T, Zouaoui Boudjeltia K. Low-density lipoprotein modified by myeloperoxidase in inflammatory pathways and clinical studies. Mediators Inflamm 2013;2013. doi:10.1155/2013/971579.

[57] van der Veen BS, de Winther MP, Heeringa P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxidants Redox Signal 2009;11:2899–937. doi:10.1089/ARS.2009.2538.

[58] Cui L, Iwamoto A, Lian J, Neoh H, Maruyama T, Horikawa Y, et al. Novel Mechanism of Antibiotic Resistance Originating in Vancomycin-Intermediate Staphylococcus aureus Novel Mechanism of Antibiotic Resistance Originating in Vancomycin-Intermediate Staphylococcus aureus. Antimicrob Agents Chemother 2006;50:428–38. doi:10.1128/AAC.50.2.428.

[59] Richter WF, Bhansali SG, Morris ME. Mechanistic determinants of biotherapeutics absorption following SC administration. AAPS J 2012;14:559–70. doi:10.1208/s12248-012-9367-0.

[60] Charman SA, McLennan DN, Edwards GA, Porter CJ. Lymphatic absorption is a significant contributor to the subcutaneous bioavailability of insulin in a sheep model. Pharm Res 2001;18:1620–6.

[61] Oussoren C, Storm G. Liposomes to target the lymphatics by subcutaneous administration. Adv Drug Deliv Rev 2001;50:143–56. doi:10.1016/S0169-409X(01)00154-5.

[62] Liu W, Cao Y. Application of scaffold materials in tissue reconstruction in immunocompetent mammals: Our experience and future requirements. Biomaterials 2007;28:5078–86. doi:10.1016/j.biomaterials.2007.07.028.

[63] Naito Y, Takagi T, Yoshikawa T. Molecular fingerprints of neutrophil-dependent oxidative stress in inflammatory bowel disease. J Gastroenterol 2007;42:787–98. doi:10.1007/s00535-007-2096-y.

4 CONCLUSÃO

No presente estudo, nós avaliamos a biocompatibilidade de scaffolds nanofibrosos de polidioxanona obtidos pela técnica de eletrospinning com a incorporação de ciprofloxacina ou metronidazol em modelo de implantação subcutânea em ratos Wistar. Scaffolds de PDS resultaram em maior produção de ROS, maior atividade de enzimas inflamatórias (MPO, NAG), e maior dano celular mensurado pela peroxidação lipídica e proteína carbonilada, quando comparado aos animais SHAM em 3 dias. Em 30 dias, o scaffold de PDS apresentou um comportamento similar ao observado em animais SHAM. Em geral, a inclusão de antibióticos reduziu os desfechos inflamatórios quando comparado ao scaffold de PDS em 3 dias, e, em 30 dias, scaffolds com antibióticos demonstraram um efeito protetor contra a inflamação quando comparados a matriz de PDS ou aos animais SHAM, especialmente em animais que receberem dois scaffolds contendo ciprofloxacina.

Extrapolação de dados obtidos a partir de estudos pré-clínicos não significa que os valores numéricos possam ser diretamente transpostos ao homem, mas significa que um comportamento similar daquele observado no animal também possa ser esperado no homem. Assim, esforços extensivos, envolvendo gestão de recursos humanos e materiais, e princípios éticos devem ser considerados na busca por excelente precisão dos achados. Isso significa que uma metodologia envolvendo exagerado número de lâminas, por exemplo, na busca de informações precisas de número de células, não é necessário, e não promove relevância adicional comparada com a obtida a partir de avaliações randomizadas e representativas.

Com a relação a pergunta de pesquisa: scaffolds nanofibrosos de PDS com metronidazol ou ciprofloxacina são biocompatíveis? Podemos, alicerçados em nossos dados, concluir que a adição de antibióticos a matriz de PDS melhorou a biocompatibilidade desta. Contudo, ressaltamos que outros desfechos podem ainda ser avaliados dentro desse contexto, como por exemplo, resposta vascular e alterações sistêmicas associadas a esses biomateriais. Outro aspecto que devemos ressaltar é que baseado em dados prévios das propriedades físicas, mecânicas, e antimicrobianas desses scaffolds (Bottino et al. 2013, 2014), associado com os promissores resultados de biocompatibilidade apresentados por nós, o estudo desses scaffolds em modelos animais de regeneração periodontal pode ser considerado.

REFERÊNCIAS

AEBI, U.; CHIU, W.; MILLIGAN, R. Role of catalase on antioxidative defenses. **Journal of Structural Biology**, v. 2, p. 117-8, 1995.

AGARWAL, S.;GREINER, A.; WENDORFF, J.H.Electrospinning of Manmade and Biopolymer Nanofibers – progress in technique, materials, and applicantions. **Advanced Functional Matererials,** v. 19, p. 2863-2879, sep. 2009.

AKALIN, F.A. et al. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. **Journal Clinical Periodontology**, v. 34, n. 7, p. 558-565, jul. 2007.

ALBANDAR, J. M.; BRUNELLE J. A.; KINGMAN, A. Destructive periodontal disease in adults 30-years of age and older in the United States, 1988 -1994. **Jounal of Periodontology**, v. 70, n. 1, p. 13-29, jan. 1999.

AMINOV, R.I. The role of antibiotics and antibiotic resistance in nature. **Environmental Microbiology**, v. 11, p. 2970-2988, jul. 2009.

ANUSAVICE, K.J. **Phillips Materiais Dentários**. 11ed. Rio de Janeiro: Elsevier, 2005.

ANUSAVICE,K.J.**Biocompatibilidade dos materiais dentários.** In: Anusavice, KJ, editor. Phillips – materiais dentários. 10 ed. Rio de Janeiro: Guanabara Koogan, 1998.

ARAUJO, A.A. et al. Effect of telmisartan on levels of IL-1, TNF- a, down- regulated COX-2, MMP-2, MMP-9 and RANKL\RANK in an experimental periodontitis model. **Journal Clinical of Periodontology**, v.40, p.1104–1111, 2013.

BAILEY,P.J. Sponge implants as models. **Methodology in Immunology**, v. 162, p. 327-334, 1988.

BALTACIOGLU,E. Lipid peroxidation levels and total oxidant\antioxidant status in serum ans saliva from patients with chronic and agressive periodontitis.Oxidative stress index:a new biomarker for periodontal disease. **Journal of Periodontology,** v. 85, n. 10, p.1432-1441, oct. 2014.

BAVARIYA, A.J. et al. Evaluation of biocompatibility and degradation of chitosan nanofiber membrane crosslinked with genipin. **Journal of Biomedical Materials Research part B Applied Biomaterials**, v. 102, n. 5, p. 1-9, nov. 2013.

BECKER, W. et al. Root isolation for new attachment procedures. A surgical and suturing method:three case retorts. **Journal of Periodontology**, v. 58, n. 12, p. 819-826, dec. 1987.

BEHRING, J. et al. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. **Odontology,** v. 96, n,1, p.1-11, jul. 2008.

BORREGAARD,N.;COWLAND, J.B. Granules of the human neutrophilic polymorphonuclear leukocyt. **Blood**, v. 89, n.10, p. 3503-3521,1997.

BOTTINO, M.C. et al. Biodegradable nanofibrous drug delivery systems: Effects of metronidazole and ciprofloxacin on periodontopathogens and commensal oral bacteria. **Clinical Oral Investigations,** v.18, p. 2151-2158, dec. 2014.

BOTTINO, M.C. et al. Bioactive nanofibrous scaffolds for regenerative endodontics. **Research reports – Biomaterials & Bioengineering**, v. 11, p. 963-969, 2013.

BOTTINO, M.C.; THOMAS, V.; JANOWSKI, G.M. A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. **Acto biomaterialia**, v. 7, p. 216-224, aug. 2011.

BOTTINO, M.C. et al. Recent advances in the development of GTR\GBR membranes for periodontal regeneration - A materials perspective. **Dental Materials**,v.28, p. 703-721, apr. 2012.

BRIGANTI,S.; PICARDO,M. Antioxidant activity,lipid peroxidation and skin diseases. What´s new. **Journal oh the European Academy of Dermatology and Venereology**, v.17, p.1336-1343, 2011.

BROWNING, M.B. et al. Determination of the in vivo degradation mechanism of PEGDA hydrogels. **Journal of biomaterials materials research**, v. 00A, p 1-8, jan. 2014.

CASSEL, G.G.; MEKALANOS, J. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. **Journal of American Medical Association**, v. 285, n. 5, p. 601-605, feb. 2001.

CHAPPLE,I.L.C.; MATTHEWS,J.B.The role of reactive oxygen and antioxidant species in periodontal tissue destruction. **Periodontology 2000,** v.43,p.160-232, jan. 2007.

CHAPPLE,I.L. Reactive oxygen species and antioxidants in inlammatory diseases.**Journal of Clinical Periodontology**, v. 24, n.5, p. 287-296, may. 1997.

CHEN, F-M.; AN, Y.; ZHANG, R.;ZHANG,M. New insights into and novel applications of release technology for periodontal reconstructive therapies. **Journal of Controlled Release**, v. 149, p.92-110, oct. 2010.

CHEN, F-M. et al. A review on endogenous regenerative technology in periodontal regenerative medicine. **Biomaterials,** v. 31, p. 7892-7927, jul. 2010.

CONSOLARO, A. Inflamação e reparo: um sílabo para a compreensão clínica e implicações terapêuticas.Maringá: Dental Press, 2009.

COBB, C. M. Clinical significance of non-surgical periodontal therapy: an evidencebased perspective of scaling and root planing. **Journal of Clinical Periodontology,** v. 29, s. 2, p. 6-16, 2002.

COOK-MILLS, J.M.; DREEM, T.L. Active participation of endothelial cells in inflamation. **Journal of Leukocyte Biology,** v. 77, p. 487-495, apr. 2005.

CORTELLINI, P.; TONETTI, M.S. Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials: a randomized-controlled trial in intra-bony defects. **Journal of Clinical Periodontology**, v. 38, p. 365-373, 2011

CORTELLINI, P.; TONETTI, M.S. Clinical concepts for regenerative therapy in intrabony defects. **Periodontology 2000**, v. 68, p. 282-307, 2015.

COSTA, C.A.S. Testes de citotoxicidade em culturas de células. **Metodologia Científica Ensino e Pesquisa em Odontologia.**São Paulo: Artes médicas, p. 147-160, 2001.

COSTA, C.A.S.; HEBLING, J.; SOUZA, P.P.C. Testes de biocompatibilidade. **Metodologia científica: ensino e pesquisa em odontologia.** São Paulo: Artes médicas. Cap 10, p.162-194, 2005.

DELIORMANLI, A.M; LIU, X.; RAHAMAN, M. Evaluation of borate bioactive glass scaffolds with different pore sizes in a rat subcutaneous implantation model. **Journal of biomaterials applications**, v. 28, p. 643-653, dec. 2012.

DICKINSON, D.P. et al. Events of wound healing/ regeneration in the canine supraalveolar periodontal defect model. **Journal of Clinical Periodontology**, v. 40, p. 527-541, 2013.

DRUG INFORMATION HANDBOOK INTERNATIONAL. Lexi -Comp Inc., 2005.

EL-KAMEL, A.H; ASHRI L.Y; ALSARRA I.A. Micromatricial metronidazole benzoate film as a local mucoadhersive delivery system for treatment of periodontal diseases. **AAPS PharmSciTech.** p E1-E11, set. 2007.

ERISKEN, C; KALYON, D.M; WAND H.J. Functionally graded electrospun polycaprolactone and beta-tricalcium phosphate nanocomposites for tissue engineering applications. **Biomaterials**, p. 4065-4073, jul**.** 2008.

EYDEN,B.;TZAPHLIDOU,M. Structural variations of collagen in normal and pathological tissues:roleof electron microscopy. **Micron**, v.18, n.3, p.287-300, apr. 2001.

EKE, P. I.; WEI, L.; THORNTON-EVANS, G. O.; BORRELL, L. N.; BORGNAKKE, W. S.; DYE, B.; GENCO, R. J. Risk Indicators for Periodontitis in US Adults: National Health and Nutrition Examination Survey (NHANES) 2009 - 2012. **Journal of Periodontology,** p. 1-18, 2016.

FAURSCHOU,M.; BORREGAARD,N. Nutrophil granules and secretory vesicles in inflammation. **Microbes and Infection.** v.5, n.14, p.1317-1327, 2003.

FREEMAN, C.D; KLUTMAN N.E; LAMB K.C. Metronidazole – a therapeutic review and update. **Drugs.** p 679 – 708, nov. 1997.

FU, Y.C. et al. Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/Hap composite scaffolds loaded with BMP-2. **Biotechnology and Bioengineering**, v. 99, p. 996-1006, 2008.

GALLEY, H.; DAVIES, M.J.; WEBSTER, N.R. Ascorbil radical formation in patients with sepsis: effects of ascorbate loading. **Free Radical Biology & Medicine**, v. 20, p. 139-43, 1996.

GARCIA, V. G. et al. Treatment of experimental periodontitis in rats using repeated adjunctive antimicrobial photodynamic therapy. **Lasers in Medical Science,** v. 28, n. 1, p. 143-150, 2012

GENTILE, P. et al. Polymeric membranes for guided bone regeneration. **Biotechnology Journal**, 2011.

GHANAATI, S. et al. The chemical composition of synthetic bone substitutes influences tissue reactions in vivo: histological and histomorphometrical analysis of the cellular inflammatory response to hydroxyapatite, beta-tricalcium phosphate and biphasic calcium phosphate ceramics. **Biomedical Materials**, v.7, p. 1-14, 2012.

GHANNATI, S. et al. Fine-tuning scaffolds for tissue regeneration: effects of formic acid processing on tissue reaction to silk fibroin. **Journal of tissue engineering and regenerative medicine**, v.4, p.464-472, jan. 2010.

GOONOO, N. et al. Polydioxanone-based bio-materials for tissue engineering and drug\gene delivery application.European **Journal of Pharmaceutics and Biopharmaceutics,** v.97, p. 371-391, nov. 2015.

GOTTLOW,J. et al. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. **Journal of Clinical Periodontology**. v.13, p.604-616, jul.1986.
GOTTLOW, J.; KARRING, T.; NYMAN, S. Guided tissue regeneration following treatment of recession-like defects in the monkey. **Journal of Periodontology**, v. 61, p. 680-685, 1990.

GUTTERIDGE, J.M.C.;HALLIWELL, B. Antioxidants:molecules,medicines and myths.**Biochemical and Biophysical Research Communications**, v.394, p.561-564, 2010.

HAFFAJEE, A.D.; SIGMUND, S. Microbial etiological agents of destructive periodontal diseases. **Periodontology 2000**, v.5, p.78-111, jun.1994.

HALLIWELL, B. Reactive oxygen species and the central nervous system. **Journal of Neurochemistry**, v.59, p.609-623,1992.

HALLIWELL, B.; GUTTERIDGE, J.M.C. Oxigen toxicity, oxygen radicals, transition metals and disease. **Biochemical Journal,** v. 219, p.1-14, 1984.

HALLIWELL, B.; GUTTERIDGE, J.M.C.Role of free radicals and catalytic metal ions in human disease:an overview. **Methods in Enzymology**, v.186, p. 1-85, 1990.

HANKS, C.T.; WATAHA, J.C.;SUN,Z. In vitro models of biocompatibility:a review. **Dental Materials**, v.12,n.3, p.186-193, 1996.

HANEY, J.M. et al. Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. **Journal of Periodontology**, v. 64, p. 883-890, 1993.

HAUMAN, C.H.; LOVE, R.M.Biocompatibility of dental materials used in contempory endodontic therapy: a review.**International Endodontic Journal**, v.36, p.75-85, 2003.

HEBBEL, R.P.Erythrocyte antioxidants and membrane vulnerability. **Journal of Laboratory and Clinical Medicine**, v.107, p.401-404, 1986.

HOSOKAWA, R. et al. Controlled local application of basic fibroblast growth factor(FGF-2) accelerates the healing of GBR – An experimental study in beagle dogs. **Clinical Oral Implants Research,** v.11, p. 345-353, aug. 2000.

HU, N. et al. Long-term outcome of the repair of 50 nm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. **Biomaterials**, v. 34, p. 100-111, 2013.

IKADA,Y. Challenges in tissue engineering. **Journal of the Royal Society Interface**, v.3, n.10, p.589-601, apr. 2006.

Internacional Organization for standardization. **ISO 7405: dentistry-preclinical evaluation of biocompability of medical devices used in dentistry - test methods for dental materials**. Genève, ISO, 1997.

JACQUES-SILVA, M.C. *et al*. Diphenyldisselenides and ascorbic acid changes deposition of selenium and ascorbic acid in brain of mice. **Pharmacology Toxicology**, v. 44, p. 119-25, 2001.

JOVANOVIC, S.A.; NEVINS,M. Bone formation utilizing titanium-reinforced barrier membranes. **The International Journal of Periodontontics and Restorative Dentistry**, v.15, n.1, p.56-69, feb.1995.

JUNG, R.R. et al. Effect of rhBMP-2 on guided bone regeneration in humans- a randomized,controlled and histomorphometric study.**Clinical Oral Implantes Research**, v.14, p. 556-568, oct.2003.

JUNQUEIRA,L.C.U;COSSERMELLI,W.;BRENTANI,R.R.Differential staining of collagen type I,II,III by sirius red and polarization microscopy.**Archivum Histologicum Japonicum**, v.11, p.267-274, 1978.

KARRING, T. et al. Development of the biological concept of guided tissue regeneration – animal and human studies. **Periodontology 2000**, v. 01, p. 26-35, feb.1993.

KIM, C.K. et al. Periodontal repair in dogs: effect of allogenic freeze-dried demineralized bone matrix implants on alveolar bone and cementum regeneration. **Journal of Periodontology**, v. 69, p. 26-33, 1998.

KIM, K. et al. **I**ncorporation and controlled release of a hydrophilic antibiotic using poly(lactide-co-glycolide)-based eletrospun nanofibrous scaffolds. **Journal Control Release**, v. 98, p. 47-56, jul. 2004.

KIKUCHI, M. et al. Development of guided bone regeneration membrane composed of betatricalcium phosphate and poly (L-lactide-co-glycolide–epsilon-caprolactone) composites. **Biomaterials**, v.25, p. 5979–86, 2004.

KLEBANOFF, S.Myeloperoxidase:friend and foe. **Journal of Leukocyte Biology,** v.77, p.598-635, may. 2005.

KOSSEBAUM, N.J. et al. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. **Journal of Dental Research**, v. 93, p. 1045-1053, 2014.

KORNMAN, K. S. Mapping the pathogenesis of periodontitis: a new look. **Journal of Peridontology,** v. 79, n. 8, p. 1560-1568, 2008.

KWON, D.H. et al. Periodontal wound healing/regeneration following the application of rhGDR-5 in a beta TCL/PLGA carrier in critical-size supra-alveolar periodontal defects in dogs. **Journal of Clinical Periodontology**, v. 37, p. 667-674, 2010.

LANGER,R.; VACANTI, J.P. Tissue engineering. **Science**, v.260, n. 5110, p.920-926, may. 1993.

LAU, D.; BALDUS, S. Myeloperoxidse and its contributoryrole in inflammatory vascular disease. **Pharmacology & therapeutic**, v. 111, n.1, p.16-26, 2006

LAWRENCE, T.;GILROY, D.W. Chronic inflammation: a failure of resolution. **International Journal of Experimental Pathology**, v.88, p.84-94, 2007.

LI,W.J. et al. Electrospun nanofibrous structure: a novel scaffold for tissue engineering. **Journal of Biomedical Materials Research**, v. 15, p.613-621, jun. 2002.

LI, M.Y. et al. Electrospinning polvaniline-contained gelatin nanofibers for tissue engineering applications. **Biomaterials**, v. 27, p.2705-2715, 2006.

LIAU, S. et al. The degradation oh the three layered nano-carbonated hydroxyapatite\collagen\PLGA composite in vitro. **Dental Materials,** v. 23, n.9, p. 1120-1128., sep. 2007.

LIM, S.H.; MAO, H.Q. Electrospun scaffolds for stem cell engineering. **Advanced Drug Delivery Review**, v.61, n.12, p.1984-1096, oct. 2009.

LIN, K. et al. Evaluation of host inflammatory responses of B-tricalcium phosphate bioceramics caused by calcium pyrophosphate impurity using a subcutaneous model. **Journal of biomedical materials research**, v.99B, p.1-9, 2011.

LINDIE, J.; LANG, N.P.;KARRING,T. **Tratado de Periodontia Clínica e Implantologia Oral**. 5. Ed.Rio de Janeiro. 2008.

LIU, W.; CAO, Y. Application of scaffold materials in tissue reconstruction in immunocompetent mammals:Our experience and future requirements. **Biomaterials,** v.28, n.34, p.5078-5086, dec. 2007.

LIU,X. et al. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. **Proceedings of the National Academy of Science of the United States of America**, v.94, p.1852-1854, mar.1997.

LOWRY, O. H. et al. Protein measurement with the Folin- Phenol reagents. **The Journal of Biological Chemistry**, v. 193, p. 265-275, nov. 1951.

MATULIENE, G. et al. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. **Journal Clinical Periodontology**, v. 35, p. 685-695, 2008.

MAYADAS,T.N.;CULLURE,X.;LOWELL,C.A. The multifaceted functions of neutrophils. **Annual Reviews Connect with our experts**, v.9, p.181-218, sep. 2014.

MELLO, A.C.; HOFFMAN, M.E.; MENEGHINI,R. Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. **The Biochemical Journal**, v. 218, p. 273-275, 1983.

MILELLA, E. et al. Physicochemial, mechanical, and biological properties of commercial membranes for GTR. **Journal of Biomedical Materials Research**., v.58, n.4, p.427-435, may. 2001**.**

MYAGAKI, D.C. **Avaliação da biocompatibilidade de cimentos endodônticos em tecido subcutâneo de ratos, e sua relação com o subtipo de macrófago M1**. 2011. Dissertação (Mestrado em Clínica Odontológica, área de concentração em Endodontia)-Universidade Federal de Uberlândia, Uberlândia, 2011.

MONTES, G. S.; JUNQUEIRA, L. C. U. The use of the pirosirius-polasirius method for the study of the biopathology of collagen. **Memórias do Instituto Oswaldo Cruz**, v. 86, p. 1-11, 1991.

MULDER, E.L.W. et al. Effect of polyurethane scaffold architecture on ingrowth speed and collagen orientation in a subcutaneous rat pocket model. **Biomedical Materials**, feb.2013.

MUNCHOW, E.A. et al. Development and characterization of novel ZnO-loaded electrospun membranes for periodontal regeneration. **Dental Materials**, v. 31, p. 1038-1051, 2015

MUNCHOW, E.A. et al. Synthesis and characterization of CaO-loaded electrospun matrices for bone tissue engineering. **Clinical Oral Investigation**, v. 27, p. 1-13, 2015.

MURPHY, K.G.; GUNSOLLEY, J.C. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects: a systematic review. **Ann Periodontology**, v. 8, n.1, p. 266-302, dec. 2003.

NEEDLEMAN, I.G. et al. Guided tissue regeneration for periodontal intra-bony defects. **Cochrane Database of Systematic Reviews**, v.19,n.2,p. 1-35, 2006.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Analytical Biochemistry**, v. 95, p. 351-358, 1979.

OLIVEIRA, S.M. et al. Antinoceceptive effect of 3-(4-fluorophenyl)-5-trifluoromethyl-1h-1-tosylpyrazole. A celecoxib structural analog in models of pathological pain. **Pharmacology Biochemistry and Behavior**, v.124, p. 396-404, sep. 2014.

OWENS, C.W.I.; BELCHER, J. A colorimetric micro-method for the determination of glutathione. **The Biochemical Journal**, v. 94, p. 705-711, 1965.

PAGE, R. C. et al. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. **Periodontology 2000**, v. 14, p. 216-48, 1997.

PETERSEN, P.E. et al. The global burden of oral diseases and risks to oral health. **Bulletin World Health Organization**, v. 83, p. 661-669, 2005.

PIHLSTROM, B.L.; MICHALOWICZ, B.S.; JOHNSON, N.W. Periodontal diseases. **The Lancet**, v.19, p.1809-1820, nov. 2005.

PHIPPS, M.C. et al. Mesenchymal stem cell responses to bone-mimetic electrospun matrices composed of polycaprolactone, collagen I and nanoparticulate hydroxyapatite. **Plos One**, v. 8, 2011.

POLIMENI, G. et al. Prognostic factors for alveolar regeneration: bone formation at teeth and titanium implants. **Journal of Clinical Periodontology**, v. 31, p. 927-932, 2004.

POLIMENI, G. et al. Prognostic factors for alveolar regeneration: effect of tissue occlusion on alveolar bone regeneration with guided tissue regeneration. **Journal of Clinical Periodontology**, v. 31, p. 730-735, 2004.

POLIMENI, G; SUSIN, C.; WIKESJO, U.M. Regenerative potential and healing dynamics of the periodontium: a critical-size supra-alveolar periodontal defect study. **Journal of Clinical Periodontology**, v. 36, p. 258-264, 2009.

RAJESH, J. B. et al. Calcium phosphate cement as a barrier-graft for the treatment of human periodontal intraosseous defects. **Indian Journal of Dental Research**, v. 20, p. 471-479, 2009.

RICHTER, W.F.; BHANSALI, S.G.; MORRIS, M.E. Mechanistic determinants of biotherapeutics absorption following SC administration. **The AAPS Journal**, v. 14, n. 3, p. 559-570, set. 2012.

ROBBINS; KUMAR, V.;ABBAS, A.K.; FAUSTO, N.; MITCHELL, R.N. **Patologia Básica.** Rio de Janeiro: Ed.8, 2008.

ROMAN, R.M.; WENDLAND, A. E.; POLANCZYK, C.A. Myeloperoxidase and coronary arterial disease:from research to clinical practice. **Arquivos Brasileiros de Cardiologia**, v.91, n.1, p.e12-e19, 2018.

RUCKH, T.T. et al. Animicrobial effects of nanofiber poly(coprolactone) tissue scaffolds releasing rifampicin. **Journal of Materials Science: Material in Medicine**, v.23, p. 1411-1420, jun. 2012.

SCHAFER, F.Q.; BUETTNER, G.R.Redox environment of the cell as viewed through the redox state of the glutathione disulfide\glutathione couple. **Free Radical Biology & Medicine**, v.30, n.11,p.1191-1212, jun. 2001.

SCHAFER,M.;WERNER,S. Oxidativ stress in normal and impaired wound repair. **Pharmacological Research**, v.58, p.165-171, aug. 2008.

SCHMALZ, G. Materials science: biological aspects. **Journal of Dental Research**, v.81, n.10, p.660-663, 2002.

SHERWOOD, E.R.; TOLIVER-KINSKY,T. Mechanisms of the inflammatory response. **Best Practice & Research Clinical Anaesthesiology,** v.18,p.385-405, sep. 2004.

SIGURDSSON, T.J. et al. Periodontal repair in dogs: evaluation of rhBMP-2 carriers. **The International Journal of Periodontics and Restorative Dentistry**, v. 16,p. 524-537, 1996.

SOUSA, C.J. et al. Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ, and Epiphany root canal sealers. **Journal of Endodontics,** v.32, p.656-562, 2006.

SPANGBERG, L.; LANGELAND, K.Biologic effects of dental materials.Toxicity of root canal filling materials on Hela cells in vitro. **Oral surgery, Oral Medicine, Oral Pathology, Oral Radiology,** v.35, p.402-414, 1973.

SROUJI, S. et al. Slow-release human recombinant bone morphogenetic protein-2 embedded within electrospun scaffolds for regeneration of bone defect: In vitro and in vivo evaluation. **Tissue engineering**, v. 17, p. 269-277.

SUSIN, C.; WIKESJO, U.M.E. Regenerative periodontal therapy: 30 years of lessons learned and unlearned. **Periodontology 2000**, v. 62, p. 232-242, 2013.

SUSIN, C. et al. Periodontal attachment loss in an urban population of brazilian adults: effect of demographic, behavioral, and environmental risk indicators. **Jounal of Periodontology**, v. 75, n. 7, p. 1033-41, jul. 2004.

SUSIN, C. et al. Wound healing following surgical and regenerative periodontal therapy. **Periodontology 2000**, v. 68, p. 83-98, 2015.

STAVROPOULOS, A; WIKESJO, U.M. Growth and differentiation factors for periodontal regeneration: a review on factors with clinical testing. **Journal Periodontal Res**, v.47, p. 545-553,2012.

TAHERI, M. et al. An evaluation of bovine derived xenograft with and without a bioabsorbable collagen membrane in the treatment of mandibular class II furcation defects. **Australian Dental Journal**, v. 54, p. 220-227, 2009.

TENG, S.H. et al.Three-layered membranes of collagen\hydroxyapatite and chitosan for guided bone regeneration. **Journal of Biomedical Materials Reaserach,** v.87, p.132-138, oct. 2008.

THOMAS.D.W.;O`NEILL,L.D.;HARDING,K.G.Cutaneous wound healing: a current perspective. **Journal of Oral and Maxillofacial Surgery,** v. 53, p.442-447,1995.

TROMBELLI, L. et al. Single flap approach with ans without guided tissue regeneration and a hydroxyapatite biomaterial in the management of intraosseous periodontal defects. **Journal of Periodontology**, v.81, n.9, p. 1256-1263, sep.2010.

TSAI,C.C. et al. Lipid peroxidation:a possible role in the induction and progression of chronic periodontitis.**Journal Clinical Periodontology,** v.40, n.5, p. 378-384, oct.2005.

UNNITHAN, A.R. et al. Electrospun antibacterial polyurethane-cellulose acetate-zein composite mats for wound dressing. **Carbohydrate Polymers**, v. 102, p. 884-892, 2014.

VENUGOPAL, J. et al. Nanotechnology for nanomedicine and delivery drogs. **Current Pharmaceutical Biotechnology**, v. 14, p. 2184-2200, 2008.

WALTER, C. et al. Three-dimensional imaging as a pre-operative tool in decision making for furcation surgery. **Journal of Clinical Periodontology**, v.36, p. 250-257, 2009.

WANG,H.L.Periodontal regeneration.**Journal of Periodontology,** v.76, n.9, p.1601-1622, sep.2005.

yWELLS, M.Y. et al. Histomorphology and vascular lesions in dorsal rat skin used as injection sites for a subcutaneous toxicity study. **Toxicologic Pathology,** v.38, n.02, p.258-266, 2010.

YAN, L.Y.; TRABER, M.G.; PACKER, L. Spectrophotometric method for determination of carbonyls in oxydatively modifiedapolipoprotein B of human low-density lipoproteins. **Analytical Biochemistry**, v. 228, p. 349-351, 1995.

YANG, F.et al. Development of an electrospun nano-apatite\PCL composite membrane for GTR\GBR application. **Acta Biomaterials**, v.5, n.9, p.3295-3304, nov. 2009.

ZAMANI, F. et al. Promotion of spinal cord axon regeneration by 3D nanofibrous core-sheath scaffolds. **Journal of Biomedical Materials Research**, v. 102, p. 506-513, 2014.

ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *DENTAL MATERIALS*

GUIDE FOR AUTHORS

Authors are requested to submit their original manuscript and figures via the online submission and editorial system for Dental Materials. Using this online system, authors may submit manuscripts and track their progress through the system to publication. Reviewers can download manuscripts and submit their opinions to the editor. Editors can manage the whole submission/review/revise/publish process. Please register at: http://ees.elsevier.com/dema.

Dental Materials now only accepts online submissions.

The Artwork Quality Control Tool is now available to users of the online submission system. To help authors submit high-quality artwork early in the process, this tool checks the submitted artwork and other file types against the artwork requirements outlined in the Artwork Instructions to Authors on http://www.elsevier.com/artworkinstructions. The Artwork Quality Control Tool automatically checks all artwork files when they are first uploaded. Each figure/file is checked only once, so further along in the process only new uploaded files will be checked.

Manuscripts

The journal is principally for publication of Original Research Reports, which should preferably investigate a defined hypothesis. Maximum length 6 journal pages (approximately 20 double-spaced typescript pages) including illustrations and tables.

Systematic Reviews will however be considered. Intending authors should communicate with the Editor beforehand, by email, outlining the proposed scope of the review. Maximum length 10 journal pages (approximately 33 double-spaced typescript pages) including figures and tables. Three copies of the manuscript should be submitted: each accompanied by a set of illustrations. The requirements for submission are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", Annals of Internal Medicine, 1997,126, 36-47. All manuscripts must be written in American English. Authors are urged to write as concisely as possible. The Editor and Publisher reserve the right to make minimal literary corrections for the sake of clarity. Authors for whom English is not the first language should have their manuscripts read by colleagues fluent in English. If extensive English corrections are needed, authors may be charged for the cost of editing. For additional reference, consult issues of Dental Materials published after January 1999 or the Council of Biology Editors Style Manual (1995 ed.).

All manuscripts should be accompanied by a letter of transmittal, signed by each author, and stating that the manuscript is not concurrently under consideration for publication in another journal, that all of the named authors were involved in the work leading to the publication of the paper, and that all the named authors have read the paper before it is submitted for publication. Always keep a backup copy of the electronic file for reference and safety. Manuscripts not conforming to the journal style will be returned. In addition, manuscripts, which are not written in fluent English, will be rejected automatically without refereeing.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

This must be presented in a structured format, covering the following subjects, although actual subheadings should not be included:

• succinct statements of the issue in question;
• the essence of existing knowledge and understanding pertinent to the issue (reference);
• the aims and objectives of the research being reported relating the research to dentistry, where not obvious.

Materials and methods

• describe the procedures and analytical techniques.

• only cite references to published methods.
• include at least general composition details and batch numbers for all materials.
• identify names and sources of all commercial products e.g.
"The composite (Silar, 3M Co., St. Paul, MN, USA)..."
"... an Au-Pd alloy (Estheticor Opal, Cendres et Metaux, Switzerland)."
• specify statistical significance test methods.
Results
• refer to appropriate tables and figures.
• refrain from subjective comments.
• make no reference to previous literature.
• report statistical findings.

Discussion

• explain and interpret data.
• state implications of the results, relate to composition.
• indicate limitations of findings.
• relate to other relevant research.

Conclusion (if included)

• must NOT repeat Results or Discussion
• must concisely state inference, significance, or consequences

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address.
Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
• Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract (structured format)

• 250 words or less.
• subheadings should appear in the text of the abstract as follows: Objectives, Methods, Results, Significance. (For Systematic Reviews: Objectives, Data, Sources, Study selection, Conclusions). The Results section may incorporate small tabulations of data, normally 3 rows maximum.

Keywords

Up to 10 keywords should be supplied e.g. dental material, composite resin, adhesion.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Embedded math equations

If you are submitting an article prepared with Microsoft Word containing embedded math equations then please read this related support information (http://support.elsevier.com/app/answers/detail/a_id/302/).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.

A detailed guide on electronic artwork is available on our website: http://www.elsevier.com/artworkinstructions.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format. Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.

TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi. Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive

information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications that can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Captions to tables and figures

• list together on a separate page.
• should be complete and understandable apart from the text.
• include key for symbols or abbreviations used in Figures.
• individual teeth should be identified using the FDI two-digit system.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Must now be given according to the following numeric system:
Cite references in text in numerical order. Use square brackets: in-line, not superscript e.g. [23]. All references must be listed at the end of the paper, double-spaced, without indents. For example: 1.
Moulin P, Picard B and Degrange M. Water resistance of resin-bonded joints with time related to alloy surface treatments. J Dent, 1999; 27:79-87. 2. Taylor DF, Bayne SC, Sturdevant JR and Wilder AD. Comparison of direct and indirect methods for analyzing wear of posterior composite restorations. Dent Mater, 1989; 5:157-160. Avoid referencing abstracts if possible. If unavoidable, reference as follows: 3. Demarest VA and Greener EH . Storage moduli and interaction parameters of experimental dental composites. J Dent Res, 1996; 67:221, Abstr. No. 868.

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

Most Elsevier journals have a standard template available in key reference management packages. This covers packages using the Citation Style Language, such as Mendeley (http://www.mendeley.com/features/reference-manager) and also others like EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager

(http://refman.com/support/rmstyles.asp). Using plug-ins to word processing packages which are available from the above sites, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style as described in this Guide. The process of including templates in these packages is constantly ongoing. If the journal you are looking for does not have a template available yet, please see the list of sample references and citations provided in this Guide to help you format these according to the journal style. If you manage your research with Mendeley Desktop, you can easily install the reference style for this journal by clicking the link below: http://open.mendeley.com/use-citation-style/dental-materials When preparing your manuscript, you will then be able to select this style using the Mendeley plugins for Microsoft Word or LibreOffice. For more information about the Citation Style Language, visit http://citationstyles.org.

Reference style

Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given. Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....' List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:

[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.

Reference to a book:

[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z.

Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

• E-mail address
• Full postal address

All necessary files have been uploaded, and contain:

• Keywords
• All figure captions
• All tables (including title, description, footnotes)

Further considerations

• Manuscript has been 'spell-checked' and 'grammar-checked'
• References are in the correct format for this journal
• All references mentioned in the Reference list are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)

Printed version of figures (if applicable) in color or black-and-white

• Indicate clearly whether or not color or black-and-white in print is required.
• For reproduction in black-and-white, please supply black-and-white versions of the figures for printing purposes.

For any further information please visit our customer support site at http://support.elsevier.com.

ANEXO B - PARECER DO COMITÊ DE ÉTICA NO USO DE ANIMAIS

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que o Projeto intitulado "Avaliação in vivo da biocompatibilidade e degradação de scaffolds nanofibrosos contendo metronidazol e ciprofloxacina em modelo de implantação subcutânea em ratos.", protocolado sob o CEUA nº 4141250315, sob a responsabilidade de **Karla Zanini Kanforski** e equipe; *Patricia Cabral Passos; Cristiane Cademartori Danesi; Roberto Martinho Maciel* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, com o Decreto 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais Universidade Federal de Santa Maria (CEUA/UFSM) em reunião de 19/05/2015.

We certify that the proposal "In vivo biocompatibility evaluation and scaffolds nanofibrosos degradation containing metronidazole and ciprofloxacin model of subcutaneous implantation in rats.", utilizing 52 Heterogenics rats (52 males), protocol number CEUA 4141250315, under the responsibility of **Karla Zanini Kanforski** and team; *Patricia Cabral Passos; Cristiane Cademartori Danesi; Roberto Martinho Maciel* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes (or teaching) - It's in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Federal University of Santa Maria in the meeting of 05/19/2015.

Vigência da Proposta: de 04/2014 a 06/2015 — Laboratório: Departamento De Estomatologia Disciplina De Periodontia

Procedência: Biotério Central UFSM

Espécie:	Ratos heterogênicos	Gênero: Machos	Idade:	2 meses	N: 52
Linhagem:	stock Wistar		Peso:	250-300g	

Nota: A periodontite é uma doença infecto inflamatória que afeta os tecidos periodontais de suporte, resultando na perda do elemento dentário. A doença periodontal tem como fator etiológico primário o biofilme bacteriano que estimula a resposta imune-inflamatória do hospedeiro. A alta prevalência e as sequelas da periodontite como defeitos ósseos e recessão gengival, tem levado ao estudo de técnicas para restabelecer os tecidos perdidos, através da regeneração de tecidos periodontais específicos como osso alveolar, cemento, ligamento periodontal e gengiva. Modalidades terapêuticas utilizando enxertos ósseos, materiais osteocondutoresindutores, fatores exógenos de crescimento, tecnologia baseada em células e genes estão sendo estudadas. Nesse contexto, a regeneração tecidual guiada (RTG) e a regeneração óssea guiada (ROG) está sendo considerada uma técnica promissora para a regeneração periodontal. A RTG/ROG consiste no uso de uma membrana como interface entre o tecido conjuntivo ou epitelial e ligamento osso alveolar. A membrana impede que o tecido conjuntivo e epitelial migre para o defeito, favorecendo que células progenitoras localizadas do ligamento remanescente ou células sanguíneas recolonizem o local, para diferenciação em novo aparato periodontal. Porém, o sucesso dessas técnicas ainda é limitado, devido à ausência de uma membranascaffold ideal, ou seja, biocompatível, capaz de promover o reparo e regeneração tecidual, estável, com propriedades mecânicas e físicas adequadas e resistentes ao colapso. Assim, pesquisadores têm avançado no conhecimento de biomateriais combinados à alta tecnologia para o desenvolvimento de membranasscaffolds que possibilitem a engenharia de tecidos periodontais. Scaffolds sintetizados a partir de polímeros naturais (p. ex colágeno, quitosana) e sintéticos (p. ex coprolactone –PCL, monofilamentos de polidioxanona -PDS) com ou sem drogas terapêuticas, fatores de crescimento ou partículas de fosfato de cálcio estão sendo estudados. A técnica do e-spinning tem demonstrado bom potecial para o processamento de membranas nanoparticuladas para regeneração periodontal, com promissoras propriedades mecânicas, biológicas e estruturais. Neste contexto, os scaffolds nanoparticulados de PDS contendo na sua composição antimicrobianos, metronidazol e ciprofloxacina, foram testadas em um estudo in vitro por Botino e colaboradores (2013). Essas membranas obtiveram redução significativa (p

Santa Maria, 19 de maio de 2015

Avenida Roraima, 1000, Reitoria, 2º andar - CEP 97105-900 Santa Maria, RS - tel: 55 (55) 3220-9362 / fax: 55 (55) 3220-8009
Horário de atendimento: das 8:30 às 11:30 e 14:00 às 16:30hs - e-mail: ceua.ufsm@gmail.com
CEUA nº 4141250315