

Marcelo Dias Moreira de Assis Costa

**Desenvolvimento e Aplicação Clínica de Concentrados
Sanguíneos de Fibrina Rica em Plaquetas**

*Development and Clinical Application of Platelet-Rich Fibrin Blood
Concentrates*

Tese apresentada à Faculdade de
Odontologia da Universidade Federal de
Uberlândia, para obtenção do Título de
Doutor em Odontologia na Área de
Concentração de Clínica Odontológica
Integrada.

Uberlândia

2023

Marcelo Dias Moreira de Assis Costa

**Desenvolvimento e Aplicação Clínica de Concentrados
Sanguíneos de Fibrina Rica em Plaquetas**

*Development and Clinical Application of Platelet-Rich Fibrin Blood
Concentrates*

Tese apresentada à Faculdade de
Odontologia da Universidade Federal de
Uberlândia, para obtenção do Título de
Doutor em Odontologia na Área de
Concentração de Clínica Odontológica
Integrada.

Orientadora: Profa. Dra. Paula Dechichi

Banca examinadora:
Profa. Dra. Paula Dechichi
Prof. Dr. Luiz Renato Paranhos
Profa. Dra. Priscilla Barbosa Ferreira Soares
Profa. Dra. Flaviana Soares Rocha
Prof. Dr. Rafael Rodrigues Lima

Uberlândia

2023

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

C837 2023	Costa, Marcelo Dias Moreira de Assis, 1981- Desenvolvimento e Aplicação Clínica de Concentrados Sanguíneos de Fibrina Rica em Plaquetas [recurso eletrônico] / Marcelo Dias Moreira de Assis Costa. - 2023. Orientadora: Paula Dechichi. Tese (Doutorado) - Universidade Federal de Uberlândia, Pós-graduação em Odontologia. Modo de acesso: Internet. Disponível em: http://doi.org/10.14393/ufu.te.2023.237 Inclui bibliografia. Inclui ilustrações. 1. Odontologia. I. Dechichi, Paula, 1965-, (Orient.). II. Universidade Federal de Uberlândia. Pós-graduação em Odontologia. III. Título. CDU: 616.314
--------------	---

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074



ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Odontologia				
Defesa de:	Tese Doutorado, nº 86, PPGODONTO				
Data:	Dezessete de Maio de Dois Mil e Vinte e Três	Hora de início:	14:00	Hora de encerramento:	17:35
Matrícula do Discente:	11913ODO006				
Nome do Discente:	Marcelo Dias Moreira de Assis Costa				
Título do Trabalho:	Desenvolvimento e aplicação clínica de concentrados sanguíneos de fibrina rica em plaquetas				
Área de concentração:	Clínica Odontológica Integrada				
Linha de pesquisa:	Processo de Reparo				
Projeto de Pesquisa de vinculação:	Processo de Reparo				

Reuniu-se em Web Conferência pela plataforma Zoom, em conformidade com a PORTARIA Nº 36, DE 19 DE MARÇO DE 2020 da COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR - CAPES, pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Odontologia, assim composta: Professores Doutores: Flaviana Soares Rocha (UnB); Rafael Rodrigues Lima (UFPA); Luiz Renato Paranhos (UFU); Priscilla Barbosa Ferreira Soares (UFU); Paula Dechichi Barbar (UFU); orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Paula Dechichi Barbar, apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovado.

Esta defesa faz parte dos requisitos necessários à obtenção do título de Doutor.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.



Documento assinado eletronicamente por **Paula Dechichi Barbar, Professor(a) do Magistério Superior**, em 18/05/2023, às 14:07, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Luiz Renato Paranhos, Professor(a) do Magistério Superior**, em 18/05/2023, às 14:13, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Priscilla Barbosa Ferreira Soares, Professor(a) do Magistério Superior**, em 18/05/2023, às 14:15, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Rafael Rodrigues Lima, Usuário Externo**, em 18/05/2023, às 15:04, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **FLAVIANA SOARES ROCHA, Usuário Externo**, em 18/05/2023, às 16:44, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **4506771** e o código CRC **27DC9ACF**.

DEDICATÓRIA

Dedico esta conquista:

À Deus, por sempre ser e agir em minha vida, proporcionando os acontecimentos conforme Seu tempo, tornando-os inesquecíveis e acima das expectativas, contribuindo para que eu possa sempre evoluir como pessoa.

Aos meus pais, José de Assis Costa e Maria Goretti Moreira Costa por sempre me proporcionarem ensino de qualidade, incentivando e mostrando a necessidade e real importância da obtenção e busca constante do conhecimento. Por zelarem pela minha criação e por sempre fazerem o melhor.

Aos meus irmãos, Júlio César Moreira de Assis Costa e Fabiana Moreira de Assis Costa que sempre foram constantes em minha vida, me ensinando a conviver, apesar das diferenças. Pelo apoio constante, incentivo e admiração.

À minha sobrinha, Júlia, que me estimula a cada dia ser uma pessoa melhor, para dar exemplo e incentivá-la a lutar e buscar sempre seus ideais.

À minha família abençoada e maravilhosa, que é, e sempre será a minha base.

Ao amor da minha vida Lia Dietrich, por todos esses anos de amor, de companheirismo, de lutas e de apoio incondicional em todas as certezas e incertezas dessa vida. Obrigado por me mostrar possibilidades acima das minhas expectativas.

Aos meus meninos, amor incondicional, Bolacha, Baunilha, Espirro, Biscoito, Cenoura, Palmito, Banguela e Paçoca, que sempre tem o carinho certo para cada momento, principalmente nos mais difíceis. Aprendo sempre com todos vocês.

AGRADECIMENTOS

À Professora Doutora Paula Dechichi,

Por me aceitar como seu aluno de doutorado, por me ensinar constantemente com suas ações tanto no convívio pessoal quanto nas suas práticas como docente e pesquisadora. Por me ouvir, entender minhas limitações e incentivar sempre a crescer como profissional e pessoa. Pelas ótimas conversas, que enriquecem a vida!!!

Ao Professor Doutor Luiz Renato Paranhos

Amigo que a pós-graduação e a profissão me proporcionou, muito obrigado pela sua dedicação, disponibilidade, companheirismo e atenção. Obrigado pelo acolhimento, pelas ótimas conversas e momentos!

À Professora Doutora Priscilla Soares,

Você sempre demonstrou apoio e alegria diante das minhas vitórias. Você sempre esteve disponível para me escutar e me incentivou a buscar e perseguir objetivos maiores do que aqueles que eu poderia conceber. Sou grato por tudo que você fez por mim. Muito obrigado!

Aos demais professores da banca Profa. Dra. Flaviana Soares Rocha e Prof. Dr. Rafael Rodrigues Lima obrigado por aceitarem o convite, contribuírem com o trabalho e participarem deste momento.

À Equipe da CTBMF-UFU

Darceny, Cláudia, Lair, Marcelo Caetano, Jonas, Lívia, Larissa, Luiz Fernando, Felipe e Saulo. Muito Obrigado por existirem e fazerem parte da minha vida, contribuindo para meu aprendizado diário!

Ao Programa de Pós-graduação (PPGO) da Faculdade de Odontologia da Universidade Federal de Uberlândia (UFU),

Pela oportunidade de participar do programa pela segunda vez, agora no doutorado e antes no mestrado, me dando a possibilidade de crescer como profissional. Às funcionárias do departamento: Graça, Brenda e Laís, por sempre serem educadas, prestativas e carinhosas. Vocês são a base do programa, contribuem ativamente por todo o sucesso e prestígio.

Parabéns por serem profissionais empenhadas e dedicadas, e serem humanos maravilhosos.
Aos colegas de doutorado, pela incrível jornada que traçamos juntos, compartilhando vitórias, frustrações e momentos de felicidade.

Ao Laboratório de Pesquisa – CPBio,

Por existir, e permitir respostas a tantos questionamentos, e assim, incentivar ideias e parcerias, despertar vocações e consolidar talentos. Principalmente aos funcionários técnicos administrativos, à equipe de limpeza e vigilância que humanizam este ambiente, trazendo conforto, carinho e praticidade.

Aos professores da Faculdade de Odontologia da Universidade Federal de Uberlândia (FOUFU) pela dedicação, amizade e ensinamentos transmitidos a mim ao longo destes anos.

Aos funcionários da FOUFU, pela amizade e disponibilidade de ajuda sempre que precisei.

À Universidade Federal de Uberlândia e Faculdade de Odontologia – FOUFU,

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro.

À Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), pelo apoio financeiro.

A todos que torceram por mim e que de alguma forma contribuíram para a realização deste trabalho!!!

EPÍGRAFE

"Há três caminhos para o fracasso: não ensinar o que se sabe, não praticar o que se ensina e não perguntar o que se ignora."

Benjamin Disraeli

SUMÁRIO

RESUMO/PALAVRAS-CHAVE	12
ABSTRACT/KEYWORDS	14
1. INTRODUÇÃO E REFERENCIAL TEÓRICO	17
2. OBJETIVOS	22
3. CAPÍTULOS	23
3.1 Capítulo 1 - <i>Platelet-Rich Fibrin Progressive Protocol: Third Generation of Blood Concentrates</i>	24
3.2 Capítulo 2 - <i>Are non-PRF specific plastic tubes without additives a feasible alternative to S-PRF© PROCESS tubes in PRF membranes production?</i>	32
3.3 Capítulo 3 – <i>Do blood concentrates influence inflammatory signs and symptoms after mandibular third molar surgery? A systematic review and network meta-analysis of randomized clinical trials</i>	50
4. CONSIDERAÇÕES FINAIS	96
REFERÊNCIAS	98
ANEXOS	105

Resumo

RESUMO

A origem dos concentrados sanguíneos ricos em plaquetas surgiu da coleta de plaquetas em soluções de plasma para serem utilizados em sítios cirúrgicos. O objetivo era aumentar a liberação de fatores de crescimento e citocinas locais a fim de promover a melhora do reparo. O concentrado de fibrina rica em plaquetas (PRF) é considerado como uma evolução na geração de concentrados sanguíneos devido à sua produção sem aditivos. Além disso, a capacidade do PRF de formar uma densa rede de fibrina permeada por células e plaquetas, juntamente com a alta concentração de moléculas ativas liberadas de forma lenta, o torna uma ferramenta promissora para melhorar a reparação tecidual. Vários tipos de preparações de PRF têm sido propostos na literatura com base em diferentes tempos de centrifugação, forças relativas de centrifugação e tubos de diferentes materiais. Essas alterações nos parâmetros de produção permitiu a produção de concentrados com características específicas de acordo com o objetivo de sua utilização. Assim, esta tese teve como objetivo geral comparar protocolos de concentrados sanguíneos de PRF, tipos de tubos de coleta e aplicação clínica do PRF na redução dos sinais e sintomas inflamatórios pós-operatórios em pacientes submetidos a exodontias de terceiros molares. Objetivo específico 1: Avaliar um novo protocolo de PRF obtido por variação escalonada da força centrífuga relativa, produzido em tubos de coleta de sangue de plástico sem aditivos. Objetivo específico 2: Comparar o desempenho dos tubos Vacuette® e S-PRF® em relação à eficiência de coleta, resistência à tração, celularidade e composição química do tubo, na produção de membranas gigantes por meio do protocolo PRF progressivo (PRO-PRF). Objetivo específico 3: Investigar, por meio de meta-análise de rede, a eficácia dos concentrados sanguíneos na redução da percepção de dor, trismo e edema, após a extração de terceiros molares inferiores. Após análise dos dados obtidos, foi possível concluir que: 1) O novo protocolo de centrifugação progressiva de fibrina rica em plaquetas (PRO-PRF), permite a produção de membranas gigantes de PRF, com maior resistência à tração e com distribuição celular adequada. Além disso, permite a incorporação dos biomateriais durante sua produção e o controle clínico da espessura e tamanho da membrana, conforme a necessidade clínica. Considerando assim parâmetros suficientes para uma nova geração de concentrados sanguíneos. 2) Os tubos Vacuette® são equivalentes aos tubos S-PRF® em desempenho de coleta, composição química e

membranas produzidas. Portanto, os tubos Vacuette© representam uma alternativa segura e de baixo custo para a produção de membranas PRF. 3) Considerando a baixa certeza das evidências, dentre os concentrados sanguíneos avaliados, o A-PRF teve melhor desempenho e pareceu eficaz na redução da dor pós-operatória, após extrações de terceiros molares inferiores, e o trismo diminuiu no pico inflamatório.

Palavras-chave: Dor; Engenharia tecidual; Fibrina rica em plaquetas; Inflamação; Trismo; Terceiros molares.

ABSTRACT

Platelet-rich blood concentrates originates from platelet collections in plasma solutions for surgical site use. The objective was to increase the release of growth factors and local cytokines for improved tissue repair. Platelet-rich fibrin (PRF) concentrate is considered an evolution in the generation of blood concentrates due to its additive-free production. Moreover, PRF ability to form a dense fibrin network permeated by cells and platelets, along with the high concentration of slow-release active molecules, makes it a promising tool for tissue repair improvement. The literature has proposed several PRF preparations based on different centrifugation times, relative centrifugal forces (RCF), and tubes of different materials. These parameter changes allowed the production of concentrates with specific characteristics according to their use goal. Thus, the general objective of this thesis was to compare protocols of PRF blood concentrates, collection tube types, and clinical applications to reduce postoperative inflammatory signs and symptoms in patients subjected to third molar extractions. Specific objective 1: To evaluate a new PRF protocol obtained through stepped variation of relative centrifugal force, produced in additive-free plastic blood collection tubes. Specific objective 2: To compare the performance of Vacuette® and S-PRF® tubes regarding collection efficiency, tensile strength, cellularity, and chemical composition of the tube in producing giant membranes using the progressive PRF protocol (PRO-PRF). Specific objective 3: To investigate, through a network meta-analysis, the effectiveness of blood concentrates in reducing pain perception, trismus, and edema after lower third molar extraction. After analyzing the obtained data, it was possible to conclude that: 1) The new progressive centrifugation protocol for platelet-rich fibrin (PRO-PRF), allows the production of giant PRF membranes with higher tensile strength and adequate cell distribution. Additionally, it enables the incorporation of biomaterials during production and clinical control of membrane thickness and size according to clinical need. Thus, providing sufficient parameters for a new generation of blood concentrates. 2) Vacuette® tubes are equivalent to S-PRF® tubes in collection performance, chemical composition, and produced membranes. Therefore, Vacuette® tubes represent a safe and cost-effective alternative for PRF membrane production. 3) Considering the low certainty of evidence, among the evaluated blood

concentrates, A-PRF performed better and appeared to be effective in reducing postoperative pain after lower third molar extractions, and trismus decreased at the inflammatory peak.

Keywords: Pain; Tissue engineering; Platelet-rich fibrin; Inflammation; Trismus; Third molar.

Introdução & Referencial teórico

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

A engenharia tecidual é um campo interdisciplinar que envolve a aplicação de princípios de engenharia, biologia e medicina para desenvolver tecidos funcionais. Destina-se a reparar, manter ou melhorar a função do tecido por meio da criação de arcabouços, biologicamente compatíveis, que suportem o crescimento e diferenciação de células em tecido funcional (1). Os danos teciduais podem ser causados por diversas condições biológicas, como doenças degenerativas ou congênitas, lesões traumáticas, e mesmo o próprio processo de envelhecimento (2). Abordagens de engenharia tecidual envolvem o uso de biomateriais, fatores de crescimento e células, isoladamente ou em combinação, para aprimorar o microambiente dos tecidos lesados, com o objetivo de restaurar sua morfologia e função originais. Para alcançar esse objetivo, tem sido propostos diferentes recursos como enxertos, arcabouços, culturas de células, impressão 3D, fatores de crescimento e citocinas, que podem ser utilizados individualmente ou em conjunto, de acordo com a necessidade específica de cada caso (3–6). O avanço do conhecimento sobre os eventos celulares e moleculares que ocorrem nos tecidos lesados, assim como os processos biológicos envolvidos no processo de reparo, despertou interesse para a utilização de concentrados sanguíneos em áreas de tecidos danificados (7,8).

O conceito original que deu origem aos concentrados sanguíneos ricos em plaquetas surgiu da ideia de coletar plaquetas e fatores de crescimento autólogos em soluções de plasma para serem utilizados em sítios cirúrgicos. O objetivo era promover a melhora do reparo local, como inicialmente foi utilizado o adesivo de fibrina na década de 1970 (9). Resultados promissores levaram à novas pesquisas que culminaram no desenvolvimento dos concentrados sanguíneos atuais, a partir de avanços na tecnologia de processamento do sangue e na compreensão dos seus componentes (10).

O concentrado sanguíneo denominado de plasma rico em plaquetas (PRP) foi introduzido no final dos anos 90 por Whitmann, sendo o primeiro concentrado sanguíneo realmente difundido no meio clínico (10,11). Essa primeira geração de concentrados sanguíneos o PRP utiliza dupla centrifugação, sendo uma centrifugação inicial em tubos com anticoagulante, normalmente o citrato de sódio, seguida de uma segunda centrifugação onde,

ao final do processo, é adicionado coagulantes, como trombina bovina e/ou cloreto de cálcio. A primeira centrifugação separa os glóbulos vermelhos do plasma da camada leucocitária. Posteriormente, o tampão de plaquetas é separado do plasma pobre em plaquetas, após a segunda centrifugação (2).

Apesar da difusão da utilização do PRP, desvantagens foram relatadas, como apresentar consistência gelatinosa, liberação rápida e curta das substâncias bioativas e o uso de fatores anticoagulantes e procoagulantes, que retardam os eventos normais de reparo de feridas (10). Pouco tempo depois, Anitua e colaboradores, (1999) formularam um novo concentrado de plaquetas, denominado fator de crescimento rico em plaquetas (PRGF) que apresentava características semelhantes ao PRP, mas com a possibilidade de formação de uma estrutura mais resistente, mas ainda com utilização de anticoagulante e procoagulante na sua produção (12).

A segunda geração de concentrados sanguíneos, denominada fibrina rica em plaquetas e leucócitos (LPRF) proposta por Choukroun (13,14), é obtida a partir do sangue periférico em uma única centrifugação, sem utilização de aditivos na sua produção. Após a centrifugação, é obtido um arcabouço de fibrina resistente com arquitetura tridimensional, que retém leucócitos e plaquetas no seu interior (10,13,14). Esta matriz de fibrina contém uma variedade de fatores de crescimento, citocinas e interleucinas que são liberadas lentamente durante os 10 primeiros dias após a sua utilização (13,15). Assim, os concentrados sanguíneos de fibrina rica em plaquetas e leucócitos, apresentam potencial terapêutico para uma ampla gama de condições médicas (7,8,16,17), como na cirurgia neurológica (18), dermatologia (19), otorrinolaringologia (20), estética (21), e várias áreas da odontologia principalmente a cirurgia, implantodontia e harmonização orofacial (22–24).

A partir do concentrado sanguíneo LPRF original, outros tipos de protocolos de PRF têm sido propostos na literatura, com base na variação de tempo de centrifugação, força de centrifugação relativas (*Relative Centrifugal Force*) e uso de tubos de diferentes materiais (25,26). Nesse contexto, surgiu o conceito de centrifugação a baixa velocidade com o objetivo de melhorar as propriedades biológicas dos concentrados de PRF. Assim, reduzindo a força relativa de centrifugação (RCF) e o tempo de centrifugação, obtêm-se matrizes sólidas

com maior retenção e melhor distribuição de leucócitos na rede de fibrina e maior liberação de fatores de crescimento e citocinas. Em contrapartida, maiores RCFs geram matrizes PRF com maior densidade de fibrina, portanto mais resistentes. Essas alterações nos parâmetros de produção permitiu a produção específica de concentrados de acordo com o objetivo de sua utilização clínica (2,27–32).

Outro fator importante na produção de PRF é o tipo de material dos tubos de coleta (10). Em geral, os tubos de vidro são utilizados para a produção de matrizes sólidas (membranas) e tubos plásticos, sem aditivo, para a produção de matrizes fluidas (29,30). No entanto, o custo dos tubos originalmente projetados para preparar matrizes sólidas e a descontinuidade do fornecimento de tubos de vidro comuns, levaram ao uso de tubos de plástico revestidos de sílica (33–35). A sílica imita o efeito do vidro acelerando o processo de coagulação do sangue. No entanto, essas micropartículas desprendem-se dos tubos e são incorporadas à membrana de PRF (35), e apresentam citotoxicidade para as células periosteais humanas (36). Considerando possíveis riscos para a saúde humana, tubos plásticos revestidos de sílica não devem ser utilizados para a produção de concentrados sanguíneos (36).

Os diferentes tipos de PRF's têm recebido atenção pela sua rede de fibrina que abriga principalmente plaquetas e leucócitos, e consequente liberação lenta e contínua de fator de crescimento endotelial vascular (VEGF), fator de crescimento derivado de plaquetas (PDGF), fator de crescimento transformador (TGF) e fator de crescimento semelhante a insulina (IGF) (37,38). In vitro, o PRF é capaz de suprimir a secreção de citocinas pró-inflamatórias como IL-1 β , TNF- α e IL-6 (39), modular a transição da posição M1 para M2 (fenótipo anti-inflamatório) da polarização dos macrófagos (40) e diminuir a resposta inflamatória de fibroblastos gengivais, células dendríticas (41) e células de Schwann (42). Todas essas características levaram sua utilização como meio de modular a resposta inflamatória em procedimentos cirúrgicos odontológicos.

A extração de terceiros molares inferiores é um dos procedimentos mais realizados pelos cirurgiões-dentistas na área de cirurgia oral (43). No entanto, complicações inflamatórias pós-operatórias, como dor, edema e trismo, podem ocorrer como resultado

dessa intervenção (44). Esses sintomas normalmente manifestam-se nos primeiros dias após a remoção cirúrgica dos terceiros molares mandibulares, exigindo a implementação de medidas eficazes para controlar e minimizar esses efeitos. Importante ressaltar que essas complicações são consequência de uma resposta inflamatória desencadeada pelo trauma local e estão diretamente associadas ao grau de manipulação dos tecidos duros e moles durante o procedimento cirúrgico (45).

Diversos métodos são empregados com o objetivo de controlar esses efeitos indesejáveis, com destaque para abordagens farmacológicas, como o uso de anti-inflamatórios esteroides, anti-inflamatórios não esteroides e analgésicos (46). A fim de alcançar um controle mais efetivo da resposta inflamatória pós-operatória após a extração dos terceiros molares, uma variedade de terapias coadjuvantes têm sido exploradas, incluindo laserterapia de baixa potência (46,47), irrigação com água ozonizada (48), aplicação de kinesio tape (49,50), crioterapia (51), dreno cirúrgico (52) e uso de diversos tipos de concentrados sanguíneos nos alvéolos dos terceiros molares extraídos (53–56).

Atualmente, os concentrados sanguíneos-PRF são amplamente utilizado para cirurgia de terceiros molares mandibulares a fim de desenvolver a estratégia personalizada para reduzir o risco de complicações e melhorar os sinais e sintomas inflamatórios pós-operatórios (57–59), no entanto, não estão claros seus efeitos sobre possíveis complicações inflamatórias pós-cirúrgicas (60).

Objetivos

2. OBJETIVOS

Objetivo Geral

Comparar diferentes protocolos de concentrados sanguíneos de PRF, tipos de tubos para sua produção e sua aplicação clínica na redução dos sinais e sintomas inflamatórios pós-operatórios em pacientes submetidos a exodontias de terceiros molares.

Objetivos Específicos

Objetivo específico 1

Capítulo 1 – *Platelet-Rich Fibrin Progressive Protocol: Third Generation of Blood Concentrates*

Avaliar um novo protocolo de PRF obtido por variação escalonada da força centrífuga relativa, produzido em tubos de coleta de sangue de plástico sem aditivos.

Objetivo específico 2

Capítulo 2 – *Are non-PRF specific plastic tubes without additives a feasible alternative to S-PRF® PROCESS tubes in PRF membranes production?*

Comparar o desempenho dos tubos Vacuette® e S-PRF® em relação à eficiência de coleta, resistência à tração, celularidade e composição química do tubo, na produção de membranas gigantes através do protocolo PRF progressivo (PRO-PRF).

Objetivo específico 3

Capítulo 3 – *Do blood concentrates influence inflammatory signs and symptoms after mandibular third molar surgery? A systematic review and network meta-analysis of randomized clinical trials*

Investigar, por meio de uma meta-análise de rede, a eficácia dos concentrados sanguíneos na redução da percepção de dor, trismo e edema após a extração de terceiros molares inferiores.

Capítulos

3. CAPÍTULOS

3.1 CAPÍTULO 1

Artigo publicado no periódico Journal of Oral and Maxillofacial Surgery (JOMS)

Platelet-Rich Fibrin Progressive Protocol: Third Generation of Blood Concentrates



Carlos José Saboia-Dantas, DDS, MS, PhD,*
Pedro Henrique Justino Oliveira Limirio, DDS, MS, PhD,†
Marcelo Dias Moreira de Assis Costa, DDS, MS,‡
Camila Rodrigues Borges Linhares, DDS, MS,‡
Maria Adelia Faleiro Santana Silva, DDS, MS,‡
Hany Angelis Abadia Borges de Oliveira, DDS, MS,§ and Paula Dechichi, DDS, MS, PhD||

Purpose: Platelet-rich fibrin (PRF) has been used in several fields of dentistry to improve tissue healing. However, PRF from glass tubes results in a limited number of small membranes, increasing clinical difficulty and work time. The aim of this study was to evaluate cell and platelet amounts and biomechanical strength of PRF-giant membranes produced from plastic tubes without additives.

Material and Methods: The investigators designed an ex vivo study, to compare 3 different centrifugation protocols for obtaining PRF: $700 \times g/12$ minutes (leukocyte and PRF [L-PRF]), $350 \times g/14$ minutes (GM350), and $60-700 \times g$ more than 15 minutes total (progressive PRF [PRO-PRF]). We collected blood samples from 5 volunteers aged 25-54 years, over 3 different time periods (triplicate and paired study). From each venipuncture, 4 mL of blood was collected in vacutainers with ethylenediamine tetraacetic acid (EDTA) and approximately 104 mL in 12 plastic tubes without additives, which were separated into 3 groups, as per the centrifugation protocols ($n = 5$): L-PRF, GM350, and PRO-PRF. The PRF from the tubes of the same protocol was aspirated and 9 mL were placed in polylactic acid (PLA) forms and 3 mL were placed in a glass receptacle. The membranes from PLA forms were tested for tensile strength and the membranes from glass receptacles were evaluated by histomorphometry, while platelets and leukocytes were counted for those in tubes with EDTA. Statistical analyses were performed using Shapiro-Wilk normality test and then a one-way repeated measures analysis followed by Tukey multiple comparisons test ($\alpha < 0.05$).

Results: In tensile analyses, PRO-PRF (0.85 ± 0.23 N) showed a significantly higher maximum breaking strength than L-PRF (0.61 ± 0.26 N, $P = .01$) and GM350 (0.58 ± 0.23 N, $P < .01$). The histomorphometry revealed no significant statistical difference in cell counts between the groups ($P = .52$). Furthermore, there was no significant difference between the leukocyte ($P = .25$) and platelet counts ($P = .59$) in whole blood between the groups.

*Tissue Repair Research Laboratory, Brain Storm Academy, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

†Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

‡Department of Cell Biology, Histology and Embryology, Biomedical Science Institute, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

§Department of Oral and Maxillofacial Surgery, Brazilian Association of Dentistry (ABO), Uberlândia, Minas Gerais, Brazil.

||Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil; Department of Cell Biology, Histology and Embryology, Biomedical Science Institute, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

Conflict of Interest Disclosures: None of the authors have any relevant financial relationship(s) with a commercial interest.

Address correspondence and reprint requests to Dr Dechichi: Biomedical Science Institute, Federal University of Uberlândia, Avenida Pará 1720, Campus Umuarama, Bloco 2B, Departamento de histologia, Bairro Umuarama, Uberlândia, Minas Gerais, Brazil, 38.400-902; e-mail: pauladechichi@ufu.br

Received May 3 2022

Accepted September 5 2022

© 2022 American Association of Oral and Maxillofacial Surgeons

0278-2391/22/008357

<https://doi.org/10.1016/j.joms.2022.09.002>

Conclusion: The progressive protocol (PRO-PRF) enabled the production of PRF giant membranes with greater tensile strength and adequate cell distribution. Moreover, it allows biomaterial incorporation during production and enables clinical control of membrane thickness and size as per the surgical procedure.

© 2022 American Association of Oral and Maxillofacial Surgeons

J Oral Maxillofac Surg 81:80-87, 2023

New strategies in tissue engineering have been proposed to predictably regenerate or restore damaged, diseased, injured, aged, and supporting tissues.^{1,2} Platelet-rich fibrin (PRF) has been used in several fields of dentistry³⁻⁵ alone or in combination with materials and drugs to protect the surgical site or bone grafts,⁶ improve surgical recovery,⁷ reduce the risk of postoperative infections^{8,9} and improve soft and hard tissue repair.¹⁰⁻¹²

PRF is considered the second generation of platelet concentrates, differing in many aspects from the first generation, which is represented by platelet-rich plasma.¹³ The platelet-rich plasma protocol involves the double centrifugation of whole blood samples in the presence of nonautologous anticoagulants and clotting by the addition of coagulation factors.¹⁴⁻¹⁶ In contrast, PRF preparation involves the immediate subjection of blood samples to a single centrifugation cycle without the addition of exogenous substances.^{14,16} The fibrin clot is obtained through the activation of the intrinsic coagulation pathway^{14,15} with a functional 3-dimensional matrix in which leukocytes,¹⁷ circulating stem cells,¹⁸ growth factors, and platelets are entrapped.¹⁵ In addition, it does not dissolve within a few hours after application but is slowly replaced, similar to a natural blood clot.¹⁰

The low-speed centrifugation concept (LSCC)¹⁹ was proposed to improve the biological properties of PRF concentrates, based on centrifugation speed reduction, to obtain fibrin clots. This approach generates a variety of PRF protocols to enhance the tissue repair potential of blood concentrates in regenerative medicine.²⁰⁻²³ Variations in centrifugation speed and time affect the physicochemical and biological properties of PRF, favoring fibrin mesh density and resistance, cellularity, cell distribution, and cell entrapment.^{24,25} It further modulates tissue reactions toward an increased angiogenic potential^{24,26,27} at the surgical site. PRF preparation with lower g-forces results in increased vascular endothelial growth factor release, greater proliferation of endothelial cells, and increased migration of these cells.²⁸

Based on the LSCC, PRF membranes can be produced from aspirated blood concentrates that are still in a fluid state in glass tubes. Therefore, the blood concentrate can be aspirated and placed in recipients for clotting or injected into the area of interest.²⁹ However, handling the material is challenging due to the accelerated coagulation process, which occurs when

in contact with the glass tube walls.^{30,31} This reduces the clinical working time and limits the frequency and quantity of use, thereby influencing the predictability of the surgical procedure.

Thus, the purpose of this study was to obtain a new generation of blood concentrates in plastic blood collection tubes, with a staggered variation in the relative centrifugal force (RCF) over the same total time interval. PRF giant membranes can be produced from several collection tubes, thereby opening a range of new possibilities for the use of blood concentrates in regenerative surgery. Here, we hypothesized that giant membranes generated using the progressive protocol (progressive PRF [PRO-PRF]) must have equal or superior resistance and cellularity to leukocyte and PRF (L-PRF) membranes, or those obtained with a constant RCF during a centrifugation time interval (GM350). Therefore, the specific aims of the study were to compare the tensile strength, leukocytes, and platelet counts of giant membranes obtained from plastic blood-collection tubes without additives, produced from different centrifugation protocols.

Materials and Methods

STUDY DESIGN/SAMPLE

To address the research purpose, the investigators designed and implemented an ex vivo study. The study population was composed of 5 healthy individuals aged 25-54 years. To be included in the study sample, the individuals had to be healthy nonsmokers who had not used anticoagulant, anti-inflammatory, or antibiotic drugs in the last 3 months. Exclusion criteria included the use of anticoagulant medication, anti-inflammatory drugs or antibiotics in the 3 months prior to the study, active systemic infections, and smokers. The Brazilian Human Research Ethics Committee (CAEE - 31,441,520.2.0000.5152) approved this study. All individuals were informed of the procedures and signed a consent form prior to testing. Volunteer blood samples were collected at 3 different times (triplicate and paired study) by peripheral venipuncture in one of the antecubital fossa veins and immediately transferred to a fixed-angle rotor centrifuge (SpinPlus Titan, Daiki, Ribeirão Preto, SP, Brazil). For each venipuncture, 4 mL of blood was placed in 1 tube with ethylenediamine tetraacetic acid (EDTA) (complete blood count) and approximately 104 mL in 12 plastic tubes without additives, separated into 3 groups as per the

centrifugation protocol ($n = 5$): leukocyte and PRF (L-PRF: 700 g/12 min), giant membrane 350 (GM350: 350 g/15 min), and progressive giant membrane (PRO-PRF: 60 g/5 min, 200 g/5 min, and 700 g/5 min) groups. The G-force of the centrifuge for the PRO-PRF group was increased as soon as the determined time limit for each RCF was reached, with a total centrifugation time of 14 minutes. The supernatant fluid PRF from plastic tubes using the same protocol was aspirated using a 40/12 needle and a 20-mL syringe (Fig 1A) and placed in polylactic acid (PLA) forms (9 mL) for tensile strength analysis (Fig 1B1), a glass receptacle (3 mL) for histomorphometric analysis (Fig 1B2), and a tube with EDTA (4 mL) for platelet and leukocyte counts.

DATA COLLECTION METHODS

The aspirated fluid was dispensed into a standard PLA form fixed on the central portion of a 6-mm wide glass plate with 15 mm at each end and a total extension of 70 mm (EN ISO 527-2: 2012) for tensile

testing of the ductile material. After polymerization (approximately 45 minutes), the formed fibrin clot was gently detached from the edges and removed from the PLA form (Fig 2A,B). The clot was transferred to the PRF-BOX and pressed for 5 minutes (Fig 2C) to drain excess serum and form a membrane. Each membrane was then positioned vertically in 2 fixation accessories on a universal testing machine (EMIC DL 2000, EMIC Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, Brazil). One of the accessories was coupled to a 20-N load cell to perform the tensile test at a displacement of 5 mm/min with the membrane rupture as the maximum point (Fig 3A). The analyzed parameter was the mechanical disruption strength (N) of the evaluated membranes.

For the histomorphometric analysis, the clot formed from the 4-mL fluid PRF dispensed in the glass receptacle (after 15 minutes) was transferred to the PRF-BOX and pressed for 5 minutes. The obtained membrane was fixed in 10% formaldehyde in 0.1 M PBS for 24 hours and embedded in paraffin. Subsequently,

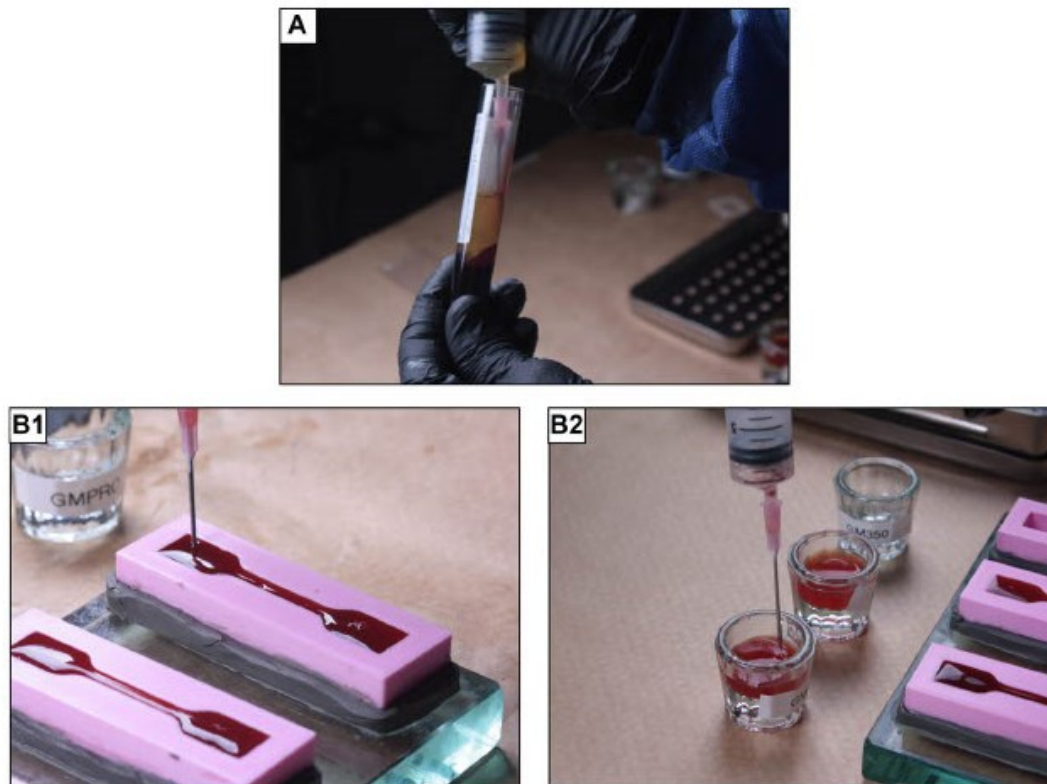


FIGURE 1. Supernatant fluid platelet-rich fibrin (PRF) aspiration from the buffy coat region above the red cell junction (A). The PRF fluid was dispensed in the polylactic acid (PLA) forms (B1) and a glass recipient (B2).

Sabola-Dantas et al. Platelet-Rich Fibrin Progressive Protocol. J Oral Maxillofac Surg 2023.

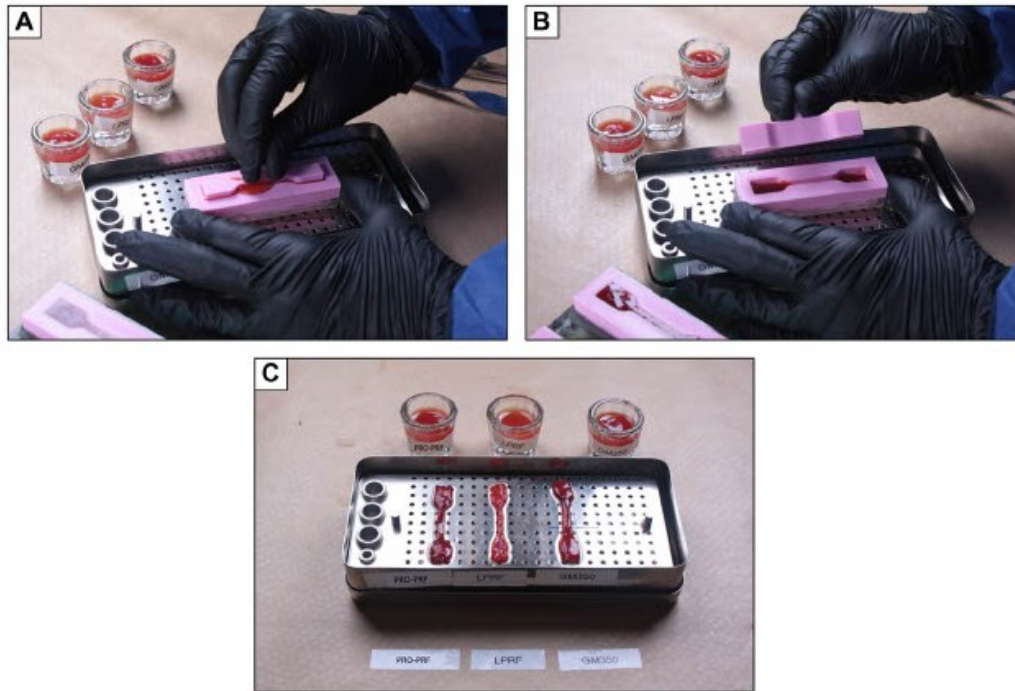


FIGURE 2. After polymerization, the clot was gently compressed for 5 seconds by the PLA device (A) and removed from the form (B). Membranes softly compressed into PRF-Box for 5 minutes (C).

Sabola-Dantas et al. Platelet-Rich Fibrin Progressive Protocol. J Oral Maxillofac Surg 2023.

5 μ m thickness slices were obtained using a microtome (Leica Biosystems RM2245, Leica, Nussloch, Germany) and stained with hematoxylin and eosin (H&E). Histological images were digitized using the Aperio AT

Turbo Scanner device (Copyright 2013, Leica Biosystems Imaging, Inc.) at 20 $\times$ magnification. For the analysis, 3 slides from each membrane were selected. The digitized histological images were visualized using

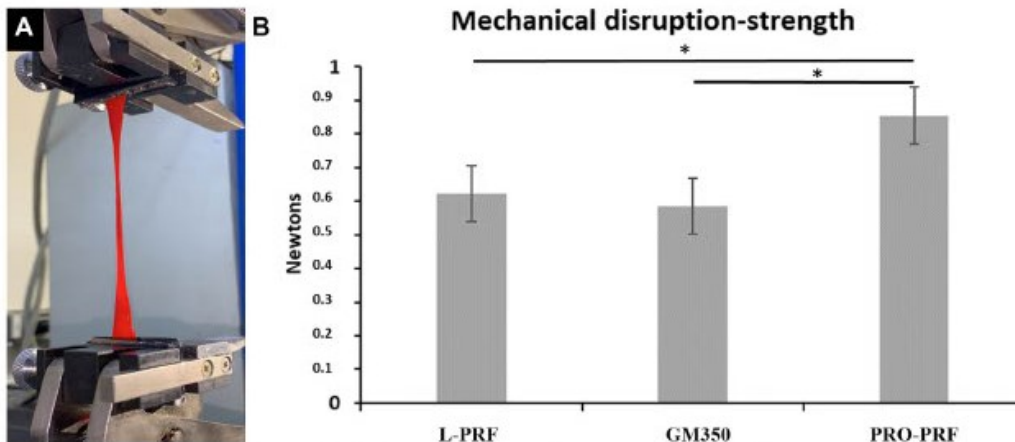


FIGURE 3. PRF giant membrane on the testing machine (EMIC DL 2000) (A). Results of tensile strength analysis (B). * $P < .05$.

Sabola-Dantas et al. Platelet-Rich Fibrin Progressive Protocol. J Oral Maxillofac Surg 2023.

the Aperio ImageScope image reading program (Copyright Aperio Technologies, Inc. 2003-2014). Thirty 600×600 images were obtained from each membrane and the cells were counted using ImageJ software.³²

Blood count analysis was performed with the fluid PRF from 1 plastic tube without additives in each protocol, which was aspirated and dispensed in a tube with anticoagulants (EDTA) (Greiner Bio-One, Americana, SP, Brazil). The tubes were immediately subjected to an automated hemocytometer (ABC VET Animal Blood Counter, Scil Animal Care Company Veterinary Diagnostics, Orange City, Florida) to count platelets and leukocytes in each protocol for each volunteer.

DATA ANALYSES

Statistical analyses were performed using Sigma Plot v 13.1 (Systat Software Inc., San Jose, California). The results obtained were first subjected to the Shapiro-Wilk normality test and then a one-way repeated measures analysis followed by a Tukey multiple comparisons test, and the results are presented as mean and standard deviation (mean $\pm$ SD). Differences were considered statistically significant at $\alpha < 0.05$.

Results

The tensile test showed that PRO-PRF (0.85 ± 0.23 N) had a higher mechanical disruption-strength than L-PRF (0.61 ± 0.26 N, $P = .01$) and GM350 (0.58 ± 0.23 N, $P < .01$) (Fig 3B). Qualitative histological analysis showed a delicate fibrin network with cells distributed throughout the network. In addition, many red blood cells were observed in the fibrin mesh in all the groups studied (Fig 4). The histomorphometric analysis revealed no statistically significant difference in cell counts between the groups ($P = .52$). Furthermore, there was no significant statistical difference between the leukocyte ($P = .25$) and platelet counts ($P = .59$) in the whole blood analysis between the groups (Table 1).

Discussion

The purpose of this study was to obtain a new PRF protocol, produced in plastic tubes, opening a range of possibilities for the use of blood concentrates in regenerative surgery. We hypothesized that giant membranes generated from the PRO-PRF protocol would have equal or superior resistance and cellularity to L-PRF and GM350 membranes. Therefore, the specific aims of the study were to evaluate the tensile strength and the number of leukocytes and platelets of new PRF protocols obtained in constant RCF and time or in progressive RCF over the same time interval. The fibrin from PRO-PRF had a higher tensile strength than the other studied protocols. However, there

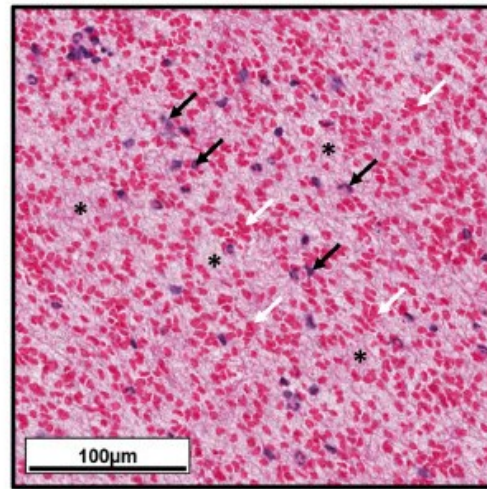


FIGURE 4. Representative histological image of PRF giant membrane showing red blood cells (white arrow), cells (black arrow), and fibrin network (*) stained in hematoxylin-eosin.

Sabola-Dantas et al. Platelet-Rich Fibrin Progressive Protocol. *J Oral Maxillofac Surg* 2023.

was no difference in the number of leukocytes and platelets among the different protocols.

In this study, we proposed 2 protocols for obtaining giant membranes from plastic tubes without additives: GM350 (350 g RCF/14 min) and PRO-PRF (progressive RCF variation/15 min). The tensile strength, leukocytes, and platelet numbers in the giant membranes were compared to those of the standard PRF protocol L-PRF (700 g RCF/12 min). This method of giant membrane (GM) generation enables for the production of membranes that could provide varied clinical needs with only 1 thick membrane, thereby simplifying the clinical procedure.

The greater tensile strength of PRO-PRF can be attributed to the higher concentration of fibrin molecules and the establishment of equilateral junctions, resulting in a flexible network. The lower forces in the progressive RCF of PRO-PRF favored the slow and progressive polymerization of fibrin molecules, by increasing lateral aggregation and forming thicker fibers.³³ However, lower G-forces also promote a decrease in fibrin-fiber diameter.³⁴ Considering that the fibrin protofibrils only associate laterally after reaching the determined length and that these interactions are weak,³³ it is possible that progressive centrifugation favors this interaction. Thus, it would be possible to obtain a delicate but integrated mesh with a greater number of chemical bonds. This mesh could facilitate the infiltration of growth factors and chemotaxis of other cells to the site¹⁵ and increase membrane resistance.

Table 1. RESULTS OF HISTOMORPHOMETRIC AND WHOLE BLOOD COUNT ANALYSIS

Analysis	Blood Count	L-PRF	GM350	PRO-PRF	P Values
Cells numbers*		99.49 ± 66.86	79.61 ± 47.78	88.37 ± 41.49	.52
Platelet counts (platelets/ μ L)	209,600 ± 25,264.6	153,000 ± 152,369.6	265,600 ± 147,801.6	127,000 ± 38,098.5	.59
Leukocyte counts (leukocytes/ μ L)	5,640 ± 585.6	6,200 ± 953.9	5,080 ± 1,706.4	5,640 ± 1,487.6	.25

* Histomorphometric.

Sabola-Dantas et al. Platelet-Rich Fibrin Progressive Protocol. J Oral Maxillofac Surg 2023.

The histomorphometric and blood count analyses showed no difference in the number of leukocytes and platelet counts among the studied protocols. However, a large standard deviation was observed in the total means between the groups. Thus, we performed a one-way repeated measures test to compare the different protocols based on blood samples from the same individual. This is because there is great variability in blood composition between genders, age, and even period blood.³⁵ In addition, the variation in cell count may have been due to technical challenges or variation in access to the buffy coat during PRF aspiration. However, it is important to emphasize that aspirating the blood concentrate from several plastic tubes without additives and dispensing it in a container allow for better cell distribution in the produced membrane. Despite the variation in cell count in the PRF matrix, all had expressive and homogeneous cellularity. Thus, in this context, the fibrin scaffold played a more relevant role in the surgical environment because it is a 3-dimensional structure that supports tensional forces and allows cell migration, which optimizes the tissue repair process.¹⁵

In wound closure and ridge reconstruction, the volume and strength of the membranes determine their efficiency as a framework. In our comparative study, the methodology for obtaining the membranes efficiently increased membrane resistance and cell distribution, without compromising cellularity. Here, we suggested an innovative way to manipulate blood concentrates to produce a new type of PRF membrane, the GM, for clinical purposes. In this technique, the fluid blood concentrate was aspirated from several plastic tubes without additives and transferred to a container, where it formed a single fibrin clot, with the size and shape of the receptacle. This allowed the combination of the GM with materials or drugs of clinical interest. The clot was then pressed into a PRF box to drain the serum and bring fibrin fibrils closer together to form the GM.

The use of plastic blood-collection tubes without additives avoids the acceleration of coagulation, which occurs when blood contacts the walls of glass tubes.^{30,31} This process facilitates concentrate handling

without limiting the working time and increases the predictability of obtaining the GM in surgical procedures. Furthermore, PRF contamination by silica particles from silica-coated tubes is avoided³⁶⁻⁴⁰ as silica microparticles, depending on their amorphousness and size, can be harmful to health.^{36,41} Manufacturers advise against the use of silica-coated plastic tubes, including plastic tubes containing silica-coated film, for the preparation of PRF for regenerative therapy because these tubes have not been approved for this purpose by regulatory agencies.³⁶

Thus, we suggested a new protocol for obtaining blood concentrates by varying the RCF progressively in the centrifugation from lower to higher values within the same time interval. The new protocol represents a new generation of blood concentrates, generating a giant membrane (PRO-PRF). This PRO-PRF demonstrated a more resistant fibrin matrix than that in the L-PRF and GM350 membranes. Therefore, this material was called PRO-PRF and was considered the third generation of blood concentrates. It represents a new modality of PRF for clinical use, obtained through a progressive protocol, which provides an excellent association between resistance and cellularity. It likely improves the migration of the surrounding cells and replacement of the fibrin matrix and features as a conductive and temporary scaffold. Moreover, PRO-PRF could be produced in glass tubes to obtain solid clots following centrifugation and membranes after pressing in the box, similar to the original L-PRF. Its resistance and cellularity were not evaluated in this study.

Considering the evolution of second-generation concentrates, as per the LSCC, biological properties such as conduction and induction were favored over the scaffold effect. Furthermore, several membranes obtained from glass tubes were limited in thickness and shape, as their dimensions were defined by the tube volume. Moreover, it is difficult to place and maintain the membranes at the site during wound closure. Thus, we devised a progressive protocol for obtaining giant membranes in plastic tubes without additives (PRO-PRF) that increased working time (without glass tubes), making it possible to control the dimensions

and shape of the membrane to increase the scaffolding effect and facilitate their application at the surgical site.

Our initial clinical observations suggest that PRO-PRF acts as a physical and biochemical barrier, probably by releasing growth factors as per the tissue signaling pattern, similar to a reciprocal induction mechanism.⁴² The resistance and control of dimensions (mostly thickness) allow it to be stabilized by sutures and surgical tacks. Thus, PRO-PRF protects bone grafts or substitutes and wound closures by first intention bone regeneration or by means of an open wound technique.⁴³ In addition, the inducing effect of PRO-PRF can be optimized by improving the spatial distribution of leukocytes and platelets. However, these characteristics need to be confirmed by analyzing the release of growth factors over time and through controlled clinical trials. The superior biological properties of PRO-PRF, when used as a conductive and inductive scaffold for tissue repair, also need to be confirmed by additional laboratory analysis and clinical trials.

The progressive protocol (PRO-PRF) enables the production of PRF giant membranes with a greater tensile strength and adequate cell distribution. Moreover, it enables biomaterial incorporation during its production and clinical control of membrane thickness and size as per the surgical procedure.

Acknowledgments

We acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) and Research Support Foundation of the State of Minas Gerais (FAPEMIG/Brazil) for granting us the scholarship and resources for the purchase of materials needed. This study was performed at the CPBio-Biomechanics, Biomaterials, and Cell Biology Research Center-UFU.

References

- Dai R, Wang Z, Samanipour R, Koo KI, Kim K: Adipose-derived stem cells for tissue engineering and regenerative medicine applications. *Stem Cells Int* 2016;67373-45, 2016
- Rouwkema J, Khademhosseini A: Vascularization and Angiogenesis in tissue engineering: Beyond Creating static networks. *Trends Biotechnol* 34:733, 2016
- Lyris V, Millen C, Besi E, Pace-Balzan A: Effect of leukocyte and platelet rich fibrin (L-PRF) on stability of dental implants. A systematic review and meta-analysis. *Br J Oral Maxillofac Surg* 59: 1130-1139, 2021
- Miron RJ, Zucchelli G, Pikos MA, et al: Use of platelet-rich fibrin in regenerative dentistry: A systematic review. *Clin Oral Investig* 21:1913, 2017
- Narendran N, Anegundi RV, Shenoy SB, Chandran T: Autologous platelet-rich fibrin as an adjunct to non-surgical periodontal therapy-a follow up clinical pilot study. *Wound Repair Regen* 30:140-145, 2021
- Simonpietri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM: The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part I: A new grafting protocol. *Implant Dent* 18:102, 2009
- Bao M, Du G, Zhang Y, Ma P, Cao Y, Li C: Application of platelet-rich fibrin derivatives for mandibular third molar extraction related post-operative sequelae: a systematic review and network meta-analysis. *J Oral Maxillofac Surg* 79:2421-2432, 2021
- Eshghpour M, Danacifar N, Kermani H, Nejat AH: Does intra-alveolar application of chlorhexidine gel in combination with platelet-rich fibrin have an advantage over application of platelet-rich fibrin in decreasing alveolar osteitis after mandibular third molar surgery? a double-blinded randomized clinical trial. *J Oral Maxillofac Surg* 76:939.c1, 2018
- Kuchnel RU, Schroeter F, Mueller T, Ostovar R, Albes JM: Platelet-rich fibrin in combination with local antibiotics optimizes wound healing after deep sternal wound problems and prevents reinfection. *Surg Technol Int* 39:313-316, 2021
- Miron RJ, Fujioka-Kobayashi M, Bisham M, Zhang Y, Hernandez M, Choukroun J: Platelet-rich fibrin and soft tissue wound healing: A systematic review. *Tissue Eng B Rev* 23:83, 2017
- Nowak JM, Surma S, Romanczyk M, Wojtowicz A, Filipiak KJ, Czemliuk MR: Assessment of the effect of A-PRF application during the surgical Extraction of third Molars on healing and the concentration of C-reactive Protein. *Pharmaceutics* 13:1471, 2021
- Sharma R, Sharma P, Sharma SD, Chhabra N, Gupta A, Shukla D: Platelet-rich fibrin as an Aid to soft- and hard-tissue healing. *J Maxillofac Oral Surg* 20:496, 2021
- Kawase T, Mubarak S, Mourão CF: The platelet concentrates therapy: From the Biased Past to the Anticipated Future. *Bioengineering (Basel)* 7, 2020
- Dohan DM, Choukroun J, Diss A, et al: Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e37, 2006
- Dohan DM, Choukroun J, Diss A, et al: Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e45, 2006
- Choukroun J, Adda F, Schoeffler C, Vervelle A: Une opportunité? En parodontologie: Le PRF Implantodontie 42:55, 2001
- Dohan DM, Choukroun J, Diss A, et al: Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e51, 2006
- Choukroun J, Diss A, Simonpietri A, et al: Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e56, 2006
- Choukroun J, Ghanaati S: Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: The first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg* 44:87, 2018
- Ghanaati S, Booms P, Orlowska A, et al: Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 40:679, 2014
- Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandam U, Zhang Y, Choukroun J: Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, Biocompatibility, and cellular Response. *J Periodontol* 88:112, 2017
- Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al: Injectable platelet rich fibrin (i-PRF): Opportunities in regenerative dentistry? *Clin Oral Investig* 21:2619, 2017
- El Bagdadi K, Kubesch A, Yu X, et al: Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: A proof of concept of LSCC (low speed centrifugation concept). *Eur J Trauma Emerg Surg* 45:467, 2019
- Kubesch A, Barbeck M, Al-Maawi S, et al: A low-speed centrifugation concept leads to cell accumulation and vascularization of solid platelet-rich fibrin: An experimental study in vivo. *Platelets* 30:329, 2019
- Pascoal MANC, Dos Santos NBM, Completo AMG, Fernandes GVO: Tensile strength assay comparing the resistance between two different autologous platelet concentrates (leucocyte-platelet rich fibrin versus advanced-platelet rich fibrin): A pilot study. *Int J Implant Dent* 7:1, 2021
- Herrera-Vizcaino C, Dohle E, Al-Maawi S, et al: Platelet-rich fibrin secretome induces three dimensional angiogenic activation in vitro. *Eur Cell Mater* 37:250, 2019

27. Blatt S, Thiem DGE, Pabst A, Al-Nawas B, Kämmerer PW: Does platelet-rich fibrin enhance the Early Angiogenic potential of different bone substitute materials? An in vitro and in vivo analysis. *Biomedicine* 9, 2021
28. Caruana A, Savina D, Macedo JP, Soares SC: From platelet-rich plasma to advanced platelet-rich fibrin: Biological Achievements and clinical advances in modern surgery. *Eur J Dent* 13:280, 2019
29. Nacopoulos C, Vesala AM: Lower facial regeneration with a combination of platelet-rich fibrin liquid matrices based on the low speed centrifugation concept-Cleopatra technique. *J Cosmet Dermatol* 19:185, 2020
30. Margolis J: Initiation of blood coagulation by glass and related surfaces. *J Physiol* 137:95, 1957
31. Kratz A, Stanganelli N, Van Cott EM: A comparison of glass and plastic blood collection tubes for routine and specialized coagulation assays: A comprehensive study. *Arch Pathol Lab Med* 130:39, 2006
32. Linhares CRB, Rabelo GD, Límrio P, Venancio JF, Ribeiro Silva IG, Dechichi P: Automated bone healing evaluation: New approach to histomorphometric analysis. *Microsc Res Tech* 85:3339-3346, 2022
33. Weisel JW, Litvinov RI: Fibrin formation, structure and properties. *Subcell Biochem* 82:405, 2017
34. de Oliveira LA, Borges TK, Soares RO, Buzzi M, Kuckelhaus SAS: Methodological variations affect the release of VEGF in vitro and fibrinolysis' time from platelet concentrates. *PLoS One* 15: e0240134, 2020
35. Miron RJ, Dham A, Dham U, Zhang Y, Pikos MA, Sculean A: The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes. *Clin Oral Investig* 23:2179, 2019
36. Tsujino T, Takahashi A, Yamaguchi S, et al: Evidence for contamination of silica microparticles in advanced platelet-rich fibrin matrices prepared using silica-coated plastic tubes. *Biomedicine* 7, 2019
37. Tsujino T, Masuki H, Nakamura M, et al: Striking differences in platelet distribution between advanced-platelet-rich fibrin and concentrated growth factors: Effects of silica-containing plastic tubes. *J Funct Biomater* 10, 2019
38. Yamaguchi S, Aizawa H, Sato A, et al: Concentrated growth factor matrices prepared using silica-coated plastic tubes are Distinguishable from those prepared using glass tubes in platelet distribution: Application of a Novel Near-Infrared Imaging-based, Quantitative technique. *Front Bioeng Biotechnol* 8:600, 2020
39. Masuki H, Isobe K, Kawabata H, et al: Acute cytotoxic effects of silica microparticles used for coating of plastic blood-collection tubes on human periosteal cells. *Odontology* 108:545, 2020
40. Miron RJ, Kawase T, Dham A, Zhang Y, Fujioka-Kobayashi M, Sculean A: A technical note on contamination from PRF tubes containing silica and silicone. *BMC Oral Health* 21:135, 2021
41. Steenland K, Ward E: Silica: A lung carcinogen. *CA Cancer J Clin* 64:63, 2014
42. Kang YH, Jeon SH, Park JY, et al: Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. *Tissue Eng A* 17:349, 2011
43. Saboia-Dantas CJ, Límrio PHJO, Campos LEC, Dechichi P: Use of second-generation blood concentrates for Alveolar Preservation and increase of Keratinized Mucosa in Extraction and Peri-Implant sites: The open wound technique. *Odontologia: da ciência da saúde às ciências humanas e Soc* 102, 2021

3.2 CAPÍTULO 2

Artigo a ser enviado para publicação no periódico Journal of Oral and Maxillofacial Surgery (JOMS)

Are non-PRF specific plastic tubes without additives a feasible alternative to S-PRF[®] PROCESS tubes in PRF membranes production?

Marcelo Dias Moreira de Assis Costa DDS, MS [†],
Pedro Henrique Justino Oliveira Limirio DDS, MS, PhD [†],
Carlos José Saboia-Dantas DDS, MS, PhD ^{*},
Camila Rodrigues Borges Linhares DDS, MS [‡],
Maria Adelia Faleiro Santana Silva DDS, MS [‡],
Hany Angelis Abadia Borges de Oliveira DDS, MS [§],
Paula Dechichi DDS, MS, PhD [†]

[†] Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

^{*} Tissue Repair Research Laboratory, Brain Storm Academy, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

[‡] Department of Cell Biology, Histology and Embryology, Biomedical Science Institute, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

[§] Department of Oral and Maxillofacial Surgery, Brazilian Association of Dentistry (ABO), Uberlândia, Minas Gerais, Brazil

[†] Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil; Department of Cell Biology, Histology and Embryology, Biomedical Science Institute, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

Abstract

Purpose: Platelet-rich fibrin (PRF), in the form of membranes, has been proposed to predictably repair tissues. This study aimed to compare non-PRF specific plastic tubes (Vacuette[®]) with tubes originally designed to produce PRF (S-PRF[®]), both without additives, providing an economically viable alternative for their production.

Material and Methods: Blood samples were collected from five volunteers, over three different periods (triplicate and paired study). From each venipuncture, approximately 48

mL of blood was collected using plastic tubes without additives of two different brands: S-PRF[®] (n=3) and Vacuette[®] (n=3). The filling times of the tubes were registered, and the PRF was aspirated from the same brand tubes using a needle and syringe, with the resultant volume being recorded. Subsequently, 9 mL was placed into polylactic acid (PLA) forms, and 4 mL was transferred to a glass recipient. The membranes from PLA forms were subjected to a tensile strength test. The membranes from glass recipients were subjected to cell counts under light microscopy; scanning electron microscope (SEM) and transmission electron microscopy (TEM) qualitative analysis. The chemical composition of the inner wall of the tubes was analyzed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Statistical analyses were performed using the Shapiro-Wilk normality test and then Paired Samples T-Test analysis ($\alpha < 0.05$).

Results: Tube filling time ($p=0.364$) and volume of PRF ($p=0.068$) were not statistically different between tubes. There was no difference in maximum breaking strength tensile between S-PRF[®] Tubes and Vacuette[®] ($p=0.389$). Moreover, no statistically significant difference was observed in the amount of cell ($p=0.083$) between S-PRF[®] and Vacuette[®], and cell integrity was confirmed by TEM analysis in both groups. SEM analysis revealed that membranes from both tubes contained cells and platelets trapped in a delicate fibrin network with varying thickness. Additionally, FTIR analysis showed that both tubes exhibited similar chemical compositions.

Conclusion: The membranes produced from both Vacuette[®] and S-PRF[®] plastic tubes demonstrated equivalent tensile strength, morphology, and amount of cell. Furthermore, the tubes exhibited similar chemical composition, filling performance and yielded comparable volumes of PRF.

Introduction

Platelet-rich fibrin (PRF) has emerged as a promising tool to enhance tissue repair owing to its ability to form a dense fibrin network that is permeated by cells and platelets, as well as a high concentration of active molecules that are locally released.¹ PRF has been proposed for tissue repair in various medical fields such as neurological surgery², dermatology³, otorhinolaryngology⁴, aesthetics⁵, and several specialties of dentistry⁶⁻⁸. The blood concentrate proposed by Choukroun^{9,10}, Leukocyte and Platelet Rich Fibrin

(L-PRF), is produced from peripheral blood in glass tubes through single centrifugation without any type of additive. This protocol results in the formation of a fibrin scaffold with leukocytes, platelets, and growth factors^{9–11}. PRF is used alone or in combination with materials and drugs to protect the surgical site or bone graft¹², reduce postoperative infections^{13,14} and accelerate and improve soft and hard tissue repair^{15–20}.

From the original L-PRF blood concentrate, several types of PRF preparations have been proposed in the literature based on different centrifugation times, relative centrifugation forces (RCF) and tubes of different materials^{21,22}. These preparations include solid (membranes) and fluid (injectable) matrices. Initially, plastic tubes without additives were used to produce injectable PRF, but recently they have also been proposed for the production of larger membranes from multiple collection tubes. Consequently, numerous new applications for blood concentrates have emerged²³.

The use of plastic tubes without additives, which exhibit a higher degree of hydrophobicity in comparison to glass, delays the adsorption of proteins onto the tube wall surfaces. This leads to a postponement in the conversion of fibrinogen and thrombin into fibrin clots, that facilitates the generation of membranes from multiple tubes and the incorporation of biomaterials and drugs²⁴. Saboia-Dantas et al (2023)²³ suggested a novel approach to produce giant PRF membranes with enhanced resistance and cellularity, utilizing a progressive protocol.

At present, PRF production requires the use of specific collection tubes (plastic S-PRF[®] or glass A-PRF[®]), considering that conventional hematology analysis tubes would not be suitable for PRF production. The present study was designed with the concept of facilitating the production of PRF blood concentrates and making it more accessible to professionals. Given the high cost of tubes S-PRF[®], it was hypothesized that Vacuette[®] tubes (non-PRF specific plastic tubes) would exhibit comparable performance, and the membranes generated from them would maintain similar biomechanical and histological properties to those produced using S-PRF[®] tubes (PRF specific plastic tubes). Therefore, the aim of the present study was to compare the performance of Vacuette[®] and S-PRF[®] tubes in relation to collection efficiency, tensile strength, cellularity and tube chemical composition (ATR-FTIR), in the production of giant membranes through the progressive PRF protocol (PRO-PRF).

Materials and Methods

Study design/sample

To address the research purpose, the investigators designed and implemented an *ex vivo* study. This study involved the participation of five healthy volunteers aged between 25 and 55. Exclusion criteria included the use of anticoagulant medication, anti-inflammatory drugs, or antibiotics within three months prior to the study, active systemic infections, and smokers. All individuals were informed of the procedures and signed a consent form prior to testing. This study was approved by the Brazilian Human Research Ethics Committee (CAEE – 31441520.2.0000.5152).

Peripheral venous blood samples were collected, alternately, in two types of plastic vacuum tubes without additives: 3 S-PRF tubes (Process, Nice, France) e 3 Vacuette tubes (Greiner Bio-One, Americana, Brazil). The samples were centrifuged using a fixed-angle rotor centrifuge Driller CS-TOUCH (Driller, Carapicuíba, Brasil), and the centrifugation process was initiated no later than 4 minutes after the start of blood collection. The centrifuge was sequentially programmed with 60 *g* for 5 minutes, followed by 200 *g* for 5 minutes, and with 700 *g* for 5 minutes, without interruption, to produce membranes, according to the progressive protocol (PRO-PRF). Then, the PRF supernatant fluid from the same protocol was aspirated using a 40/12 needle and a 20 mL syringe (Fig 1a). The fluid PRF was placed in polylactic acid (PLA) forms (9 mL) for tensile strength analysis (Fig 1b1) and into a glass recipient (4 mL) for histomorphometric analysis (Fig 1b2). This procedure was performed in triplicate from each volunteer with a minimum interval of seven days between collections.

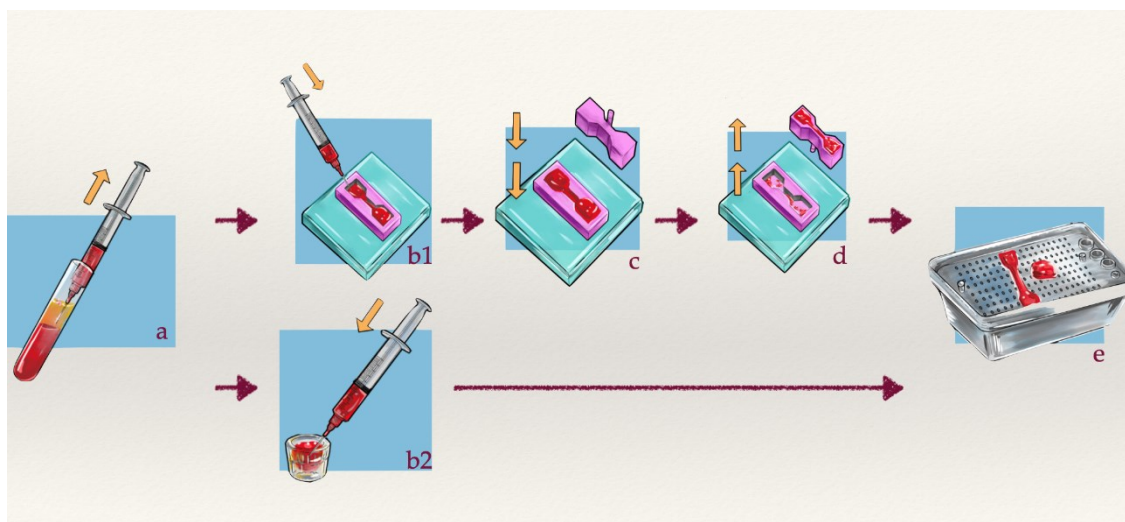


Figure 1 – (a) Aspiration of PRF supernatant fluid; (b1) 9 mL of aspirated PRF fluid was placed in the standardized PLA form and (b2) 4 mL was dispensed into the glass recipient; (c) after coagulation, the PRF membrane produced in the PLA form was pressed and (d) removed from the form; (e) membranes softly compressed into PRF-Box for 5 minutes.

Data collection methods

To evaluate the tubes performance, the filling time and the volume of concentrate produced were measured and recorded. Then, the aspirated fluid (Fig 1a) was dispensed into a standard (EN ISO 527-2: 2012) polylactic acid form (PLA)²³ (Fig 1b1), fixed on a wide glass plate, for tensile testing of the ductile material. After polymerization (approximately 45 min) the PRF membrane produced was pressed (Fig 1c), the fibrin clot formed was gently detached from the edges and removed from the PLA form (Fig 1d). The clot was transferred to a PRF Box and pressed for 5 min to drain excess serum and form a membrane (Fig 1e). Each membrane was positioned vertically in two fixation accessories on a universal testing machine (EMIC DL 2000, EMIC, São José dos Pinhais, Brazil). One of the accessories was coupled to a 20N load cell to perform the tensile test at a displacement of 5 mm/min with the membrane rupture as the maximum point. The analyzed parameter was the mechanical disruption strength (N) of the evaluated membranes (Fig 2).

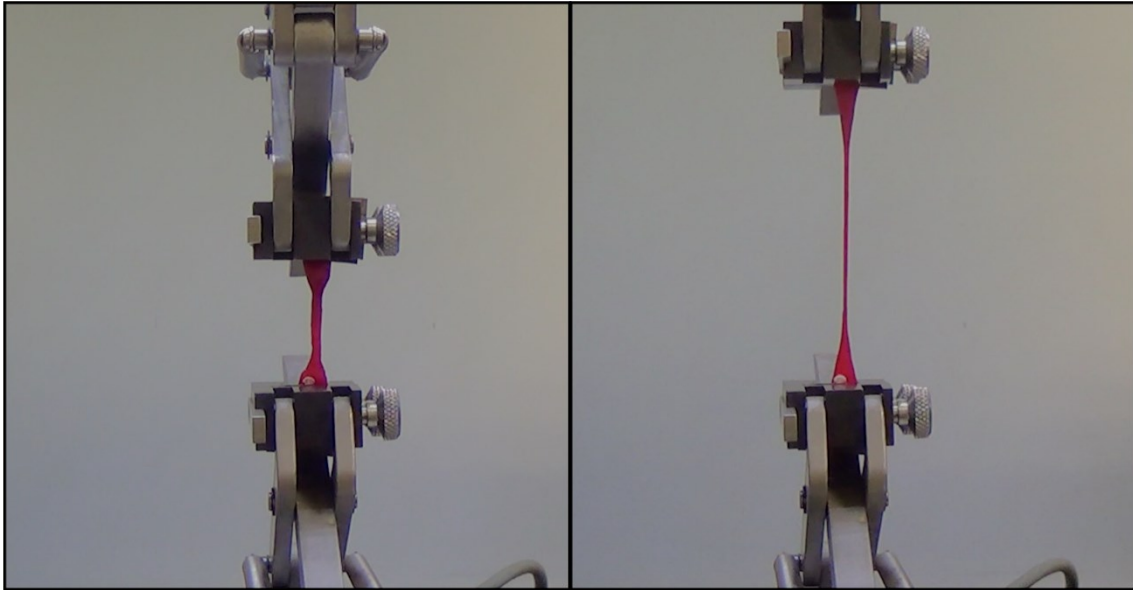


Figure 2 - PRF membrane on the testing machine (EMIC DL 2000). (a) The membrane was fixed vertically before the application of traction. (b) Elongation of the membrane before rupturing upon application of traction.

For histomorphometric analysis, 4mL of PRF fluid was transferred to glass containers to accelerate the fibrin network polymerization process. After 15 minutes, the generated membrane was gently compressed with a PRF Box (Fig 1e). Each membrane was fixed in a solution of 10% phosphate-buffered formaldehyde and 0.1% glutaraldehyde for 24 hours. Then, the membrane was divided into 2 parts, one processed for analysis at light microscope (LM) and the other divided into smaller fragments and processed to scanning electron microscope (SEM) and transmission electron microscopy (TEM) analysis.

For LM analysis, the paraffin-embedded membranes were cut into 5 μm thick sections using a microtome (Leica Biosystems RM2245, Leica[®], Nussloch, Germany) and stained with hematoxylin and eosin (HE). The histological images were captured at Aperio AT Turbo Scanner (Copyright © 2013 Leica Biosystems Imaging, Inc. All Rights Reserved) at x20 magnification. Following this, the images were visualized in the software Aperio ImageScope (Copyright © Aperio Technologies, Inc.). Thirty 600 $\times$ 600 images were obtained from each membrane, and the cells were automated counted using a macro as a plugin in the ImageJ software²⁵.

For SEM analysis the membranes were mounted on metallic stubs, sputter-coated with gold, and examined using field emission scanning electron microscopy (EVO MA 10, Carl Zeiss, London, UK) at a voltage of 20 kV after dehydrated an increasing series

of alcohol (50; 75; 90; 100%) and dried in a device (critical point device) (Balzers CPD -300 -Leica Microsystems Vienna, Austria). Then, the samples were pulverized with gold in a vacuum metallizing machine (Bal-Tec SCD-050, Leica Microsystems; Wetzlar, Germany). At a 1000x magnification, images of each specimen were captured for SEM analysis. The images were analyzed considering the morphology of the fibrin network and trapped cells.

For TEM analysis, the membranes fragments were embedded in Epon and ultrathin 60 μm sections were obtained (Reichert-Jung Ultra-cut microtome 701701) and double contrasted with uranyl acetate 3% and lead citrate 1%. The sections were analyzed under a transmission electron microscope (HT7-700 Hitachi), considering cellular membrane, organelles, and nuclei integrity.

For Fourier Transform Infrared Spectroscopy analysis, each tube was sectioned with a diamond disk under constant irrigation to obtain three fragments measuring 2x2 mm, with the total thickness of the tube wall for the attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis. The mean values of three spectra in each tube were obtained on its inner surface of three tubes. The tube fragment was placed against the diamond crystal of the ATR-FTIR unit and a force gauge at a constant pressure to facilitate contact. Data were recorded and analyzed using the OPUS 6.5 software (Bruker, Ettlingen, Germany). The tube composition was analyzed using Fourier Transform Infrared Spectroscopy (ATR-FTIR, Vertex 70 –Bruker, Ettlingen, Germany) equipped with an accessory that allowed spectrum acquisitions in the Attenuated Reflectance (ATR) mode. The ATR spectrums were recorded in the range of 400– 4,000 cm^{-1} at a 4 cm^{-1} resolution. Vector normalization and baseline correction were performed in all spectrums and these were considered absorbance height ratios. The ATR-FTIR spectra (our spectrums) were further analyzed by comparing the equivalence of the absorption bands of the peaks between the tubes.

Data analyses

Statistical analysis was performed using JAMOVI (Version 2.3.18). The results obtained were subjected to the Shapiro-Wilk normality test and Paired Samples T-Test, and the results are presented as mean and standard deviation (mean $\pm$ SD). Differences were considered statistically significant at $\alpha < 0.05$.

Results

The mean filling time of the tubes showed that there was no statistically significant difference between the S-PRF (69.5 ± 11.7 sec) and Vacuette tubes (72.6 ± 14.0 sec) ($p=0.364$). The mean volume of blood concentrate produced in the S-PRF (12.8 ± 2.02 mL) and Vacuette (12.4 ± 2.14 mL) tubes did not differ statistically ($p = 0.068$). The tensile test showed that there was no statistical difference between S-PRF Tubes (0.599 ± 0.202 N) and Vacuette (0.547 ± 0.197 N) ($p=0.389$) in mechanical disruption strength. Histological qualitative analysis showed a delicate fibrin network with cells dispersed throughout the network. No statistical difference was found in cell counts between the groups S-PRF and Vacuette in histomorphometric analysis ($p=0.083$) (Table 1).

Table 1. PERFORMANCE RESULTS OF TUBES AND ANALYSIS OF PRODUCED MEMBRANES

Analysis	Tube filling time (seconds)	Volume of concentrate produced (mL)	Cell count	Mechanical disruption-strenght (N)
S-PRF®	69.5 ± 11.7	12.8 ± 2.02	2534 ± 1085	0.599 ± 0.202
Vacuette®	72.6 ± 14.0	12.4 ± 2.14	2141 ± 764	0.547 ± 0.197
<i>P</i> Values	.364	.068	.083	.389

SEM ultrastructural analyses of S-PRF and Vacuette membranes did not reveal qualitative differences. Both membranes exhibited a fibrin network with cells, platelets, and a large number of entrapped red blood cells. Fibrin bundles showed variations in thickness and irregular arrangement, configuring a three-dimensional network (Fig 3).

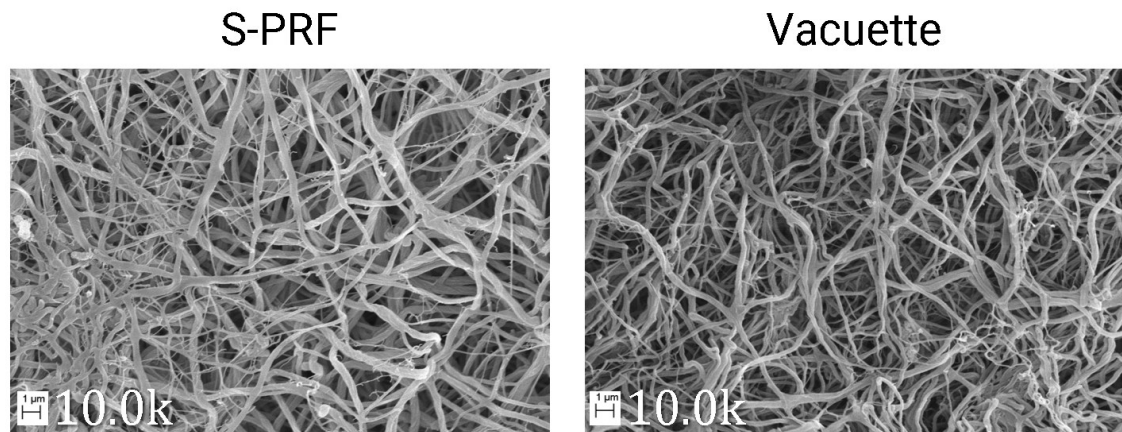


Figure 3. Three-dimensional fibrin network, showing the fibrin bundles variations in thickness and irregular arrangement.

TEM ultrastructural analyses of S-PRF and Vacuette indicated the presence of cells with preserved morphological integrity. The nucleus and organelles were observed with maintained membranes and components, as well as normal morphological features. There were no signs of ruptured or apoptosis-like cells. Moreover, platelet aggregates of varying sizes associated with fibrin bundles were observed in both tubes (Fig 4). In TEM images, the interaction between platelets and fibrin can be observed as the presence of platelets and fibrin fibers in close proximity to each other.

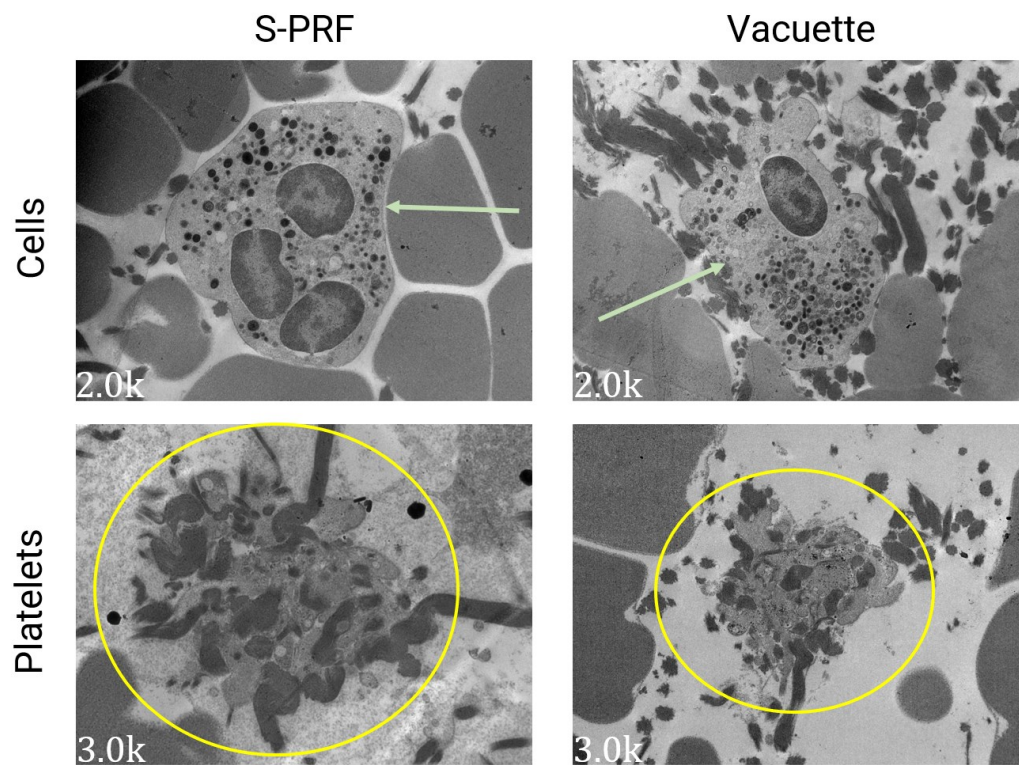


Figure 4. Transmission electron microscopy images showing leukocytes (green arrow), red cells and platelet aggregates between bundles of fibrin (yellow circle).

The FTIR analysis revealed similarities in the spectra of the tubes, indicating that they possess a similar chemical composition (Fig 5).

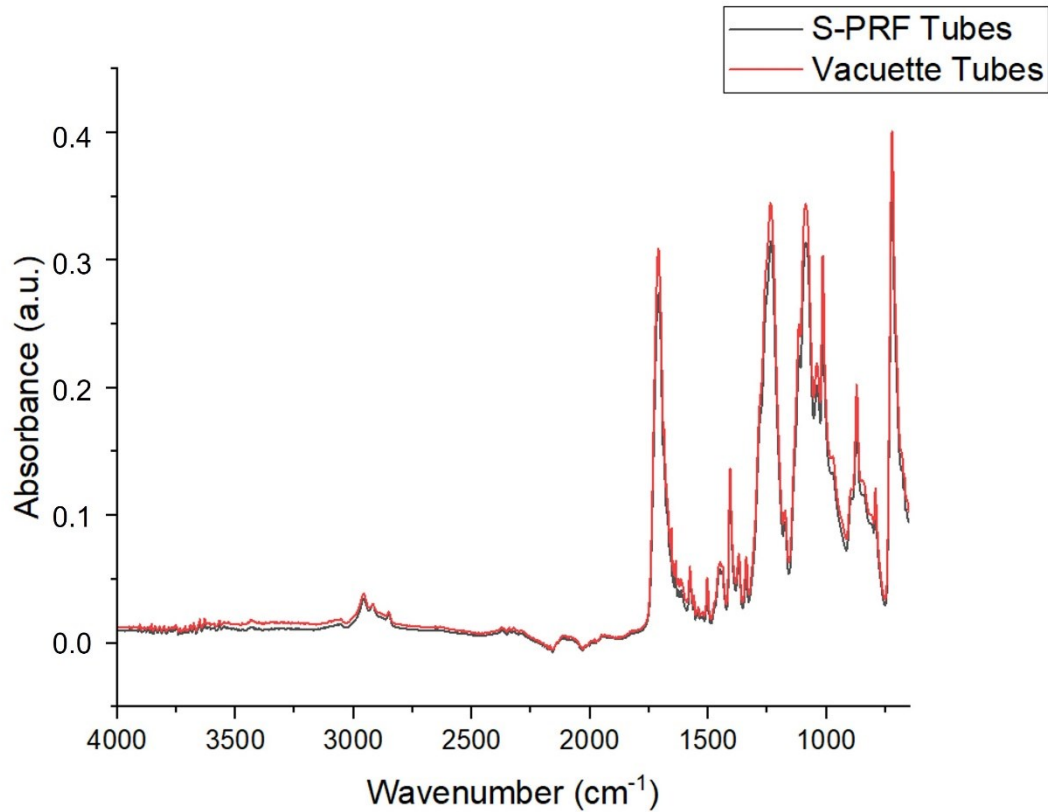


Figure 5. Superposition of spectra showing similarity between the chemical composition of S-PRF[®] and Vacuette[®] tubes.

Discussion

This study aimed to compare non-PRF specific plastic tubes with tubes originally designed to produce PRF, both without additives, providing a viable alternative for their production. Our hypothesis was confirmed once the Vacuette[®] and the S-PRF[®] showed similar chemical composition, blood collection performance, and produced PRF membranes with equivalent resistance and cellularity.

Traditionally, PRF membranes were obtained using glass tubes, while plastic tubes without additives were utilized to produce fluid matrices²³. The collection tubes employed were the same ones utilized in hematological analyses. However, due to concerns regarding the efficacy and quality of the PRF produced using these tubes, specific collection tubes for PRF production were proposed. Parameters as performance during collection and chemical composition may impact the quality of the PRF produced.

To our knowledge, the present study is the first attempt to compared specific and non-specific tubes for PRF production.

The primary parameter examined in this study was the performance of the collection tubes, as measured by the filling time and the volume of PRF produced after centrifugation. There were no significant differences in either filling times or PRF production volumes between the S-PRF[®] and Vacuette[®] tubes. Since the development of second-generation blood concentrates, anticoagulants are no longer required for their production²⁶. Consequently, a concise and fast collection and production protocol is required²⁷. The efficiency of blood collection and transfer to the centrifuge is essential to the success of the technique¹¹. The components and bioactivity of PRF are directly influenced by every parameter used during its preparation²⁸. These parameters are important because there is a working time limit from the moment the collection starts to the start of centrifugation, which can make it impractical to use the concentrate produced. The longer the collection time, the more fibrin diffusely polymerizes in the tube before and during centrifugation, resulting in a blood clot that lacks consistency and is dysfunctional²⁹. The volume of concentrate produced is directly related to the size and thickness of the membranes.

The present study employed the progressive protocol, which allows slow polymerization, resulting in enhanced lateral aggregation between the protofibrils and increased membrane resistance³⁰. The high tensile strength of PRO-PRF is due to the higher concentration of fibrin molecules and the establishment of equilateral junctions, resulting in a flexible network²³. The results indicate that there were no statistically significant differences in the tensile strength of the membranes produced from S-PRF[®] and Vacuette[®] tubes. Strength and flexibility are criteria for a membrane to be able to retain biomaterials, be fixed to neighboring or underlying tissues, be exposed to the oral environment, and adapt to cavities or to the tissues contours³¹.

The histomorphometric analysis found no significant difference in cell counts between the studied tube brands. However, a high standard deviation was observed in the means of groups. It is possible that gender and age differences among participants, as well as the time interval between data collections, could have contributed to the observed variance in the data. To address this variability, a paired-samples t-test was performed to compare the same blood sample protocols from each participant. It is important to

consider all potential sources of variance in the data in order to ensure that any conclusions drawn from the analysis are reliable and accurate. This variation in cell amount may be due to technical challenges or variation in access to the buffy coat during PRF aspiration²³. It is to emphasize that the membranes generated from PRO-PRF in plastic tubes without additives are the result of multiple collected tubes. This homogenization of the concentrate prior to its polymerization prevents cell concentration in the membrane end of the membrane, a phenomenon observed in glass tubes.

Plastic tubes made of more hydrophobic materials, such as polyethylene terephthalate (PET), such as S-PRF and Vacuette tubes, delay the adsorption of proteins to the tube wall surfaces, where clotting begins²⁴. The similarity between the spectra obtained in the AT-FTIR suggests similar chemical composition between the S-PRF and Vacuette tubes. These findings support the quantitative and qualitative results obtained, which do not show significant differences in the evaluated aspects.

Regarding the cellular morphology assessed by transmission electron microscopy, no significant morphological variations were observed from both tubes. The fibrin fibers may appear as long, thin strands that form a mesh-like network around the platelets, whereas the platelets may appear as pseudopodia or membrane fragments embedded within the fibrin network. These results corroborate the similarity in the chemical composition, indicating that the coagulation process was not different between the tubes. The formation of a dense fibrin network is crucial for creating a stable membrane, and the presence of platelets inside is important for hemostasis and tissue repair³².

Several factors were investigated to better understand the clinical feasibility of the progressive centrifugation protocol in producing large membranes from blood concentrates using the Vacuette tube. The use of plastic blood collection tubes without additives for the production of PRO-PRF prevents the acceleration of coagulation^{33–35}, facilitates the handling of the concentrate without limiting the working time, allows the production of customized membranes in terms of size and thickness²³. Moreover, allows the incorporation of drugs and biomaterials³⁶, and additionally prevents PRF contamination by silica particles from silica-coated tubes^{37–41}.

Vacuette[®] tubes are equivalent to S-PRF[®] plastic tubes in collection performance, chemical composition, membranes produced. Therefore, Vacuette[®] tubes represent a safe and low cost alternative for producing PRF membranes.

Acknowledgments

We acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Research Support Foundation of the State of Minas Gerais (FAPEMIG/Brazil) for granting us the scholarship and resources for the purchase of materials needed. This study was performed at the CPbio–Biomechanics, Biomaterials, and Cell Biology Research Center–UFU.

References

1. Mihaylova Z, Mitev V, Stanimirov P, Isaeva A, Gateva N, Ishkitiev N: Use of platelet concentrates in oral and maxillofacial surgery: an overview. *Acta Odontol Scand* 75: 1, 2017.
2. Vasilikos I, Beck J, Ghanaati S, Grauvogel J, Nisyrios T, Grapatsas K, Hubbe U: Integrity of dural closure after autologous platelet rich fibrin augmentation: an in vitro study. *Acta Neurochir (Wien)* 162: 737, 2020.
3. Shashank B, Bhushan M: Injectable Platelet-Rich Fibrin (PRF): The newest biomaterial and its use in various dermatological conditions in our practice: A case series. *J Cosmet Dermatol* 20: 1421, 2021.
4. Huang J, Teh BM, Zhou C, Shi Y, Shen Y: Tympanic membrane regeneration using platelet-rich fibrin: a systematic review and meta-analysis. *Eur Arch Oto-Rhino-Laryngol Off J Eur Fed Oto-Rhino-Laryngol Soc EUFOS Affil Ger Soc Oto-Rhino-Laryngol - Head Neck Surg* 279: 557, 2022.
5. Nacopoulos C: Use of Platelet Rich Fibrin in Facial Aesthetics and Rejuvenation. In: *Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications*. John Wiley & Sons, Ltd, 2017:215–235.
6. Lyris V, Millen C, Besi E, Pace-Balzan A: Effect of leukocyte and platelet rich fibrin (L-PRF) on stability of dental implants. A systematic review and meta-analysis. *Br J Oral Maxillofac Surg*: S0266, 2021.
7. Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, Guillemette V, Fujioka-Kobayashi M, Bishara M, Zhang Y, Wang H-L, Chandad F, Nacopoulos C, Simonpieri A, Aalam AA, Felice P, Sammartino G, Ghanaati S, Hernandez MA, Choukroun J: Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clin Oral Investig* 21: 1913, 2017.
8. Narendran N, Anegundi RV, Shenoy SB, Chandran T: Autologous platelet-rich fibrin as an adjunct to non-surgical periodontal therapy-A follow up clinical pilot

- study. *Wound Repair Regen Off Publ Wound Heal Soc Eur Tissue Repair Soc* 30: 140, 2022.
9. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101: e45, 2006.
 10. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101: e51, 2006.
 11. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101: e37, 2006.
 12. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM: The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part I: a new grafting protocol. *Implant Dent* 18: 102, 2009.
 13. Eshghpour M, Danaeifar N, Kermani H, Nejat AH: Does Intra-Alveolar Application of Chlorhexidine Gel in Combination With Platelet-Rich Fibrin Have an Advantage Over Application of Platelet-Rich Fibrin in Decreasing Alveolar Osteitis After Mandibular Third Molar Surgery? A Double-Blinded Randomized Clinical Trial. *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg* 76: 939.e1, 2018.
 14. Kuehnel R-U, Schroeter F, Mueller T, Ostovar R, Albes JM: Platelet-Rich Fibrin in Combination with Local Antibiotics Optimizes Wound Healing After Deep Sternal Wound Problems and Prevents Reinfection. *Surg Technol Int* 39: 313, 2021.
 15. Joshi SR, Palekar AU, Pendyala GS, Mopagar V, Padmawar N, Shah P: Clinical Success of Platelet-rich Fibrin and Mineral Trioxide Aggregate (MTA) or MTA-like Agents in Healing of Periapical Lesion in Nonsurgically Treated Pulpless Immature Permanent Teeth: A Systematic Review. *J Int Soc Prev Community Dent* 10: 379, 2020.
 16. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J: Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. *Tissue Eng Part B Rev* 23: 83, 2017.
 17. Nowak JM, Surma S, Romańczyk M, Wojtowicz A, Filipiak KJ, Czerniuk MR: Assessment of the Effect of A-PRF Application during the Surgical Extraction of Third Molars on Healing and the Concentration of C-Reactive Protein. *Pharmaceutics* 13: 1471, 2021.
 18. Ortega-Mejia H, Estrugo-Devesa A, Saka-Herrán C, Ayuso-Montero R, López-López J, Velasco-Ortega E: Platelet-Rich Plasma in Maxillary Sinus Augmentation: Systematic Review. *Mater Basel Switz* 13, 2020.

19. Sharma R, Sharma P, Sharma SD, Chhabra N, Gupta A, Shukla D: Platelet-Rich Fibrin as an Aid to Soft- and Hard-Tissue Healing. *J Maxillofac Oral Surg* 20: 496, 2021.
20. Bao M, Du G, Zhang Y, Ma P, Cao Y, Li C: Application of Platelet-Rich Fibrin Derivatives for Mandibular Third Molar Extraction Related Post-Operative Sequelae: A Systematic Review and Network Meta-Analysis. *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg*: S0278, 2021.
21. Marchetti E, Mancini L, Bernardi S, Bianchi S, Cristiano L, Torge D, Marzo G, Macchiarelli G: Evaluation of Different Autologous Platelet Concentrate Biomaterials: Morphological and Biological Comparisons and Considerations. *Mater Basel Switz* 13, 2020.
22. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Yaprak E, Toker H, Fıratlı E: A novel platelet concentrate: titanium-prepared platelet-rich fibrin. *BioMed Res Int* 2014: 209548, 2014.
23. Saboia-Dantas CJ, Limirio PHJO, Costa MDM de A, Linhares CRB, Santana Silva MAF, Borges de Oliveira HAA, Dechichi P: Platelet-Rich Fibrin Progressive Protocol: Third Generation of Blood Concentrates. *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg* 81: 80, 2023.
24. Miron RJ, Horrocks NA, Zhang Y, Horrocks G, Pikos MA, Sculean A: Extending the working properties of liquid platelet-rich fibrin using chemically modified PET tubes and the Bio-Cool device. *Clin Oral Investig* 26: 2873, 2022.
25. Linhares CRB, Rabelo GD, Limirio PHJO, Venâncio JF, Ribeiro Silva IG, Dechichi P: Automated bone healing evaluation: New approach to histomorphometric analysis. *Microsc Res Tech* 85: 3339, 2022.
26. Miron RJ, Xu H, Chai J, Wang J, Zheng S, Feng M, Zhang X, Wei Y, Chen Y, Mourão CF de AB, Sculean A, Zhang Y: Comparison of platelet-rich fibrin (PRF) produced using 3 commercially available centrifuges at both high (~ 700 g) and low (~ 200 g) relative centrifugation forces. *Clin Oral Investig* 24: 1171, 2020.
27. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T: Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 27: 158, 2009.
28. Al-Maawi S, Dohle E, Kretschmer W, Rutkowski J, Sader R, Ghanaati S: A Standardized g-Force Allows the Preparation of Similar Platelet-Rich Fibrin Qualities Regardless of Rotor Angle. *Tissue Eng Part A* 28: 353, 2022.
29. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier J-B: Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 110: 418, 2010.

30. Weisel JW, Litvinov RI: Fibrin Formation, Structure and Properties. In: Parry DAD, Squire JM, eds. *Fibrous Proteins: Structures and Mechanisms*. Cham: Springer International Publishing, 2017:405–456.
31. Chan BP, Leong KW: Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *Eur Spine J* 17: 467, 2008.
32. Eisinger F, Patzelt J, Langer HF: The Platelet Response to Tissue Injury. *Front Med* 5: 317, 2018.
33. Asher R, Oren F, Meir T, Shapira L, Assad R, Polak D: Tranexamic acid integrated into platelet-rich fibrin produces a robust and resilient antihemorrhagic biological agent: a human cohort study. *Oral Surg Oral Med Oral Pathol Oral Radiol*: S2212, 2022.
34. Castro AB, Cortellini S, Temmerman A, Li X, Pinto N, Teughels W, Quirynen M: Characterization of the Leukocyte- and Platelet-Rich Fibrin Block: Release of Growth Factors, Cellular Content, and Structure. *Int J Oral Maxillofac Implants* 34: 855, 2019.
35. Kratz A, Stanganelli N, Van Cott EM: A comparison of glass and plastic blood collection tubes for routine and specialized coagulation assays: a comprehensive study. *Arch Pathol Lab Med* 130: 39, 2006.
36. Miron RJ, Zhang Y: Autologous liquid platelet rich fibrin: A novel drug delivery system. *Acta Biomater* 75: 35, 2018.
37. Tsujino T, Takahashi A, Yamaguchi S, Watanabe T, Isobe K, Kitamura Y, Tanaka T, Nakata K, Kawase T: Evidence for Contamination of Silica Microparticles in Advanced Platelet-Rich Fibrin Matrices Prepared Using Silica-Coated Plastic Tubes. *Biomedicines* 7: 45, 2019a.
38. Yamaguchi S, Aizawa H, Sato A, Tsujino T, Isobe K, Kitamura Y, Watanabe T, Okudera H, Mourão CF, Kawase T: Concentrated Growth Factor Matrices Prepared Using Silica-Coated Plastic Tubes Are Distinguishable From Those Prepared Using Glass Tubes in Platelet Distribution: Application of a Novel Near-Infrared Imaging-Based, Quantitative Technique. *Front Bioeng Biotechnol* 8: 600, 2020.
39. Tsujino T, Masuki H, Nakamura M, Isobe K, Kawabata H, Aizawa H, Watanabe T, Kitamura Y, Okudera H, Okuda K, Nakata K, Kawase T: Striking Differences in Platelet Distribution between Advanced-Platelet-Rich Fibrin and Concentrated Growth Factors: Effects of Silica-Containing Plastic Tubes. *J Funct Biomater* 10: E43, 2019b.
40. Masuki H, Isobe K, Kawabata H, Tsujino T, Yamaguchi S, Watanabe T, Sato A, Aizawa H, Mourão CF, Kawase T: Acute cytotoxic effects of silica microparticles used for coating of plastic blood-collection tubes on human periosteal cells. *Odontology* 108: 545, 2020.

41. Miron RJ, Kawase T, Dham A, Zhang Y, Fujioka-Kobayashi M, Sculean A: A technical note on contamination from PRF tubes containing silica and silicone. BMC Oral Health 21: 135, 2021.

3.3 CAPÍTULO 3

Artigo enviado para publicação no periódico Clinical Oral Investigations (CLOI)

Do blood concentrates influence inflammatory signs and symptoms after mandibular third molar surgery? A systematic review and network meta-analysis of randomized clinical trials

PROSPERO: CRD42022371616

Marcelo Dias Moreira de Assis Costa¹

Luiz Renato Paranhos²

Vinicius Lima de Almeida³

Leandro Machado Oliveira⁴

Walbert de Andrade Vieira⁵

Paula Dechichi⁶

1. Postgraduate Program in Dentistry, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. <https://orcid.org/0000-0001-9148-3674>, e-mail: marcelodmac@yahoo.com.br
2. Division of Preventive and Community Dentistry, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. <https://orcid.org/0000-0002-7599-0120>, e-mail: paranhos.lrp@gmail.com
3. Postgraduate Program in Dentistry, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. <https://orcid.org/0000-0002-2135-1040>, e-mail: viniciusl.dealmeida91@gmail.com
4. Division of Stomatology, School of Dentistry, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. <https://orcid.org/0000-0003-3706-5687>, e-mail: leandromachadooliv@gmail.com
5. Department of Restorative Dentistry, Endodontics Division, School of Dentistry of Piracicaba, State University of Campinas, Piracicaba, São Paulo, Brazil. <https://orcid.org/0000-0001-8872-2865>, e-mail: walbert.vieira18@gmail.com
6. Department of Cell Biology, Histology, and Embryology, Biomedical Science Institute, Federal University of Uberlândia, Uberlândia, Brazil. <https://orcid.org/0000-0001-5509-8138>, e-mail: pauladechichi@ufu.br

Corresponding author:

Luiz Renato Paranhos

Department of Preventive and Community Dentistry, Federal University of Uberlândia, Campus Umuarama

Av. Pará, 1720, Bloco 2G, sala 1

ZIP code: 38405-320, Uberlândia, MG, Brazil

paranhos.lrp@gmail.com

Abstract

Objectives: To investigate, through a network meta-analysis, the effectiveness of blood concentrates in reducing pain perception, trismus, and edema after mandibular third molar extraction.

Materials and Methods: An electronic search was performed in nine databases to locate randomized clinical trials comparing blood concentrate use after mandibular third molar extraction. Two authors selected and extracted the data independently. The individual risk of bias in the studies was assessed with the RoB v2.0 tool. A network meta-analysis compared postoperative pain and trismus scores after applying different blood concentrates, using the mean difference (MD) as an effect estimate. The GRADE approach assessed the certainty of evidence.

Results: Thirty-one randomized clinical trials were included in the review and 18 in the meta-analysis. Leukocyte- and platelet-rich fibrin (L-PRF) was the most used blood concentrate, followed by platelet-rich plasma (PRP). The network meta-analysis showed that A-PRF performed better among the analyzed blood concentrates, decreasing postoperative pain in one, two, three, and seven days and reducing trismus up to the inflammatory peak compared to blood clots. Only two studies had a low risk of bias.

Conclusions: Based on very low certainty of evidence, using concentrates seemed efficient compared to blood clots in reducing pain and trismus after mandibular third molar surgeries. A-PRF decreased postoperative pain throughout the evaluated time and trismus during the acute inflammatory peak.

Clinical relevance: A-PRF after mandibular third molar extractions performed better among the analyzed blood concentrates and seemed efficient in improving postoperative quality by decreasing inflammatory signs and symptoms.

Keywords: Platelet-rich fibrin; Leukocyte- and platelet-rich fibrin; Pain; Postoperative pain; Trismus; Edema; Third molar surgery.

Introduction

Third molar removal is a routine surgery in dentistry [1] and is related to postoperative inflammatory complications [2]. Pain, edema, and trismus are usually reported after surgery, decreasing the patients quality of life [3–5]. Therapies to control pain, edema, and trismus have been proposed [6–8], showing satisfactory results in minimizing the inflammatory sequelae from third molar extractions [9, 10]. Using different blood concentrates is one of these treatments, and it is accepted but does not show established efficacy [11–13].

Applying different blood derivatives to modulate the repair process started over 40 years ago [14]. Platelet-rich plasma (PRP) was the first autologous blood concentrate extensively used, but due to the need for additives and the numerous production steps, studies were performed for its improvement [15–18].

Second-generation platelet concentrates are autologous biomaterials from a fibrin clot rich in platelets, leukocytes, cytokines, and stem cells, without any additive [19]. The second-generation blood concentrate known as platelet-rich fibrin (PRF) later originated the leukocyte- and platelet-rich fibrin (L-PRF) [15, 16, 20, 21]. Studies on the low-speed centrifugation concept and relative centrifugal force application allowed the development of new blood concentrates from L-PRF. These elements are known as A-PRF and A-PRF+, showing a fibrin mesh with more leukocytes, more homogeneous platelet distribution, and increased concentration of growth factors [20, 22].

Blood concentrates have stood out because of their fibrin network that houses mainly platelets and leukocytes and the slow and continuous release of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor (IGF) [23, 24]. *In vitro*, blood concentrates can suppress the secretion of pro-inflammatory cytokines, such as IL-1 β , TNF- α , and IL-6 [25], modulate the transition from position M1 to M2 (anti-inflammatory phenotype) of macrophage polarization [26], and decrease the inflammatory response of gingival fibroblasts, dendritic cells [27], and Schwann cells [28].

Previous systematic reviews [11-13, 28-31] evaluated the role of blood concentrates as an alternative therapy to control inflammatory signs and symptoms of mandibular third molar surgeries but were only direct comparisons between L-PRF and A-PRF blood concentrates and blood clots, showing contradictory results. Our alternative hypothesis is that replacing the blood clot in the alveolus of extracted mandibular third molars with a blood concentrate may significantly decrease postoperative signs and symptoms. Therefore, this systematic review of randomized clinical trials aimed to assess the efficacy of different blood concentrates in controlling inflammatory signs and symptoms after mandibular third molar extractions.

Methods

2.1 Protocol and registration

The review protocol was created and reported according to the PRISMA-P guidelines [32] and then registered in the PROSPERO database (CRD42022371616). There were no changes or departures from the protocol when performing the review.

This systematic review was reported according to the PRISMA [34] and PRISMA-NMA [35] guidelines and conducted following the manual of the Joanna Briggs Institute (JBI) [36].

2.2 Research question and eligibility criteria

The research question was developed according to the PVO acronym (Population, Variable, and Outcome): "In patients subjected to mandibular third molar extraction surgery (P), which blood concentrate applied to the alveolus (V) is more effective in reducing pain perception, edema, and trismus (O)?"

Inclusion criteria:

The study included only randomized clinical trials with healthy no smoking adult patients subjected to the extraction of mandibular third molars, impacted or not, comparing at least one blood

concentrate to another or blood clots, and reporting the protocol for blood concentrate preparation. Articles that evaluated at least one of the three postoperative inflammatory parameters - pain perception (Visual Analog / Numerical Scale), facial edema (linear facial measurements), or trismus (interincisal distance) - were considered. There was no restriction on language or year of publication.

Exclusion criteria:

- a) Studies that did not describe the data adequately;
- b) Studies involving only exodontia with unconventional techniques, such as piezosurgery.

2.3 Sources of information and search

An electronic search was performed up to March 2022 in the MedLine (via PubMed), EMBASE, Web of Science, Scopus, the Cochrane Library, LILACS, and SciELO databases. Moreover, the OpenGrey and MedRxiv platforms were accessed for partially searching the "grey literature". The MedLine search was updated constantly by PubMed through warnings until June 2022. The search strategies were created using the Medical Subject Headings (MeSH), Descriptors in Health Science (DeCS), and Embase Subject Headings (Emtree). Boolean operators (AND and OR) combined the descriptors and improved the search strategy through different combinations (**Table 1**), respecting the syntax rules of each database. Previous systematic reviews and the references of eligible studies were analyzed to capture qualified articles potentially neglected in the search process.

Table 1. Strategies for database search.

Database	Search Strategy (March, 2022)
PubMed http://www.ncbi.nlm.nih.gov/pubmed	#1 "Molar, Third"[MeSH] OR "Lower Third Molar"[tw] OR "Wisdom Teeth"[tw] OR "Wisdom Tooth"[tw] OR "Third Molar"[tw]) #2 "Pain, Postoperative"[Mesh] OR "Pain"[tw] OR "Edema"[MeSH] OR "Swelling"[tw] OR "Trismus"[MeSH] OR "Recovery"[tw] OR "Healing"[tw] OR "Wound"[tw] OR "Outcomes"[tw] OR "Efficacy"[tw] OR "Morbidity"[tw] OR "Comparison"[tw] #3 "Platelet-Rich Fibrin"[Mesh] OR "PRF"[tw] OR "CGF"[tw] OR "PRP"[tw] OR "Platelet Rich Fibrin"[tw] OR "Leukocyte and Platelet Rich Fibrin"[tw] OR "Concentrated Growth Factor"[tw] OR "Platelet-Rich Plasma"[tw] OR "Platelet-Rich"[tw] OR "Platelet Rich"[tw] #1 AND #2 AND #3
EMBASE	#1 "Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar" #2 "Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison" #3 "PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich" #1 AND #2 AND #3
Web of Science	#1 TS=("Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar") #2 TS=("Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison") #3 TS=("PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich") #1 AND #2 AND #3
Scopus	#1 "Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar" #2 "Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison"

	#3 "PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich" #1 AND #2 AND #3
Cochrane Library	#1 "Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar" #2 "Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison" #3 "PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich" #1 AND #2 AND #3
LILACS http://lilacs.bvsalud.org/	((("Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar") AND ("Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison") AND ("PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich"))
SciELO http://www.scielo.org/	((("Lower Third Molar" OR "Wisdom Teeth" OR "Wisdom Tooth" OR "Third Molar") AND ("Pain" OR "Swelling" OR "Trismus" OR "Recovery" OR "Healing" OR "Wound" OR "Outcomes" OR "Efficacy" OR "Morbidity" OR "Comparison") AND ("PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich"))
MedRxiv	"PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich"
OpenGrey	"PRF" OR "CGF" OR "PRP" OR "Platelet Rich Fibrin" OR "Leukocyte and Platelet Rich Fibrin" OR "Concentrated Growth Factor" OR "Platelet-Rich Plasma" OR "Platelet-Rich" OR "Platelet Rich"

2.4 Selection of studies

The research findings were exported to EndNote Web™ software (Thomson Reuters, Toronto, Canada) for cataloging and removing duplicates. The "grey literature" was exported to Microsoft Word™ (Microsoft™, Ltd, Washington, USA) to remove duplicates manually.

Before selecting the studies, a calibration exercise was performed, in which the examiners discussed the eligibility criteria. Next, the results were exported to Rayyan QCRI software (Qatar Computing Research Institute, Doha, Qatar) [37], in which the titles and abstracts were read using the previous eligibility criteria. Subsequently, the full texts of the preliminary eligible studies were obtained and evaluated. Two eligibility reviewers (V.L.A. and M.D.M.A.C.) performed the entire process independently. Disagreements were solved after consulting with a third reviewer (L.R.P.). The articles excluded in the full-text analysis were listed separately, and the reasons for exclusion were specified. In the cases of articles not found, other study centers were contacted to retrieve the studies in their libraries via COMUT (Bibliographic Commutation Program). In the case of publications in languages other than English or Portuguese, the full text was translated.

2.5 Data collection

To ensure consistency between the reviewers (V.L.A. and M.D.M.A.C.), they performed a calibration exercise before data collection, which consisted of jointly extracting information from an eligible study. Next, the texts of the selected studies were reviewed. The data were systematically extracted, including authorship, publication year, country of origin, design (split-mouth or parallel), study sample characteristics (sample size, sex, and age), lower third molar classification, surgical flap design, need for

osteotomy and/or odontosection, used medication (postoperative drugs), blood concentrate type, the protocol for blood concentrate preparation, outcome analyzed in each study (pain, edema, and mouth opening), and primary conclusions. In case of missing data, the corresponding authors were contacted via e-mail up to three times in seven days.

2.6 Critical assessment of individual evidence sources

Two reviewers (V.L.A. and M.D.M.A.C.) independently assessed the risk of bias and individual quality of the selected studies using the Cochrane Collaboration Risk of Bias tool (version 2.0) (RoB2) for randomized clinical trials [38]. Overall assessments and those for each domain followed the algorithms proposed in the tool manual [38]. Disagreements between the reviewers were solved by discussing and consulting with a third reviewer (L.R.P.).

2.7 Synthesis of results

Pairwise comparisons were made with the available head-to-head data. Independent analyses were performed for two review outcomes (postoperative pain and trismus). A normalization protocol was followed to ensure the comparability of the data from the included studies. Postoperative pain was assessed across studies using VAS normalized to a 100-point scale. Additionally, trismus occurrence was measured using (1) the percentage of interincisal opening reduction, (2) interincisal opening distance, and (3) the difference between the pre- and postoperative interincisal opening. The studies were only grouped when the outcome assessment was the same. Given the variability of facial swelling assessment protocols, findings for this outcome were synthesized narratively.

Next, frequentist random-effects network meta-analyses (NMA) compared multiple concentrates via shared comparators by integrating direct and indirect estimates [39] when at least three studies were available for comparison. Random-effects models with the Der-Simonian and Laird variance estimator [40] were preferred from fixed-effects models based on the deviance information criterion (DIC). Treatments were grouped into common nodes to satisfy the transitivity assumption. **Figure 1** displays the building of each NMA. Direct comparisons among the different concentrates were represented by edges with sizes proportional to the number of arms included in the corresponding pairwise meta-analysis.

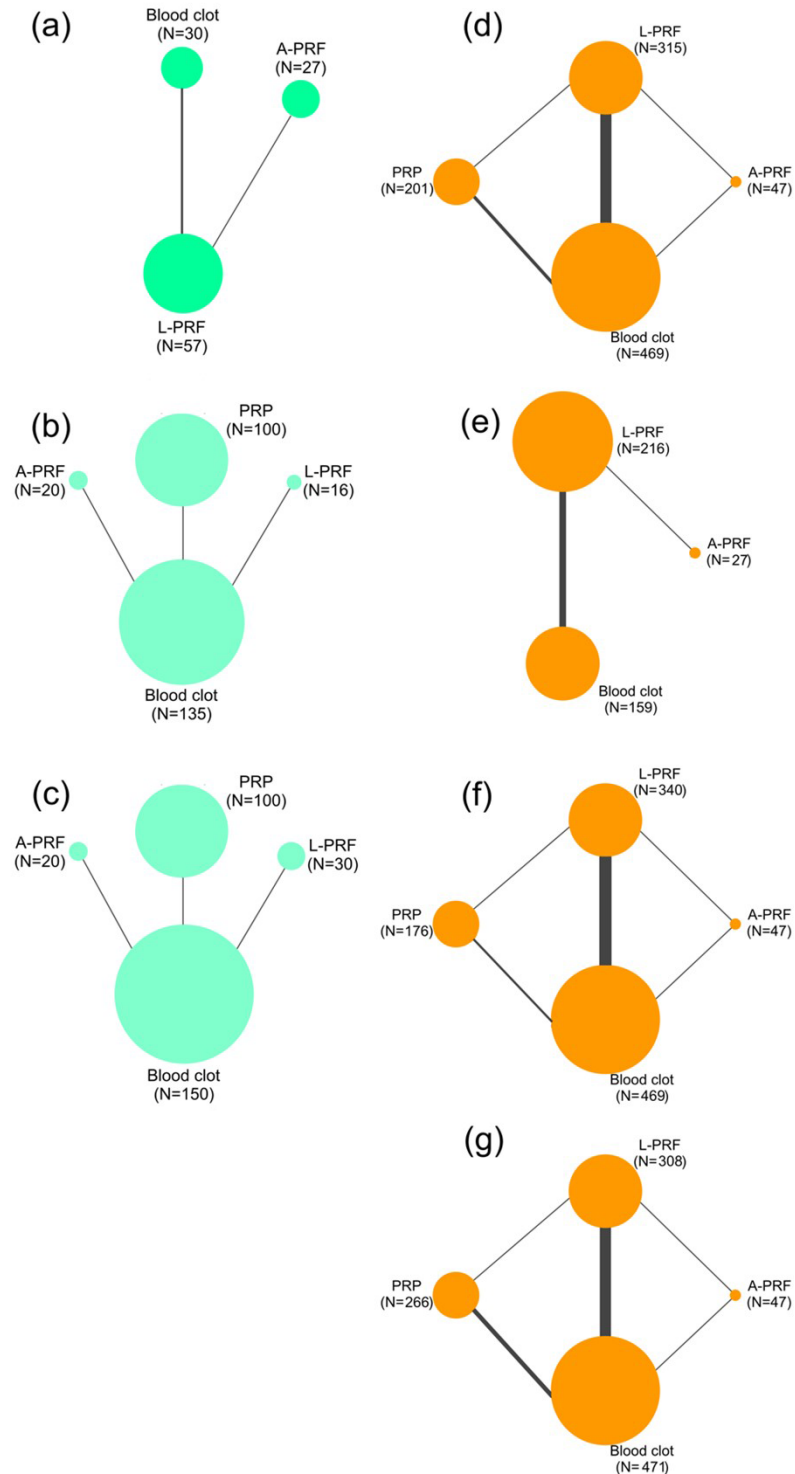


Fig. 1 Network plot geometries for (a) the percentage of interincisal opening reduction (one, two, three, and seven days after surgery); (b) interincisal opening distance (one and seven days after surgery); (c) the difference between pre- and postoperative interincisal opening (seven days after surgery); (d) postoperative pain one, (e) two, (f) three, and (g) seven days after surgery.

The effect estimate was the mean difference (MD) instead of standardized MD (SMD), as this study grouped only comparable scales for the outcomes to prevent the standard deviation (SD) effect on

SMD estimates. A 95% confidence interval (CI) was calculated for all evaluations. Heterogeneity was assessed using the Cochran Q test and I^2 statistics. Local incoherence was assessed by comparing direct, indirect, and network estimates (0.05 significance level). Inconsistency was impossible to analyze for postoperative pain (two days after surgery) and trismus, given the low number of comparisons. It is also worth noting that seven days after surgery, four studies reported zero postoperative pain events [11, 41–43], thus, proposed corrections were made [44, 45].

Results were presented in league tables, and each treatment was ordered from best to worst based on ranking probabilities of treatment effects. When possible, sensitivity analyses were performed, excluding studies with parallel design, as most were split-mouth. All analyses used Stata software (version 14.0) and MetaInsight (version 4.0.2 beta).

2.8 Certainty of evidence

The GRADE system classified the certainty of evidence of treatment effect estimates for the network meta-analysis [46, 47]. First, the certainty of evidence of each direct comparison was evaluated to verify the risk of bias, inconsistency, indirectness, and publication bias. For indirect comparisons, a first-order loop comparison with the lowest certainty was considered, and intransitivity was evaluated. Finally, the certainty of evidence was downgraded in case of concerns of imprecision or incoherence for the network meta-analysis [46]. The certainty of evidence could be high, moderate, low, or very low [47].

Results

3.1 Study selection

The electronic search showed 1,097 results, of which 868 continued for the title and abstract analyses after excluding duplicates. After a detailed analysis, 50 studies went to the third phase, of which ten were identified as randomized clinical trial records and three were not found. Thus, 37 studies were fully read. Seven articles did not meet the eligibility criteria (**Supplementary Table 1**). Additionally, the manual search in the references revealed an eligible study not located in the electronic search. Therefore, 31 studies were included in the systematic review (**Figure 2**).

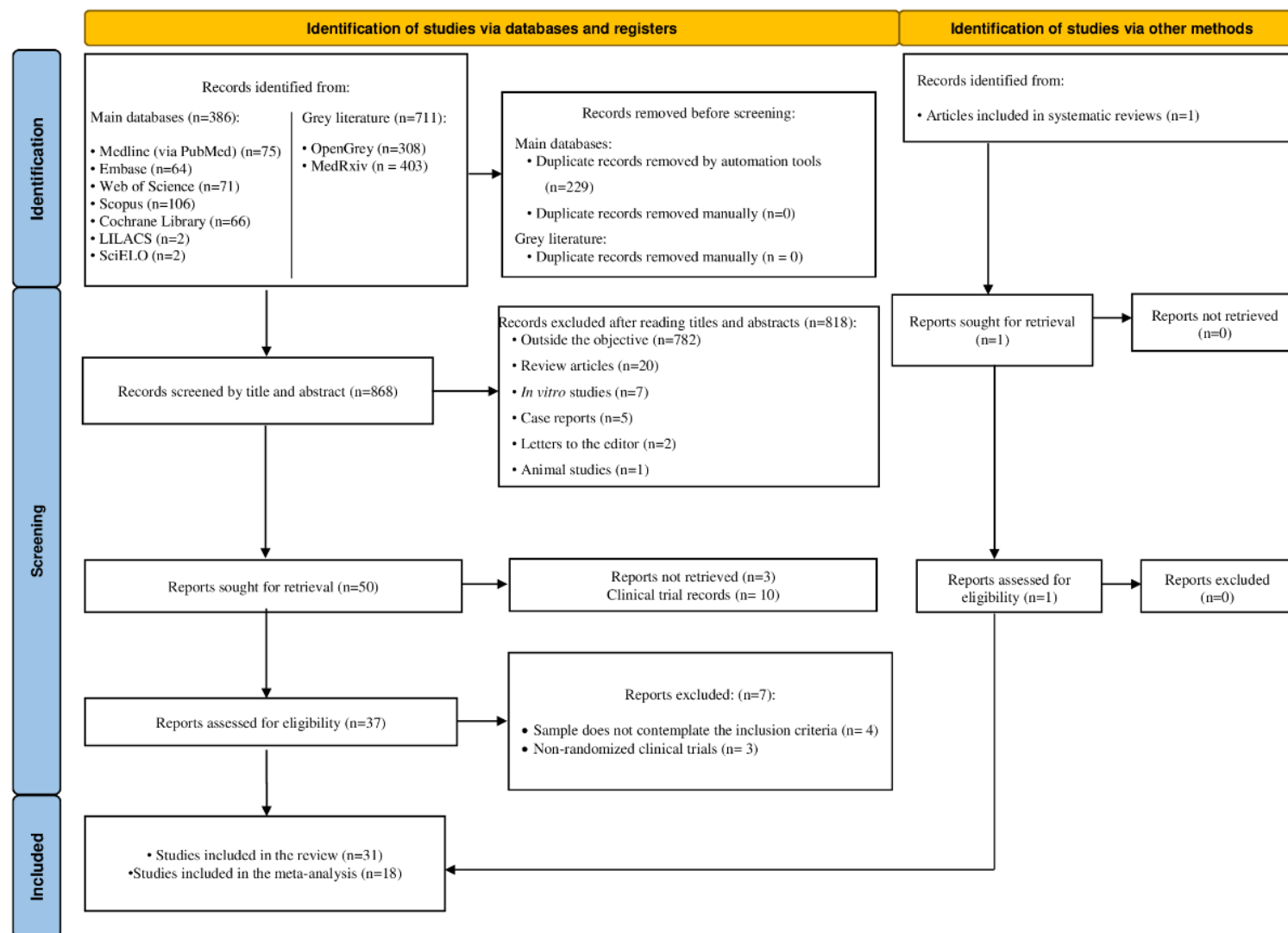


Fig. 2 Flowchart of the search strategy.

3.2 Characteristics of eligible studies

Most studies were performed in Asia, published between 2011 and 2021, included a total sample of 1,276 patients aged between 16 and 60 years, and presented a split-mouth design [41, 43, 48–65]. Mandibular molars were categorized according to Pell and Gregory or Winter classifications. Platelet-rich fibrin (PRF) was the most used blood concentrate (21 studies) [11, 41, 42, 48, 50–54, 56, 58–60, 62–64, 66–70], followed by platelet-rich plasma (PRP) (eight studies) [41, 43, 49, 57, 67, 71–73], advanced platelet-rich fibrin (A-PRF) (four studies) [50, 55, 65, 74], and concentrated growth factor (CGF) (2 studies) [61, 74] (**Table 2**).

Except for the study by Dutta et al. (2016), which used a visual analog scale (VAS) based on the six-point facial Wong-Baker scale, the other articles evaluated postoperative pain intensity with the VAS. The methods for measuring edema used several anatomic references: tragus to the labial commissure, tragus to the pogonion, lateral corner of the eye to the gonion, lateral corner of the eye to the mandibular angle, ear lobe to the corner of the mouth, gonion to the labial commissure, and tragus to the subnasal point. Moreover, studies showed linear measures according to each analyzed distance, and others calculated the values of each distance to determine a final score. All investigations assessing trismus used interincisal distance during mouth opening.

Table 2. Main data of the eligible studies.

Author, year of publication Country	Study design	Sample size	Range Age (mean - SD)	Type of third molar	Type of flap	Bone removal	Co-interventions	Type of blood concentrate	Centrifuge	Blood concentrate protocol	Tubes	Outcomes
Ogundipe et al., 2011 Nigeria [71]	Parallel	25 ♂ 35 ♀	19 – 35 (24.7 ± 3.6)	31 mesioangular, 14 distoangular, 9 vertical, and 6 horizontal	Triangular	Yes	n.r.	PRP	PowerSpin Fx Centrifuge (Single)	1200 rpm for 10 min and 1000 rpm for 10 min	10-mL tubes with citrate phosphate dextrose (no material) (no brand)	Pain, swelling, and trismus
Kumar et al., 2015 India [66]	Parallel	31*	19 – 35 **	19 mesioangular and 12 horizontal	Triangular	Yes	ATB, NSAID, ANA, and AMW	L-PRF	n.r.	3000 rpm for 10 min	5-mL tubes (no material) (no brand)	Pain, swelling, and trismus
Ozgul et al., 2015 Turkey [60]	Split-mouth	23 ♂ 33 ♀	18 – 28 **	20 horizontal, 15 mesioangular, and 21 vertical	Triangular	Yes	ATB and ANA	L-PRF	n.r.	3000 rpm for 10 min	10-ml of glass-coated plastic tubes (plastic) [no brand]	Pain and swelling
Uyanik et al., 2015 Chipre [64]	Split-mouth	4 ♂ 6 ♀	19 – 35 **	Class IC by Pell and Gregory	Triangular	Yes	ATB, ANA, and AMW	L-PRF	Elektro-mag M415P (Turkey)	3000 rpm for 10 min	10-ml of glass-coated plastic tubes (plastic) [no brand]	Pain, swelling, trismus
Bilginaylar and Uyanik, 2016 Chipre [42]	Parallel	17 ♂ 23 ♀	18 – 35 **	Class IC by Pell and Gregory	Triangular	Yes	ATB, ANA, and AMW	L-PRF	Elektro-mag M415P (Turkey)	3000 rpm for 10 min	10-ml of glass-coated plastic tubes (plastic) [no brand]	Pain, swelling, and trismus
Dutta et al., 2016 India [67]	Parallel	30*	17 – 36 (27 ± 5)	n.r.	Triangular or envelope	n.r.	n.r.	PRP and L-PRF	n.r.	2000 rpm for 15 min +3000 rpm for 10 min (PRP) and 3000 rpm for 10 min (PRF)	PRP: first centrifugation: 4 mL tubes with citrate phosphate dextrose adenine solution (no material) (no brand). Second centrifugation: test tube (no material) (no brand)	Pain and swelling
Kumar et al., 2016 India [58]	Split-mouth	34*	18 – 40 **	n.r.	Triangular	Yes	n.r.	L-PRF	n.r.	3000 rpm for 10 min	PRF: test tube (no material) (no brand)	Pain
Al-Hamed et al., 2017 Egypt [11]	Parallel	13 ♂ 37 ♀	18-48 **	Vertical, mesioangular, horizontal, and distoangular	Envelope	Yes	ATB, NSAID, and AMW	L-PRF	80-1 electric centrifuge	3000 rpm for 10 min	NR	Pain
Asutay et al., 2017 Turkey [48]	Split-mouth	6 ♂ 24 ♀	18 – 29 (20.32)	Full bone impacted, symmetric, bilateral, and mesioangular	Triangular	Yes	ATB and ANA	L-PRF	n.r.	2700 rpm for 12 min	5-ml tubes without anticoagulant (no material) (no brand)	Pain, swelling, and trismus
Gandevivala et al., 2017 India [43]	Split-mouth	25	16 – 60 **	n.r.	Triangular	Yes	n.r.	PRP	n.r.	1300 rpm for 10 min and 2000 rpm for 10 min	10-mL tubes without anticoagulant (no material) (no brand)	Pain and swelling
Gulsen and Senturk, 2017 Turkey [54]	Split-mouth	21 ♂ 9 ♀	17 – 27 (20.03)	Vertical (Winter), Class I B (Pell and Gregory)	Triangular	Yes	ATB, NSAID, and AMW	L-PRF	NUVE NF 200,	3000 rpm for 10 min	First centrifugation: 10-mL tubes with citrate phosphate dextrose adenine solution (no material)	Pain and swelling

[no brand]. Second centrifugation: no information												
Afat et al., 2018 Turkey [68]	Parallel	15 ♂ 25 ♀	18-30 **	Class II B (Pell and Gregory)	n.r.	Yes	n.r.	L-PRF	Elektro-mag M615P (Turkey)	3000 rpm for 10 min	10-ml of glass	Pain, swelling, and trismus
Bhujbal et al., 2018 Iran [49]	Split-mouth	5 ♂ 15 ♀	25.2 ± 7.19 **	Identical bilateral impaction	Triangular	Yes	n.r.	PRP	Elektro-mag M615P (Turkey)	1200 rpm for 10 min and 2000 rpm for 10 min	Coated plastic tubes (plastic) [BD]	Pain and swelling
Dar et al., 2018 India [52]	Split-mouth	13 ♂ 17 ♀	18 -50 23.6 ± 4.385	Mesioangular	Triangular	Yes	ATB and NSAID	L-PRF	n.r.	3000 rpm for 12 min	10-mL tubes without anticoagulant (no material) (no brand)	Pain and swelling
Daugela et al., 2018 Lithuania [53]	Split-mouth	14 ♂ 20 ♀	18 - 60 22.76 ± 2.02	Identical bilateral impaction	Triangular	Yes	NSAID	L-PRF	n.r.	2800 rpm for 12 min	First centrifugation: 10-mL tubes with citrate phosphate dextrose adenine solution (no material) [BD]	Pain and swelling
Jeyaraj and Chakranarayan, 2018 India [69]	Parallel	60*	n.r.	n.r.	n.r.	Yes	ATB, NSAID, and ANA	L-PRF	Hettich Zentrifugen centrifuge EBA 20 (Andreas Hettich GmbH& Co, KG, Germany)	2700 rpm for 12 min	Second centrifugation 12-mL tubes (borosilicate glass tube) [no brand]	Pain, swelling, and trismus
Caymaz and Uyanic, 2019 Turkey [50]	Spli-mouth	12 ♂ 15 ♀	18 – 26 **	Class I C by Pell and Gregory	Triangular	Yes	ATB, ANA and AMW	L-PRF and A-PRF	n.r.	L-PRF: 3000 RPM for 10 min A-PRF: 1500 rpm for 14 min	10-mL tubes without anticoagulant (no material) (no brand)	Pain, swelling, and trismus
Kapse et al., 2019 India [56]	Split-mouth	13 ♂ 17 ♀	18 - 40 25.47 ± 0.9	Identical bilateral impaction	n.r.	Yes	n.r.	L-PRF	Elektro-mag	2700 rpm for 12 min	9-mL glass-coated tubes (no material) (Intra Lock)	Pain and swelling
Ritto et al., 2019 Brazil [62]	Spli-mouth	10 ♂ 7 ♀	16 – 29 (21.8) **	n.r.	Envelope	Yes	NSAID, SAID, and ANA	L-PRF	Elektro-mag M 415 P (Turkey)	2700 rpm for 12 min	10-mL tubes without anticoagulant (no material) (no brand)	Pain
Zahid and Nadershah, 2019 Saudi Arabia [65]	Split-mouth	10 ♂	(24) **	Mesioangular and vertical	Envelope	n.r.	ATB, NSAID, and AMW	A-PRF	R-4C DX, REMI	1300 rpm for 13 min	L-PRF: 10 ml of glass	Pain and swelling
Aftab et al., 2020 India [72]	Parallel	38 ♂ 62 ♀	18 – 42 (26.12 ± 5.92)	Class B by Pell and Gregory	Envelope	Yes	n.r.	PRP	A-PRF 12	1200 rpm for 10 min 100 rpm for 10 min	coated plastic tubes (plastic) [BD]	Pain, swelling, and trismus
Bhujbal et al., 2020 India [41]	Split-mouth	8 ♂ 12 ♀	22 (26 ± 6.9) **	n.r.	Triangular	Yes	ATB and NSAID	PRP and L-PRF	DUO centrifuge (Process for PRF, Nice, France)	1200 rpm for 10 min and 2000 rpm for 10 min (PRP) 3000 rpm for 10 min (PRF)	A-PRF: 10-mL tubes without anticoagulant (glass)[A-PRF+ by Choukroun]	Pain and swelling

Ozveri Koyuncu et al., 2020 Turkey [61]	Split-mouth	21 ♂ 39 ♀	18 – 35 (25.82)	Vertical (Winter), Class I B (Pell and Gregory)	n.r.	Yes	ATB and ANA	CGF	n.r.	2700 rpm in 2min, 2400 rom in 4 min, 2700 rpm in 4 min, and 3000 rpm in 3 min	10-mL tubes without anticoagulant (glass) [no brand]	Pain, swelling, and trismus
Kumar et al., 2020 India [57]	Split-mouth	68 ♂ 32 ♀	20 – 50 **	Class I bilateral, A or B by Pell and Gregory	n.r.	*	ATB and NSAID	PRP	n.r.	2000 rpm for 15 min and 3000 rpm for 10 min	10-mL tubes without anticoagulant (no material) (no brand)	Pain, swelling, and trismus
Miyamoto et al., 2020 Japan [59]	Split-mouth	14 ♂ 18 ♀	20 – 51 **	17 IA, 5 IB, 15 2A, 23 2B, 4 2C by Pell and Gregory	n.r.	Yes	ATB and NSAID	L-PRF	Medifuge	400 g for 10 min	10-mL tubes without anticoagulant (glass)[A-PRF+ by Choukroun]	Pain, swelling
Sybil et al., 2020 India [63]	Split-mouth	14 ♂ 11 ♀	18 – 55 (32.3) **	30 mesioangular and 50 horizontal	n.r.	*	n.r.	L-PRF	n.r.	3000 rpm for 10 min	10-mL tubes with citrate (no material) (no brand)	Pain and swelling
Torul et al., 2020 Turkey [74]	Parallel	23 ♂ 52 ♀ SG 1: 25 SG 2: 25 CG: 25	18 – 37 (22.31 ± 4.65)	Vertical or 2 B by Pell and Gregory	n.r.	Yes	n.r.	SG 1: A-PR F SG 2: CGF	n.r.	1300 rpm for 14 min (A-PRF) 2700 rpm in 2min, 2400 rom in 4 min, 2700 rpm in 4 min, and 3000 rpm in 3 min (CGF)	PRP: first centrifugation: 6-mL tubes with citrate phosphate dextrose adenine	Pain, swelling, and trismus
Gupta and Agarwal, 2021 India [55]	Split-mouth	8 ♂ 12 ♀	18 – 35 **	n.r.	Triangular	Yes	n.r.	A-PRF		1500 rpm for 14 min	Solution (no material) [BD]	Pain, swelling, and trismus
Hanif and Sheik, 2021 Pakistan [73]	Parallel	52 ♂ 78 ♀	19 – 43 (27.95 ± 6.05)	n.r.	n.r.	n.r.	ANA	PRP	A-PRF 12	1200 rpm for 10 min and 2000 rpm for 10 min	Second centrifugation: 12-mL tubes (borosilicate glass tube) [no brand]	Pain
Nourwali, 2021 Saudi Arabia [70]	Parallel	20*	18 – 40 **	n.r.	Envelope	Yes	ATB, NSAID, and SMW	L-PRF	n.r.	3000 rpm for 10 min		Pain and Swelling
Da Silva et al., 2021 Brazil [51]	Split-mouth	6 ♂ 14 ♀	18 – 29 (23 ± 3.28)	Class I A by Pell and Gregory	Envelope	n.r.	NSAID, ANA, and AMW	L-PRF	n.r.	2700 rpm for 12 min	PRF:6-mL tubes without anticoagulant (no material) [BD]	Pain

♂ - male. ♀ - female. * It was impossible to measure the sample by gender. ** It was impossible to measure the range or standard deviation. Abbreviations: n.r., not reported; ATB, antibiotic drug; NSAID, nonsteroidal anti-inflammatory drug; SAID, steroidal anti-inflammatory drug; ANA, analgesic drug; AMW, antiseptic mouthwash; SMW, normal saline mouthwash; PRP, platelet-rich plasma; L-PRF, leukocyte-and platelet-rich fibrin; A-PRF, advanced-platelet-rich fibrin; A-PRF+, advanced-platelet-rich fibrin plus; CGF, concentrated growth factor; SD, standard deviation; rpm, rotations per minute; min, minutes; mL, milliliters; g, relative centrifugal force.

3.3 Individual results of eligible studies

Postoperative pain

All studies evaluated postoperative pain intensity after third molar extraction (**Table 3**). Twelve studies comparing L-PRF to blood clots [11, 42, 51–53, 53, 56, 58, 59, 63, 64, 66, 67] showed significantly lower pain values in patients treated with L-PRF in at least one of the evaluated times. When comparing PRP and blood clots, five studies showed statistically favorable results for the group treated with the blood concentrate [57, 67, 71–73]. Two articles statistically favored A-PRF [55, 65], compared to blood clots. Pain severity in the group treated with CGF was significantly lower than with blood clots in only one study [61].

Two articles also did not find differences between PRP and L-PRF [41, 67]. Finally, one study [50] showed significantly higher pain scores in patients treated with L-PRF compared to A-PRF.

Table 3. Main outcomes of eligible studies.

Study	Groups	Outcomes		
		Pain	Edema	Trismus
Afat et al., 2018 [68]	L-PRF L-PRF + HA Blood clot	There was no significant difference among groups in VAS pain scores.	The results suggest that L-PRF, particularly combined with HA, is an effective approach to minimize postoperative edema after M3 surgery.	There was no significant difference among groups.
Aftab et al., 2020 [72]	PRP gel Blood clot	A significant lower pain score was observed in the experimental group compared to the control group at all time intervals ($p < 0.05$).	There was a significant difference in both groups in relation to facial swelling at postoperative 1st, 3rd, 5 th , and 7th days ($p < 0.05$).	There was an obvious increase in interincisal opening ($p = 0.001$) in the experimental group compared to the control group.
Al-Hamed et al., 2017 [11]	PRF Blood clot	PRF patients significantly reported less pain for the fifth, sixth, and seventh postoperative days ($p < 0.05$), and there were no differences for the second, third, and fourth postoperative days ($p > 0.05$).	n.r.	n.r.
Asutay et al., 2017 [48]	PRF Blood clot	There was no significant difference among groups in VAS pain scores.	There was no significant difference among groups.	There was no significant difference among groups.
Bhujbal et al., 2018 [49]	PRP Blood clot	There was no significant difference between immediate postoperative pain and after 1, 3, and 7 days between experimental and control groups.	Swelling significantly decreased on the experimental side on the 1st ($p=0.0059$) and 3rd ($P=0.0001$) days. However, on the 7th day, there was no statistically significant difference.	n.r.
Bhujbal et al., 2020 [41]	PRP PRF	There was no significant difference for the immediate postoperative period ($p < 0.15$), 1st day ($p < 0.96$), 3rd day ($p < 0.58$), and 7th day ($p < 0.78$).	Measurement of swelling on the 1st ($p < 0.0020$) and 3rd ($p < 0.0010$) days showed significant differences on the PRF site, but it ceased without a significant difference on the 7th day ($p < 1.00$).	n.r.
Bilginaylar & Uyanik, 2016 [42]	PRF Blood clot	There was a significant ($p < 0.05$) pain reduction on days 1, 2, and 3 in the PRF group.	There was no significant difference among groups.	There was no significant difference among groups.
Caymaz & Uyanik, 2019 [50]	L-PRF A-PRF	VAS pain scores were significantly higher in the L-PRF group than the ones in the A-PRF group during the first, second, and third days ($p < 0.05$). On the seventh day, there was no significant difference between A-PRF and L-PRF groups.	There was no significant difference among groups.	There was no significant difference among groups.
Dar et al., 2018 [52]	PRF Blood clot	Postoperative pain score (VAS) was lower for the PRF group at all times compared with the blood clot control.	There was different and higher swelling score for the blood clot groups as compared to PRF group at all time periods	n.r.
Daugela et al., 2018 [53]	L-PRF Blood clot	Sites treated with L-PRF resulted in lower pain VAS scores ($p = .001$) in the first postoperative week.	Facial swelling significantly decreased on the first ($p = .035$) and third ($p = .023$) postoperative days in L-PRF sites versus controls, ceasing without a significant difference at day 7 ($p = .224$).	n.r.
Dutta et al., 2016 [67]	PRP PRF Blood clot	The results show pain intensity reduction in all groups from day 1 up to maximum pain reduction in PRF- and PRP-treated patients ($p < 0.05$).	The maximum swelling reduction was in PRF- and PRP-treated patients ($p < 0.05$).	n.r.
Gandevivala et al., 2017 [43]	PRP Blood clot	There was no significant difference among groups.	There was no significant difference among groups.	n.r.
Gulsen and Sertuk, 2017 [54]	PRF Blood clot	There was no significant difference among groups.	There was no significant difference among groups.	n.r.

Gupta and Agarwal, 2021 [55]	A-PRF Blood clot	Data from day1 does not show statistically significant differences between test and control groups, but values from days 3 and 7 were statistically significant, favoring the A-PRF group.	At day 1, the mean scores for swelling were 2.43 and 2.61, with a p-value of 0.061, indicating no statistical significance. However, the scores at day 3 (3.46 and 3.71) were significant.	At day 1, the values were not statistically significant. The values at day 3 were highly significant with $p = 0.0001$, indicating a noticeable improvement in mouth opening when using A-PRF.
Hanif and Sheikh, 2021 [73]	PRP Blood clot	Pain was lower in the PRP group compared to non-PRP group according to the Visual Analog scale, on the 7th follow-up visit ($p < 0.05$).	n.r.	Trismus was lower in the PRP group than in the non-PRP group ($p < 0.05$).
Jeyaraj and Chakranarayan, 2018 [69]	PRF Blood clot	Pain was lower in the PRF group compared to non-PRF group.	Swelling was lower in the PRF group than in the non-PRF group.	Trismus was lower in the PRF group than in the non-PRF group.
Kapse et al., 2018 [56]	PRF Blood clot	Pain was lower in the PRF group compared to the blood clot group ($p < 0.05$) in all periods (1 st , 3 rd , 7 th , 14 th days).	Swelling was lower in the PRF group than in the blood clot group ($p < 0.05$) at the 1 st , 3 rd , 7 th , 14 th days.	n.r.
Ozveri Koyuncu et al., 2020 [61]	CGF Blood clot	Postoperative pain severity for the CGF group was lower than the non-CGF group for 7 days ($p < 0.001$).	The total swelling in the CGF group was lower than in the non-CGF group, and there was a significant difference between them in time interactions ($P < 0.001$).	The mean values of mouth opening were significantly different for the groups on days 3 and 7, with better results for the CGF-group ($p < 0.001$).
Kumar et al., 2015 [66]	PRF Blood clot	Pain was significantly lower in the PRF group ($p = 0.017$).	Swelling was significantly lower in the PRF group ($p = 0.022$).	Interincisal distance was significantly high in the PRF group ($p = 0.022$).
Kumar et al., 2016 [58]	PRF Blood clot	Pain scores significantly differed between groups ($p = 0.00$) at all follow-up intervals (day 1, day 3, and week 1) except for the 4th week.	n.r.	n.r.
Kumar et al., 2020 [57]	PRP Blood clot	The pain score of patients in non-PRP group was higher than the PRP group.	Swelling at Tragus-Subnasal and Tragus-Pogonion was higher among patients of non-PRP group compared to the PRP group.	The mean trismus of patients in the PRP group was significantly lower ($p < 0.001$) than that of non-PRP group.
Miyamoto et al., 2020 [59]	PRF Blood clot	There were no significant differences from the day of tooth extraction to day 2 after the surgery, but at days 3 and 4 after the surgery, the PRF group showed a significantly lower value than the control group.	The PRF group showed swelling relief slightly earlier but the difference was mild, and there was no statistically significant effect of PRF on alleviating swelling.	n.r.
Nourwali, 2021 [70]	PRF Blood clot	There was no significant difference among groups.	There was no significant difference among groups.	n.r.
Ogundipe et al., 2011 [71]	PRP Blood clot	The mean postoperative pain score (visual analog scale) was lower for the PRP group at all times, and it was statistically significant ($p < 0.05$).	There was no significant difference among groups.	There was no significant difference among groups.
Ozgul et al., 2015 [60]	PRF Blood clot	There were no statistically significant differences for pain among the groups.	There was more swelling on the control side ($p < 0.05$).	n.r.
Ritto et al., 2019 [62]	L-PRF Blood clot	There were no statistically significant differences for pain among the groups.	n.r.	n.r.

Da Silva et al., 2021 [51]	L-PRF Blood clot	The mean postoperative pain score (visual analog scale) was lower for the L-PRF group at all times, and it was statistically significant ($p < 0.05$).	n.r.	n.r.
Sybil et al., 2020 [63]	PRF Blood clot	There was a statistically significant difference for pain intensity in the control and test sides at days 1, 3, and 7 ($p < 0.001$).	Edema was always statistically significantly less ($p < 0.001$) on the test side compared to that of the control side.	n.r.
Torul et al., 2020 [74]	A-PRF CGF Blood clot	There were no statistically significant differences for pain among the groups.	The change in tragus to labial commissure measurements was significantly different at baseline-7th day between the control and CGF groups ($p = 0.038$). There were significant differences between the tragus to pogonion measurements at baseline-7th day between the control-CGF groups ($p = 0.014$) and A-PRF-CGF groups ($p = 0.038$).	There was no significant difference among groups.
Uyanik et al., 2015 [64]	PRF Blood clot	The statistical analysis revealed a significant postoperative pain reduction (1st, 2nd, 3 rd , and 7th days).	Postoperative days 1, 3, and 7, did not show significant group differences in swelling ($p > 0.05$).	There was a significant difference in the extent of trismus between groups ($p < 0.05$).
Zahid and Nadershah, 2019 [65]	A-PRF Blood clot	The statistical analysis revealed a significant postoperative pain reduction on the 7 th day.	The statistical analysis revealed a significant swelling reduction on the 7 th day.	n.r.

Abbreviations: n.r., not reported; PRP, platelet-rich plasma; L-PRF, leukocyte-and platelet-rich fibrin; A-PRF, advanced-platelet-rich fibrin; A-PRF+, advanced-platelet-rich fibrin plus; CGF, concentrated growth factor; HA, hydroxyapatite.

Edema

Twenty-six articles assessed edema, of which 16 showed favorable results in patients who received blood concentrates compared to those in the blood clot group (**Table 3**). In eight studies [52, 53, 56, 60, 63, 66–68], the group treated with L-PRF presented significantly lower edema than those treated with blood clots in at least one evaluation time. Six articles [42, 48, 54, 59, 64, 70] did not show differences between L-PRF and blood clots. Only four studies [49, 57, 67, 72] concluded that PRP significantly decreased edema compared to blood clots, and two did not find differences between PRP and blood clots [43, 71]. All studies [55, 65, 74] comparing A-PRF and blood clots presented favorable results for blood concentrates. Two articles [61, 74] associated CGF use with lower edema compared to blood clots.

Among studies directly comparing blood concentrates, one did not find differences between L-PRF and A-PRF [50]. However, one article found favorable results for L-PRF compared to PRP in the first three days but without difference on the seventh day [41].

Trismus

Only 13 studies evaluated trismus, of which seven had satisfactory results for blood concentrates compared to blood clots (**Table 3**). Two articles [64, 66] showed lower trismus in patients treated with L-PRF compared to blood clots, and three [42, 48, 68] did not find differences between using L-PRF or blood clots. PRP was superior to blood clots in three studies [57, 72, 73], and one did not find differences between the groups [71]. Only one study showed superior A-PRF [55] and CGF [61] compared to blood clots. Finally, one article [50] also did not find differences between A-PRF and L-PRF.

3. Risk of individual bias in the eligible studies

Two studies [53, 62] presented a low risk of bias, 12 showed "some concerns", and 17 had a high risk of bias [41–43, 49, 50, 52, 54–59, 66, 67, 69, 72, 73] (**Figure 3**). The randomization process was the domain with the highest prevalence of high risk of bias. Moreover, most eligible studies were classified as "some concerns" in the "selection bias of reported results" domain because they did not perform or present the randomized clinical trial registration.

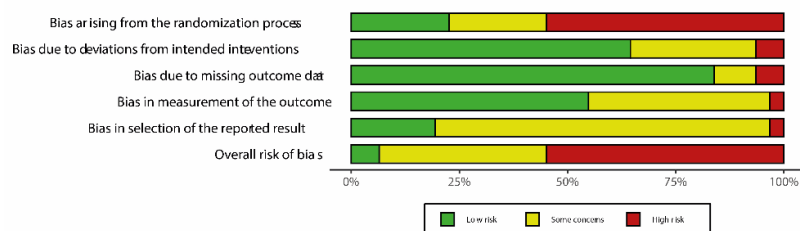
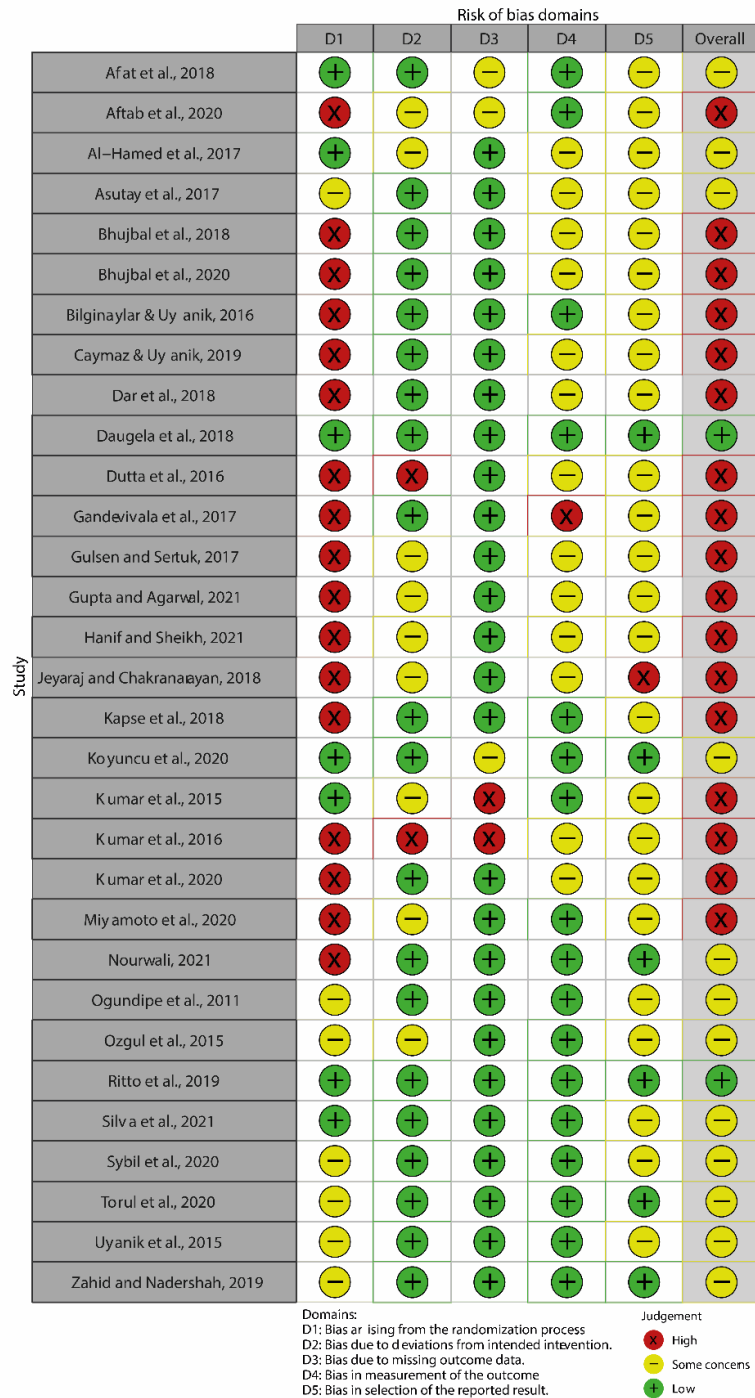


Fig. 3 Risk of bias and the percentage of all included studies according to the domain.

3.5 Network meta-analysis (NMA)

Postoperative pain

Day 1

This NMA included 16 studies, with four treatments and five pairwise comparisons, totaling 1,032 mandibular third molar extractions. **Supplementary Figure 1** presents direct evidence results. A-PRF (MD: -12.60; 95% CI: -22.95 – -2.26) and L-PRF (MD: -5.87; 95% CI: -10.66 – -1.08) showed statistically significant lower postoperative pain than blood clots (**Figure 4a**). The difference among direct, indirect, and NMA evidence suggested inconsistency in the comparisons between A-PRF and blood clots and A-PRF and L-PRF (**Supplementary Figure 2**).

(a) one day				
A-PRF		L-PRF		
-6.74 [-17.05; 3.57]		-0.49 [-8.59; 7.61]		
-7.23 [-19.72; 5.27]			PRP	
-12.60 [-22.95; -2.26]	-5.87 [-10.66; -1.08]		-5.38 [-12.85; 2.09]	Blood clot

(b) two days				
A-PRF		L-PRF		
-16.77 [-28.36; -5.18]				
-21.33 [-34.37; -8.29]		-4.56 [-10.53; 1.41]		Blood clot

(c) three days				
A-PRF		L-PRF		
-8.22 [-21.95; 5.50]		-3.72 [-15.20; 7.75]		
-11.94 [-29.35; 5.46]			PRP	
-16.91 [-30.79; -3.03]	-8.69 [-14.28; -3.10]		-4.97 [-16.09; 6.16]	Blood clot

(d) seven days				
A-PRF		L-PRF		
-0.26 [-4.88; 4.37]				
-0.65 [-6.09; 4.78]		-0.40 [-3.74; 2.94]		
-5.14 [-9.82; -0.46]	-4.88 [-6.98; -2.79]		-4.49 [-7.45; -1.52]	Blood clot

Fig. 4 League tables with postoperative pain results from NMA one (a), two (b), three (c), and seven (d) days after surgery. Treatments were ordered from best to worst according to the ranking probabilities of treatment effects. Significant results are in bold.

The sensitivity analysis showed similar findings, including only studies with a split-mouth design (**Supplementary Figure 3**), and comparisons between A-PRF and blood clots and A-PRF and L-PRF remained inconsistent (**Supplementary Figure 4**).

Day 2

This NMA included seven studies, with three treatments and two pairwise comparisons, totaling 402 mandibular third molar extraction procedures. **Supplementary Figure 5** presents direct evidence results and **Figure 4b** describes NMA outcomes. A-PRF showed statistically significant lower postoperative pain than L-PRF (MD: -16.77; 95% CI: -28.36 – -5.18) and blood clot (MD: -21.33; 95% CI: -34.37 – -8.29).

Day 3

This NMA included 16 studies, with four treatments and five pairwise comparisons, totaling 1,032 mandibular third molar extractions. **Supplementary Figure 6** presents direct evidence results. Postoperative pain was significantly lower for PRP, L-PRF, and A-PRF than for blood clots and for A-PRF than L-PRF. **Figure 4c** describes NMA results, and there were no inconsistencies (**Supplementary Figure 7**).

The sensitivity analysis detected similar findings, including only studies with a split-mouth design (**Supplementary Figure 8**), showing no inconsistencies (**Supplementary Figure 9**).

Day 7

This NMA included 17 studies, with four treatments and five pairwise comparisons, totaling 1,092 mandibular third molar extractions. **Supplementary Figure 10** presents direct evidence results and **Figure 4d** shows NMA estimates. There was a statistically significant lower postoperative pain for A-PRF (MD: -5.14; 95% CI: -9.82 – -0.46), L-PRF (MD: -4.88; 95% CI: -6.98 – -2.79) and PRP (MD: -4.49; 95% CI: -7.45 – -1.52) than for blood clots. There were no inconsistencies (**Supplementary Figure 11**).

The sensitivity analysis detected similar findings, including only studies with a split-mouth design (**Supplementary Figure 12**), showing no inconsistencies (**Supplementary Figure 13**).

Trismus

Day 1

The NMA of percentage results of interincisal opening reduction included three studies, with three treatments and two pairwise comparisons, totaling 114 mandibular third molar extractions (**Figure 1a**). **Supplementary Figure 14** presents direct evidence results and **Figure 5a** shows NMA estimates.

The NMA of interincisal opening distance results included three studies, with four treatments and three pairwise comparisons, totaling 271 mandibular third molar extraction procedures (**Figure 1b**). **Supplementary Figure 15** presents direct evidence results and **Figure 6a** shows NMA estimates.

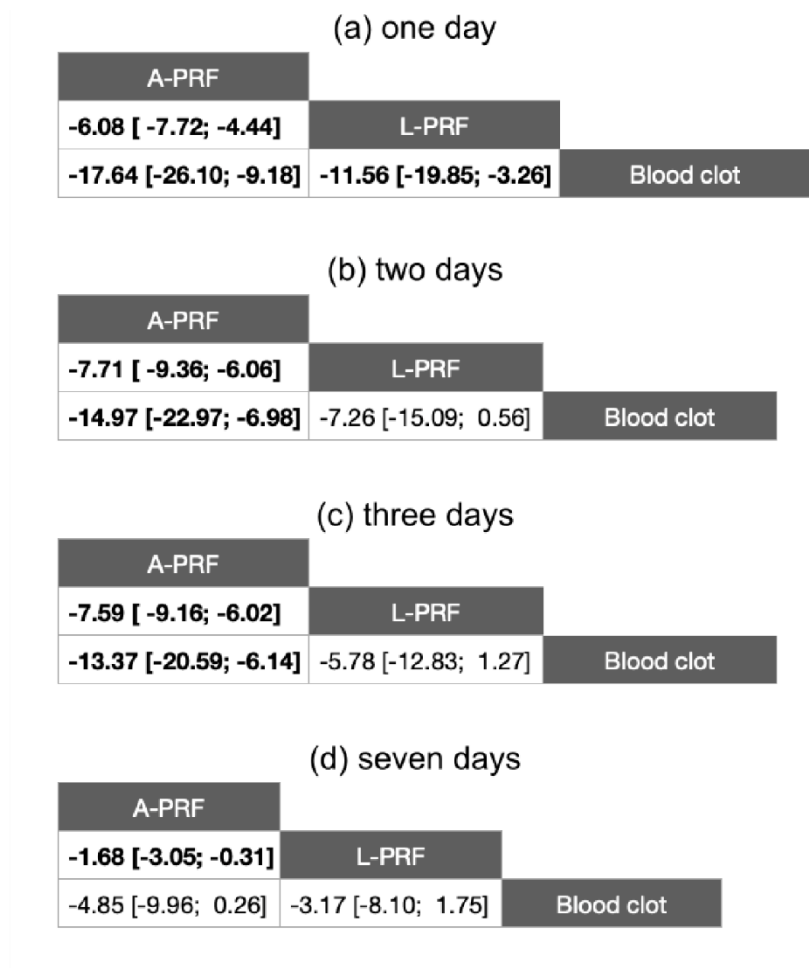


Fig. 5 League tables with trismus results (measured as the percentage of interincisal opening reduction) from NMA after one (a), two (b), three (c), and seven (d) days after surgery. Treatments were ordered from best to worst according to the ranking probabilities of treatment effects. Significant results are in bold.

Day 2

This NMA included three studies, with four treatments and two pairwise comparisons, totaling 114 mandibular third molar extractions. **Supplementary Figure 14** presents direct evidence results and **Figure 5b** describes NMA outcomes.

Day 3

This NMA included three studies, with four treatments and two pairwise comparisons, totaling 114 mandibular third molar extractions. **Supplementary Figure 14** presents direct evidence results and **Figure 5c** describes NMA outcomes.

Day 7

The NMA of percentage results of interincisal opening reduction included three studies, with three treatments and two pairwise comparisons, totaling 114 mandibular third molar extractions (**Figure 1a**). **Supplementary Figure 14** presents direct evidence results and **Figure 5d** shows NMA estimates. There

was a statistically significant lower percentage of interincisal opening reduction for A-PRF (MD: -1.68; 95% CI: -3.05 – -0.31) than for L-PRF.

The NMA of interincisal opening distance results included three studies, with three treatments and two pairwise comparisons, totaling 300 mandibular third molar extractions (**Figure 1c**). **Supplementary Figure 15** presents direct evidence results. There was no difference between treatments. **Figure 6b** shows NMA estimates and no difference between treatments.

(a) one day			
PRP		L-PRF	
2.73 [0.77; 4.69]		0.52 [-1.83; 2.87]	
3.25 [1.54; 4.96]			A-PRF
4.66 [3.87; 5.45]	1.93 [0.13; 3.73]	1.41 [-0.10; 2.92]	Blood clot

(b) seven days			
A-PRF		PRP	
1.03 [-0.67; 2.73]			
1.40 [-0.02; 2.82]		0.37 [-0.58; 1.32]	
			Blood clot
2.18 [-1.87; 6.23]	1.15 [-2.76; 5.06]	0.78 [-3.01; 4.57]	L-PRF

Fig. 6 League tables with trismus results (measured as interincisal opening distance) from NMA after one (a) and seven (b) days after surgery. Treatments were ordered from best to worst according to the ranking probabilities of treatment effects. Significant results are in bold.

3.6 Certainty of evidence

All comparisons were considered "very low certainty of evidence" (**Table 4**). The reasons for downgrading the certainty of evidence included the risk of bias, incoherence, and imprecision.

Table 4. GRADE assessment for direct and indirect certainty of evidence comparisons.

Postoperative period	Comparison	Direct evidence				Direct certainty of evidence	Indirect evidence comparison from the closest loop	Indirect evidence comparison from the closest loop	Lowest between both	Intransitivity	Incoherence for NMA	Imprecision for NMA	Final certainty of evidence	
		Risk of bias	Inconsistency	Indirectness	Publication bias									
Pain														
1 day	Blood clot vs PRP	Very serious ¹	Very serious ³	None	None	Very low	Blood clot vs L-PRF	L-PRF vs PRP	vs	Very low	None	None	Very serious ⁶	Very low
	Blood clot vs L-PRF	Very serious ¹	Very serious ³	None	None	Very low	Blood clot vs PRP	L-PRF vs PRP	vs	Very low	None	Serious ⁵	Serious ⁷	Very low
	Blood clot vs A-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	A-PRF vs L-PRF	vs	Very low	None	Serious ⁵	Serious ⁷	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs A-PRF	vs	Very low	None	Serious ⁵	Very serious ⁶	Very low
	L-PRF vs PRP	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs PRP	vs	Very low	None	None	Very serious ⁶	Very low
2 days	Blood clot vs L-PRF	Very serious ¹	Serious ⁴	None	None	Very low	-	-		-	-	-	Very serious ⁶	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	-	-		-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	-	-	-	-	-	Blood clot vs L-PRF	A-PRF vs L-PRF	vs	Low	None	-	Serious ⁷	Very low
3 days	Blood clot vs L-PRF	Very serious ¹	Very serious ³	None	None	Very low	Blood clot vs PRP	L-PRF vs PRP	vs	Low	None	None	Serious ⁷	Very low
	Blood clot vs PRP	Very serious ¹	None	None	None	Low	Blood clot vs L-PRF	L-PRF vs PRP	vs	Very Low	None	None	Very serious ⁶	Very low
	Blood clot vs A-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	A-PRF vs L-PRF	vs	Very Low	None	None	Serious ⁷	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs A-PRF	vs	Very Low	None	Serious ⁵	Very serious ⁶	Very low
	L-PRF vs PRP	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs PRP	vs	Very Low	None	None	Very serious ⁶	Very low
7 days	Blood clot vs L-PRF	Very serious ¹	Very serious ³	None	None	Very low	Blood clot vs PRP	L-PRF vs PRP	vs	Low	None	None	Serious ⁷	Very low
	Blood clot vs PRP	Very serious ¹	None	None	None	Low	Blood clot vs L-PRF	L-PRF vs PRP	vs	Very low	None	None	Serious ⁷	Very low
	Blood clot vs A-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	A-PRF vs L-PRF	vs	Very low	None	None	Very serious ⁶	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs A-PRF	vs	Very low	None	None	Very serious ⁶	Very low
	L-PRF vs PRP	Very serious ¹	-	None	None	Low	Blood clot vs L-PRF	Blood clot vs PRP	vs	Very low	None	None	Very serious ⁶	Very low
Trismus (measured as a percentage of interincisal opening reduction)														

1 day	Blood clot vs L-PRF	Very serious ¹	None	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	-	-	-	-	-	Blood clot vs L-PRF	A-PRF vs L-PRF	Low	None	-	Serious ⁷	Very low
2 days	Blood clot vs L-PRF	Very serious ¹	None	None	None	Low	-	-	-	-	-	Very serious ⁶	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	-	-	-	-	-	Blood clot vs L-PRF	A-PRF vs L-PRF	Low	None	-	Serious ⁷	Very low
3 days	Blood clot vs L-PRF	Very serious ¹	None	None	None	Low	-	-	-	-	-	Very serious ⁶	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	-	-	-	-	-	Blood clot vs L-PRF	A-PRF vs L-PRF	Low	None	-	Serious ⁷	Very low
7 days	Blood clot vs L-PRF	Very serious ¹	None	None	None	Low	-	-	-	-	-	Very serious ⁶	Very low
	A-PRF vs L-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Very serious ⁶	Very low
	Blood clot vs A-PRF	-	-	-	-	-	Blood clot vs L-PRF	A-PRF vs L-PRF	Low	None	-	Serious ⁷	Very low
Trismus (measured as an interincisal opening distance)													
1 day	Blood clot vs L-PRF	None	-	None	None	High	-	-	-	-	-	Very serious ⁶	Low
	Blood clot vs PRP	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Very serious ⁶	Very low
	A-PRF vs PRP	-	-	-	-	-	Blood clot vs PRP	Blood clot vs A-PRF	Low	None	-	Serious ⁷	Very low
	A-PRF vs L-PRF	-	-	-	-	-	Blood clot vs L-PRF	Blood clot vs A-PRF	Low	None	-	Very serious ⁶	Very low
	L-PRF vs PRP	-	-	-	-	-	Blood clot vs L-PRF	Blood clot vs PRP	Low	None	-	Very serious ⁶	Very low
7 days	Blood clot vs L-PRF	Serious ²	-	None	None	Moderate	-	-	-	-	-	Very serious ⁶	Very low
	Blood clot vs PRP	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	Blood clot vs A-PRF	Very serious ¹	-	None	None	Low	-	-	-	-	-	Serious ⁷	Very low
	A-PRF vs L-PRF	-	-	-	-	-	Blood clot vs A-PRF	Blood clot vs L-PRF	Low	None	-	Very serious ⁶	Very low

A-PRF PRP	vs	-	-	-	-	-	Blood clot vs A-PRF	Blood clot vs PRP	Low	None	-	Very serious ⁶	Very low
L-PRF PRP	vs	-	-	-	-	-	Blood clot vs L-PRF	Blood clot vs PRP	Low	None	-	Very serious ⁶	Very low

1 – High risk of bias due to problems in the randomization process and/or outcome assessment. 2 – Some concerns due to problems in the randomization process and/or outcome assessment. 3 - I^2 for direct comparisons if they were moderate (30-60%), substantial (50-90%), or considerable (75%-100%), and there was no 95%CI overlap. 4 - I^2 for direct comparisons if they were moderate (30-60%), substantial (50-90%), or considerable (75%-100%), and there was 95%CI overlap. 5 - Global inconsistency statistics had a $p < 0.05$ or the evidence was downgraded one level if the direct and indirect evidence favored opposite treatments, but the direct effect estimate agreed with the NMA effect estimate; and two levels if the direct effect estimate was incoherent with the indirect and NMA effect estimates. 6 - Downgraded one level if the 95%CI of the NMA effect estimate crossed the large effect threshold (>0.8 SD); and two levels if the NMA 95%CI also crossed the null effect line. 7 - Whenever OIS was not achieved, one point was downgraded.

Discussion

Blood concentrates increase the local concentration of cytokines and growth factors rapidly and sustainably, depending on the type, which is among the characteristics that biologically distinguish these concentrates [75]. Releasing such substances causes - *in vitro* - pro-inflammatory cytokine suppression [25–28]. Therefore, this network meta-analysis (NMA) aimed to examine the efficacy of applying blood concentrates after mandibular third molar removal regarding postoperative signs and symptoms. The study hypothesized that postoperative inflammatory signs and symptoms would be minimized by applying blood concentrates compared to blood clots to the alveolus of extracted mandibular third molars. Hence, in this network systematic review, the patient's blood clot, as a normal process, was the more adequate and reasonable reference group for indirect comparisons to assess PRF potential for controlling pain perception, edema, and trismus.

According to the qualitative analysis of the included studies, blood concentrate actions were contradictory. There was no consensus on the efficacy of these elements in promoting a postoperative period with lower pain perception, lower edema, and larger mouth opening. Studies indicating the effectiveness of PRP for pain control are based on the ability of this concentrate to reduce the rate of prostaglandins, the primary mediator responsible for pain perception [76, 77]. Investigations with postoperative outcomes favoring L-PRF use for pain control evaluate this concentrate not exclusively as a source of growth factors because it is also a fibrin-based biomaterial containing cells, matrix proteins, and anti-inflammatory mediators released for a longer time and working throughout the inflammatory repair phase [53]. Thus, L-PRF inserted in the alveolus would modulate the inflammatory response and promote higher hemostasis, chemotaxis, and angiogenesis, working as a bioactive scar matrix for the complex repair cascade that would occur later [17, 78]. However, lower pain levels cannot be explained only by PRF action because patients also used medications in different regimens, which may affect postoperative pain perception. The network meta-analysis showed that the analyzed blood concentrates for pain perception (A-PRF, L-PRF, and PRP) were better for reducing inflammatory signs and symptoms than blood clots. A-PRF was statistically superior to blood clots in all evaluated times and L-PRF in the third and seventh postoperative days. The release of growth factors and cytokines from A-PRF and L-PRF predominantly occurs between the first and third days, coinciding with the acute inflammatory peak. That may be due to the extension of this release to the tenth day, and A-PRF was the concentrate with the highest total release of these factors throughout the evaluated time [75, 79]. Second-generation blood concentrates, such as L-PRF and A-PRF, have a three-dimensional network structure that provides a favorable site for cell migration, fixation, and differentiation but also retain a high number of growth factors and cytokines that remain trapped in the fibrin mesh and probably even in fibrin polymers [16, 80]. Progressive polymerization would mean a higher circulating cytokine incorporation into fibrin meshes, allowing a continuous release of these factors and causing the anti-inflammatory effect sustained during the evaluated time. Using anti- and procoagulants to produce PRP would promote lower incorporation of these cytokines and a short-term release at lower amounts [18, 75]. Pain decreased more using A-PRF than blood clots in the second and third days, coinciding with the acute inflammatory peak.

As for trismus, the NMA was divided according to the measurement method. The evaluated concentrates for the interincisal opening percentage were L-PRF and A-PRF, providing evidence for the

positive effect of A-PRF compared to L-PRF at all evaluated times and A-PRF for the control group at one, three, and seven days. L-PRF was superior to blood clots only for the measurement on the first day. When measuring interincisal opening distance, the first-generation concentrate (PRP) was added to the comparisons and surpassed all other treatments on the first day. There were no differences between the treatments and the control group on the seventh day. The difference between PRP and the other concentrates may be due to the higher release of cytokines and growth factors in the initial times after its use compared to the slow and continuous release of second-generation concentrates [75].

Studies evaluating blood concentrate action related to mouth opening showed a consensus regarding CGF efficacy in reducing trismus [61, 74]. Such findings should be interpreted carefully because these were the only included studies using CGF. Moreover, TORUL et al. (2020) did not show statistically significant differences among patients treated with CGF. The findings of KOYUNCU et al. (2020) were statistically significant, indicating the success of CGF as a fibrin matrix working as a support material and reservoir for growth factors, such as PDGF, TGF, and VEGF and inflammatory cytokines.

Edema assessments presented heterogeneous results, hindering a quantitative evaluation, especially for edema and trismus, which made it impossible to group the data for the network analysis. The low number of patients receiving some concentrates for the outcomes may affect estimate precisions in the data analyses.

Blood concentrates are prepared by varying the relative centrifugal force (RCF), centrifugation time, and tubes [15, 20]. Centrifuge characteristics and correctly using the centrifugation protocol significantly affect the quality of the produced concentrate [81]. Although RCF is the reference standard to produce blood concentrates, only three studies described it in the methodology [51, 59, 64]. Among the main limitations in the literature on blood concentrates is the absence of a standard method to report RCF values [82, 83]. RCF may be obtained by measuring the maximum rotor radius and rotations per minute. Most studies cannot determine the exact RCF to produce blood concentrates because none informed the rotor radius and only a few described the centrifuge used to produce the concentrates. All articles mentioned the centrifugation time. Considering that each of these aspects is relevant for blood concentrate production, the absence of such information hinders the knowledge of whether the concentrates were produced correctly (standardized). Standardizing blood concentrate protocols and their description is required for correct production and improved reproducibility of new randomized clinical trials - the most reliable clinical study for making decisions about therapy applications in the health field.

Considering their significance, these interventions must be described through high-quality reports that provide transparency and allow readers to assess the study findings critically [84]. Hence, execution failures in this study type interfere with results, causing conclusion errors regarding the effectiveness or safety of the evaluated interventions [38]. The main goal of randomization in treatment arms is to stop researchers, clinicians, and patients from predicting and influencing the appointment of a given intervention group [85]. Most studies showed a high risk of bias, especially regarding the randomization process. Among the limitations of this systematic review is the low certainty of evidence caused by the high risk of bias in most eligible studies, mainly referring to randomization. There were only a few studies included in each outcome, and the methodologies applied to the outcomes showed heterogeneity. Therefore, most analyzed studies presented major methodological problems.

Postoperative sequelae after lower third molar extraction varied according to several factors, such as age, comorbidities, tooth difficulty level, surgeon experience, oral hygiene level, and surgical procedure [86, 87]. The studies included in this review presented limitations regarding these comparative effects. There was no standardization among the factors that might affect the analyzed outcomes, such as the position and difficulty of extracted lower third molars, the flap used, the interval between procedures in the study/control (washout), and medications used before and after surgery. Additionally, different study designs, measurement scales, and surgical protocols might explain the heterogeneity. Sensitivity tests were performed to minimize these biases, including only split-mouth studies for pain perception, and all results were similar at one, three, and seven days. Regarding the analyzed outcomes, even though the measurements simulated comparison conditions, several results were non-consonants due to their forms of expression. These data acquisition limitations indicate the need for improving reporting quality and standardization in further studies.

Numerous factors can affect the analyzed outcomes, such as prior toothache experience, medications used before and after surgery, hygiene, surgeon experience, and surgical duration and difficulty, complicating the interpretation of results. The low number of patients receiving some concentrates for the outcomes may affect estimate precisions in the data analyses. Therefore, new clinical studies with higher standardization, improved methodological quality, and a larger scale are required to establish the actual effect of blood concentrates on pain perception, edema, and trismus. Improving these factors may help further reviews determine the efficacy of blood concentrates in controlling inflammatory signs and symptoms after lower third molar surgeries, thus establishing the potential cost-effectiveness of their clinical application to the routine of this procedure.

Conclusion

Considering the low certainty of evidence, A-PRF after mandibular third molar extractions performed better among the analyzed blood concentrates and seemed effective in reducing postoperative pain at all analyzed times. Trismus decreased when using A-PRF at the inflammatory peak. Considering the relevance of third molar extractions in the clinical routine of dentists and the evidence level of the evaluated articles, further randomized clinical trials are required, presenting better designs and supporting solid guidelines for recommending blood concentrates for reducing inflammatory signs and symptoms.

Declarations Section:

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Marcelo Dias Moreira de Assis Costa, Luiz Renato Paranhos, Vinícius Lima de Almeida, Leandro Machado Oliveira, Walbert de Andrade Vieira and Paula Dechichi. The first draft of the manuscript was written by Marcelo Dias Moreira de Assis Costa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. We are also thankful for the support of Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil (CNPq) and of Fundação de Amparo à Pesquisa do Estado de Minas Gerais – Brazil (FAPEMIG).

Ethical Approval

Not Applicable

References

1. Duarte-Rodrigues L, Miranda EFP, Souza TO, de Paiva HN, Falci SGM, Galvão EL (2018) Third molar removal and its impact on quality of life: systematic review and meta-analysis. *Qual Life Res* 27:2477 – 2489. <https://doi.org/10.1007/s11136-018-1889-1>
2. Bailey E, Kashbour W, Shah N, Worthington HV, Renton TF, Coulthard P (2020) Surgical techniques for the removal of mandibular wisdom teeth. *Cochrane Database Syst Rev* 7:CD004345. <https://doi.org/10.1002/14651858.CD004345.pub3>
3. Albuquerque AFM, Fonteles CSR, do Val DR, Chaves HV, Bezerra MM, Pereira KMA, de Barros Silva PG, de Lima BB, Soares ECS, Ribeiro TR, Costa FWG (2017) Effect of pre-emptive analgesia on clinical parameters and tissue levels of TNF- α and IL-1 β in third molar surgery: a triple-blind, randomized, placebo-controlled study. *Int J Oral Maxillofac Surg* 46:1615 – 1625. <https://doi.org/10.1016/j.ijom.2017.05.007>
4. Costa FWG, Soares ECS, Esses DFS, Silva PG deB, Bezerra TP, Scarparo HC, Ribeiro TR, Fonteles CSR (2015) A split-mouth, randomized, triple-blind, placebo-controlled study to analyze the pre-emptive effect of etoricoxib 120 mg on inflammatory events following removal of unerupted mandibular third molars. *Int J Oral Maxillofac Surg* 44:1166 – 1174. <https://doi.org/10.1016/j.ijom.2015.06.012>
5. Silva PUJ, Meneses-Santos D, Vieira W de A, Ramacciato JC, Silva RP da, Silva MCP da, Rode S de M, Paranhos LR (2021) Preemptive use of intravenous ibuprofen to reduce postoperative pain after lower third molar surgery: a systematic review of randomized controlled trials. *Clinics (Sao Paulo)* 76:e2780. <https://doi.org/10.6061/clinics/2021/e2780>
6. Jaroń A, Preuss O, Grzywacz E, Trybek G (2021) The Impact of Using Kinesio Tape on Non-Infectious Complications after Impacted Mandibular Third Molar Surgery. *Int J Environ Res Public Health* 18:E399. <https://doi.org/10.3390/ijerph18020399>
7. Landim FS, Laureano Filho JR, Nascimento J, do Egito Vasconcelos BC (2020) Effectiveness of nicotine patch for the control of pain, oedema, and trismus following third molar surgery: a randomized clinical trial. *Int J Oral Maxillofac Surg* 49:1508 – 1517. <https://doi.org/10.1016/j.ijom.2019.08.013>
8. Sáez-Alcaide L-M, Molinero-Mourelle P, González-Serrano J, Rubio-Alonso L, Bornstein M-M, López-Quiles J (2020) Efficacy of a topical gel containing chitosan, chlorhexidine, allantoin and dexpantenol for pain and inflammation control after third molar surgery: A randomized and placebo-controlled clinical trial. *Med Oral Patol Oral Cir Bucal* 25:e644 – e651. <https://doi.org/10.4317/medoral.23661>

9. Kaplan V, Hasanoglu Erbasar GN, Cigerim L, Altay Turgut H, Cerit A (2021) Effect of St. John's wort oil and olive oil on the postoperative complications after third molar surgery: randomized, double-blind clinical trial. *Clin Oral Investig* 25:2429 – 2438. <https://doi.org/10.1007/s00784-020-03639-0>
10. Quesada-Bravo FJ, García-Carricondo AR, Espín-Gálvez F, Fernández-Sánchez C, Fernández-Ginés D, Requena-Mullor MDM, Alarcón-Rodríguez R (2021) Comparative Study between the Combination of Dexamethasone and Bupivacaine for Third Molar Surgery Postoperative Pain: A Triple-Blind, Randomized Clinical Trial. *J Clin Med* 10:5081. <https://doi.org/10.3390/jcm10215081>
11. Al-Hamed FS, Tawfik MA-M, Abdelfadil E, Al-Saleh MAQ (2017) Efficacy of Platelet-Rich Fibrin After Mandibular Third Molar Extraction: A Systematic Review and Meta-Analysis. *J Oral Maxillofac Surg* 75:1124 – 1135. <https://doi.org/10.1016/j.joms.2017.01.022>
12. Canellas JVDS, Ritto FG, Medeiros PJD (2017) Evaluation of postoperative complications after mandibular third molar surgery with the use of platelet-rich fibrin: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 46:1138 – 1146. <https://doi.org/10.1016/j.ijom.2017.04.006>
13. Zhu J, Zhang S, Yuan X, He T, Liu H, Wang J, Xu B (2021) Effect of platelet-rich fibrin on the control of alveolar osteitis, pain, trismus, soft tissue healing, and swelling following mandibular third molar surgery: an updated systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 50:398 – 406. <https://doi.org/10.1016/j.ijom.2020.08.014>
14. Mihaylova Z, Mitev V, Stanimirov P, Isaeva A, Gateva N, Ishkitiev N (2017) Use of platelet concentrates in oral and maxillofacial surgery: an overview. *Acta Odontol Scand* 75:1 – 11. <https://doi.org/10.1080/00016357.2016.1236985>
15. Choukroun J, Ghanaati S (2018) Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg* 44:87 – 95. <https://doi.org/10.1007/s00068-017-0767-9>
16. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e37-44. <https://doi.org/10.1016/j.tripleo.2005.07.008>
17. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e51-55. <https://doi.org/10.1016/j.tripleo.2005.07.010>
18. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e45-50. <https://doi.org/10.1016/j.tripleo.2005.07.009>
19. Choukroun J, Diss A, Simonpieri A, Girard M-O, Schoeffler C, Dohan SL, Dohan AJJ, Mouhyi J, Dohan DM (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101:e56-60. <https://doi.org/10.1016/j.tripleo.2005.07.011>
20. Choukroun J, Ghanaati S (2017) Introducing the Low-Speed Centrifugation Concept. In: *Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications*. John Wiley & Sons, Ltd, pp 33 – 46

21. Sato A, Kawabata H, Aizawa H, Tsujino T, Isobe K, Watanabe T, Kitamura Y, Miron RJ, Kawase T (2020) Distribution and quantification of activated platelets in platelet-rich fibrin matrices. *Platelets* 1 – 6. <https://doi.org/10.1080/09537104.2020.1856359>
22. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, Landes C, Sader R, Kirkpatrick C, Choukroun J (2014) Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 40:679 – 689. <https://doi.org/10.1563/aaid-joi-D-14-00138>
23. Bai M-Y, Wang C-W, Wang J-Y, Lin M-F, Chan WP (2017) Three-dimensional structure and cytokine distribution of platelet-rich fibrin. *Clinics (Sao Paulo)* 72:116 – 124. [https://doi.org/10.6061/clinics/2017\(02\)09](https://doi.org/10.6061/clinics/2017(02)09)
24. Yanmin Z (2017) Biological Additives and Platelet Concentrates for Tissue Engineering on Regenerative Dentistry: Basic Science and Concise Review. *Asian Journal of Pharmaceutics (AJP)*: Free full text articles from Asian J Pharm 11:. <https://doi.org/10.22377/ajp.v11i04.1621>
25. Mudalal M, Sun X, Li X, Zhou Y (2019) The evaluation of leukocyte-platelet rich fibrin as an antiinflammatory autologous biological additive: A novel in vitro study. *Saudi Medical Journal* 40:657 – 669. <https://doi.org/10.15537/smj.2019.7.24302>
26. Kargarpour Z, Nasirzade J, Strauss FJ, Di Summa F, Hasannia S, Müller H-D, Gruber R (2020) Platelet-rich fibrin suppresses in vitro osteoclastogenesis. *J Periodontol* 91:413 – 421. <https://doi.org/10.1002/JPER.19-0109>
27. Zhang J, Yin C, Zhao Q, Zhao Z, Wang J, Miron RJ, Zhang Y (2020) Anti-inflammation effects of injectable platelet-rich fibrin via macrophages and dendritic cells. *J Biomed Mater Res A* 108:61 – 68. <https://doi.org/10.1002/jbm.a.36792>
28. Wang Z, Mudalal M, Sun Y, Liu Y, Wang J, Wang Y, Sun X, Zhou Y (2020) The Effects of Leukocyte-Platelet Rich Fibrin (L-PRF) on Suppression of the Expressions of the Pro-Inflammatory Cytokines, and Proliferation of Schwann Cell, and Neurotrophic Factors. *Sci Rep* 10:2421. <https://doi.org/10.1038/s41598-020-59319-2>
29. Bao M, Du G, Zhang Y, Ma P, Cao Y, Li C (2021) Application of Platelet-Rich Fibrin Derivatives for Mandibular Third Molar Extraction Related Post-Operative Sequelae: A Systematic Review and Network Meta-Analysis. *J Oral Maxillofac Surg* S0278-2391(21)00661 – 3. <https://doi.org/10.1016/j.joms.2021.07.006>
30. Barona-Dorado C, González-Regueiro I, Martín-Ares M, Arias-Irimia O, Martínez-González J-M (2014) Efficacy of platelet-rich plasma applied to post-extraction retained lower third molar alveoli. A systematic review. *Med Oral Patol Oral Cir Bucal* 19:e142-148. <https://doi.org/10.4317/medoral.19444>
31. Vitenson J, Starch-Jensen T, Bruun NH, Larsen MK (2022) The use of advanced platelet-rich fibrin after surgical removal of mandibular third molars: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 51:962 – 974. <https://doi.org/10.1016/j.ijom.2021.11.014>
32. Xiang X, Shi P, Zhang P, Shen J, Kang J (2019) Impact of platelet-rich fibrin on mandibular third molar surgery recovery: a systematic review and meta-analysis. *BMC Oral Health* 19:163. <https://doi.org/10.1186/s12903-019-0824-3>
33. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, PRISMA-P Group (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 4:1. <https://doi.org/10.1186/2046-4053-4-1>
34. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T,

- Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 10:89. <https://doi.org/10.1186/s13643-021-01626-4>
35. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JPA, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D (2015) The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med* 162:777 – 784. <https://doi.org/10.7326/M14-2385>
 36. Aromataris E, Munn Z (2020) JBI Manual for Evidence Synthesis. JBI
 37. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A (2016) Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews* 5:210. <https://doi.org/10.1186/s13643-016-0384-4>
 38. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng H-Y, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 366:l4898. <https://doi.org/10.1136/bmj.l4898>
 39. Dias S, Ades T, Welton NJ, Jansen J, Sutton AJ (2018) Network meta-analysis for decision-making. Wiley, New Jersey
 40. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. *Control Clin Trials* 7:177 – 188. [https://doi.org/10.1016/0197-2456\(86\)90046-2](https://doi.org/10.1016/0197-2456(86)90046-2)
 41. Bhujbal R, Veerabhadrapa SK, Yadav S, Chappi M, Patil V (2020) Evaluation of platelet-rich fibrin and platelet-rich plasma in impacted mandibular third molar extraction socket healing and bone regeneration: A split-mouth comparative study. *European Journal of General Dentistry* 9:96 – 102
 42. Bilginaylar K, Uyanik LO (2016) Evaluation of the effects of platelet-rich fibrin and piezosurgery on outcomes after removal of impacted mandibular third molars. *Br J Oral Maxillofac Surg* 54:629 – 633. <https://doi.org/10.1016/j.bjoms.2016.03.016>
 43. Gandeivala A, Sangle A, Shah D, Tejnani A, Sayyed A, Khutwad G, Patel AA (2017) Autologous Platelet-rich Plasma after Third Molar Surgery. *Ann Maxillofac Surg* 7:245 – 249. https://doi.org/10.4103/ams.ams_108_16
 44. Sweeting MJ, Sutton AJ, Lambert PC (2004) What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Stat Med* 23:1351 – 1375. <https://doi.org/10.1002/sim.1761>
 45. Wei J-J, Lin E-X, Shi J-D, Yang K, Hu Z-L, Zeng X-T, Tong T-J (2021) Meta-analysis with zero-event studies: a comparative study with application to COVID-19 data. *Mil Med Res* 8:41. <https://doi.org/10.1186/s40779-021-00331-6>
 46. Brignardello-Petersen R, Bonner A, Alexander PE, Siemieniuk RA, Furukawa TA, Rochwerg B, Hazlewood GS, Alhazzani W, Mustafa RA, Murad MH, Puhan MA, Schünemann HJ, Guyatt GH, GRADE Working Group (2018) Advances in the GRADE approach to rate the certainty in estimates from a network meta-analysis. *J Clin Epidemiol* 93:36 – 44. <https://doi.org/10.1016/j.jclinepi.2017.10.005>
 47. Puhan MA, Schünemann HJ, Murad MH, Li T, Brignardello-Petersen R, Singh JA, Kessels AG, Guyatt GH, GRADE Working Group (2014) A GRADE Working Group approach for rating the quality of treatment effect estimates from network meta-analysis. *BMJ* 349:g5630. <https://doi.org/10.1136/bmj.g5630>

48. Asutay F, Yolcu Ü, Geçör O, Acar AH, Öztürk SA, Malkoç S (2017) An evaluation of effects of platelet-rich-fibrin on postoperative morbidities after lower third molar surgery. *Niger J Clin Pract* 20:1531 – 1536. <https://doi.org/10.4103/1119-3077.181400>
49. Bhujbal R, A Malik N, Kumar N, Kv S, I Parkar M, Mb J (2018) Comparative evaluation of platelet rich plasma in socket healing and bone regeneration after surgical removal of impacted mandibular third molars. *J Dent Res Dent Clin Dent Prospects* 12:153 – 158. <https://doi.org/10.15171/joddd.2018.024>
50. Caymaz MG, Uyanik LO (2019) Comparison of the effect of advanced platelet-rich fibrin and leukocyte- and platelet-rich fibrin on outcomes after removal of impacted mandibular third molar: A randomized split-mouth study. *Niger J Clin Pract* 22:546 – 552. https://doi.org/10.4103/njcp.njcp_473_18
51. da Silva MT, de Almeida Barros Mourão CF, Mello-Machado RC, Montemezzi P, de Lima Barbosa R, Sartoretto SC, Leite PEC, Javid K, Kawase T, Alves GG, Calasans-Maia MD (2021) Effects of Leukocyte-Platelet-Rich Fibrin (L – PRF) on Pain, Soft Tissue Healing, Growth Factors, and Cytokines after Third Molar Extraction: A Randomized, Split-Mouth, Double-Blinded Clinical Trial. *Applied Sciences* 11:1666. <https://doi.org/10.3390/app11041666>
52. Dar MM, Shah AA, Najjar AL, Younis M, Kapoor M, Dar JI (2018) Healing Potential of Platelet Rich Fibrin in Impacted Mandibular Third Molar Extraction Sockets. *Ann Maxillofac Surg* 8:206 – 213. https://doi.org/10.4103/ams.ams_181_18
53. Daugela P, Grimuta V, Sakavicius D, Jonaitis J, Juodzbaly G (2018) Influence of leukocyte- and platelet-rich fibrin (L-PRF) on the outcomes of impacted mandibular third molar removal surgery: A split-mouth randomized clinical trial. *Quintessence Int* 49:377 – 388. <https://doi.org/10.3290/j.qi.a40113>
54. Gülşen U, Şentürk MF (2017) Effect of platelet rich fibrin on edema and pain following third molar surgery: a split mouth control study. *BMC Oral Health* 17:79. <https://doi.org/10.1186/s12903-017-0371-8>
55. Gupta N, Agarwal S (2021) Advanced-PRF: Clinical evaluation in impacted mandibular third molar sockets. *J Stomatol Oral Maxillofac Surg* 122:43 – 49. <https://doi.org/10.1016/j.jormas.2020.04.008>
56. Kapse S, Surana S, Satish M, Hussain SE, Vyas S, Thakur D (2019) Autologous platelet-rich fibrin: can it secure a better healing? *Oral Surg Oral Med Oral Pathol Oral Radiol* 127:8 – 18. <https://doi.org/10.1016/j.oooo.2018.08.010>
57. Kumar A, Singh G, Gaur A, Kumar S, Numan M, Jacob G, Kiran K (2020) Role of Platelet-rich Plasma in the Healing of Impacted Third Molar Socket: A Comparative Study on Central India Population. *J Contemp Dent Pract* 21:986 – 991
58. Kumar YR, Mohanty S, Verma M, Kaur RR, Bhatia P, Kumar VR, Chaudhary Z (2016) Platelet-rich fibrin: The benefits. *British Journal of Oral and Maxillofacial Surgery* 54:57 – 61
59. Miyamoto H, Nakamura T, Takashima H, Mizutani T, Morita M, Hirose M, Kurata M, Maeda M, Aoki N, Kato S, Shibuya Y (2020) Investigation of the analgesic effect of platelet-rich fibrin on postoperative pain after mandibular impacted wisdom tooth extraction. *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology* 32:237 – 240. <https://doi.org/10.1016/j.ajoms.2020.03.001>
60. Ozgul O, Senses F, Er N, Tekin U, Tuz HH, Alkan A, Kocyigit ID, Atil F (2015) Efficacy of platelet rich fibrin in the reduction of the pain and swelling after impacted third molar surgery: randomized multicenter split-mouth clinical trial. *Head Face Med* 11:37. <https://doi.org/10.1186/s13005-015-0094-5>

61. Özveri Koyuncu B, Işık G, Özden Yüce M, Günbay S, Günbay T (2020) Effect of concentrated growth factor (CGF) on short-term clinical outcomes after partially impacted mandibular third molar surgery: A split-mouth randomized clinical study. *J Stomatol Oral Maxillofac Surg* 121:118 – 123. <https://doi.org/10.1016/j.jormas.2019.07.002>
62. Ritto FG, Pimentel T, Canellas JVS, Junger B, Cruz M, Medeiros PJ (2019) Randomized double-blind clinical trial evaluation of bone healing after third molar surgery with the use of leukocyte- and platelet-rich fibrin. *Int J Oral Maxillofac Surg* 48:1088 – 1093. <https://doi.org/10.1016/j.ijom.2019.01.020>
63. Sybil D, Sawai M, Faisal M, Singh S, Jain V (2020) Platelet-Rich Fibrin for Hard- and Soft-Tissue Healing in Mandibular Third Molar Extraction Socket. *Ann Maxillofac Surg* 10:102 – 107. https://doi.org/10.4103/ams.ams_228_19
64. Uyanık LO, Bilginaylar K, Etikan İ (2015) Effects of platelet-rich fibrin and piezosurgery on impacted mandibular third molar surgery outcomes. *Head Face Med* 11:25. <https://doi.org/10.1186/s13005-015-0081-x>
65. Zahid TM, Nadershah M (2019) Effect of Advanced Platelet-rich Fibrin on Wound Healing after Third Molar Extraction: A Split-mouth Randomized Double-blind Study. *J Contemp Dent Pract* 20:1164 – 1170
66. Kumar N, Prasad K, Ramanujam L, K R, Dexith J, Chauhan A (2015) Evaluation of treatment outcome after impacted mandibular third molar surgery with the use of autologous platelet-rich fibrin: a randomized controlled clinical study. *J Oral Maxillofac Surg* 73:1042 – 1049. <https://doi.org/10.1016/j.joms.2014.11.013>
67. Dutta SR, Passi D, Singh P, Sharma S, Singh M, Srivastava D (2016) A randomized comparative prospective study of platelet-rich plasma, platelet-rich fibrin, and hydroxyapatite as a graft material for mandibular third molar extraction socket healing. *Natl J Maxillofac Surg* 7:45 – 51. <https://doi.org/10.4103/0975-5950.196124>
68. Afat İM, Akdoğan ET, Gönül O (2018) Effects of Leukocyte- and Platelet-Rich Fibrin Alone and Combined With Hyaluronic Acid on Pain, Edema, and Trismus After Surgical Extraction of Impacted Mandibular Third Molars. *J Oral Maxillofac Surg* 76:926 – 932. <https://doi.org/10.1016/j.joms.2017.12.005>
69. Jeyaraj PE, Chakranarayan A (2018) Soft Tissue Healing and Bony Regeneration of Impacted Mandibular Third Molar Extraction Sockets, Following Postoperative Incorporation of Platelet-rich Fibrin. *Ann Maxillofac Surg* 8:10 – 18. https://doi.org/10.4103/ams.ams_185_17
70. Nourwali I (2021) The effects of platelet-rich fibrin on post-surgical complications following removal of impacted wisdom teeth: A pilot study. *J Taibah Univ Med Sci* 16:521 – 528. <https://doi.org/10.1016/j.jtumed.2021.02.004>
71. Ogundipe OK, Ugboko VI, Owotade FJ (2011) Can autologous platelet-rich plasma gel enhance healing after surgical extraction of mandibular third molars? *J Oral Maxillofac Surg* 69:2305 – 2310. <https://doi.org/10.1016/j.joms.2011.02.014>
72. Aftab A, Joshi UKM, Patil SKG, Hussain E, Bhatnagar S (2020) Efficacy of autologous platelet rich plasma gel in soft and hard tissue healing after surgical extraction of impacted mandibular third molar - A prospective study. *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology* 32:241 – 246. <https://doi.org/10.1016/j.ajoms.2020.03.008>
73. Hanif M, Sheikh MA (2021) Efficacy of platelet rich plasma (PRP) on mouth opening and pain after surgical extraction of mandibular third molars. *J Oral Med Oral Surg* 27:9. <https://doi.org/10.1051/mbcb/2020045>

74. Torul D, Omezli MM, Kahveci K (2020) Evaluation of the effects of concentrated growth factors or advanced platelet rich-fibrin on postoperative pain, edema, and trismus following lower third molar removal: A randomized controlled clinical trial. *J Stomatol Oral Maxillofac Surg* 121:646 – 651. <https://doi.org/10.1016/j.jormas.2020.02.004>
75. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, Miron R (2016) Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clinical Oral Investigations* 20:1 – 8. <https://doi.org/10.1007/s00784-016-1719-1>
76. Everts P, Onishi K, Jayaram P, Lana JF, Mautner K (2020) Platelet-Rich Plasma: New Performance Understandings and Therapeutic Considerations in 2020. *Int J Mol Sci* 21:7794. <https://doi.org/10.3390/ijms21207794>
77. Kuffler DP (2018) Variables affecting the potential efficacy of PRP in providing chronic pain relief. *J Pain Res* 12:109 – 116. <https://doi.org/10.2147/JPR.S190065>
78. Bielecki T, Dohan Ehrenfest DM (2012) Platelet-rich plasma (PRP) and Platelet-Rich Fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. *Curr Pharm Biotechnol* 13:1121 – 1130. <https://doi.org/10.2174/138920112800624292>
79. Wang X, Fok MR, Pelekos G, Jin L, Tonetti MS (2022) In Vitro and Ex Vivo Kinetic Release Profile of Growth Factors and Cytokines from Leucocyte- and Platelet-Rich Fibrin (L-PRF) Preparations. *Cells* 11:2089. <https://doi.org/10.3390/cells11132089>
80. Castro AB, Herrero ER, Slomka V, Pinto N, Teughels W, Quirynen M (2019) Antimicrobial capacity of Leucocyte-and Platelet Rich Fibrin against periodontal pathogens. *Sci Rep* 9:8188. <https://doi.org/10.1038/s41598-019-44755-6>
81. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang B-S, Nally M, Lanata N, Wang H-L, Quirynen M (2018) The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. *Platelets* 29:171 – 184. <https://doi.org/10.1080/09537104.2017.1293812>
82. Miron R, Choukroun J, Ghanaati S (2018) Controversies related to scientific report describing g-forces from studies on platelet-rich fibrin: Necessity for standardization of relative centrifugal force values. *International Journal of Growth Factors and Stem Cells in Dentistry* 1:80. https://doi.org/10.4103/GFSC.GFSC_23_18
83. Miron RJ, Choukroun J, Ghanaati S (2019) Reply from authors: RE: Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response: Necessity for standardization of relative centrifugal force values in studies on platelet-rich fibrin. *J Periodontol* 90:122 – 125. <https://doi.org/10.1002/JPER.18-0329>
84. Cuschieri S (2019) The CONSORT statement. *Saudi J Anaesth* 13:S27 – S30. https://doi.org/10.4103/sja.SJA_559_18
85. Lambert J (2011) Statistics in brief: how to assess bias in clinical studies? *Clin Orthop Relat Res* 469:1794 – 1796. <https://doi.org/10.1007/s11999-010-1538-7>
86. Al-Moraissi EA, Elmansi YA, Al-Sharaee YA, Alrmali AE, Alkhutari AS (2016) Does the piezoelectric surgical technique produce fewer postoperative sequelae after lower third molar surgery than conventional rotary instruments? A systematic review and meta analysis. *Int J Oral Maxillofac Surg* 45:383 – 391. <https://doi.org/10.1016/j.ijom.2015.10.005>
87. Yamada S-I, Hasegawa T, Yoshimura N, Hakoyama Y, Nitta T, Hirahara N, Miyamoto H, Yoshimura H, Ueda N, Yamamura Y, Okuyama H, Takizawa A, Nakanishi Y, Iwata E, Akita D, Itoh R, Kubo K, Kondo S, Hata H, Koyama Y, Miyamoto Y, Nakahara H, Akashi M, Kirita T, Shibuya

Y, Umeda M, Kurita H (2022) Prevalence of and risk factors for postoperative complications after lower third molar extraction: A multicenter prospective observational study in Japan. *Medicine (Baltimore)* 101:e29989. <https://doi.org/10.1097/MD.00000000000029989>

Supplementary Material

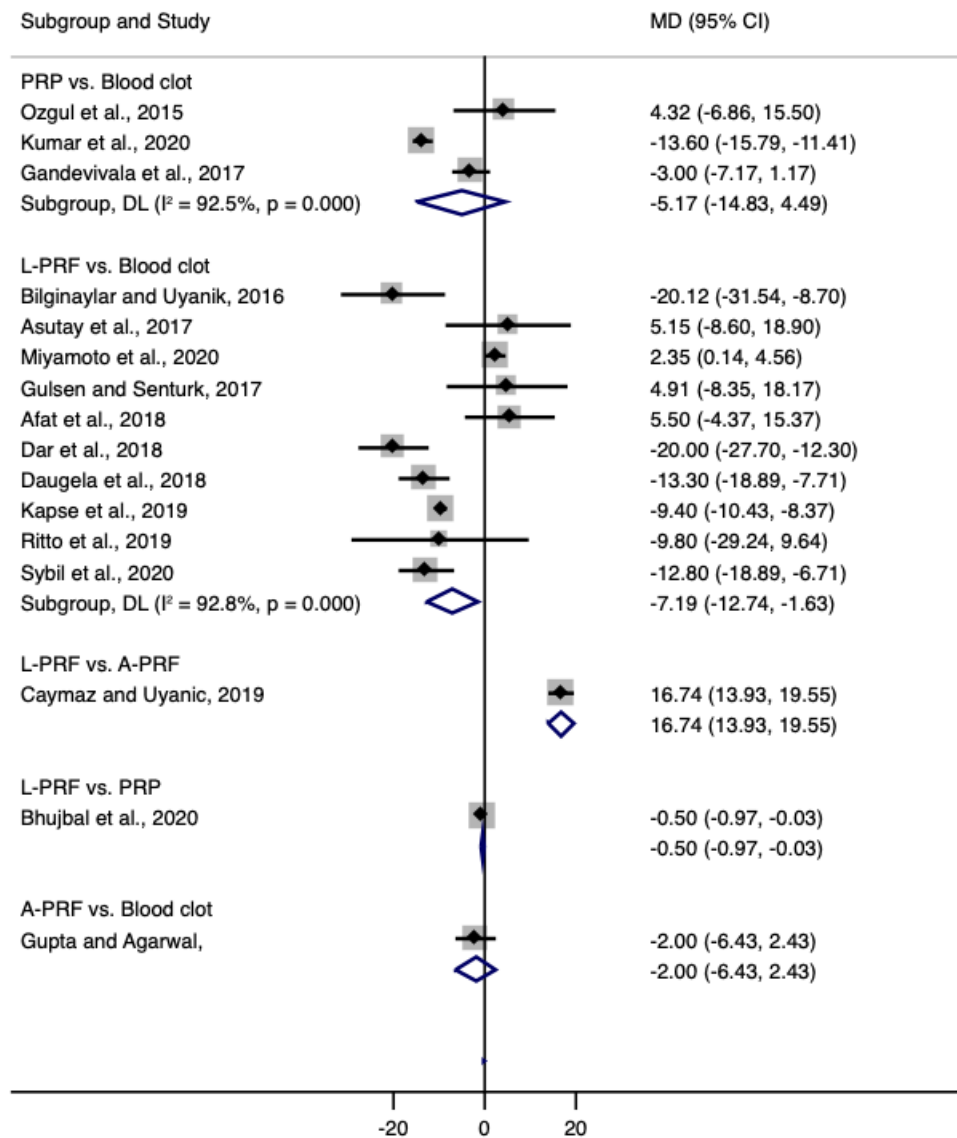
Supplementary Table 1. Studies excluded after reading the full texts, and the reasons for exclusion (n=7).

	<i>Excluded study</i>	<i>Reason for exclusion</i>
1.	Vivek and Sripathi, 2009	The study design is not a randomized clinical trial.
2.	Rutkowski et al., 2010	The study design is not a randomized clinical trial.
3.	Haraji et al., 2012	The sample consists of patients who use contraceptives, are smokers, and have bruxism or alveolar osteitis.
4.	Singh et al., 2012	The study design is not a randomized clinical trial.
5.	Unsal and Erbasar, 2018	Sample composed of smokers.
6.	Yuce and Komerik, 2019	The sample consists of patients with alveolar osteitis.
7.	Alfarraj and Karabit, 2021	Sample composed of smokers.

REFERENCES

1. Vivek GK, Sripathi RBH. Potential for osseous regeneration of platelet rich plasma: a comparative study in mandibular third molar sockets. J Maxillofac Oral Surg. 2009; 8(4):308-11.
2. Rutkowski JL et al. Platelet Rich Plasma to Facilitate Wound Healing Following Tooth Extraction. Journal of Oral Implantology. 2010; 36(1):11-33.
3. Haraji A et al. Effect of plasma rich in growth factors on alveolar osteitis. National Journal of Maxillofacial Surgery. 2012; 3(1):38-41.
4. Singh A, Kohli M, Gupta N. Platelet Rich Fibrin: A Novel Approach for Osseous Regeneration. J Maxillofac Oral Surg. 2012; 11(4):430-434.
5. Unsal H, Erbasar GNH. Evaluation of the Effect of Platelet-Rich Fibrin on the Alveolar Osteitis Incidence and Periodontal Probing Depth after Extracting Partially Erupted Mandibular Third Molars Extraction. Nigerian Journal Of Clinical Practice. 2018; 21: 201-5.
6. Yuce E, Komerik N. Potential Effects of Advanced Platelet Rich Fibrin as a Wound-Healing Accelerator in the Management of Alveolar Osteitis: A Randomized Clinical Trials. Nigerian Journal Of Clinical Practice. 2019; 22:1189-95.
7. Alfarraj M, Karabit Z. Evaluation of the Efficacy of Platelet Rich Fibrin on the Following Complications After Surgical Extraction of the Lower Third Molar in Smoker Patients. The New American Medical Journal. 2021;15(1):44-52.

Supplementary Figure 1. Pairwise comparisons for postoperative pain one day after surgery.



Supplementary Figure 2. Assessment of inconsistency between direct and indirect comparisons of interventions (postoperative pain one day after surgery).

	Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue
1	A-PRF:Blood_clot	1	-12.6036013423496		-2	-23.8478968863388	1.15116416111428	42.5446296115634	0.0385481223966034
2	A-PRF:L-PRF	1	-6.73701661167276	16.74	5.10789688633887	-21.8478968863389	-42.5446296115634	-1.1511641611143	0.0385481223966033
3	A-PRF:PRP	0	-7.22541585636881	NA	-7.22541585636881	NA	NA	NA	NA
4	L-PRF:Blood_clot	10	-5.86658473067688	-7.18230152489153	2.26725260182995	-9.49955412672149	-23.1551552555185	4.15604700207557	0.172739472934712
5	PRP:Blood_clot	3	-5.37818548598082	-5.16283601660808	-5.36503711012615	-0.0177989064819206	-17.0218687054571	16.9862708924933	0.99836307685244
6	L-PRF:PRP	1	-0.488399244696053	-0.5	-0.48220109351808	-0.0177989064819196	-17.0218687054571	16.9862708924933	0.99836307685244

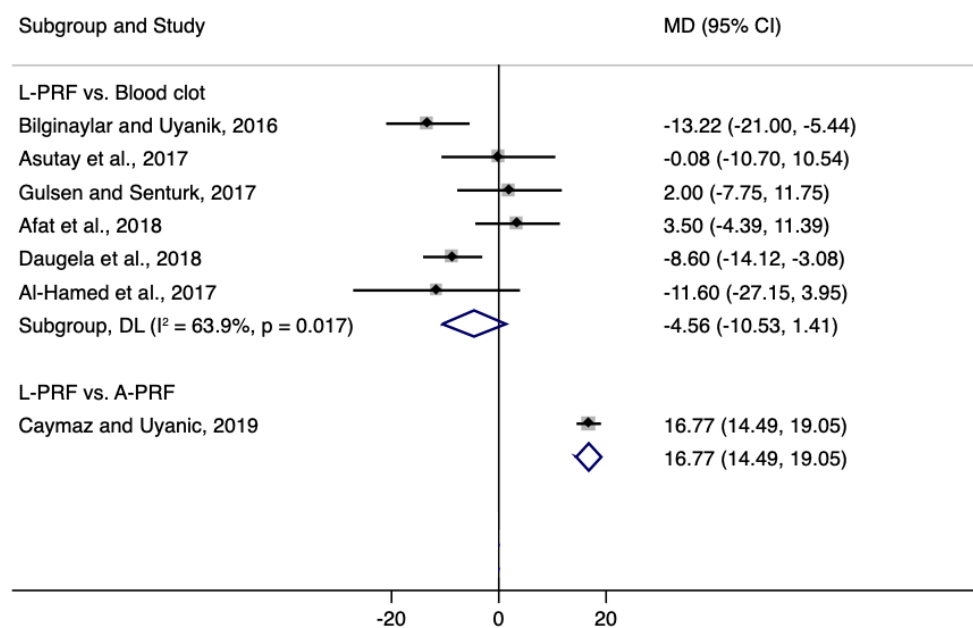
Supplementary Figure 3. Sensitivity analysis (postoperative pain one day after surgery). Significant results are in bold.

A-PRF			
-6.81 [-17.06; 3.45]	L-PRF		
-7.19 [-19.57; 5.19]	-0.38 [-8.53; 7.77]	PRP	
-12.54 [-22.84; -2.25]	-5.74 [-10.90; -0.58]	-5.36 [-12.78; 2.07]	Blood clot

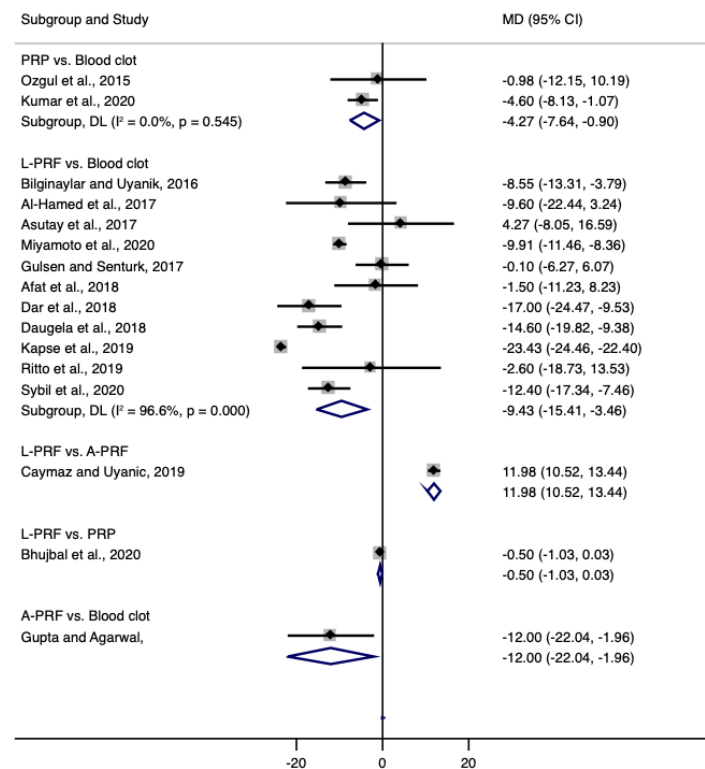
Supplementary Figure 4. Assessment of inconsistency between direct and indirect comparisons of interventions after removing studies with a parallel design (postoperative pain one day after surgery).

Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue
1 A-PRF:Blood_clot	1	-12.5431159620504	-2	-23.9524674454913	21.9524674454913	1.34697033072421	42.5579645602584	0.036790097319184
2 A-PRF:L-PRF	1	-6.8057327675274	16.74	5.21246744549132	-21.9524674454913	-42.5579645602584	-1.34697033072424	0.0367900973191837
3 A-PRF:PRP	0	-7.18777799014718	NA	-7.18777799014718	NA	NA	NA	NA
4 L-PRF:Blood_clot	8	-5.73738319452299	-7.37485070076869	2.25218906885863	-9.62703976962732	-23.3576567477248	4.10357720847015	0.16937875594435
5 PRP:Blood_clot	3	-5.3553379719032	-5.16283601660808	-5.21841456594787	-0.184347606053011	-17.1638430643226	16.7951478522166	0.983022720497
6 L-PRF:PRP	1	-0.382045222619783	-0.5	-0.315652393947	-0.184347606053	-17.1638430643226	16.7951478522166	0.983022720497001

Supplementary Figure 5. Pairwise comparisons for postoperative pain two days after surgery.



Supplementary Figure 6. Pairwise comparisons for postoperative pain three days after surgery.



Supplementary Figure 7. Assessment of inconsistency between direct and indirect comparisons of interventions (postoperative pain three days after surgery).

	Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue
1	A_PRFBlood_clot	1	-16.9102262246741		-12	-21.0498340793851	-18.8020413664548	36.9017095252248	0.524225314225848
2	A_PRFL_PRFBlood_clot	1	-8.22112490763487	11.98		-2.930165920615	-9.049834079385	-36.9017095252248	18.8020413664548
3	A_PRFBlood_clot	0	-11.9442674069877	NA		-11.9442674069877	NA	NA	NA
4	L_PRFBlood_clot	11	-8.68910131703919	-9.42658622556892		-2.11305270256245	-7.33353352300647	-25.7054631542295	11.0383961082166
5	PRFBlood_clot	2	-4.96595881768639	-4.27141162695456		-8.52414120401119	5.47272957705664	-17.850183245078	28.7956423991913
6	L_PRFBlood_clot	1	-3.7231424993528	-0.5		-5.97272957705665	5.47272957705665	-17.850183245078	28.7956423991913

Supplementary Figure 8. Sensitivity analysis (postoperative pain three days after surgery). Significant results are in bold.

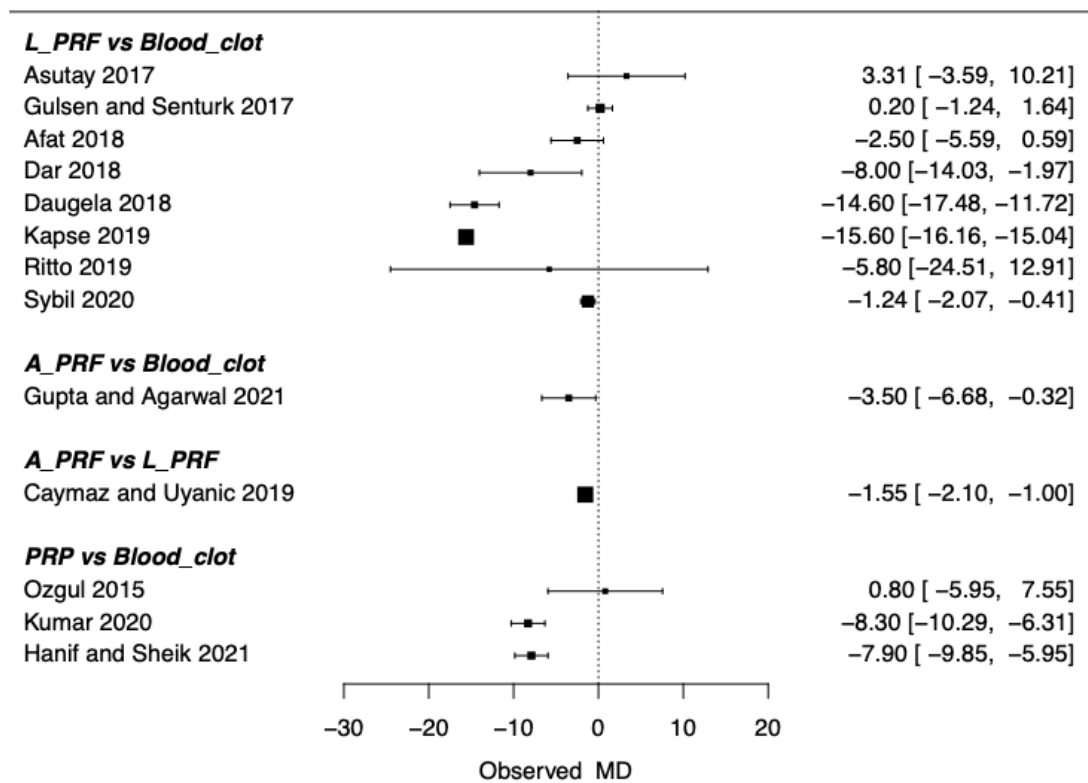
A-PRF			
-7.94 [-21.82; 5.95]	L-PRF		
-12.06 [-29.60; 5.48]	-4.13 [-15.84; 7.59]	PRP	
-17.26 [-31.34; -3.18]	-9.32 [-15.74; -2.90]	-5.20 [-16.46; 6.06]	Blood clot

Supplementary Figure 9. Assessment of inconsistency between direct and indirect comparisons of interventions after removing studies with a parallel design (postoperative pain three days after surgery).

Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue	
1 A_PRF:Blood_clot	1	-17.2621201093814		-12	-21.8435005688038	9.84350056880382	-18.3808532769531	38.0678544145608	0.494256020558576
2 A_PRF:L_PRF	1	-7.93736813608725	11.98		-2.13649943119623	-9.84350056880377	-38.0678544145607	18.3808532769532	0.494256020558578
3 A_PRF:PRP	0	-12.0642590425719	NA		-12.0642590425719	NA	NA	NA	NA
4 L_PRF:Blood_clot	8	-9.32475197329417	-10.4437328121654		-2.10999095082872	-8.33374186133671	-27.1631928483922	10.4957091257188	0.385688903707806
5 PRP:Blood_clot	2	-5.19786106680953	-4.2714116		-9.32392821037363	6.27581120805801	-17.4502348960493	30.0018573121653	0.604156234035813
6 L_PRF:PRP	1	-4.12689090648463	-0.5		-6.77581120805806	6.27581120805806	-17.4502348960492	30.0018573121653	0.604156234035811

Supplementary Figure 10. Pairwise comparisons for postoperative pain seven days after surgery.

Individual study results (for all studies) grouped by treatment comparison



Supplementary Figure 11. Assessment of inconsistency between direct and indirect comparisons of interventions (postoperative pain seven days after surgery).

	Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue
1	A_PRF:Blood_clot	1	-5.1400777051297	-3.5	-6.59811493620816	3.09811493620816	-6.27397982333029	12.4702096957466	0.517048699012995
2	A_PRF:L_PRF	1	-0.255299184386681	1.55	1.54811493620815	-3.09811493620815	-12.4702096957466	6.2739798233303	0.517048699012997
3	A_PRF:PRP	0	-0.654643128166252	NA	-0.654643128166252	NA	NA	NA	NA
4	L_PRF:Blood_clot	10	-4.88477858612629	-4.95784684828745	-3.5674901373237	-1.54035671096375	-7.49190841547646	4.41119499354895	0.611965379994602
5	PRP:Blood_clot	4	-4.48543464234671	-4.13344961430157	-4.82447364649468	0.431024032193107	-6.81217619317434	7.67422425756056	0.907151388408841
6	L_PRF:PRP	1	-0.399343943779571	-0.1	-0.531024032193108	0.431024032193108	-6.81217619317435	7.67422425756056	0.90715138840884

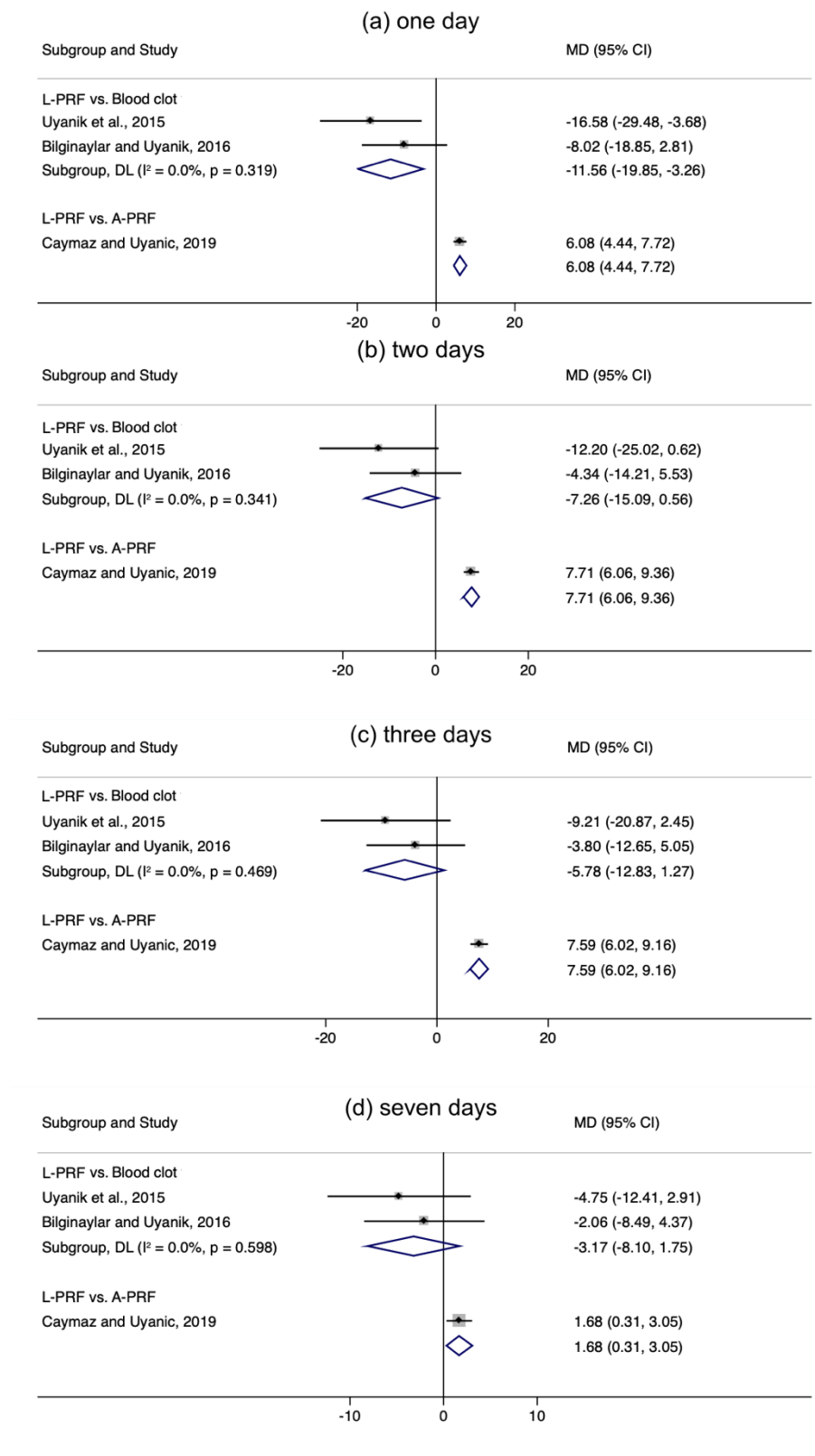
Supplementary Figure 12. Sensitivity analysis (postoperative pain seven days after surgery). Significant results are in bold.

A-PRF			
-0.24 [-5.11; 4.63]	L-PRF		
-0.66 [-6.38; 5.07]	-0.42 [-3.93; 3.10]	PRP	
-5.12 [-10.05; -0.20]	-4.88 [-7.08; -2.68]	-4.47 [-7.59; -1.34]	Blood clot

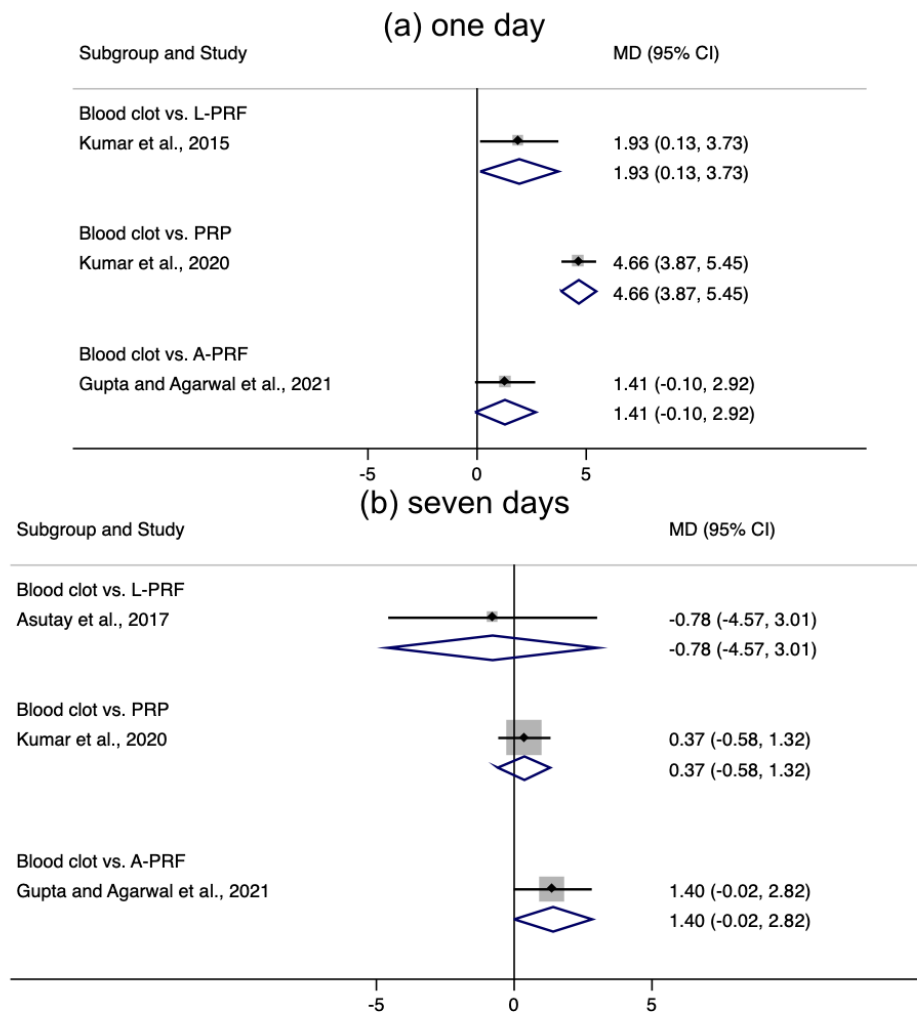
Supplementary Figure 13. Assessment of inconsistency between direct and indirect comparisons of interventions after removing studies with a parallel design (postoperative pain seven days after surgery).

	Comparison	No.Studies	NMA	Direct	Indirect	Difference	Diff_95CI_lower	Diff_95CI_upper	pValue
1	A_PRF:Blood_clot	1	-5.12221072103197	-3.5	-6.59548631226926	3.09548631226926	-6.76827648000906	12.9592491045476	0.538499313996355
2	A_PRF:L_PRF	1	-0.239328565789362	1.55	1.54548631226925	-3.09548631226925	-12.9592491045476	6.76827648000906	0.538499313996355
3	A_PRF:PRP	0	-0.656469292779753	NA	-0.656469292779753	NA	NA	NA	NA
4	L_PRF:Blood_clot	9	-4.88288215524261	-5.10652051906213	-3.54631528608639	-1.56020523297574	-7.8399225115503	4.71951204559882	0.626289505885098
5	PRP:Blood_clot	3	-4.46574142825222	-4.36971455025036	-4.82414919724379	0.454434646993427	-7.20388509250431	8.11275438649116	0.907413444913963
6	L_PRF:PRP	1	-0.41714072699039	-0.1	-0.554434646993439	0.454434646993439	-7.2038850925043	8.11275438649118	0.907413444913961

Supplementary Figure 14. Pairwise comparisons for trismus (percentage of interincisal opening reduction) one day after surgery.



Supplementary Figure 15. Pairwise comparisons for trismus (interincisal opening distance) one day after surgery.



Considerações finais

4 – CONSIDERAÇÕES FINAIS

O novo protocolo de fibrina rica em plaquetas proposto pela equipe de pesquisa, PRO-PRF, demonstrou ser capaz de produzir membranas gigantes de PRF com maior resistência sem perda da celularidade. Além disso, a utilização de tubos de plásticos sem aditivos proporcionou vantagens significativas, permitindo a personalização do tamanho e da espessura da membrana produzida, bem como a incorporação de biomateriais e/ou fármacos durante o processo de produção. Esses resultados apontam para um avanço no campo dos concentrados sanguíneos e indicam que o PRO-PRF pode ser considerado uma nova geração de concentrados sanguíneos, com parâmetros que justificam sua utilização em diversas aplicações clínicas.

Os tubos de plástico sem aditivos Vacuette® se mostraram como uma alternativa viável e segura para a produção de membranas de PRF. Esses tubos têm a vantagem de serem mais acessíveis, financeiramente, do que os tubos S-PRF® PROCESS, que, atualmente, são considerados tubos de referência para produção de PRF.

Apesar da baixa certeza de evidência foi possível observar que, dentre os concentrados sanguíneos avaliados, o A-PRF foi o que apresentou o melhor desempenho na redução da dor pós-operatória e trismo, após extrações de terceiros molares inferiores. Considerando o nível de evidência muito baixo e os vieses dos ensaios clínicos randomizados atualmente existentes na literatura, comparando as sequelas inflamatórias das extrações de terceiros molares, nossa próxima etapa será realizar ensaio clínico randomizado com essa temática, apoiado em diretrizes sólidas para ajudar na elucidação da temática.

Referências

REFERÊNCIAS*

1. Alonzo M, Primo FA, Kumar SA, Mudloff JA, Dominguez E, Fregoso G, et al. Bone tissue engineering techniques, advances and scaffolds for treatment of bone defects. *Curr Opin Biomed Eng.* março de 2021;17:100248. <https://doi.org/10.1016/j.cobme.2020.100248>
2. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. *Tissue Eng Part B Rev.* fevereiro de 2017;23(1):83–99. <https://doi.org/10.1089/ten.teb.2016.0233>
3. Endy D. Foundations for engineering biology. *Nature.* 24 de novembro de 2005;438(7067):449–53. <https://doi.org/10.1038/nature04342>
4. Sammartino G, Tia M, Marenzi G, di Lauro AE, D'Agostino E, Claudio PP. Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. *J Oral Maxillofac Surg.* junho de 2005;63(6):766–70. <https://doi.org/10.1016/j.joms.2005.02.010>
5. Mikos AG, Herring SW, Ochareon P, Elisseeff J, Lu HH, Kandel R, et al. Engineering complex tissues. *Tissue Eng.* dezembro de 2006;12(12):3307–39. <https://doi.org/10.1089/ten.2006.12.3307>
6. Ansari MAA, Golebiowska AA, Dash M, Kumar P, Jain PK, Nukavarapu SP, et al. Engineering biomaterials to 3D-print scaffolds for bone regeneration: practical and theoretical consideration. *Biomater Sci.* 31 de maio de 2022;10(11):2789–816. <https://doi.org/10.1039/D2BM00035K>
7. Rouwkema J, Khademhosseini A. Vascularization and Angiogenesis in Tissue Engineering: Beyond Creating Static Networks. *Trends Biotechnol.* setembro de 2016;34(9):733–45. <https://doi.org/10.1016/j.tibtech.2016.03.002>
8. Padilla S, Sánchez M, Orive G, Anitua E. Human-Based Biological and Biomimetic Autologous Therapies for Musculoskeletal Tissue Regeneration. *Trends Biotechnol.* março de 2017;35(3):192–202. <https://doi.org/10.1016/j.tibtech.2016.09.008>
9. Mihaylova Z, Mitev V, Stanimirov P, Isaeva A, Gateva N, Ishkitiev N. Use of platelet concentrates in oral and maxillofacial surgery: an overview. *Acta Odontol Scand.* janeiro de 2017;75(1):1–11. <https://doi.org/10.1080/00016357.2016.1236985>
10. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* março de 2006;101(3):e37–44. <https://doi.org/10.1016/j.tripleo.2005.07.008>
11. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg.* abril de 2004;62(4):489–96. <https://doi.org/10.1016/j.joms.2003.12.003>
12. Anitua E, Prado R, Troya M, Zalduendo M, de la Fuente M, Pino A, et al. Implementation of a more physiological plasma rich in growth factor (PRGF) protocol: Anticoagulant removal and reduction in activator concentration. *Platelets.* julho de 2016;27(5):459–66. <https://doi.org/10.3109/09537104.2016.1143921>

13. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* março de 2006;101(3):e45-50. <https://doi.org/10.1016/j.tripleo.2005.07.009>
14. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* março de 2006;101(3):e51-55. <https://doi.org/10.1016/j.tripleo.2005.07.010>
15. Zwitter K, Kirnbauer B, Jakse N, Schlenke P, Mischak I, Ghanaati S, et al. Growth Factor Release within Liquid and Solid PRF. *J Clin Med.* 29 de agosto de 2022;11(17):5070. <https://doi.org/10.3390/jcm11175070>
16. Burnouf T, Goubran HA, Chen TM, Ou KL, El-Ekiaby M, Radosevic M. Blood-derived biomaterials and platelet growth factors in regenerative medicine. *Blood Rev.* março de 2013;27(2):77–89. <https://doi.org/10.1016/j.blre.2013.02.001>
17. Dai R, Wang Z, Samanipour R, Koo KI, Kim K. Adipose-Derived Stem Cells for Tissue Engineering and Regenerative Medicine Applications. *Stem Cells Int.* 2016;2016:6737345. <https://doi.org/10.1155/2016/6737345>
18. Vasilikos I, Beck J, Ghanaati S, Grauvogel J, Nisyrios T, Grapatsas K, et al. Integrity of dural closure after autologous platelet rich fibrin augmentation: an in vitro study. *Acta Neurochir (Wien).* abril de 2020;162(4):737–43. <https://doi.org/10.1007/s00701-020-04254-4>
19. Shashank B, Bhushan M. Injectable Platelet-Rich Fibrin (PRF): The newest biomaterial and its use in various dermatological conditions in our practice: A case series. *J Cosmet Dermatol.* maio de 2021;20(5):1421–6. <https://doi.org/10.1111/jocd.13742>
20. Huang J, Teh BM, Zhou C, Shi Y, Shen Y. Tympanic membrane regeneration using platelet-rich fibrin: a systematic review and meta-analysis. *Eur Arch Otorhinolaryngol.* fevereiro de 2022;279(2):557–65. <https://doi.org/10.1007/s00405-021-06915-1>
21. Nacopoulos C. Use of Platelet Rich Fibrin in Facial Aesthetics and Rejuvenation. Em: *Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications* [Internet]. John Wiley & Sons, Ltd; 2017 [citado 7 de abril de 2019]. p. 215–35. <https://doi.org/10.1002/9781119406792.ch13>
22. Lyrís V, Millen C, Besi E, Pace-Balzan A. Effect of leukocyte and platelet rich fibrin (L-PRF) on stability of dental implants. A systematic review and meta-analysis. *Br J Oral Maxillofac Surg.* 19 de janeiro de 2021;S0266-4356(21)00015-2. <https://doi.org/10.1016/j.bjoms.2021.01.001>
23. Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, Guillemette V, et al. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clin Oral Invest.* 1º de julho de 2017;21(6):1913–27. <https://doi.org/10.1007/s00784-017-2133-z>
24. Narendran N, Anegundi RV, Shenoy SB, Chandran T. Autologous platelet-rich fibrin as an adjunct to non-surgical periodontal therapy-A follow up clinical pilot study.

- Wound Repair Regen. janeiro de 2022;30(1):140–5.
<https://doi.org/10.1111/wrr.12979>
25. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Yaprak E, Toker H, et al. A novel platelet concentrate: titanium-prepared platelet-rich fibrin. Biomed Res Int. 2014;2014:209548. <https://doi.org/10.1155/2014/209548>
 26. Marchetti E, Mancini L, Bernardi S, Bianchi S, Cristiano L, Torge D, et al. Evaluation of Different Autologous Platelet Concentrate Biomaterials: Morphological and Biological Comparisons and Considerations. Materials (Basel). 15 de maio de 2020;13(10):2282. <https://doi.org/10.3390/ma13102282>
 27. Anitua E, Nurden P, Prado R, Nurden AT, Padilla S. Autologous fibrin scaffolds: When platelet- and plasma-derived biomolecules meet fibrin. Biomaterials. fevereiro de 2019;192:440–60. <https://doi.org/10.1016/j.biomaterials.2018.11.029>
 28. Choukroun J, Ghanaati S. Introducing the Low-Speed Centrifugation Concept. Em: Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications [Internet]. John Wiley & Sons, Ltd; 2017 [citado 7 de abril de 2019]. p. 33–46. <https://doi.org/10.1002/9781119406792.ch3>
 29. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept. Eur J Trauma Emerg Surg. fevereiro de 2018;44(1):87–95. <https://doi.org/10.1007/s00068-017-0767-9>
 30. El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orlowska A, Dias A, et al. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: a proof of concept of LSCC (low speed centrifugation concept). Eur J Trauma Emerg Surg. junho de 2019;45(3):467–79. <https://doi.org/10.1007/s00068-017-0785-7>
 31. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandam U, Zhang Y, Choukroun J. Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. J Periodontol. 2017;88(1):112–21. <https://doi.org/10.1902/jop.2016.160443>
 32. Kubesch A, Barbeck M, Al-Maawi S, Orlowska A, Booms PF, Sader RA, et al. A low-speed centrifugation concept leads to cell accumulation and vascularization of solid platelet-rich fibrin: an experimental study in vivo. Platelets. 2019;30(3):329–40. <https://doi.org/10.1080/09537104.2018.1445835>
 33. Kawase T, Okuda K. Comprehensive Quality Control of the Regenerative Therapy Using Platelet Concentrates: The Current Situation and Prospects in Japan. Biomed Res Int. 2018;2018:6389157. <https://doi.org/10.1155/2018/6389157>
 34. Tsujino T, Masuki H, Nakamura M, Isobe K, Kawabata H, Aizawa H, et al. Striking Differences in Platelet Distribution between Advanced-Platelet-Rich Fibrin and Concentrated Growth Factors: Effects of Silica-Containing Plastic Tubes. J Funct Biomater. 17 de setembro de 2019;10(3):E43. <https://doi.org/10.3390/jfb10030043>
 35. Tsujino T, Takahashi A, Yamaguchi S, Watanabe T, Isobe K, Kitamura Y, et al. Evidence for Contamination of Silica Microparticles in Advanced Platelet-Rich

- Fibrin Matrices Prepared Using Silica-Coated Plastic Tubes. *Biomedicines*. 19 de junho de 2019;7(2):45. <https://doi.org/10.3390/biomedicines7020045>
36. Masuki H, Isobe K, Kawabata H, Tsujino T, Yamaguchi S, Watanabe T, et al. Acute cytotoxic effects of silica microparticles used for coating of plastic blood-collection tubes on human periosteal cells. *Odontology*. outubro de 2020;108(4):545–52. <https://doi.org/10.1007/s10266-020-00486-z>
 37. Bai MY, Wang CW, Wang JY, Lin MF, Chan WP. Three-dimensional structure and cytokine distribution of platelet-rich fibrin. *Clinics (Sao Paulo)*. 1º de fevereiro de 2017;72(2):116–24. [https://doi.org/10.6061/clinics/2017\(02\)09](https://doi.org/10.6061/clinics/2017(02)09)
 38. Yanmin Z. Biological Additives and Platelet Concentrates for Tissue Engineering on Regenerative Dentistry: Basic Science and Concise Review. *Asian Journal of Pharmaceutics (AJP)*: Free full text articles from Asian J Pharm [Internet]. 15 de dezembro de 2017 [citado 7 de janeiro de 2022];11(04). <https://doi.org/10.22377/ajp.v11i04.1621>
 39. Mudalal M, Sun X, Li X, Zhou Y. The evaluation of leukocyte-platelet rich fibrin as an antiinflammatory autologous biological additive: A novel in vitro study. *Saudi Medical Journal*. 1º de julho de 2019;40(7):657–69. <https://doi.org/10.15537/smj.2019.7.24302>
 40. Kargarpour Z, Nasirzade J, Strauss FJ, Di Summa F, Hasannia S, Müller HD, et al. Platelet-rich fibrin suppresses in vitro osteoclastogenesis. *J Periodontol*. março de 2020;91(3):413–21. <https://doi.org/10.1002/JPER.19-0109>
 41. Zhang J, Yin C, Zhao Q, Zhao Z, Wang J, Miron RJ, et al. Anti-inflammation effects of injectable platelet-rich fibrin via macrophages and dendritic cells. *J Biomed Mater Res A*. janeiro de 2020;108(1):61–8. <https://doi.org/10.1002/jbm.a.36792>
 42. Wang Z, Mudalal M, Sun Y, Liu Y, Wang J, Wang Y, et al. The Effects of Leukocyte-Platelet Rich Fibrin (L-PRF) on Suppression of the Expressions of the Pro-Inflammatory Cytokines, and Proliferation of Schwann Cell, and Neurotrophic Factors. *Sci Rep*. 12 de fevereiro de 2020;10(1):2421. <https://doi.org/10.1038/s41598-020-59319-2>
 43. Kaewkumnert S, Phithaksinsuk K, Changpoo C, Nochit N, Muensaiyat Y, Wilaipornsawai S, et al. Comparison of intraosseous and submucosal dexamethasone injection in mandibular third molar surgery: a split-mouth randomized clinical trial. *Int J Oral Maxillofac Surg*. abril de 2020;49(4):529–35. <https://doi.org/10.1016/j.ijom.2019.10.006>
 44. Falci SGM, Lima TC, Martins CC, Santos CRR dos, Pinheiro MLP. Preemptive Effect of Dexamethasone in Third-Molar Surgery: A Meta-Analysis. *Anesthesia Progress*. 1º de setembro de 2017;64(3):136–43. <https://doi.org/10.2344/anpr-64-05-08>
 45. Herrera-Briones FJ, Prados Sánchez E, Reyes Botella C, Vallecillo Capilla M. Update on the use of corticosteroids in third molar surgery: systematic review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol*. novembro de 2013;116(5):e342–351. <https://doi.org/10.1016/j.oooo.2012.02.027>
 46. Hadad H, Santos AFP, de Jesus LK, Poli PP, Mariano RC, Theodoro LH, et al. Photobiomodulation Therapy Improves Postoperative Pain and Edema in Third

- Molar Surgeries: A Randomized, Comparative, Double-Blind, and Prospective Clinical Trial. *J Oral Maxillofac Surg.* janeiro de 2022;80(1):37.e1-37.e12.
<https://doi.org/10.1016/j.joms.2021.08.267>
47. Momeni E, Kazemi F, Sanaei-Rad P. Extraoral low-level laser therapy can decrease pain but not edema and trismus after surgical extraction of impacted mandibular third molars: a randomized, placebo-controlled clinical trial. *BMC Oral Health.* 20 de setembro de 2022;22(1):417. <https://doi.org/10.1186/s12903-022-02461-2>
 48. Glória JCR, Douglas-de-Oliveira DW, E Silva LDA, Falci SGM, Dos Santos CRR. Influence of ozonized water on pain, oedema, and trismus during impacted third molar surgery: a randomized, triple blind clinical trial. *BMC Oral Health.* 5 de fevereiro de 2020;20(1):41. <https://doi.org/10.1186/s12903-020-1029-5>
 49. Jaroń A, Preuss O, Grzywacz E, Trybek G. The Impact of Using Kinesio Tape on Non-Infectious Complications after Impacted Mandibular Third Molar Surgery. *Int J Environ Res Public Health.* 6 de janeiro de 2021;18(2):E399.
<https://doi.org/10.3390/ijerph18020399>
 50. Menziletoglu D, Guler AY, Basturk F, Isik BK. Comparison of Surgical Drain Placement With Use of Kinesiologic Tape on Postoperative Pain, Swelling, and Trismus in Impacted Mandibular Third Molar Surgery. *J Oral Maxillofac Surg.* novembro de 2020;78(11):1919.e1-1919.e7.
<https://doi.org/10.1016/j.joms.2020.06.023>
 51. Larsen MK, Kofod T, Starch-Jensen T. Therapeutic efficacy of cryotherapy on facial swelling, pain, trismus and quality of life after surgical removal of mandibular third molars: A systematic review. *J Oral Rehabil.* junho de 2019;46(6):563–73.
<https://doi.org/10.1111/joor.12789>
 52. Katz MS, Peters F, Elvers D, Winterhalder P, Kniha K, Möhlhenrich SC, et al. Effect of drain application on postoperative complaints after surgical removal of impacted wisdom teeth-a randomized observer-blinded split-mouth clinical trial. *Clin Oral Investig.* janeiro de 2021;25(1):345–53. <https://doi.org/10.1007/s00784-020-03464-5>
 53. Afat İM, Akdoğan ET, Gönül O. Effects of Leukocyte- and Platelet-Rich Fibrin Alone and Combined With Hyaluronic Acid on Pain, Edema, and Trismus After Surgical Extraction of Impacted Mandibular Third Molars. *J Oral Maxillofac Surg.* maio de 2018;76(5):926–32. <https://doi.org/10.1016/j.joms.2017.12.005>
 54. Kim TH, Kim SH, Sándor GK, Kim YD. Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing. *Arch Oral Biol.* maio de 2014;59(5):550–8.
<https://doi.org/10.1016/j.archoralbio.2014.02.004>
 55. Nowak JM, Surma S, Romańczyk M, Wojtowicz A, Filipiak KJ, Czerniuk MR. Assessment of the Effect of A-PRF Application during the Surgical Extraction of Third Molars on Healing and the Concentration of C-Reactive Protein. *Pharmaceutics.* 15 de setembro de 2021;13(9):1471.
<https://doi.org/10.3390/pharmaceutics13091471>
 56. da Silva MT, de Almeida Barros Mourão CF, Mello-Machado RC, Montemezzi P, de Lima Barbosa R, Sartoretto SC, et al. Effects of Leukocyte-Platelet-Rich Fibrin (L-PRF) on Pain, Soft Tissue Healing, Growth Factors, and Cytokines after Third

- Molar Extraction: A Randomized, Split-Mouth, Double-Blinded Clinical Trial. *Applied Sciences*. janeiro de 2021;11(4):1666. <https://doi.org/10.3390/app11041666>
57. Al-Hamed FS, Tawfik MAM, Abdelfadil E, Al-Saleh MAQ. Efficacy of Platelet-Rich Fibrin After Mandibular Third Molar Extraction: A Systematic Review and Meta-Analysis. *J Oral Maxillofac Surg*. junho de 2017;75(6):1124–35. <https://doi.org/10.1016/j.joms.2017.01.022>
58. Aftab A, Joshi UKM, Patil SKG, Hussain E, Bhatnagar S. Efficacy of autologous platelet rich plasma gel in soft and hard tissue healing after surgical extraction of impacted mandibular third molar - A prospective study. *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology*. 1º de julho de 2020;32(4):241–6. <https://doi.org/10.1016/j.ajoms.2020.03.008>
59. Barona-Dorado C, González-Regueiro I, Martín-Ares M, Arias-Irimia O, Martínez-González JM. Efficacy of platelet-rich plasma applied to post-extraction retained lower third molar alveoli. A systematic review. *Med Oral Patol Oral Cir Bucal*. 1º de março de 2014;19(2):e142-148. <https://doi.org/10.4317/medoral.19444>
60. Xiang X, Shi P, Zhang P, Shen J, Kang J. Impact of platelet-rich fibrin on mandibular third molar surgery recovery: a systematic review and meta-analysis. *BMC Oral Health*. 25 de julho de 2019;19(1):163. <https://doi.org/10.1186/s12903-019-0824-3>

Anexos

6. ANEXOS

Anexo 1: Parecer Consubstanciado do Comitê de Ética em Pesquisa



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: CARACTERIZAÇÃO MORFOLÓGICA DE CONCENTRADOS SANGUÍNEOS DE A-PRF. ESTUDO MORFOLÓGICO POR MICROSCOPIA DE LUZ E ELETRÔNICA DE TRANSMISSÃO

Pesquisador: Paula Dechichi

Área Temática:

Versão: 1

CAAE: 13857519.0.0000.5152

Instituição Proponente: Universidade Federal de Uberlândia/ UFU/ MG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.347.524

Apresentação do Projeto:

Conforme apresenta o protocolo: Os concentrados sanguíneos consistem em uma matriz autóloga de fibrina rica em plaquetas e leucócitos, composta por uma estrutura molecular, com citocinas e células tronco que favorecem o desenvolvimento da microvascularização e, consequentemente, a neoformação óssea e a regeneração tecidual. Podem ser utilizados de maneira isolada ou em associação com enxerto ósseo como material de preenchimento em alveolos pós-extração ou técnicas de levantamento do seio, sendo que com a sua arquitetura de fibrina e lenta liberação de fatores de crescimento e glicoproteínas ao longo de vários dias, pode melhorar o reparo de tecidos moles e duros enquanto protege sítios cirúrgicos e materiais enxertados.

METODOLOGIA

=>População: O estudo será conduzido no Laboratório de Biologia Celular localizado no Centro de Pesquisas Odontológico, Biomecânica, Biomateriais e Biologia Celular (CPbio) da Universidade Federal de Uberlândia (UFU). Os voluntários serão selecionados a partir de chamada através de cartazes e informativos no âmbito do hospital odontológico e faculdade de odontologia da UFU de 30/06/2019 a 30/10/2019.

Endereço: Av. João Naves de Ávila 2121- Bloco "1A", sala 224 - Campus Sta. Mônica
Bairro: Santa Mônica **CEP:** 38.408-144
UF: MG **Município:** UBERLÂNDIA
Telefone: (34)3239-4131 **Fax:** (34)3239-4335 **E-mail:** cep@propp.ufu.br

Anexo 2: Parecer Consubstanciado do Comitê de Ética em Pesquisa



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: CARACTERIZAÇÃO MORFOLÓGICA E MECÂNICA DE CONCENTRADOS SANGÜÍNEOS

Pesquisador: Paula Dechichi

Área Temática:

Versão: 3

CAAE: 31441520.2.0000.5152

Instituição Proponente: Universidade Federal de Uberlândia/ UFU/ MG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.115.144

Apresentação do Projeto:

Trata-se de análise de respostas que os(as) pesquisadores(as) apresentaram às pendências apontadas no parecer consubstanciado número 4.103.373.

Conforme apresenta o protocolo: Os concentrados sanguíneos tipo PRF consistem em uma matriz autóloga de fibrina rica em plaquetas e leucócitos, composta por uma estrutura molecular, com citocinas e células tronco, que favorecem o desenvolvimento da microvascularização e, conseqüentemente, a regeneração tecidual. Podem ser utilizados de maneira isolada ou em associação com enxerto, como material de preenchimento, sendo que com a sua arquitetura de fibrina e lenta liberação de fatores de crescimento, pode melhorar o reparo de tecidos moles e duros, além de proteger os sítios cirúrgicos e materiais enxertados. A hipótese apresentada pelos autores reflete sob a variação de força centrífuga relativa e tempo de centrifugação do sangue para obtenção de membranas L-PRF, A-PRF e dois protocolos de GM-PRF para verificar se haverá diferença entre elas, em relação ao número de plaquetas e leucócitos, rede de fibrina, resistência e dimensões.

Objetivo da Pesquisa:

O objetivo dos autores conforme descrição é analisar a morfologia, utilizando os aparelhos de

Endereço: Av. João Naves de Ávila 2121- Bloco "1A", sala 224 - Campus Sta. Mônica
Bairro: Santa Mônica **CEP:** 38.408-144
UF: MG **Município:** UBERLÂNDIA
Telefone: (34)3239-4131 **Fax:** (34)3239-4131 **E-mail:** cep@propp.ufu.br