

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-13982- -2	FECHA: 2015-08-26 09:54:42
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7
ACT: 476 TRASTITULARSOLI	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
LUIS FELIPE CASTILLO GIBSON

Asunto: Radicación: 14-13982- -2
 Trámite: 11
 Evento: 378
 Actuación: 476
 Oficio: 10146
 Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/ES2012/000196				
Fecha de Presentación Internacional	13/07/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N°14-13982-00000-0000	24/Enero/2014
Reivindicaciones:	Radicado N°14-13982-00000-0000	24/Enero/2014
Dibujos:	Radicado N°14-13982-00000-0000	24/Enero/2014
Secuencias:	Radicado N°14-13982-00000-0000	24/Enero/2014
Prioridad:	ES P 201100867	11/Julio/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las

reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 19 del capítulo reivindicatorio (Radicado N°. 14-13982-00000-0000).

Título de la solicitud: “PROCEDIMIENTO DE OBTENCIÓN DE UNA COMPOSICIÓN QUE CONTIENE FACTORES DE CRECIMIENTO A PARTIR DE UN COMPUESTO SANGUÍNEO Y COMPOSICIÓN OBTENIBLE POR DICHO PROCEDIMIENTO”

El problema planteado en esta solicitud consiste en la necesidad de obtener nuevos métodos de obtención de una composición para tratar de forma regular patologías crónicas y, particularmente, patologías crónicas de naturaleza autoinmune.

Para resolver este problema técnico la solicitud en estudio proporciona un método de obtención de una composición que contiene factores de crecimiento a partir de un compuesto sanguíneo y composición obtenible por dicho procedimiento.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 38/18.

4. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara puesto que menciona un “*Procedimiento de obtención de una composición que contiene factores de crecimiento, que comprende las fases de: a) disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados, b) aplicar un tratamiento térmico al plasma rico en plaquetas o al sobrenadante de plasma rico que en plaquetas, durante el cual se aumenta la temperatura del mismo, c) liofilizar dicho plasma rico en plaquetas o sobrenadante de plasma rico en plaquetas*” y hace referencia que las etapas b) y c) “*se realizan en orden indistinto*”, lo cual es impreciso y no permite distinguir el objeto de la invención del estado de la técnica. Se sugiere a la solicitante mencionar las características técnicas estructurales, es decir, las condiciones específicas de cada etapa, las cuales se encuentren debidamente sustentadas por medio de pruebas técnicas en el capítulo descriptivo.

La reivindicación 19 no es clara ni posee sustento puesto que menciona una composición que contiene factores de crecimiento, caracterizando esta por el procedimiento por el cual es obtenida la composición. Se recuerda a la solicitante que una composición se caracteriza estructuralmente, es decir, por los componentes y proporciones de los mismos, los cuales se encuentren debidamente sustentados en el capítulo descriptivo.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 29 de julio de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	EP 1066838	Bone tissue regenerating composition	10/Enero/2001
D2	Pietramaggiore G, Kaipainen A- Wound Repair Regen	Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds	2006

D3	EP 2077118	Concentré coagulo-actif de facteurs de croissance de plaquettes et son procédé de préparation	8/Julio/2009
D4	Weida Huang, Takashi Kimura- The Journal of Biochemistry	Purification and Properties of Epithelial Growth Inhibitor (EGI) from Human Platelets: Its Separation from Type β Transforming Growth Factor (TGF- β)	18/Noviembre/2008

El documento D1¹ divulga una composición para la regeneración de tejido óseo y un método para preparar dicha composición, que se utiliza principalmente pero no exclusivamente en cirugía oral y consiste en un gel rico en factores de crecimiento plasma (PRGF gel) obtenida a partir de la sangre del paciente. Con la ayuda de dicho gel, más rápido y más eficaz la regeneración ósea de las cavidades a ser tratada se obtiene en comparación con materiales y técnicas conocidas (Resumen).

El documento D2² divulga una composición que contiene factores de crecimiento de plaquetas para uso terapéutico y/o cosmético, que comprende preferiblemente los factores de crecimiento PDGF, TGF, IGF, EGF, CTGF, bFGF y VEGF (Resumen).

El documento D3³ divulga un método para preparar un concentrado de factores de crecimiento coagulable de plaquetas (Resumen).

El documento D3⁴ divulga métodos para purificar TGF- β a fin de obtener tal, en altos rendimientos (Resumen).

6. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 19 consiste en: *“Composición que contiene factores de crecimiento, obtenible según el procedimiento de cualquiera de las reivindicaciones 1 a 18”* (Radicado N°. 14-13982-00000-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Composición	Composición
factores de crecimiento	factores de crecimiento (reivindicación 1)

Las características esenciales de la composición de la reivindicación 19 habían sido divulgadas previamente en el documento D1, el cual da a conocer una composición la cual comprende factores de crecimiento (reivindicación 1).

En consecuencia, la reivindicación 19 carece de novedad, según lo divulgado en el documento D1.

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en: *“Procedimiento de obtención de una composición que contiene factores de crecimiento, que comprende las fases de:”* (Radicado N°. 14-

1 <http://worldwide.espacenet.com/publicationDetails/biblio?CC=EP&NR=1066838A1&KC=A1&FT=D>

2 <http://www.ncbi.nlm.nih.gov/pubmed/17014669>

3 <http://worldwide.espacenet.com/publicationDetails/biblio?CC=EP&NR=2077118A1&KC=A1&FT=D>

4 [http://www.researchgate.net/publication/19690059_Purification_and_properties_of_epithelial_growth_inhibitor_\(EGI\)_from_human_platelets_its_separation_from_type_beta_transforming_growth_factor_\(TGF-beta\)](http://www.researchgate.net/publication/19690059_Purification_and_properties_of_epithelial_growth_inhibitor_(EGI)_from_human_platelets_its_separation_from_type_beta_transforming_growth_factor_(TGF-beta))

13982-00000-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D3
Procedimiento	Procedimiento	Procedimiento
a) Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados	Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Resumen, Pág. 573, Col. 1).	Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Párr. [0011]).
b) Aplicar un tratamiento térmico al plasma rico en plaquetas o al sobrenadante de plasma rico en plaquetas	No se menciona	Aplicar un tratamiento térmico al plasma rico en plaquetas o al sobrenadante de plasma rico en plaquetas (Párr. [0028]).
c) Liofilizar dicho plasma rico en plaquetas o sobrenadante de plasma rico en plaquetas	Liofilizar dicho plasma rico en plaquetas o sobrenadante de plasma rico en plaquetas (Resumen, Pág. 574, Col. 3).	No se menciona

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque enseña un procedimiento de obtención de una composición que contiene factores de crecimiento, que comprende las fases de disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Resumen, Pág. 573, Col. 1) y la etapa de liofilización del plasma (Resumen, Pág. 574, Col. 3).

La diferencia entre la solicitud, definida en la reivindicación 1 y el procedimiento del documento D2 consiste en que la solicitud menciona la etapa de aplicación de un tratamiento térmico al plasma rico en plaquetas.

El efecto técnico asociado a la etapa de aplicar un tratamiento térmico al plasma rico en plaquetas es que permite eliminar el complemento y reducir la presencia de algunas de las inmunoglobulinas más frecuentes en el plasma o el sobrenadante que juegan un papel crítico en reacciones cruzadas desde el punto de vista inmunológico.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo proveer un procedimiento alternativo para la obtención de una composición que contiene factores de crecimiento.”

Sin embargo, el documento D3 enseña un procedimiento de obtención de una composición que contiene factores de crecimiento que comprende la etapa de aplicar un tratamiento térmico al plasma rico en plaquetas a una temperatura entre 2°C y 50°C (Párr. [0028]).

En consecuencia, la persona del oficio normalmente versada en la materia, modificaría el procedimiento de obtención de una composición que contiene factores de crecimiento enseñado en el documento D2, con la etapa de aplicar un tratamiento térmico al plasma rico en plaquetas divulgada en el documento D3, con el fin de proveer un procedimiento alternativo, para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Las reivindicaciones 2-5, 7-18 no mencionan características técnicas adicionales por lo tanto tampoco tienen nivel inventivo.

La reivindicación 6 consiste en: “Procedimiento según la reivindicación 5, que se

caracteriza por que: -el plasma rico en plaquetas o el sobrenadante de plasma rico en plaquetas se somete a una temperatura de 56°C durante un tiempo entre 30 y 60 minutos” (Radicado N°. 14-13982-00000-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D4
Procedimiento	Procedimiento	Procedimiento
a) Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados	Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Resumen, Pág. 573, Col. 1).	Disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Resumen, Pág. 689, Col. 5).
b) Aplicar un tratamiento térmico el plasma rico en plaquetas o el sobrenadante de plasma rico en plaquetas se somete a una temperatura de 56°C durante un tiempo entre 30 y 60 minutos	No se menciona	Aplicar un tratamiento térmico el plasma rico en plaquetas o el sobrenadante de plasma rico en plaquetas se somete a una temperatura de 56°C durante un tiempo entre 30 minutos (Tabla 3).
c) Liofilizar dicho plasma rico en plaquetas o sobrenadante de plasma rico en plaquetas	Liofilizar dicho plasma rico en plaquetas o sobrenadante de plasma rico en plaquetas (Resumen, Pág. 574, Col. 3).	No se menciona

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 6 porque enseña un procedimiento de obtención de una composición que contiene factores de crecimiento, que comprende las fases de disponer de un plasma rico en plaquetas o de un sobrenadante de plasma rico en plaquetas que contiene factores de crecimiento liberados (Resumen, Pág. 573, Col. 1).y la etapa de liofilización del plasma (Resumen, Pág. 574, Col. 3).

La diferencia entre la solicitud, definida en la reivindicación 6 y el procedimiento del documento D2 consiste en que la solicitud menciona la etapa de someter el plasma rico en plaquetas o el sobrenadante del plasma rico en plaquetas a una temperatura de 56°C durante un tiempo entre 30 y 60 minutos la etapa de aplicación de un tratamiento térmico al plasma rico en plaquetas.

El efecto técnico asociado al someter el plasma rico en plaquetas o el sobrenadante del plasma rico en plaquetas a una temperatura de 56°C durante un tiempo entre 30 y 60 minutos es que permite eliminar el complemento y reducir la presencia de algunas de las inmunoglobulinas más frecuentes en el plasma o el sobrenadante.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo proveer un procedimiento alternativo para la obtención de una composición que contiene factores de crecimiento”

Sin embargo, el documento D4 enseña un procedimiento de obtención de una composición que contiene factores de crecimiento que comprende la etapa de aplicar un tratamiento térmico al plasma rico en plaquetas; el plasma rico en plaquetas o el sobrenadante de plasma rico en plaquetas a una temperatura de 56°C durante un tiempo entre 30 minutos (Tabla 3).

En consecuencia, la persona del oficio normalmente versada en la materia, modificaría el procedimiento de obtención de una composición que contiene factores de crecimiento enseñado en el documento D2, con la etapa de aplicar un tratamiento térmico al plasma

rico en plaquetas a una temperatura de 56°C durante un tiempo entre 30 divulgada en el documento D4, con el fin de proveer un procedimiento alternativo, para llegar así al objeto de la reivindicación 6 de la solicitud. Por lo cual se considera obvia.

7. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	EP 1066838	19	Novedad
D2	Pietramaggiore G, Kaipainen A- Wound Repair Regen	1-18	Nivel inventivo
D3	EP 2077118	1-5,7-18	Nivel inventivo
D4	Weida Huang, Takashi Kimura- The Journal of Biochemistry	6	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-09-01

Elaboró: Juanita Andrea Carvajal
Revisó: Sandra Carolina Crespo

INFORME DE BÚSQUEDA DE PATENTES PARA EL No de Radicación: 14-13982-00000-0000						
CRITERIOS PARA LA BÚSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)						
Palabras clave utilizadas		Composition factor of a growth, Freeze dried platetelet rich plasma, Purification and Properties of factor Growth				
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA		A61K 38/18				
BASES DE DATOS CONSULTADAS.						
NCBI						
ispch						
Patentscope						
Google/patents						
INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS (documentos patentes, literatura no patente etc...)						
Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
N.A						
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
Actualización de la Técnica de Obtención y Uso del Plasma Rico en Factores de Crecimiento (P.R.G.F.)						
BÚSQUEDA DE SECUENCIAS						
Número de depósito / patente y N° de secuencia % de identidad						
SEQ ID NO (de la solicitud)	NCBI	The Lens	EMBL-EBI	DDJB	Genome Quest	
N.A						

CRITERIOS DE BUSQUEDA

Composition factor of a growth

Web Imágenes Noticias Videos Más ▾ Herramientas de búsqueda

Cerca de 254,000,000 resultados (0.70 segundos)

Artículos académicos para Composition factor of a growth

...-like **growth factor-I** in **growth** hormone-deficient adults ... - Svensson -
Mencionado por 123
... **factor-I** and **growth** hormone on body **composition** in ... - Thompson -
Mencionado por 166
... **growth factor-beta** depends upon the **composition** and ... - Madri - Mencionado
por 501

Lack of an association between insulin-like growth factor-I ...

www.ncbi.nlm.nih.gov/pubmed/9670867 ▾ Traducir esta página
por DP Kiel - 1998 - Mencionado por 48 - Artículos relacionados
Lack of an association between insulin-like **growth factor-I** and body **composition**,
muscle strength, physical performance or self-reported mobility among older ...

Deficient insulin-like growth factor I in chronic heart failure ...

www.ncbi.nlm.nih.gov/pubmed/9708466 ▾ Traducir esta página
por J Niebauer - 1998 - Mencionado por 184 - Artículos relacionados
BACKGROUND: Recent studies of **growth** hormone supplementation in ... with low
insulin-like **growth factor I** levels show signs of altered body **composition**, ...

One year of insulin-like growth factor I treatment does not ...

www.ncbi.nlm.nih.gov/pubmed/11297574 ▾ Traducir esta página
por AL Friedlander - 2001 - Mencionado por 92 - Artículos relacionados
One year of insulin-like **growth factor I** treatment does not affect bone density, body
composition, or psychological measures in postmenopausal women.

Circulating levels of fibroblast growth factor-21 increase with ...

www.sciencedirect.com/.../pii/S2214623715000472 ▾ Traducir esta página
por LJ Hanks - 2015
Circulating levels of fibroblast **growth factor-21** increase with age independently of
body **composition** indices among healthy individuals. Lynae J. Hanks · ...

Deficient insulin-like growth factor I in chronic heart failure ...

www.sciencedirect.com/.../pii/S0735109798002265 - Traducir esta página
por J Niebauer - 1998 - Mencionado por 184 - Artículos relacionados
Patients with low insulin-like **growth factor I** levels show signs of altered body
composition, cytokine and neuroendocrine activation, to a greater extent than ...

Deficient insulin-like growth factor I in chronic heart failure ...

content.onlinejacc.org/article.aspx?articleid=1125300 ▾ Traducir esta página
por J Niebauer - 1998 - Mencionado por 184 - Artículos relacionados
Patients with low insulin-like **growth factor I** levels show signs of altered body
composition, cytokine and neuroendocrine activation, to a greater extent than ...

[PDF] On the formation, growth and composition of nucleation ...

www.researchgate.net/.../growth...composition.../09e... ▾ Traducir esta página
por M KULMALA - 2001 - Mencionado por 355 - Artículos relacionados
This method, when used with hygroscopic **growth-factors**, can also estimate basic
composition properties of these recently-formed particles. From size spectra ...

[PDF] Effects of Environmental Factors and Nutrient Availability ...

www.mdpi.com/1996-1073/6/9/4607/pdf ▾ Traducir esta página
por A Juneja - 2013 - Mencionado por 29 - Artículos relacionados
3 sept. 2013 - the Biochemical **Composition** of Algae for Biofuels Production: on
the impacts of environmental and nutritional **factors** on algal **growth** rates.

Chapter 3. Factors that Influence Microbial Growth - Food ...

www.fda.gov/Food/.../ucm094145.htm ▾ Traducir esta página
19 mar. 2015 - Table 3-2 lists the approximate minimum aw values for the **growth** of
.... **growth** or toxin production based on the nutrient **composition** of the ...

One Year of Insulin-Like Growth Factor I Treatment Does ...

press.endocrine.org/doi/full/10.../jem.86.4.7377 ▾ Traducir esta página
por AL Friedlander - 2001 - Mencionado por 92 - Artículos relacionados
One Year of Insulin-Like **Growth Factor I** Treatment Does Not Affect Bone Density,
Body **Composition**, or Psychological Measures in Postmenopausal Women.

La Web ▾ Antes del 1 jul. 2011 ▾ Cualquier oficina de patentes ▾ Cualquier estado de presentac

Composition for delivery of hematopoietic growth factor



www.google.com.co/.../EP1351696A2?cl... - Traducir esta página
Solicitud - Presentada el 26 Jun. 2001 - Publicada el 15 Oct. 2003 -
Jeffrey B. Etter - **Rxkinetix, Inc.**
A hematopoietic **growth factor** delivery **composition** includes a
hematopoietic **growth factor**, a liquid vehicle, a first biocompatible
polymer and a second ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Composition of whey growth factor extract for reducing ...



www.google.com.co/.../EP1940426A1?cl... - Traducir esta página
Solicitud - Presentada el 8 Sep. 2006 - Publicada el 9 Jul. 2008 -
Michelle Rowney - **Murray Goulburn Co-Operative Co Limited**
More specifically, it relates to **compositions** containing whey **growth**
factor extract, as well as their use in methods for reducing muscle
inflammation in individuals ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Vaccine composition comprising autologous epidermal growth...



www.google.com.co/patents/US5894016 - Traducir esta página
Otorgada - Presentada el 21 Feb. 1996 - Emitido el 13 Abr. 1999 -
Augustin Bienvenido Lage Davila - **Centro De Immunologia Molecular**
Vaccine **composition** comprising autologous epidermal **growth factor** or
a fragment or a derivative thereof having anti-tumor activity and use
thereof in the ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Use of pharmaceutical composition containing epidermal ...



www.google.com.co/patents/US7799760 - Traducir esta página
Otorgada - Presentada el 22 Ago. 2008 - Emitido el 21 Sep. 2010 - **Jorge**
Berlanga Acosta - **Centro De Ingenieria Genetica Y Biotecnologia**
The invention relates to the use of Epidermal **Growth Factor** (EGF) in a ...
Said **composition** can be administered to recently-created surgical
surfaces damaged ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Synergistic composition comprising a fibroblast growth ...



www.google.com.co/patents/US5288704 - Traducir esta página
Otorgada - Presentada el 31 Ene. 1992 - Emitido el 22 Feb. 1994 -
Domenico Ungheri - **Farmitalia Carlo Erba S.R.L.**
The **composition** comprises a fibroblast **growth factor**, a sulfated ... The
fibroblast **growth factor** may be a basic fibroblast **growth factor** or an
analogue thereof, ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Synergistic composition comprising a fibroblast growth ...



www.google.com.co/.../EP0497341A2?cl... - Traducir esta página
Solicitud - Presentada el 30 Ene. 1992 - Publicada el 5 Ago. 1992 -
Domenico Ungheri - **FARMITALIA CARLO ERBA S.r.l.**
The **composition** comprises a fibroblast **growth factor**, a sulfated ... The
fibroblast **growth factor** may be a basic fibroblast **growth factor** or an
analogue thereof, ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Methods and compositions for delivery of growth factor to ...



www.google.com.co/.../US20090098175 - Traducir esta página
Solicitud - Presentada el 4 Sep. 2008 - Publicada el 16 Abr. 2009 -
Benjamin Marcus Buehrer - **Affinergy, Inc.**
The presently disclosed subject matter relates to a peptide **composition**
for non-covalently localizing fibrous connective tissue-inducing **growth**
factor ...

[Descripción general](#) - [Información relacionada](#) - [Debatir](#)

Compositions for inducing secretion of insulin-like growth ...



www.google.com.co/patents/US6984405 - Traducir esta página
Otorgada - Presentada el 14 Dic. 2001 - Emitido el 10 Ene. 2006 - **Jae-**
Soo Kim - **Naturalendo Tech Co., Ltd.**
The present invention relates to a pharmaceutical or a food **composition**
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Purpose: The purpose of this study was to assess the efficacy of demineralized bone powder (DBP) alone or combined in a mixture with **platelet-rich plasma** (PRP) used to enhance osseointegration of dental implants in a dog model. Materials and Methods: Tissue ...

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ABSTRACT: The repair of confined trabecular bone defects in rabbits treated by autologous bone marrow stromal cells (BMSC), **platelet-rich plasma** (PRP), **freeze-dried** bone allografts (FDBA) alone and in combination (BMSC+ PRP; FDBA+ BMSC; FDBA+ PRP; FDBA+ ...

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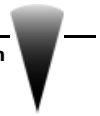
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G Pietramaggiori, A Kaipainen... - Wound repair and ..., 2006 - Wiley Online Library

ABSTRACT Fresh **platelet** concentrates are used in many centers to treat recalcitrant wounds. To extend the therapeutic shelf-life of **platelets**, we analyzed the wound-healing effects of fresh-frozen and **freeze-dried** (FD) **platelet-rich plasma** (PRP) using a diabetic ...

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Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds

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ABSTRACT

Fresh platelet concentrates are used in many centers to treat recalcitrant wounds. To extend the therapeutic shelf-life of platelets, we analyzed the wound-healing effects of fresh-frozen and freeze-dried (FD) platelet-rich plasma (PRP) using a diabetic mouse model. Db/db mice with 1.0 cm² dorsal excisional wounds ($n=15$ /group) were treated with a single application of FD PRP (1.2×10^6 platelets/ μ L) with or without a stabilization solution, and compared with wounds treated with fresh-frozen, sonicated PRP, and untreated wounds. Granulation tissue area, thickness, and wound size were analyzed 9 days posttreatment. Immunostained sections were quantified for vascularity and proliferation using antiplatelet endothelial cell adhesion molecule I and antiproliferating cell nuclear antigen antibodies. The results showed that all PRP preparations increased granulation tissue formation as assessed by surface coverage, thickness, and angiogenic response, when compared with untreated wounds. In addition, wounds treated with FD PRP, and biochemically stabilized FD PRP, exhibited higher proliferative levels. The possibility to deliver growth factors using platelets, and the potential to extend the shelf-life of platelet concentrates makes freeze-drying methods particularly suitable for enhanced wound care.

Platelet aggregation initiates coagulation in bleeding vessels¹ and releases growth factors that regulate wound-healing events. Platelets carry over 20 growth factors² including platelet-derived growth factor-BB (PDGF-BB), epidermal growth factor (EGF), transforming growth factor- β (TGF- β), and vascular endothelial growth factor (VEGF), which have all been shown to improve wound healing in healing-impaired animal models and in human chronic wounds.³⁻⁵ Conversely, reduced levels or absence of these same growth factors, and some of their associated receptors, have been found in difficult-to-heal wounds.^{6,7}

The temporal changes in growth factor concentrations in wound beds are indicative of a complex process involving multiple pathways that orchestrates wound healing. Demonstration of enhanced wound healing through synergistic growth factor application supports this concept.⁸ The underlying practical difficulty with designing a multiple growth factor treatment is to predict the proper time-dependent growth factor ratios for optimal healing. An alternative approach is to replicate and possibly enhance the natural response to wounding through the application of platelet-rich plasma (PRP). PRP preparations can concentrate platelets and their growth factors up to eight times the basal levels in whole blood without inducing platelet activation.⁹ PRP is currently used in combination with cancellous bone grafts to treat osseous defects and has been used extensively in plastic surgery.¹⁰⁻¹² The use of autologous PRP eliminates the potential for disease transmission and immunogenicity;

however, preparation requires time to draw and process patient blood. Furthermore, the quality of autologous platelet preparations is inconsistent due to individual patient characteristics,¹³ many of whom have systemic diseases and are debilitated. This inconsistency may explain in part the mixed results seen in clinical studies.¹⁴⁻¹⁷ Healthy, allogeneic platelets may potentially circumvent this problem by allowing process standardization, but current blood-banking standards require platelets to be stored at room temperature and discarded after 5 days¹⁸

DG	Dermal gap
EG	Epithelial gap
EGF	Epidermal growth factor
FD	Freeze-dried
FDp	FD platelets
FFs	Fresh frozen, sonicated
GM	Growth medium
H&E	Hematoxylin & eosin
NT	Nontreated
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PECAM-1	Platelet endothelial cell adhesion molecule-1
POD	Postoperative day
PRP	Platelet-rich plasma
SRM	Serum reduced medium
TGF- β	Transforming growth factor- β
VEGF	Vascular endothelial growth factor

thereby limiting the availability of these cells for wound-healing applications. Platelets stored for as long as 21 days retain their proliferative activity,¹⁹ but are at risk for bacterial proliferation²⁰ and accumulation of pyrogenic cytokines.^{21,22} Refrigerated preservation (4 °C) or cryopreservation methods (−80 or −196 °C) reduce the potential for bacterial proliferation,²³ but shorten cell lifespan²⁴ by inducing irreversible platelet activation, commonly known as the cold lesion.^{25,26} Improved understanding of the cold lesion enables preparation of platelets in such a way as to preserve their physiological properties following cold storage.^{23,27} One method of platelet stabilization is to directly target intracellular biochemical pathways modulated by reduced temperatures using specific second messenger effectors. Such a formulation containing amiloride, adenosine, and sodium nitroprusside has been shown to protect platelets from damage during cold and cryopreserved storage and, thereby, prevent platelet activation.^{28,29} Platelets cryopreserved using this biochemical stabilization method show retention of *in vitro* platelet function compared with fresh control cells,²⁸ maintain circulation residence time *in vivo*,³⁰ and provide clinically effective correction of platelet count following transfusion in chemotherapeutically induced thrombocytopenia.³¹ Storage modality plays a significant role in acceptance of any potential therapy. With respect to wound-healing treatments, cryopreserved storage of a platelet-based product at either −80 or −196 °C would be difficult at most wound care clinics and hospitals. An effective, freeze-dried (FD) therapeutic would provide simpler storage requirements and possibly, self-application. This study investigated the effectiveness of platelet storage options by applying a single dose of FD PRP, with or without biochemical stabilization, to full-thickness wounds and compared the results with control wounds treated with fresh-frozen, sonicated PRP, and nontreated wounds.

MATERIALS AND METHODS

Single donor units of human platelets were purchased from an American Association of Blood Banks-accredited blood bank, stored with agitation at room temperature, and used within 5 days of donation. All required donor-screening and release-testing were performed by the blood bank in accordance with American Association of Blood Bank requirements.³² The single donor unit was divided into three aliquots to prepare the platelet-based therapeutic materials, each containing 1.2×10^6 platelets/ μ L. Fresh-frozen, sonicated (FFs) PRP was prepared by adjusting the platelet concentration of one aliquot with a physiologic buffer (Buffer B; 136 mM NaCl, 11.9 mM NaHCO₃, 5.6 mM glucose, 5 mM HEPES, 2.7 mM KCl, 2.0 mM MgCl₂, 0.42 mM NaH₂PO₄; pH 7.4), sonicating the cell suspension to disrupt cellular structure and release intracellular constituents (~10 seconds; VibraCell250, Sonics & Materials, Newtown, CT), and stored frozen at −80 °C until used. FD PRP was prepared by adjusting the second aliquot with Buffer B and drying in a LyoStar freeze-dryer (FTS Kinetics Systems, Stone Ridge, NY). The final aliquot was adjusted using a combination of Buffer B and a biochemical stabilization solution (250 μ M amiloride, 100 μ M adenosine, 50 μ M sodium nitroprusside, 1% (v/v) dimethyl sulfoxide, 4% (w/v) polyvinyl pyrrolidone [Plas-

done C-15, International Specialty Products, Wayne, NJ], and 50 mM mannitol) and freeze-drying as above to prepare a biochemically preserved FD platelet (FDp) sample. All manipulations of the platelet materials were performed using the standard aseptic technique and all solutions were filter sterilized using filters having 0.2 μ m diameter pores (Millipore, Billerica, MA). Platelet concentrations were verified using a CellDyn 1700 hematology analyzer (Abbott Laboratories, Abbott Park, IL). The FDp products were packed under dry nitrogen in heat-sealed foil pouches and stored at −80 °C until use.

In vitro analysis of growth factor load and distribution

Growth factor levels for PDGF-AB, TGF- β , VEGF, and EGF were measured in sonicated PRP samples and in platelet-free supernatants to determine total growth factor load, plasma-based growth factor levels, and the relative distribution of growth factors between the intracellular and extracellular compartments. Comparison of pre- and postprocessing plasma-based growth factors specifies load redistribution due to processing. Platelet ability to release growth factors was also quantified by determining plasma-based growth factor concentration before and following platelet activation with a mixture of 2 μ g/mL collagen and 20 μ M adenosine diphosphate for 5 minutes.

Growth factor levels were determined using the enzyme-linked immunosorbent assay method, and were tested according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). The intensity of the color development was measured using a microplate reader (BioRad Laboratories, Hercules, CA).

In vitro cell proliferation assay

Swiss Albino 3T3 fibroblast cells (CCL-92; American Type Tissue Collection, Manassas, VA) were seeded in a 24-well tissue culture plate at 10,000 cells/well and grown in growth media (GM; Dulbecco's modified Eagle's medium with 4 mM glutamine, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, and 10% fetal calf serum [Invitrogen/GIBCO, Carlsbad, CA]) for 16 hours in a humidified incubator at 37 °C and 5% CO₂. In order to halt growth but maintain viability, GM was replaced with serum-reduced media (SRM; equivalent to GM but supplemented with 0.25% fetal calf serum). An 8 μ m pore Transwell® (Life Sciences, Acton, MA) insert containing 100 μ L of SRM was set in place, and 75 μ L of platelet material was added. GM and SRM conditions were included as experimental controls. Experimental groups were grown for 72 hours, after which, the Transwell®s and culture media were removed, 500 μ L of fresh SRM and 100 μ L of CellTiter96 Aqueous one solution (Promega Corporation, Madison, WI) was added per well, and the plates were returned to the incubator for an additional 3 hours. Following this reaction period, 100 μ L of the media from each well was analyzed spectrophotometrically at 540 nm using a plate reader (BioRad Laboratories). All conditions (SRM, GM, FFs, FD, and FDp) were performed in triplicate and reported as percentage increase over baseline. Baseline was established after 16 hours.

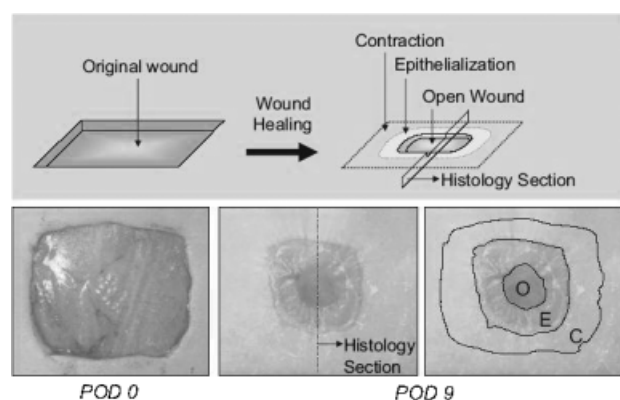


Figure 1. Methodology used to quantify wound closure: stylized illustration and clinical images. The upper panel shows an illustration of the experimental wound and the clinical parameters used to quantify wound healing. At postoperative day (POD) 0, the wound area was quantified using digital planimetry methods. At POD 9, contraction (C), epithelialization (E), and open area (O) were quantified accounting for 100% of the original (POD 0) area.

Wound model and treatment procedure

Homozygous genetically diabetic 8–12-week-old, Lep/*r*-db/db male mice (strain C57BL/KsJ-Lepr^{db}) were used under an approved animal protocol in an AAALAC-accredited facility. The day before surgery, hair was clipped and depilated (Nair[®], Church & Dwight Co., Princeton, NJ). On the day of the surgery, animals were weighed and anesthetized with 60 mg/kg Nembutal (pentobarbital). A dorsal 1.0 cm² area of skin and panniculus carnosus was excised and the wounds were photographed. Simultaneously, the platelet-based treatments were prepared: the FFs samples were thawed, while the FD samples, FD and FDp, were rehydrated with sterile deionized H₂O to the original predrying volume and separated into 250 μ L aliquots. Each aliquot was treated with 1 U/mL thrombin (Chronolog Corporation, Havertown, PA) just before application and allowed to clot in situ, thereby facilitating persistence of the platelet material in the wound. Fifteen wounds in each platelet experimental group (NT, FFs, FD, and FDp) were treated. All wounds were covered with a semi-occlusive polyurethane dressing (Tegaderm[™], 3M, St. Paul, MN). On postoperative day (POD)⁹, the animals were euthanized and the wounds were photographed, excised, and fixed in 10% neutral-buffered formalin solution.

Microscopic analysis

Central wound cross-sections were embedded in paraffin, sectioned, and stained (Figure 1) according to routine hematoxylin & eosin (H&E) protocols. Panoramic cross-sectional digital images of each wound were prepared using Adobe Photoshop CS Software (Adobe Systems Incorporated, San Jose, CA) to analyze granulation tissue area and thickness (Figure 2A).

These sections were analyzed with digital planimetry (Scion Image, Scion Corporation, Frederick, MD) by two independent observers, blinded to experimental treatment,

to quantify the area and thickness of granulation tissue. Capillary density was evaluated using three fields per slide viewed at $\times 20$ magnification: one in the middle of the lesion and one at each wound margin. The images were viewed with Adobe Photoshop CS Software and blood vessels in each high-powered field were marked and counted. Thirteen to 15 samples/group were analyzed.

The epithelial gap (EG) and dermal gap (DG) were measured using a Nikon Shadowgraph (Tokyo, Japan). EG was defined as the distance between the advancing edges of keratinocyte migration, with EG of 0 representing a completely reepithelialized wound.³³ DG was defined as the distance between uninjured dermis on both sides of the wound. Two serial sections were averaged to determine EG and DG.

Immunohistochemical protocol

Paraffin-embedded sections were rehydrated, and antigen retrieval for pan-endothelial cell marker MECA-32 and proliferating cell nuclear antigen (PCNA) analysis was accomplished by microwaving the sections in 10 mM sodium citrate (pH 6.0) for 10 minutes. Sections for endothelial cell markers platelet endothelial cell adhesion molecule 1 (PECAM-1) and CD34 were treated with 40 μ g/mL proteinase K (Roche Diagnostics Corp., Indianapolis, IN) for 25 minutes at 37 °C. PECAM-1, CD34, and MECA-32 (PharMingen, San Jose, CA) and PCNA (DAKO Corp., Carpinteria, CA) primary antibodies were incubated at 4 °C overnight. PECAM-1, MECA-32, and CD34 signal was intensified using the tyramide amplification system (PerkinElmer, Boston, MA). PCNA staining detection was performed using a Vectastain ABC Kit (Vector Laboratories, Burlingame, CA).

Vessel density and cell proliferation quantification

Digital color images of the wound sections were preprocessed before quantification to ensure uniform contrast of PECAM-1-positive areas relative to the background. A mask of positive staining was created using the color mask function of the program CorelPhotoPaint v.10 (Ottawa, ONT, Canada) by sampling five different chromogen color tones represented in positively stained areas. The masked vessel areas were converted to pure black while the background was made pure white. The black and white representations were used for area quantification in IPLab software (BD Biosciences Bioimaging, Rockville, MD) by applying the segmentation function. Tissue regions were defined by projecting the original H&E image over the processed image. Blood vessel density, quantified over the entire image, is expressed as the ratio of vessel area to total granulation tissue area. Between four and seven microscopic fields at $\times 20$ magnification were used to evaluate vessel density for each wound and treatment modality.

Wounds were analyzed for cell proliferation using image analysis of PCNA-stained sections in a manner similar to the method of vessel density quantification. High-power digital images of PCNA-stained wound sections were used to measure the number of PCNA-positive cells relative to the total number of nuclei (Figure 3). The degree of proliferation was quantified over the entire wound section using four to six fields at $\times 20$ magnification and expressed as a

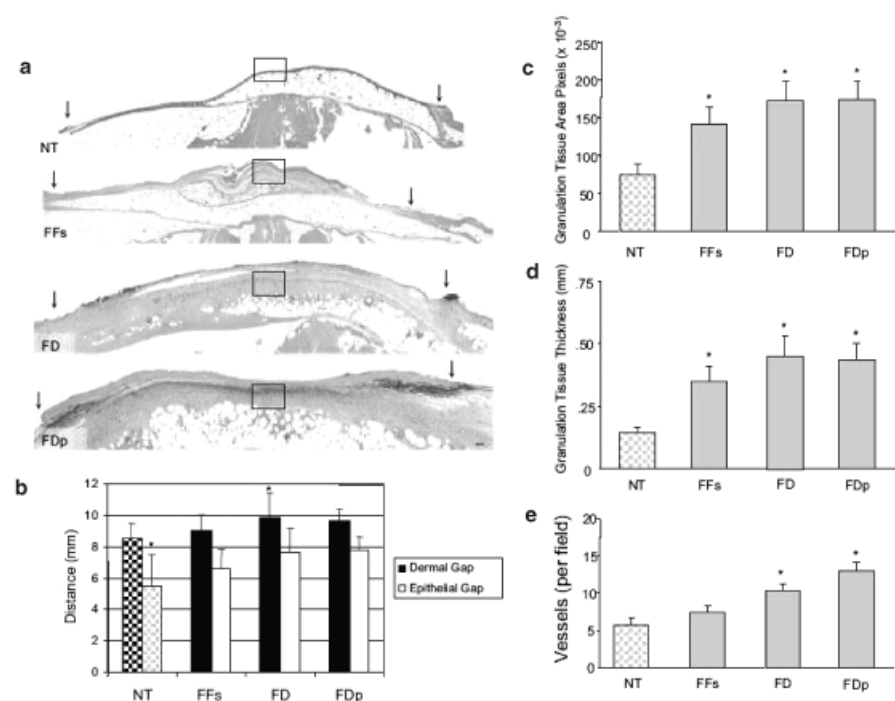


Figure 2. Analysis of granulation tissue. (A) Panoramic photomicrographs of wound beds show the different amount of granulation tissue deposition between study groups. The arrows indicate the epithelium margins and the boxes indicate where the measurements of granulation tissue area and thickness were performed. The boxes also correspond to the area that is presented at a higher magnification in the immunohistochemistry photomicrographs. Scale bar, 100 μ m. (B) Dermal gap (DG) and epithelial gap in all groups; DG was found to be significantly larger in the freeze-dried platelets (FDp) group, * Indicates $p < 0.05$. Quantification of (C) granulation tissue area, (D) thickness, and (E) blood vessel count per high-field magnification. * indicates $p < 0.01$ except where noted.

ratio of proliferating nuclei (PCNA-positive) to total nuclei.

Wound closure analysis

Digital photographs captured on POD 9 were compared with initial photographs (POD 0), by two independent observers who were blinded to the treatment mode using planimetric methods (Scion Image). Wound closure was quantified by measuring contraction, reepithelialization, and open wound as a percentage of the original wound ar-

ea. The sum of contracted, reepithelialized, and open wound areas equals 100% of the original wound size.³⁴ This method of quantifying wound closure is presented in Figure 1.

Statistical analysis

Values were expressed as means $\pm$ standard deviation in the text and figures. One-way analysis of variance and ad hoc Bonferroni tests were used to determine the significance of differences between treatment modes.

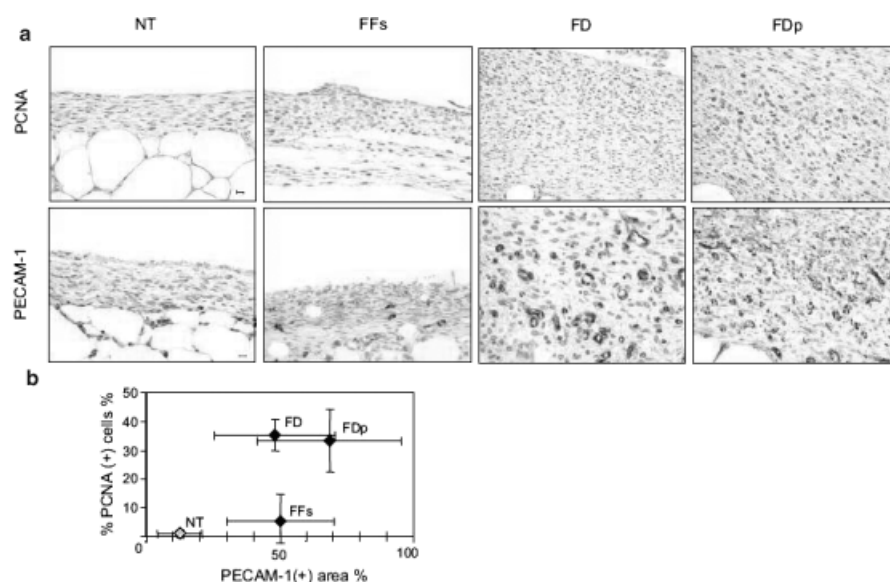


Figure 3. Photomicrographs of immunohistochemically stained sections, and histomorphometry analysis. (A) Immunohistochemical staining for PCNA (upper row) and PECAM-1 (lower row), scale bar, 10 μ m. (B) Two-dimensional plot of PECAM-1 and PCNA results of stage wound-healing modulation. The upper right position is the ideal condition, with high levels of proliferation and vascular density. The more a wound is distant from this quadrant, the less likely it is healing. Nontreated wounds combine poor vasculature with low levels of proliferation, while all treatment groups show their unique position. In this study, FD and FDp wounds combine higher vasculature and proliferation levels compared with all other groups.

Multivariate analysis was performed using Statistica v7.0 (StatSoft Inc., Tulsa, OK).

RESULTS

Growth factor concentrations were initially measured and TGF- β , PDGF-AB, VEGF, and EGF were selected for their known importance in improving wound healing. Growth factor concentrations were measured from 250 μ L aliquots of the PRP suspension before and after preparation of the distinct platelet therapeutics. The concentration of each growth factor in the different therapeutics is reported in Table 1. No significant differences were noted in either the total load or distribution of growth factors as a result of processing. However, the ability of platelets to release growth factors following freeze-drying was dependent on inclusion of the biochemical stabilization solution. For example, TGF- β release from fresh platelets was approximately 50%; from rehydrated platelets that had been FD without biochemical stabilization, FD was 16.9%, whereas, rehydrated platelets that had been FD with biochemical stabilization, FDp was significantly higher at 40.4%. Similar release profiles were noted for all growth factors analyzed (not shown, submitted for publication).

The fibroblast proliferation assay was used to determine whether preparation of samples affected the ability of platelets to stimulate cell growth in vitro. All platelet therapeutics showed statistically equivalent in vitro ability to increase cell proliferation over the SRM control condition (Table 2). Platelet-induced proliferation averaged 90% of optimal growth conditions.

Platelet therapeutics increase granulation tissue area, thickness, and vascularity

Granulation tissue formation in response to the application of the PRP preparations was measured for granulation tissue area, thickness, and number of blood vessels per high-power magnification field.

FDp, FD, and FFs treatments, respectively, induced a significant 2.3-, 2.3-, and 1.9-fold increase in granulation tissue area compared with the nontreatment (NT) group ($p < 0.01$, Figure 2C). Proportional increases were also seen in granulation tissue thickness in the center of the lesion (Figure 2D).

Increased tissue vascularization in response to treatment with platelet material was found when blood vessels were counted in standard H&E-stained sections. FDp and

Table 1. Growth factor concentrations in platelet samples as determined by enzyme-linked immunosorbent assay

Growth factor	Platelet preparation mode		
	PRP	FD	FDp
TGF- β (ng/mL)	334.4 $\pm$ 21.6	314.8 $\pm$ 10.0	245.2 $\pm$ 12.0
PDGF (pg/mL)	8672 $\pm$ 144	7,304 $\pm$ 776	7,784 $\pm$ 636
VEGF (pg/mL)	330.8 $\pm$ 37.6	346.4 $\pm$ 65.2	268.0 $\pm$ 3.2
EGF (pg/mL)	2,185.2 $\pm$ 36.4	2,016.4 $\pm$ 68.8	2,064.8 $\pm$ 23.2

Table 2. In vitro fibroblast proliferation assay as percentage increase over baseline

Condition	% Increase
Growth media	750 $\pm$ 6
Serum-reduced media	110 $\pm$ 2
FFs	620 $\pm$ 4
FD	760 $\pm$ 2
FDp	680 $\pm$ 5

FD treatments resulted in significant ($p < 0.01$) increases in mean vessel count per high-power field when compared with NT. The increase was significant ($p < 0.01$) also comparing FDp with FFs. The level of contraction of a wound can be measured by the DG. The DG was significantly larger in FD-treated wounds compared with the NT group ($p < 0.05$, Figure 2B). To assess wound closure microscopically, EG was quantified. The EG of the NT group was significantly ($p < 0.01$) smaller (Figure 2B) than the EG of both FD and FDp groups.

Although direct counts of vessels per high-power field showed statistically significant increases in platelet-treated wounds, this method of analysis fails to take into consideration the distribution of vessels within the formed granulation tissue. Vessel density in granulation tissue area was measured by high-power digital images of PECAM-1 immunohistochemically stained wound sections (Figure 3A). This analysis showed a significant ($p < 0.01$) fivefold increase in blood vessel density in both the FFs and FD groups and a sevenfold increase in the FDp group when compared with the NT group (Figure 3A). Analysis of CD34 and MECA-32 immunohistochemically stained sections revealed a pattern similar to those stained for PECAM-1 (data not shown).

The high cell density of the granulation tissue in FD and FDp wounds showed an elevated proliferative response of stromal cells. PCNA staining showed that FD and FDp groups resulted in a significant ($p < 0.01$) increase in cell proliferation compared with NT and FFs groups (Figure 3A, B).

Vascularity and proliferation are two key characteristics of granulation tissue. PECAM-1 and PCNA results were plotted together to stage wound-healing modulation and provide a quantitative assessment of granulation tissue quality (Figure 3B). In order to distinguish between combinatorial responses, multivariate statistical analysis was performed. The resulting two-dimensional plot shows that FD- and FDp-treated wounds resulted in statistically better responses compared with FFs and NT wounds. Moreover, the combinatorial analysis suggested a difference ($p=0.054$) between the FD and FDp conditions, indicating a high degree of distinction between the two conditions.

Wound closure (POD 9) was delayed and contraction reduced in FD-treated wounds

Wound closure was evaluated at POD 9 in an attempt to maximize the difference between the treatment groups

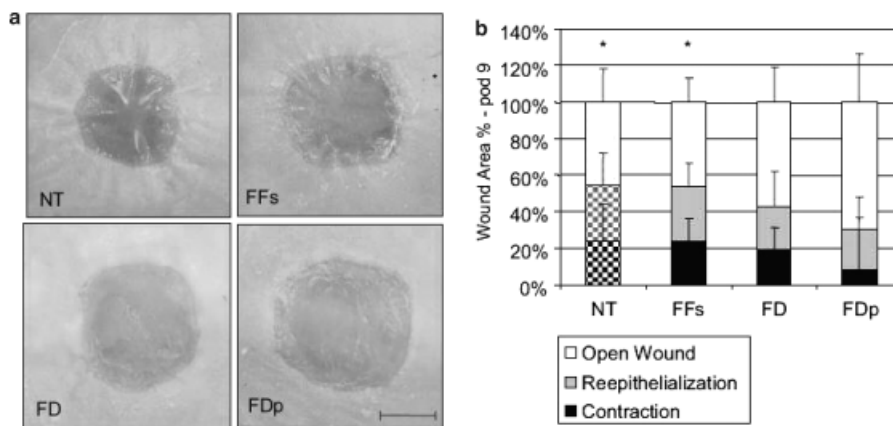


Figure 4. Macroscopic analysis of wound closure. (A) Photographs showing the macroscopic images of the wounds at postoperative day (POD) 9. Scale bar, 5 mm. (B) Overall wound closure in all study groups. NT and FFs groups showed a significantly higher closure compared with the FDp group. * Indicates $p < 0.01$.

(Figure 4A). Previous work in our laboratory showed that a 1.0 cm² wound in a diabetic mouse reaches the 50% closure point between days 8 and 12 (data submitted for publication).

The NT and FFs groups exhibited a mean closure significantly ($p < 0.01$) higher than the FDp group (Figure 4B). By POD 21, FDp wounds closure was not delayed (data not shown). Wound healing occurs by a combination of wound contraction and reepithelialization in all groups. Reepithelialization was similar in all treatment groups, while a trend toward reduced wound contraction was observed in FD platelets-treated groups, particularly the FDp group (Figure 4B).

DISCUSSION

Platelets are used clinically for a wide variety of wound-healing applications. This study showed that platelet concentrates modulate wound healing in the diabetic mouse model. Differences were observed between FD and FFs conditions: FDp preparations stimulated cell proliferation in the granulation tissue more efficiently than FFs platelets. While the FFs condition provides a bolus dose of growth factors with a short half-life before degradation, the kinetics of growth factor release from FDp is not known. This study suggests that release upon reconstitution occurs in a manner consistent with activation of fresh platelets. In vitro analyses showed that freeze-drying followed by reconstitution did not alter the intra/extracellular ratio of growth factors, especially when platelets were biochemically stabilized. In vivo results did not show significant differences between the two FD conditions; however, biochemical stabilization may be beneficial for long-term storage of platelet preparations. The stabilization solution preserves platelet membrane integrity and thereby protects the growth factors from storage-induced degradation, allowing platelet α granule release to occur in a biologically relevant manner.

Currently, individual growth factors administered for wound healing are used at much higher concentrations than those in platelet preparations in this study. The complex mixture of growth factors delivered by platelets potentially act in synergy, while also being protected against rapid degradation.^{5,8-16} In this study, the concentration of platelets in all preparations was normalized to that currently used in

clinically treated wounds.⁹⁻¹⁶ Further increases in platelet concentration progressively caused a reduction in proliferation in vitro (data not shown), probably due to saturation and down-regulation of the growth factor receptors. The question remains whether higher concentrations in vivo may be beneficial or whether multiple applications may be more favorable than a single one.

Wound closure is one of the most significant parameters in assessing wound healing; it might be argued that less wound closure is a sign of failure of the proposed therapy. The delayed wound closure observed in this study was a consequence of reduced contraction. Both FD and biochemically stabilized FDp showed the ability to modulate wound healing, enhancing proliferation of cells and vessel growth in granulation tissue, while decreasing wound contraction. The cause of the decreased contraction is unclear at this stage. The increased granulation tissue could have reduced contraction, acting like a mechanical splint or the biochemical stabilization formulation, containing adenosine, amiloride, and sodium nitroprusside, might have had a direct pharmacological effect on the fibroblasts of the wound. Because contraction causes morphological and functional deviations,³⁵ reduced contraction would be advantageous in the treatment of several wound types (e.g., burn, cavity, skin donor-sites, or nongranulating wounds); therefore, additional studies are needed to understand the mechanisms of this finding.

The rich growth factor profile of platelets has the potential to be an effective vehicle for the treatment of a variety of wound types. This study suggests that growth factors may be more effective when directly delivered by platelets, allowing a slow release, to enhance the physiological response to injury, rather than administered as a bolus dose. This may be particularly relevant because of our aging population with a growing incidence of Type 2 diabetes and obesity.

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REFERENCES

- Martinowitz U, Spotnitz WD. Fibrin tissue adhesives. *Thromb Haemostasis* 1997; 78: 661–6.
- Folkman J, Browder T, Palmblad J. Angiogenesis research: guidelines for translation to clinical application. *Thromb Haemostasis* 2001; 86: 23–33.
- Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Bunting S, Steinmetz HG, Gurtner GC. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. *Am J Pathol* 2004; 164: 1935–47.
- Kirchner LM, Meerbaum SO, Gruber BS, Knoll AK, Bulgrin J, Taylor RA, Schmidt SP. Effects of vascular endothelial growth factor on wound closure rates in the genetically diabetic mouse model. *Wound Rep Reg* 2003; 11: 127–31.
- Wieman TJ. Clinical efficacy of becaplermin (rhPDGF-BB) gel. Becaplermin Gel Studies Group. *Am J Surg* 1998; 176: 74S–9S.
- Beer HD, Longaker MT, Werner S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. *J Invest Dermatol* 1997; 109: 132–8.
- Lauer G, Sollberg S, Cole M, Flamme I, Sturzebecher J, Mann K, Krieg T, Eming SA. Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. *J Invest Dermatol* 2000; 115: 12–8.
- Marikovsky M, Vogt P, Eriksson E, Rubin JS, Taylor WG, Joachim S, Klagsbrun M. Wound fluid-derived heparin-binding EGF-like growth factor (HB-EGF) is synergistic with insulin-like growth factor-I for Balb/MK keratinocyte proliferation. *J Invest Dermatol* 1996; 106: 616–21.
- Eppey BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004; 114: 1502–8.
- Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. *Facial Plast Surg* 2002; 18: 27–33.
- Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann Surg* 1986; 204: 322–30.
- Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 2001; 24: 483–88.
- Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. *J Dent Res* 2005; 84: 434–39.
- Krupski WC, Reilly LM, Perez S, Moss KM, Crombleholme PA, Rapp JH. A prospective randomized trial of autologous platelet-derived wound healing factors for treatment of chronic nonhealing wounds: a preliminary report. *J Vasc Surg* 1991; 14: 526–32, discussion 32–36.
- Stacey MC, Mata SD, Trengove NJ, Mather CA. Randomised double-blind placebo controlled trial of topical autologous platelet lysate in venous ulcer healing. *Eur J Vasc Endovasc Surg* 2000; 20: 296–301.
- Steed D, Goslen B, Hambley R, Abell E, Hebda P, Webster M. Clinical trials with purified platelet releasate. *Prog Clin Biol Res* 1991; 365: 103–13.
- Steed DL, Goslen JB, Holloway GA, Malone JM, Bunt TJ, Webster MW. Randomized prospective double-blind trial in healing chronic diabetic foot ulcers. CT-102 activated platelet supernatant, topical versus placebo. *Diabetes Care* 1992; 15: 1598–604.
- Murphy S. Platelet storage for transfusion. *Semin Hematol* 1985; 22: 165–77.
- Chan RK, Liu P, Lew DH, Ibrahim SI, Srey R, Valeri CR, Hechtman HB, Orgill DP. Expired liquid preserved platelet releasates retain proliferative activity. *J Surg Res* 2005; 126: 55–8.
- Pietersz RN, Engelfriet CP, Reesink HW. Detection of bacterial contamination of platelet concentrates. *Vox Sang* 2003; 85: 224–39.
- Muyllé L, Joos M, Wouters E, De Bock R, Peetermans ME. Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. *Transfusion* 1993; 33: 195–9.
- Stack G, Snyder EL. Cytokine generation in stored platelet concentrates. *Transfusion* 1994; 34: 20–5.
- Vostal JG, Mondoro TH. Liquid cold storage of platelets: a revitalized possible alternative for limiting bacterial contamination of platelet production. *Transfus Med Rev* 1997; 11: 286–95.
- Slichter SJ, Harker LA. Preparation and storage of platelet concentrates. I. Factors influencing the harvest of viable platelets from whole blood. *Br J Haematol* 1976; 34: 395–402.
- Becker GA, Tuccelli M, Kunicki T, Chalos MK, Aster RH. Studies of platelet concentrates stored at 22 °C and 4 °C. *Transfusion* 1973; 13: 61–8.
- Valeri CR. Hemostatic effectiveness of liquid-preserved and previously frozen human platelets. *N Engl J Med* 1974; 290: 353–8.
- Hoffmeister KM, Josefsson EC, Isaac NA, Clausen H, Hartwig JH, Stossel TP. Glycosylation restores survival of chilled blood platelets. *Science* 2003; 301: 1531–4.
- Currie LM, Livesey SA, Harper JR, Connor J. Cryopreservation of single-donor platelets with a reduced dimethyl sulfoxide concentration by the addition of second-messenger effectors: enhanced retention of in vitro functional activity. *Transfusion* 1998; 38: 160–7.
- Rivera J, Lozano ML, Corral J, Connor J, Gonzalez-Conejero R, Ferrer F, Currie L, Vicente V. Quality assessment of platelet concentrates supplemented with second-messenger effectors. *Transfusion* 1999; 39: 135–43.
- Currie LM, Lichtiger B, Livesey SA, Tansey W, Yang DJ, Connor J. Enhanced circulatory parameters of human platelets cryopreserved with second-messenger effectors: an in vivo study of 16 volunteer platelet donors. *Br J Haematol* 1999; 105: 826–31.
- Vadhan-Raj S, Kavanagh JJ, Freedman RS, Folloder J, Currie LM, Bueso-Ramos C, Verschraegen CF, Narvios AB, Connor J, Hoots WK, Broemeling LD, Lichtiger B. Safety and efficacy of transfusions of autologous cryopreserved platelets derived from recombinant human thrombopoietin to support chemotherapy-associated severe

- thrombocytopenia: a randomised cross-over study. *Lancet* 2002; 359: 2145–52.
32. American Association of Blood Banks. *Technical manual: 50th anniversary AABB edition 1953–2003*. 14th ed. Bethesda, MD: American Association of Blood Banks, 2002.
33. Michaels JT, Dobryansky M, Galiano RD, Bhatt KA, Ashinoff R, Ceradini DJ, Gurtner GC. Topical vascular endothelial growth factor reverses delayed wound healing secondary to angiogenesis inhibitor administration. *Wound Rep Reg* 2005; 13: 506–12.
34. Yannas I. *Tissue and organ regeneration in adults*. New York: Springer, 2001.
35. Yannas IV, Burke JF, Warpehoski M, Stasikelis P, Skrabut EM, Orgill D, Giard DJ. Prompt, long-term functional replacement of skin. *Trans Am Soc Artif Intern Organs* 1981; 27: 19–23.

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