

Señor

DIRECTOR DIVISIÓN DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente No. **NC2017/0003290**

PETICION TRAMITE ACELERADO DE PATENTES

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante me permito presentar los siguientes documentos para participar en el programa de trámite acelerado de patentes.

- a. Copia de la acción oficial de patentabilidad emitida por la USPTO de fecha **26 de abril de 2016** junto con la respectiva traducción al castellano.
- b. Copia de la respuesta del solicitante a la acción oficial radicada el **28 de septiembre de 2016** junto con las reivindicaciones modificadas **1-28** y su respectiva traducción al castellano.
- c. Copia de la notificación de concesión (Notice of allowability) en donde se establece que las reivindicaciones concedidas son las **1,4,6,8-25 y 28** de la respuesta radicada el **28 de septiembre de 2016**. En esta notificación de concesión, el examinador hace modificaciones adicionales a las reivindicaciones las cuales están debidamente traducidas.
- d. Notificación de aceptación (notice of allowance) en donde se establece que la solicitud ha sido examinada y aceptada para conceder la patente.
- e. Copia de la patente **US 9,592,280** con las reivindicaciones concedidas **1-22**.
- f. Tabla de correspondencia, en donde se evidencia que las reivindicaciones modificadas en Colombia el día **28 de diciembre de 2017** son exactamente iguales a las patentadas por la USPTO.

Del Señor Director

JUAN PABLO CADENA SARMIENTO

T.P. No. 57.492 del C.S.J.

C.C. 80.410.337 de Usaquén

Calle 70 A No. 4 - 41

ACCIÓN DETALLADA

La presente solicitud, presentada en o después de marzo 16, 2013, está siendo examinada bajo el primer inventor para presentar las disposiciones de la AIA.

Las reivindicaciones 1, 4, 6, 8 -22 y 28 se dirigen a un producto permitido. De conformidad con los procedimientos dispuestos en el MPEP §821.04(b), las reivindicaciones se dirigen al proceso de preparar o usar el producto permitido, previamente retirados de consideración como un resultado de un requerimiento de restricción, están aquí reunidos y examinados completamente por patentabilidad bajo la CFR 37 1.104.

Debido a que la invención reclamada previamente retirada fue reunida, el requerimiento de restricción entre los grupos I y II como se muestra en el Oficio enviado el 3/10/2015 es retirado aquí. En vista del retiro del requerimiento de restricción como a la unión de las invenciones, se avisó al solicitante que ninguna reivindicación presentada en una solicitud divisional o de continuación es anticipada por, o incluye todas las limitaciones de, una reivindicación que es permitida en la presente solicitud, dado que la reivindicación puede estar sujeta a denegaciones de doble patente estatutario o no estatutario sobre las reivindicaciones de la presente invención.

Una vez el requerimiento de restricción es retirado, no son más aplicables las disposiciones de la 34 U.S. 121.

Una modificación realizada por el examinador para realizar aparece más adelante. Si los cambios y/o adiciones no son aceptables para el solicitante, se puede presentar una modificación por medio de la CFR 37 1.312. Para asegurar la consideración de tal modificación, se DEBE enviar no más tarde que el pago de la tasa de emisión.

La autorización para esta modificación del examinador se dio en una entrevista telefónica con Gregory M. Lefkowitz en octubre 27, 2016.

1. La solicitud ha sido modificada como sigue:

La reivindicación 1 ha sido eliminada y reemplazada con:

Una composición de desbridación de tejido que comprende:

0.1 a 99.5% en peso de un componente de amilasa y opcionalmente un componente de enzima proteolítica, en donde la proporción del componente de amilasa al componente de enzima proteolítica es al menos 10:1 en peso; y

al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente en una cantidad de hasta el 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación,

en donde dicho componente de amilasa comprende al menos 80% en peso de α -amilasa de un recurso seleccionado de un grupo que consiste de páncreas porcino y *Bacillus subtilis*, y en donde la composición de desbridación es capaz de desbridar tejido desvitalizado o necrótico.

En la reivindicación 4, “La composición de acuerdo con la reivindicación 3” en la línea 1 ha sido cambiada a “La composición de acuerdo con la reivindicación 1”.

En la reivindicación 9, “*en una cantidad de al menos*” en la línea 2 ha sido cambiada por “*en una cantidad en el rango de*”.

En las reivindicaciones 13, 14, 17 y 18, “*seleccionado del grupo*” en la línea 2 ha sido cambiado a “*seleccionado de un grupo*”.

En la reivindicación 15, “*en una cantidad de al menos 0.01% en peso, y*” en la línea 2 ha sido cambiado a “*en una cantidad en el rango de 0.01% en peso a 1.0% en peso basado en el peso de la composición de desbridación*”.

En las reivindicaciones 21 – 23, se ha insertado el término “*tejido*” antes de “*composición de desbridación*” en la línea 2.

Las reivindicaciones 2, 7 y 26 fueron canceladas.

RAZONES PARA LA CONCESIÓN

2. La siguiente es una declaración del examinador de las razones para la concesión:
El arte enseña composiciones de desbridación que comprende una cantidad sustancialmente más alta de un componente de enzima proteolítica que un componente de amilasa. Sin embargo, la composición de desbridación comprende una cantidad sustancialmente más alta de un componente de amilasa que de un componente de enzima proteolítica, en donde la composición de desbridación reclamada efectivamente desbrida tejido desvitalizado o necrótico.

Se considera necesario que sea presentado algún comentario por el solicitante no más tarde que el pago de la tasa de emisión, para evitar retardos en el procesamiento, debería estar preferiblemente acompañado con la tasa de emisión. Dicho comentario debe estar claramente etiquetado como “*Comentarios en la Declaración de las Razones para la Concesión*”.

MODIFICACIONES A LAS REIVINDICACIONES

Esta lista de reivindicaciones reemplazará todas las versiones anteriores y listas de reivindicaciones en la solicitud.

1. (Actualmente modificada) Una composición de desbridación que comprende:

0.1 a 99.5% en peso de un componente de amilasa, y

al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente en una cantidad de más del 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación,

~~en donde, si un componente de enzima proteolítica está presente,~~ una proporción de componente de amilasa a un componente de enzima proteolítica es al menos ~~[[4]]~~ 10:1, y en donde dicho componente de amilasa comprende al menos 80% en peso de α -amilasa.
2. (Presentado previamente) La composición de acuerdo con la reivindicación 1, en donde el componente de amilasa comprende una amilasa seleccionada de un grupo que consiste de amilasa de humanos, animales, bacterias, plantas, hongos y recombinación genética.
3. (Cancelada).
4. (Presentada previamente) La composición de acuerdo con la reivindicación 3, en donde dicha amilasa comprende hasta 20% en peso de una amilasa seleccionada de β -amilasa, γ -amilasa y combinaciones de las mismas.
5. (Cancelada).
6. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que además comprende hasta 20% en peso de enzimas hidrolíticas seleccionadas de proteasas, condroitinasas, hialuronidasas, lipasas, glucosidasas, heparanasas, dermatanasas, pululanasas, N-acetilglucosaminidasa, lactasas, fosfolipasas, transglucosilasas, ésterasas, tioéster-hidrolisasas, sulfatasas, escarasas, nucleasas, fosfatasas, fosfodiésterasas, mananasas, manosidasas, isoamilasas, liasas, inulinasas, queratinasas, tanasas, pentosanasas, glucanasas, arabinosidasas, pectinasas, celulasas, quitinasas, xilanasas, cutinasas, pectato liasas, hemicelulasas y combinaciones de estas, en donde el porcentaje se basa en el peso total del componente de amilasa y las enzimas hidrolíticas.
7. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que comprende α -amilasa seleccionada de un grupo que consiste de páncreas humano, páncreas animal, hongos y bacterias.
8. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que además comprende hasta el 20% en peso de otras enzimas seleccionadas de un grupo que consiste de oxidasas, peroxidasas, glucosa

oxidasas, catalasas, oxidorreductasas, fenoloxidasas, laccasas, lipoxigenasas, isomerasas y ligninasas, en donde el porcentaje está basado en el peso total del componente de amilasa y las otras enzimas.

9. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que comprende por lo menos una biguanida polimérica en una cantidad de por lo menos 0,01 por ciento en peso hasta 1,0 por ciento en peso basado en el peso de la composición de desbridación.
10. (Presentada previamente) La composición de acuerdo con la reivindicación 9, en la que dicha biguanida polimérica comprende poli (hexametileno biguanida) y sus sales.
11. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que además comprende un polímero soluble en agua en una concentración de 0.01% en peso a 50% en peso basado en el peso de la composición de desbridación.
12. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que además comprende un agente quelante en una concentración de 0.01% en peso a 1% en peso basado en un peso de la composición de desbridación.
13. (Original) La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un monoalquilglicol seleccionado del grupo que consiste de 1 ,2-propanediol (propilen glicol), 1 ,2-butanediol, 1 ,2-pentanediol, 1 ,2-hexanediol, 1 ,2-heptanediol, 1 ,2-octanediol (caprilil glicol), 1 ,2-nonanediol, 1 ,2-decanediol, 1 ,2-undecanediol, 1 ,2-dodecanediol, 1 ,2-tridecanediol, 1 ,2-tetradecanediol, 1 ,2-pentadecanediol, 1 ,2-hexadecanediol, 1 ,2-heptadecanediol, 1 ,2-octadecanediol, 2-metil-2,4-pentanediol, 1 ,3-butanediol, dietilene glicol, trietileno glicol, glicol bis(hidroxietyl) éter y combinaciones de los mismos.
14. (Original) La composición de acuerdo la reivindicación 1, que comprende alquil éter de glicerol seleccionado del grupo que consiste de 1 -O-heptilglicerol, 1 -O-octilglicerol, 1 -O-nonilglicerol, 1 -O-decilglicerol, 1 -O-undecilglicerol, 1 -O-dodecilglicerol, 1 -O-tridecilglicerol, 1 -O-tetradecilglicerol, 1 -O-pentadecilglicerol, 1 -O-hexadecilglicerol (quimil alcohol), 1 -O-heptadecilglicerol, 1 -O-octadecilglicerol (batil alcohol), 1 -O-octadec-9-enil glicerol (selaquil alcohol), glicerol 1 -(2-etilhexil) éter, 2-etilhexil glicerol, glicerol 1 -heptil éter, glicerol 1 -octil éter, glicerol 1 -decil éter, and glicerol 1 -dodecil éter, glicerol 1 -tridecil éter, glicerol 1 -tetradecil éter, glicerol 1 -pentadecil éter, glicerol 1 -hexadecil éter, glicerol 1 -octadecil éter y combinaciones de los mismos.
15. (Presentada previamente) La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos una biguanida polimérica en una cantidad de por lo menos 0,01% en peso, un agente quelación en una concentración de 0,01% en peso a 1% en peso, y un componente diol vecino que comprende seleccionado de un monoalquilglicol y un monoalquilglicerol

en una concentración de 0,05% en peso a 4% en peso, basado en el peso de la composición de desbridación.

16. (Original) La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos un agente analgésico, agente anestésico, agente de dolor neuropático, o una combinación de los mismos.
17. (Original) La composición de acuerdo con la reivindicación 16, en el que el por lo menos un agente analgésico, agente anestésico, o agente de dolor neuropático se selecciona del grupo que consiste de lidocaína, capsaicina, benzocaína, tetracaína, prilocaína, bupivacaína, levobupivacaína, procaína, carbocaína, etidocaína, mepivacaína, nortripileno, amitriptilina, pregabalina, diclofenaco, fentanilo, gabapentina, agentes antiinflamatorios no esteroideos, salicilatos y sus combinaciones.
18. (Original) La composición de acuerdo con la reivindicación 1, en el que la composición de desbridación tiene una forma seleccionada del grupo que consiste de un polvo, solución acuosa, solución líquida orgánica, silicona, gel, crema, película, látex, aerosol, suspensión, pasta, ungüento y espuma.
19. (Original) La composición de acuerdo con la reivindicación 1, en el que dicha composición de desbridación se adsorbe en un apósito o una fibra natural o sintética, malla, hidrocoloide, alginato, hidrogel, película semipermeable, película permeable o un polímero natural o sintético.
20. (Original) La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un agente de regulación.
21. (Presentada previamente) Un kit que comprende:

una composición de desbridación de acuerdo con la reivindicación 1; e

instrucciones para la desbridación de tejido desvitalizado.
22. (Original) El kit de acuerdo con la reivindicación 21, en el que dichas instrucciones comprenden poner en contacto la composición de desbridación con un área de piel en necesidad de desbridación.
23. (Retirada) Un método de desbridación de tejido desvitalizado que comprende poner en contacto una composición de desbridación de acuerdo con la reivindicación 1 con un área de la piel en necesidad de desbridación.
24. (Retirada) El método de la reivindicación 23, en el que dicha etapa de contacto se repite al menos una vez un día.
25. (Retirada) El método de la reivindicación 23, que además comprende remover el tejido desbridado de dicha área de la piel.
26. (Presentada previamente) La composición de acuerdo con la reivindicación 3, en el que dicha α -amilasa se selecciona del grupo que consiste de *Bacillus* spp. y *Aspergillus* spp.

27. (Cancelada)
28. (Presentada previamente) La composición de acuerdo con la reivindicación 1, en el que el componente de amilasa se presenta en una cantidad en el rango de 2 a 99.5% en peso basado en el peso total de la composición de desbridación.

OBSERVACIONES

Se requiere la revisión y reconsideración de las objeciones de la solicitud encontradas en el final del Oficio enviado en abril 26, 2016 (el "Oficio") en vista de las anteriores modificaciones y las siguientes observaciones. El Comisionado se autoriza aquí a cargar la tasa de \$200 por una extensión de dos meses de tiempo a la Cuenta de depósito No. 04-1679. Aunque no se cree que se deban pagar tasas adicionales, se autoriza aquí al Comisionado a cargar cualquier deficiencia o crédito de cualquier excedente a la Cuenta de depósito No. 04-1679.

En el Oficio, las reivindicaciones 1 – 4, 6 – 22 y 26 – 28 fueron redactadas para una invención elegida, con todas las reivindicaciones elegidas siendo rechazadas bajo U.S.C. 35 §103(a). Por medio de esta modificación, la reivindicación es modificada y las reivindicaciones 3 y 27 son canceladas. El soporte para la materia de las modificaciones puede encontrarse a lo largo de la descripción. *Ver, por ejemplo*, páginas 7 – 8, párrafo puente.

Las modificaciones presentadas aquí han sido realizadas solamente para agilizar el trámite de la presente solicitud para la concesión y no debería ser construido como un indicador del acuerdo o el consentimiento del solicitante con la posición del examinador. De acuerdo a esto, el solicitante expresamente mantiene el derecho a continuar con la materia más amplia a través de modificaciones subsecuentes, realizar solicitudes de continuación o divisionales, realizar procedimientos de reevaluación o reedición y todos los otros mecanismos disponibles. Las objeciones y respuestas a esto son expuestas completamente a continuación.

Resumen de la entrevista

El solicitante desea agradecer al examinador por mantener la cortesía en una entrevista telefónica realizada en agosto 31, 2016 con Gregory M. Lfkowitz y el Dr. Joseph Salamone. Se discutieron las objeciones pendientes y modificaciones potenciales y los argumentos para superar las objeciones pendientes. El solicitante explicó que procediendo en contrario con el juicio convencional en el campo se evidencia la no obviedad y que la materia reclamada es el resultado de sólo una aproximación. El solicitante provee detalles adicionales del porqué es así, mediante una declaración del Dr. Salamone. El examinador agradece y concede una consideración adicional sobre una sumisión de una respuesta formal.

Objeciones a las reivindicaciones de acuerdo a la U.S.C. 35 §103

En el Oficio, se rechazan las reivindicaciones 1, 2, 18 y 20 de acuerdo a la U.S.C. §103 siendo anticipadas por la Patente estadounidense No: US 3,681,197 por Smith ("Smith"). Como se modificó, la reivindicación 1 incluye la materia de la reivindicación 3, la cual no se rechaza basados en Smith. Por lo tanto, la reivindicación 1 y todas las reivindicaciones dependientes de esta, incluyendo las reivindicaciones 2, 18 y 20 son patentables sobre Smith. De acuerdo a esto, el solicitante requiere de manera respetuosa que la objeción sobre las reivindicaciones 1, 2, 18 y 20 sea retirada.

En el Oficio, las reivindicaciones 1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28 fueron rechazadas de acuerdo a la U.S.C. §103(a) al no ser patentables en vista de la Publicación estadounidense preconcedida No. 2013/0202581 de Fallon, et al. ("Fallon") en vista de

la patente estadounidense No. 3,003,917 de Beiler, et al. ("Beiler") y Souze, et al. (Brazilian Journal of Microbiology, Vol. 41, pags 850-861 (2010)) ("Souza"). Como se modificó, la reivindicación 1 se redactó como:

1. (Actualmente modificada) Una composición de desbridación que comprende:

0.1 a 99.5% en peso de un componente de amilasa, y

al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente en una cantidad de más del 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación,

en donde, ~~si un componente de enzima proteolítica está presente~~, una proporción de componente de amilasa a un componente de enzima proteolítica es al menos ~~[[4]] 10:1~~, y en donde dicho componente de amilasa comprende al menos 80% en peso de α -amilasa.

La composición de desbridación reclamada incluye un componente de amilasa y al menos un transportador o excipiente farmacéuticamente o cosméticamente aceptable. Adicionalmente, una proporción del componente de amilasa al componente de enzima proteolítica es al menos 10:1, y dicho componente de amilasa comprende al menos 80% en peso de α -amilasa.

Con el fin de poner la modificación a la reivindicación en contexto con las proporciones reivindicadas y la habilidad inesperada de la amilasa para incrementar la desbridación, el solicitante presenta aquí la Hoja de Datos de Seguridad (SDS) para α -amilasa #6, así como el Certificado de Análisis para α -amilasa #3 y α -amilasa #2. Esos documentos demuestran que la proporción de amilasa a la proteasa para α -amilasa #6, α -amilasa #3 y α -amilasa #2 es respectivamente 10:1, 940:1 y 240:1. Por ejemplo, (1) la SDS para α -amilasa #6 (de Dyadic) muestra que por cada gramo de polvo, hay 0.32 gramos de amilasa y 0.03 gramos de proteasa (10.67:1), (2) el certificado de análisis para α -amilasa #3 muestra que por cada miligramo de polvo, hay 0.47 miligramos de amilasa y 0.0003 miligramos de proteasa (940:1), y (3) el certificado de análisis para α -amilasa #2 muestra que por cada miligramo de polvo, hay 0.48 miligramos de amilasa y 0.002 miligramos de proteasa (240:1). La eficacia de estas composiciones se discute a lo largo de la descripción. Ver Descripción, "Ejemplos" en las páginas 28 – 37.

Por lo tanto, aunque las enzimas proteolíticas son conocidas por digerir proteínas y amilasas son conocidas para digerir almidones, la composición de desbridación reclamada se formula para incluir una cantidad sustancialmente más alta del componente amilasa que el componente de enzima proteolítica, lo cual puede no estar presente. Esto es directamente contrario a las expectativas de aquellos versados en la materia, como se evidencia en Fallon y Beiler.

Como se explicó en el Capítulo 2145.X.D.3 de la MPEP, "***procediendo contrario al juicio aceptado en el arte se evidencia no obviedad***". En re Hedges, 783 F.2d 1038 (Fed. Cir. 1986) (El proceso reclamado por el solicitante para sulfonar difenil sulfona a una temperatura sobre 127°C fue contrario al juicio aceptado porque el arte previo en su

totalidad sugería usar bajas temperaturas para óptimos resultados como evidencia para carbonización, descomposición o tasas reducidas a altas temperaturas.)”.

Por ejemplo, en *Arkie Lures v. Gene Larew Tacle*, 119 F.3d 953, 9578-58 (Fed. Cir. 1997), el Circuito Federal mantiene que, si algunos productores supieran cómo mezclar sal y plastisol, como se argumentó a la corte del distrito, no haría obvio proceder en contra de la vista general en el campo de los señuelos de peces plásticos. La advertencia de FitzSimons en contra de la adicción de ingredientes plásticos insolubles a plastisol se repitió por el Dr. Carver y el Sr. Harville, y el Sr. Larew también fue aconsejado mucho antes del surgimiento de esta litigación. Además, estos versados de los señuelos plásticos consideraban que la sal podría afectar la superficie, textura y fortalecer los señuelos. La demostración posterior de Larew indica que el producto deseado podría, incluso, ser exitosamente hecho, lo que indica que no es obvio, ni la apreciación de su valor lo hace no patentable. De esos casos, es aparente que el análisis apropiado se enfoca en el juicio en el arte como un todo, incluyendo los testimonios de aquellos versados en la materia y no se limita a divulgaciones vagas de una referencia específica.

En soporte a esta posición, el solicitante presenta la Declaración del Dr. Joseph C. Salamone de acuerdo a la C.F.R. 37 1.132 (“Declaración de Salamone”). El Dr. Salamone explica que:

Previo a la investigación que llevó a esta solicitud, la idea de maximizar la amilasa, la cual se conoce para la digestión de almidones, y minimizar las enzimas proteolíticas, las cuales se conocen para digerir proteínas, en una composición para el tratamiento de la piel fue contra-intuitivo. Esto es porque la piel está formada de proteínas y grasas, las cuales son digeridas por proteasas y lipasas, respectivamente. Por lo tanto, un versado en las composiciones enzimáticas para el cuidado de la piel habría considerado la concentración de las proteasas y la concentración de las lipasas como materia importante, mientras que el contenido de amilasa era más o menos irrelevante. Por lo tanto, mientras que un versado en las composiciones enzimáticas para el cuidado de la piel habría tolerado la contaminación de amilasa en la proteasa o lipasa para ser usado en una formulación, no habría tenido motivación para incrementar la cantidad de amilasa. Adicionalmente, hubiera sido impensable minimizar o eliminar la proteasa.

Declaración de Salomone, sección 4.

El Dr. Salamone indicó que es “directamente contrario a las expectativas de aquellos versado en la materia” tener una formulación para el cuidado de la piel ***“formulada para incluir una cantidad sustancialmente más alta del componente de amilasa, y α -amilasa en particular, que el componente de enzima proteolítica, el cual podría incluso no estar presente”***. *Id.*

El Dr. Salamone también explica que:

Los inventores estuvieron muy sorprendidos de aprender que la amilasa era más efectiva para el uso para la desbridación de tejidos necróticos. Los inventores incluso fueron más allá para conducir experimentos para demostrarse a ellos mismos que el efecto no fue resultado de la contaminación con enzimas proteolíticas. Esos experimentos son discutidos con respecto a los experimentos

de digestión con gel de colágeno en la descripción (Descripción, página 29, línea 15 – página 30, línea 31). Una comparación de las propiedades reológicas del colágeno tratado demostró que (1) la proteasa licuó completamente el gel de colágeno, y (2) el gel de colágeno tratado con α -amilasa no exhibió digestión (Descripción, página 30, líneas 7 – 10). El módulo complejo también confirmó esos resultados (Descripción, página 30, líneas 14 -16). Este estudio estableció que el efecto de desbridación de la amilasa no fue el resultado de la contaminación con enzimas proteolíticas.

Teniendo establecido que la amilasa no estaba, de hecho, contaminada con cantidades suficientes de enzimas proteolíticas para digerir proteínas, ahora podemos revisar los resultados de la digestión de la descripción (Descripción, página 30, línea 32 – página 35, línea 11). Esos experimentos usaron piel de cerdo hervida como un ejemplo para tejido desvitalizado (necrótico) presente durante la desbridación. Como puede verse en las tablas 1 – 6, las composiciones de amilasa fueron capaces de digerir efectivamente la piel de cerdo hervida (*Id.*). Debería notarse que la α -amilasa #2, la cual incluye una impureza más alta de proteasa (0.2% en peso v. 0.05% en peso) que la amilasa #3, de hecho, realizada ligeramente peor, la cual refuerza la conclusión de que la actividad de desbridación de amilasa no es el resultado de la contaminación de proteasa (Descripción, página 31, líneas 22 – 26).

Esto fue algo inesperado considerando que las amilasas catalizan la hidrólisis de los almidones en azúcares, mientras que las proteasas son enzimas que empiezan el catabolismo de las proteínas por hidrólisis de los enlaces peptídicos que unen aminoácidos en una cadena de polipéptidos. ***De hecho, mientras que es convencional utilizar las enzimas proteolíticas para tratamientos de la piel, era inesperado descubrir que las amilasas pueden ser efectivas para el uso en composiciones de desbridación.***

Id.

El Oficio asegura que los rangos reclamados son solamente el resultado de la optimización. Sin embargo, Fallon se enfoca claramente en proteasas y lipasas y no considera que la amilasa sea un componente importante de las preparaciones divulgadas allí. Además, Fallon se enfoca en composiciones en donde la cantidad de proteasa es casi el doble de la cantidad de amilasa. Por lo tanto, no habría nada en las referencias citadas que divulgue o sugiera el uso de altas proporciones de amilasa en relación a la proteasa. Por el contrario, el conocimiento de aquellos versados en la materia los habría llevado a incrementar la cantidad de proteasa y minimizar la cantidad de amilasa (si está presente del todo) en composiciones de desbridación.

Volviendo a las referencias citadas, en más detalle, Fallon se refiere a una composición para la cura de heridas que puede incluir proteasa, lipasa y amilasa. Mientras que Fallon divulga algunos increíbles rangos amplios, una persona versada en la materia reconocería que ***Fallon se basa en un estudio conducido mediante el uso de varias dosis de pancreatina.***

El Dr. Salamone provee el siguiente análisis de Fallon:

La referencia primaria citada en el Oficio es Fallín, el cual se refiere a una composición de enzimas para el uso en la curación de heridas. Fallon incluye, excepcionalmente, amplios rangos de proteasa, lipasa y amilasa. Sin embargo, en ninguna parte se divulgan cantidades específicas de proteasa, lipasa y amilasa, hay más proteasa presente que lipasa. *Ver, por ejemplo*, Fallon, párrafos [0176] – [0178]. De hecho, esta es la proporción específica presente en la pancreatina. Fallon, párrafo [0093]. Además, todos los ejemplos están basados en un “concentrado de enzimas alto en proteasa”, el cual ilustra que la proteasa en las enseñanzas de Fallon es la enzima importante. Fallon, párrafo [0175].

Otra observación importante es que Fallon incluye una enseñanza acerca de las proporciones útiles de proteasa a lipasa (Fallon, párrafo [0011]), pero nada acerca de los porcentajes útiles de proteasa a amilasa o de lipasa a amilasa.

Por lo tanto, un versado en composiciones enzimáticas para el cuidado de la piel interpretaría a Fallon como una divulgación de que la pancreatina es útil para facilitar la curación de heridas y que la proteasa es el componente más importante de la mezcla de pancreatina, lo cual también incluye lipasa y proteasa. ***No hay nada en Fallon que contradiga o cambie el conocimiento de que la amilasa es un componente menor en las composiciones para la curación de heridas.***

Salamone, sección 5.

El solicitante provee un número de puntos detallados adicionales respecto a Fallon en la respuesta previa. Esos puntos son incorporados aquí para la referencia.

Beiler divulga una composición para la curación de heridas que contiene una proporción 5:1:1 de relaxina, tripsina y amilasa (Beiler, columna 1, líneas 46 – 52). Como puede notarse en Beiler, la tripsina puede ser sustituida con ***otras enzimas proteolíticas*** (Beiler, columna 1, líneas 27 – 30 & Columna 2, líneas 1 – 22). Por lo tanto, la proporción de enzimas no proteolíticas a enzima proteolíticas es 1:1 en la composición de Beiler, lo cual es menos de la proporción 4:1 especificada en la reivindicación 1 modificada.

El Dr. Salamone provee el siguiente análisis de Beiler:

Beiler divulga una composición para la curación de heridas que contiene relaxina y aproximadamente cantidades iguales de tripsina y amilasa. Beiler, columna 1, líneas 36 -52 & tabla I. Beiler demuestra que, cuando son combinados, dichos componentes aceleran la curación, pero que ninguno de ellos por sí solos son significativamente diferentes del control. Beiler, Tabla I. Adicionalmente, la proporción preferida de componentes es 5 partes de relaxina, 1 parte de enzima proteolítica y 1 parte de enzima amilolítica. Beiler, columna 1, líneas 46 – 52. Por lo tanto, no hay nada en Beiler que contradiga o cambie el conocimiento de que la amilasa es un componente menor en las composiciones para la curación de heridas.

Declaración de Salamone, sección 6.

Además, Beiler no divulga o sugiere la composición de desbridación reclamada en donde ***una proporción del componente de amilasa a un componente de enzima proteolítica es al menos 4:1, y el componente de amilasa es al menos 80% α -amilasa***, en combinación con las otras características. De hecho, cuando se combinan Fallon y Beiler, los ejemplos de Fallon sugieren el incremento de las enzimas proteolíticas presentes (es decir, una proporción 7:4 de proteasa a amilasa) y Beiler divulga una composición en donde la cantidad de proteasa y amilasa es igual (proporción 1:1). Por lo tanto, la combinación de Fallon y Beiler no sólo falla en divulgar o sugerir la composición de desbridación reclamada, sino que sus enseñanzas están lejos de la composición de desbridación reclamada, en donde una proporción del componente de amilasa a un componente de enzima proteolítica es al menos 4:1, en combinación con las otras características.

Debería notarse que las enseñanzas de Fallon y Beiler tienen sentido, porque la amilasa es una enzima que cataliza la hidrólisis de almidones en azúcares, mientras que las proteasas son enzimas que comienzan el catabolismo de proteínas mediante la hidrólisis de enlaces peptídicos que unen aminoácidos en una cadena de polipéptidos. Por lo tanto, era totalmente inesperado cuando el solicitante descubrió que la amilasa era particularmente útil para la remoción del tejido necrótico. De hecho, el solicitante condujo estudios específicamente para descartar la posibilidad de que el efecto de desbridación fuera el resultado de la contaminación por enzimas proteolíticas (*Ver, por ejemplo*, Descripción, páginas 29 – 30; página 31, último párrafo, y páginas 35 – 37, “Resultados de digestión, piel de rata”). Mientras que no es necesario para llevar a cabo la invención, se cree que algo acerca de la transformación del tejido sano a tejido necrótico hace al tejido necrótico susceptible de escisión por amilasa. Sin embargo, como se mencionó anteriormente, esto es bastante inesperado.

Souza es usado solamente para la divulgación de recursos de α -amilasa y no corrige las deficiencias de Fallon y Beiler.

En resumen, la combinación de Fallon, Beiler y Souza falla en divulgar o sugerir la composición de desbridación reclamada que contiene 0.1 a 99.5% en peso de un componente de amilasa basado en un peso total de la composición de desbridación, ***donde una proporción del componente de amilasa a un componente de enzima proteolítica es al menos 10:1, y al menos 80% del componente de amilasa es α -amilasa***. De hecho, dicha combinación procede de manera contraria al conocimiento de los versados en las composiciones para el cuidado de la piel. Por lo tanto, la reivindicación 1 y todas las reivindicaciones dependientes de estas son patentables sobre la combinación de Fallon en vista de Beiler y Souza. De acuerdo a esto, el solicitante requiere de manera respetuosa que la objeción de las reivindicaciones 1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28, basados en Fallon en vista de Beiler y Souza, sea retirada.

En el Oficio, la reivindicación 8 fue objetada de acuerdo a la U.S.C. 35 §103(a) al ser no patentable sobre Fallon en vista de Beiler, Souza y Loo, et al (PLoS One, Vol. 7(11), e49215 (2012)) (“Loo”). Se usa Loo solamente para la divulgación de la glucosa oxidasa en la curación de heridas, pero no corrige las deficiencias de Fallon, Beiler y Souza descritas anteriormente. Por lo tanto, la reivindicación 1 y todas las reivindicaciones que dependen de ésta, son patentables sobre las combinaciones de Fallon, Beiler, Souza

y Loo. De acuerdo a esto, el solicitante requiere de manera respetuosa que la objeción de la reivindicación 8 basada en Fallon, Beiler, Souza y Loo sea retirada.

En el Oficio, las reivindicaciones 1, 9, 10, 14 y 15 fueron objetadas de acuerdo a la U.S.C. 35 §103(a) al no ser patentable sobre Fallon, Beiler y Souza en vista de la Publicación estadounidense pre-concedida No. 2004/0076671 de Tippet, et al. ("Tippet"). Se utiliza Tippet solamente para la divulgación de PHMB, un agente quelante y analgésico y no corrige las deficiencias de Fallon, Beiler y Souza descritas anteriormente. Por lo tanto, la reivindicación 1 y todas las reivindicaciones dependientes de ésta, son patentables sobre la combinación de Fallon, Beiler, Souza y Tippet. De acuerdo a esto, el solicitante requiere, de manera respetuosa, que la objeción de las reivindicaciones 1, 16 y 17 basada en Fallon, Beiler, Souza y Tippet sea retirada.

Conclusion

Por al menos las razones expuestas anteriormente, se considera que la reivindicación independiente sea permitida. Además, se considera que las reivindicaciones dependientes son permitidas debido a su dependencia en una reivindicación base permitida y para las demás características que allí se mencionan. Se considera que la solicitud está en condición de ser autorizadas de inmediato. Si ningún asunto se mantiene, el solicitante invita al Examinador a llamar a Greg Lefkowitz quien firma (línea directa: 561-962-2110) si se considera que una entrevista telefónica podría agilizar el trámite de la solicitud para una concesión.

Respetuosamente,

Día: septiembre 28, 2016

J. Rodman Steele, Jr., Reg. No. 25,931

ACCIÓN DETALLADA

La presente solicitud, presentada en o después de marzo 16, 2013, está siendo examinada de acuerdo al primer inventor para completar las disposiciones de la AIA.

La modificación del solicitante y la respuesta presentada el 4/11/2016 ha sido recibida y ha entrado al caso. La reivindicación 5 ha sido cancelada. Las reivindicaciones 1 – 4 y 6 – 28 están pendientes, las reivindicaciones 23 – 25 han sido retiradas y las reivindicaciones 1 – 4, 6 – 22 y 26 – 28 han sido consideradas en los méritos, en la medida que se leen en las especies seleccionadas de lipasa (en la reivindicación 6) y oxidadas (en la reivindicación 8). Todos los argumentos han sido considerados completamente.

Objeciones retiradas

1. Las objeciones son retiradas en vista de las modificaciones del solicitante.

Denegaciones retiradas

2. Las denegaciones de acuerdo a U.S.C. 35 §102 son retirados en vista de las modificaciones del solicitante.

Denegaciones a las reivindicaciones - U.S.C. 35 §103

En el evento de la determinación del estado de la solicitud sujeta a AIA U.S.C. 35 102 y 103 sea incorrecta, ninguna corrección de la base estatutaria para la denegación será considerada un nuevo motivo de denegación si el arte previo presentado y el soporte racional de la objeción, pudiera ser la misma bajo cualquier estado.

La siguiente es una cita de U.S.C. 35 103 que forma la base de todas las denegaciones por obviedad expuestas en el Oficio:

Podría no ser obtenida una patente para una invención reivindicada, a pesar de que la invención reivindicada no es idénticamente divulgada como se expone en la sección 102 de este título, si las diferencias entre la invención reivindicada y el arte previo son tales que la invención reclamada como un todo hubiera sido obvia, antes de la fecha de presentación efectiva de la invención reclamada, para un versado en la materia para quien la invención reclamada pertenece. La patentabilidad no debería ser negada por la manera en la cual la invención fue hecha.

3. Las reivindicaciones **1, 2, 18 y 20** son denegadas bajo la U.S.C. 35 103 al no ser patentable sobre Smith (US 3,681,197 A; 8/1/1972).

Las reivindicaciones mencionan una composición de desbridación que comprende: 0.1 – 99.5% en peso de un componente de amilasa y al menos un transportador o excipiente farmacéuticamente o cosméticamente aceptable en

una cantidad de más del 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación, en donde, si un componente de enzima proteolítica está presente, una proporción del componente de amilasa al componente de enzima proteolítica es al menos 4:1.

Smith enseña una fórmula de solución que comprende 1 g de amilasa pancreática (0.09% en peso de un componente de amilasa, de animales, columna 6, línea 71), 622 mL de agua (transportador o excipiente farmacéuticamente o cosméticamente aceptable en una cantidad de más del 99.9% en peso de la composición), y amortiguador fosfato (un agente amortiguador) (Ejemplo II), en donde la formulación es apropiada y útil en la desbridación de partículas orgánicas en una cavidad oral (columna 1, líneas 55 – 58).

Smith no enseña la concentración del componente de amilasa reclamada (reivindicación 1). Sin embargo, Smith sí enseña la fórmula de la solución que comprende 0.09% en peso de un componente de amilasa, el cual está cerca a la concentración reclamada 0.1% en peso. La referencia sí identifica que este componente es un ingrediente activo en la fórmula de la solución. Por lo tanto, el componente alcanza resultados reconocidos y es, por lo tanto, considerado como una variable de resultado efectivo. Como tal, antes de la fecha de presentación efectiva de la invención reclamada, es obvio optimizar la concentración del componente de amilasa como materia de experimentación rutinaria. Generalmente, las diferencias en la concentración no soportan la patentabilidad de materia abarcada por el arte previo hasta que haya evidencia que indique que la concentración es crítica. (MPEP 2144.05 II). Además, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia habría estado motivado por la referencia citada a optimizar la concentración del componente de amilasa con una expectativa razonable de éxito, obteniendo una fórmula de solución.

4. Las reivindicaciones **1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28** son denegadas bajo la U.S.C. 35 103 sobre Fallon et al (US 2013/0202581 A1; 8/8/2013) en vista de Beiler et al (US 3,003,917; 10/10/1961) y Souza et al (Brazilian Journal of Microbiology, 2010;41:850 – 861).

Las reivindicaciones mencionan una composición de desbridación que comprenden: 0.1 – 99.5% en peso de un componente de amilasa, y al menos un transportador o excipiente farmacéuticamente o cosméticamente aceptable en una cantidad de más del 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación, en donde, si un componente de enzima proteolítica está presente, una proporción del componente de amilasa al componente de enzima proteolítica es al menos 4:1.

Fallon enseña una composición que comprende una o más amilasas, proteasas y lipasas y con uno o más transportadores farmacéuticamente aceptables (párrafo 0066), en donde dichas una o más amilasas tienen una actividad en el rango de aproximadamente 200 – 300 unidades USP/mg, dichas proteasas tienen una actividad en el rango de aproximadamente 150 – 250 unidades USP/mg (párrafo 0095), dicho transportador está en una cantidad de aproximadamente 0.1% a aproximadamente 95% en peso de la composición (párrafo 0130), y dichas

enzimas (que comprenden una amilasa) son derivadas de un recurso animal, recurso microbiano, recurso vegetal o recurso de hongo (párrafo 0007). La composición además comprende polietilenglicoles (un polímero soluble en agua) (párrafo 0120), trietanolamina (un agente quelante) (párrafo 0127), propilenglicol (un glicol monoalquilo) (párrafo 0121), una sustancia analgésica (párrafo 0142), un agente amortiguador (párrafo 00269). La composición tiene una forma de un polvo, una solución acuosa, gel, crema o ungüento, otras formulaciones incluyen materiales de vestir (párrafo 0086).

Fallon no enseña las concentraciones reclamadas de amilasas, enzimas proteolíticas, lipasas, polímero soluble en agua (polietilenglicoles), y el agente quelante (trietanolamina) como se menciona en las reivindicaciones 1, 6, 11, 12 y 28.

Sin embargo, Fallon enseña que la composición comprende una cantidad efectiva de enzimas que incluyen amilasas, enzimas proteolíticas y lipasas y polietilenglicoles (un polímero soluble en agua) y trietanolamina (un agente quelante).

Por lo tanto, antes de la fecha de presentación efectiva de la invención reclamada, hubiera estado fuera del alcance de un versado en la materia optimizar la concentración de los presentes componentes como materia de experimentación rutinaria, porque la referencia identifica cada uno de los presentes componentes como ingredientes activos en la composición. Como tal, cada uno de los componentes alcanza un resultado reconocido y, por lo tanto, es considerado para ser una variable efectiva para los resultados. Generalmente, las diferencias en la concentración no sustentan la patentabilidad de materia abarcada por el arte previo a no ser que haya evidencia que indique que dicha concentración es crítica (MPEP 2144.05 II).

Además, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia no habría estado motivado por la referencia citada para optimizar la cantidad de los presentes componentes con una expectativa razonable de éxito para obtener una composición.

Fallon no enseña que la composición comprenda α -amilasa y β -amilasa, así como las concentraciones reclamadas de esas amilasas, como se menciona en las reivindicaciones 3 – 4, 7 y 26 – 27.

Sin embargo, Fallon no enseña la composición que comprende una cantidad efectiva de una o más amilasas (párrafo [0095]), donde la composición es útil para la curación de heridas (Título). Beiler enseña una composición útil para la curación de heridas que comprende mezclas de amilasas bacterianas, pancreáticas o α -amilasa y β -amilasa (columna 1, línea 32 – 33, tabla I). Antes de la fecha de presentación efectiva de la invención reclamada, era bien sabido en el arte que la α -amilasa es derivada en su mayoría del género *Bacillus* o *Aspergillus* para aplicaciones comerciales/industriales (página 855 columna izquierda – párrafo 1, columna derecha – párrafo 3 de Souza).

Por lo tanto, antes de la fecha de presentación efectiva de la invención reclamada, habría sido obvio para un versado en la materia incorporar α -

amilasa (amilasa pancreática) y β -amilasa teniendo en cuenta que Fallon divulga una composición que comprende amilasas y que es útil para la curación de heridas y Beiler divulga que la α -amilasa (amilasa pancreática) y la β -amilasa ayudan con la curación de heridas (Tabla I). Teniendo en cuenta que estas referencias identifican cada uno de los componentes presentes como ingredientes activos en la composición efectiva para la curación de heridas, cada uno de los componentes alcanza un resultado reconocido y es, por lo tanto, considerado una variable de resultados efectiva. Como tal, es obvio optimizar la concentración de los presentes componentes como una materia de experimentación rutinaria. Generalmente, las diferencias en la concentración no soportan la patentabilidad de la materia abarcada por el arte previo a menos de que haya evidencias que indique que dicha concentración es crítica (MPEP 2144.05 II). Además, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia habría estado motivado por las referencias citadas a incorporar α - y β -amilasas, así como optimizar sus concentraciones, con una expectativa razonable de éxito para obtener una composición.

Las referencias citadas anteriormente no enseñan un kit que comprende la composición reclamada y las instrucciones reclamadas, como se menciona en las reivindicaciones 21 – 22. Sin embargo, las referencias combinadas enseñan una composición como se reivindica, en donde la composición es para administración tópica (poner en contacto la composición con un área de la piel). Se evidencia que sólo la diferencia entre un producto del arte previo y un producto reivindicado es materia impresa que no está relacionada funcionalmente con el producto, el contenido de la materia impresa no diferenciará el producto reclamado del arte previo (MPEP 2112.01).

La limitación del término “desbridación” es considerada como un intento de limitación por uso. La reivindicación de un nuevo uso, función o propiedad desconocida no hace la composición necesariamente patentable. El intento de uso de la composición reclamada no diferencia, patentablemente, la composición, per se, teniendo en cuenta que el uso no divulgado es inherente de la composición de referencia. Con el propósito de ser limitante, el intento de uso debe crear una diferencia estructural entre la composición reclamada y la composición del arte previo. En el presente caso, las referencias combinadas enseñan una composición como se reclama. El intento de uso falla en crear una diferencia estructural, por lo tanto, el intento de uso no es limitativo. Por favor noten que cuando el solicitante reclama una composición en términos de función y la composición del arte previo parece ser la misma, el examinador puede hacer objeciones bajo ambas, U.S.C. 35 102 y 103 (MPEP 2112).

5. La reivindicación **8** es objetada bajo la U.S.C. 35 103 al ser no patentable sobre Fallon et al (US 2013/0202581 A1; 8/8/2013) en vista de Beiler et al (US 3,003,917; 10/10/1961) y Souza et al (Brazilian Journal of Microbiology, Vol. 41, pags 850-861 (2010)) como se aplicó a las anteriores reivindicaciones 1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28 en vista de Loo et al (PLoS One. 2012;7(11):e49215).

Las referencias citadas anteriormente no enseñan la composición que además comprenden oxidasas y su concentración reivindicada, como se menciona en la **reivindicación 8**.

Sin embargo, Fallon enseña que la composición es útil para la curación de heridas (título). Loo enseña que se encuentra que la glucosa oxidasa promueve la curación de heridas (párrafo e49215 columna izquierda – párrafo 2).

Por lo tanto, antes de la fecha de presentación efectiva de la invención reclamada, habría sido obvio para un versado en la materia incorporar glucosa oxidasa en la composición de Fallon, teniendo en cuenta que Fallon divulga una composición para la curación de heridas y Loo divulga que la glucosa oxidada promueve la curación de heridas. Además, antes de la fecha de presentación efectiva de la invención reclamada, era obvio variar y/u optimizar la cantidad de glucosa oxidasa proporcionada por la composición, de acuerdo a la guía proporcionada por la referencia citada, proveer una composición que tenga las propiedades deseadas como la concentración deseada de glucosa oxidasa para la curación de heridas. Se evidencia que “[D]onde las condiciones generales de una reivindicación son divulgadas en el arte previo, no es inventivo descubrir rangos óptimos o trabajables por experimentación rutinaria”. Adicionalmente, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia habría estado motivado por la referencia citada a incorporar una cantidad optimizada de glucosa oxidasa con una expectativa razonable de éxito para obtener una composición.

6. Las reivindicaciones **9, 10, 14 y 15** son rechazadas bajo la U.S.C. 34 103 al no ser patentables sobre Fallon et al (US 2013/0202581 A1; 8/8/2013) en vista de Beiler et al (US 3,003,917; 10/10/1961) y Souza et al (Brazilian Journal of Microbiology, Vol. 41, pags 850-861 (2010)) como se aplicó a las anteriores reivindicaciones 1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28 en vista de Patel et al (US 2007/0237812 A1; 10/11/2007) y Parks (WO 2013/016255 A1; 1/31/2013).

Las referencias citadas anteriormente no enseñan la composición que además comprende poli(hexametileno biguanida) y su concentración reclamada (**reivindicaciones 9 - 10 y 15**).

Sin embargo, Fallon enseña que la composición además comprende propilenglicol (un glicol monoalquilo) (párrafo 0121) y antimicrobianos (párrafo 0029), en donde la composición es un material para vendajes (párrafo 0086). Pate enseña un material para heridas el cual se construye para acelerar el proceso de curación de heridas (párrafo 0028), que comprende una cantidad adecuada de agentes antimicrobianos como biguanida de polihexametileno (párrafo 0052). Además, Parks enseña una composición y un vendaje de heridas para el tratamiento de heridas de la piel (resumen), que comprende biguanida de polihexametileno y Sensiva SC 50 (glicerol 1-(2-etilhexil) éter, un éter alquilo de glicerol) (Tabla 3).

Por lo tanto, antes de la fecha de presentación efectiva de la invención reclamada, habría sido obvio para un versado en la materia incorporar

biguanida de polihexametileno y Senciva SC 50 en la composición de Fallon, teniendo en cuenta que Fallon, Patel y Parks divulgan una composición para la curación de heridas, Fallon divulga que la composición divulga agentes antimicrobianos y Patel y Parks divulgan que la composición comprende biguanida de polihexametileno como un agente antimicrobiano que acelera el proceso de curación de heridas. Además, antes de la fecha de presentación efectiva de la invención reclamada, era obvio variar y/u optimizar la cantidad de propilenglicol (glicol monoalquilo) y agentes antimicrobianos como biguanida de polihexametileno proporcionada en la composición, de acuerdo a la guía proporcionada por las citadas referencias, para proporcionar una composición que tenga las propiedades deseadas, así como las concentraciones deseadas de propilenglicol y biguanida de polihexametileno para la curación de heridas. Se evidencia que “[D]onde las condiciones generales de una reivindicación son divulgadas en el arte previo, no es inventivo descubrir rangos óptimos o trabajables por experimentación rutinaria”. Adicionalmente, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia habría estado motivado por la referencia citada a incorporar biguanida de polihexametileno y Sensiva SC 50, así como a optimizar la cantidad de propilenglicol y biguanida de polihexametileno con una expectativa razonable de éxito para obtener una composición.

7. La reivindicación 17 es objetada bajo la U.S.C. 34 103 al no ser patentables sobre Fallon et al (US 2013/0202581 A1; 8/8/2013) en vista de Beiler et al (US 3,003,917; 10/10/1961) y Souza et al (Brazilian Journal of Microbiology, Vol. 41, pags 850-861 (2010)) como se aplicó a las anteriores reivindicaciones 1 – 4, 6, 7, 11 – 13, 16, 18 – 22 y 26 – 28 en vista de Tippet (US 20048/0076671 A1; 4/22/2004).

Las referencias citadas anteriormente no enseñan la composición que además comprenden el agente analgésico reclamado, como se menciona en la **reivindicación 17**.

Sin embargo, Fallon enseña que la composición además comprende una sustancia analgésica (párrafo 0142). Tippet enseña un vendaje para heridas que incrementa la curación de una herida, el cual comprende lidocaína (un agente analgésico) (párrafo 0011).

Por lo tanto, antes de la fecha de presentación efectiva de la invención reclamada, hubiera sido obvio para un versado en la materia incorporar lidocaína (el agente analgésico reclamado) en la composición de Fallon, teniendo en cuenta que Fallon y Tippet divulgan una composición para la curación de heridas y Tippet divulga que la composición que comprende lidocaína incrementa la curación de una herida. Además, antes de la fecha de presentación efectiva de la invención reclamada, un versado en la materia habría estado motivado por la referencia citada para incorporar lidocaína con una expectativa razonable de éxito para obtener una composición.

Respuesta a los argumentos

8. El solicitante argumenta que Smith no anticipa la reivindicación modificada 1, específicamente, 0.1 a 99.5% en peso del componente amilasa. Sin embargo, esos argumentos son irrelevantes, teniendo en cuenta que las objeciones han sido retiradas en vista de las modificaciones del solicitante.

El solicitante argumenta que Fallon se enfoca en proteasas y lipasas y no considera que la amilasa sea un componente importante de las preparaciones allí divulgadas, que Fallon se enfoca en composiciones donde la cantidad de proteasa es casi el doble de la cantidad de amilasa, que Beiler no enseña la proporción de amilasa a proteasa reclamada, que las enseñanzas de Fallon y Beiler están lejos de la proporción de amilasa a proteasa reclamada de al menos 4:1, y que Souza no corrige las deficiencias de Fallon y Beiler.

Esos argumentos no se encuentran persuasivos porque las modalidades preferidas no están sólo en la enseñanza de una referencia. “El uso de patentes como referencias no está limitado a lo que la patente describe como sus invenciones propias o los problemas que allí se discuten. Ellos son parte del arte de la literatura, relevante para todo lo que ellos contienen” *In re Heck*, 699 F. 2d 1331, 1332-33, 216 USPQ 1038, 1039 (Fed. Cir. 1983) (citando *in re Lemelson*, 397 F.2d 1006, 1009, 158 USPQ 275, 277 (CCPA 1968)). Se puede confiar en una referencia que habría sugerido razonablemente a un versado en la materia, incluyendo modalidades no preferidas. *Merck&Co. V. Biocraft Laboratories*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir), *cert. negado*, 493 U.S. 975 (1989). En el presente caso, Fallon claramente enseña una composición que comprende amilasas que tienen una actividad entre aproximadamente 200 – 300 unidades USP/mg y proteasa que tienen una actividad en el rango de aproximadamente 150 – 250 unidades USP/mg, y pueden ser útiles para modificar la cantidad de una actividad enzimática particular en la composición (párrafo 0095). Generalmente, las diferencias en la concentración no sustentan la patentabilidad de la materia abarcada en el arte previo a menos que haya evidencia que indique que dicha concentración es crítica (MPEP 2144.05 II). Hasta aquí, el solicitante ha fallado en mostrar que la criticalidad de la concentración de amilasa reclamada (o amilasa/enzima proteolítica). De acuerdo a la propia descripción del solicitante, la amilasa de diferentes recursos, o a los niveles de actividad diferentes expresados en unidades, mostraron diferentes niveles de actividad de desbridación *ex vivo*, como se demuestra en la Tabla 1, 0.1% en peso de amilasa de ciertos recursos mostró una alta eficacia de desbridación mientras que el 0.1% en peso de amilasa de otros recursos no mostró eficacia de desbridación (página 31 – 32). Además, la tabla 8 demuestra que las enzimas amilolíticas son comparables con las enzimas proteolíticas en su habilidad de digerir la piel de rata (página 35 – 36). A menos que el solicitante muestre evidencia que indique que la concentración de amilasa reivindicada (o amilasa/enzima proteolítica) es crítica, las diferencias en concentración de amilasa (o amilasa/enzima proteolítica) no soportará la patentabilidad de la materia que abarca el arte previo. Adicionalmente, Beiler se basa en demostrar que la α -amilasa y la β -amilasa pueden ser incluidas en una composición para la curación de heridas, teniendo en cuenta que la α -amilasa y la β -amilasa ayudan a la curación de heridas. Souza se basa en demostrar que la α -amilasa se deriva en su mayoría

del género *Bacillus* o *Aspergillus* para aplicaciones industriales/comerciales. Finalmente, “los ejemplos y las modalidades preferidas divulgadas no constituyen una enseñanza lejana de una divulgación más amplia o modalidades no preferidas” *In re Susi*, 440 F.2d 442, 169 USPQ 423 (CCPA 1971)”. (MPEP 2123). Además, “las divulgaciones del arte previo de más de una alternativa no constituye una enseñanza lejana de ninguna de esas alternativas porque dicha divulgación no critica, desacredita o desaprueba de otra manera la solución reclamada... “ *In re Fulton*, 391 F.3d 1195, 1201, 73 USPQ2d 1141, 1146 (Fed. Cir. 2004).” (MPEP 2141.02). En el presente caso, Fallon y Beiler no “critican, desacreditan o desaprueban de otra manera” el uso de la concentración de amilasa reclamada (o amilasa/enzima proteolítica) para el tratamiento de heridas. Por lo tanto, los ejemplos divulgados y las modalidades preferidas de Fallon y Beiler no constituye una enseñanza lejana de la concentración de amilasa reivindicada (o amilasa/enzima proteolítica).

El solicitante argumenta que es bastante inesperado descubrir que la amilasa puede ser efectiva para el uso en composiciones de desbridación.

No se encuentra que esos argumentos sean persuasivos porque la reivindicación de un nuevo uso, función o propiedad desconocida no hace necesariamente patentable la composición, teniendo en cuenta que el arte previo enseña una composición que comprende un componente de amilasa y al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente, como se reivindica. Además, la evidencia se basa en que se debería establecer que la diferencia en los resultados es, de hecho, inesperada y no obvia para la significancia estadística y práctica. La evidencia de propiedades inesperadas puede ser en la forma de una comparación directa o indirecta de la invención reclamada con el arte previo el cual es adecuado en el alcance con las reivindicaciones. En el presente caso, no hubo comparación de la invención reclamada (por ejemplo, la concentración de amilasa reivindicada (o amilasa/enzima proteolítica)) con el arte previo más cercano (por ejemplo, la concentración de amilasa (o amilasa/enzima proteolítica) divulgada en Fallon), y, por lo tanto, carece de bases para juzgar la significancia práctica o datos con respecto a los resultados inesperados divulgados. Si los resultados inesperados son el resultado de resultados inesperadamente mejorados o una propiedad no enseñada por el arte previo, “la evidencia objetiva de no obviedad debe ser conmensurada en el alcance de las reivindicaciones en las cuales la evidencia es soportada”. En otras palabras, la demostración de resultados inesperados debe ser revisada para ver si los resultados ocurren sobre el rango entero reclamado (MPEP 716.02).

El solicitante argumenta que Loo, Patel, Parks y Tippettt no corrigen las deficiencias de Fallon.

No se encuentra que estos argumentos sean persuasivos porque Loo se basa en demostrar oxidasas, Patel se basa en demostrar la biguanida de polihexametileno, Parks se basa en demostrar el éter de alquil glicerol y Tippettt se basa en demostrar un agente analgésico. Fallon se describe en el segundo párrafo bajo el título de la sección Respuesta a los Argumentos.

Conclusión

9. No se permiten las reivindicaciones.

Las modificaciones del solicitante necesitaron las nuevas etapas de objeciones presentadas en este Oficio. De acuerdo a esto, **ESTA ACCIÓN ES FINAL**. Ver MPEP §706.07(a). Se recuerda al solicitante de la política de extensión del tiempo como se expone en la CFR 37 1.136(a).

Un periodo estatutario abreviado para responder a esta acción final es ajustado para expirar en TRES MESES desde la fecha de notificación de este Oficio. En el evento de que una primera respuesta sea presentada dentro de los DOS MESES de la fecha de notificación de esta acción final y la acción asesora no sea notificada hasta el final del periodo estatutario abreviado de TRES MESES, entonces el periodo estatutario abreviado expirará en la fecha de que la acción asesora sea notificada, y ninguna tasa de extensión a la 37 CFR 1.136(a) será calculada desde la fecha de notificación de la acción asesora. En ningún evento, sin embargo, el periodo estatutario para responder expira a más tardar en SEIS MESES desde la fecha de notificación de esta acción final.

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AS. 138.780

DIRECCIÓN DE NUEVAS CREACIONES
Procedimiento acelerado de exámenes de patente (PPH)

1	NÚMERO DE SOLICITUD ANTE LA SIC	NC2017/0003290		
2	OFICINA DE PRIMERA PRESENTACIÓN (OPP)	USPTO		
3	NÚMERO(S) DE SOLICITUD O PUBLICACIÓN DE LA(S) SOLICITUD(ES) (OPP):	1. 14/511,912 2. 3.		
4	☐ REPRESENTANTE LEGAL ☒ APODERADO			
APELLIDOS CADENA SARMIENTO		NOMBRES JUAN PABLO	IDENTIFICACIÓN C.C. 80.410.337 T.P. 57492	
DIRECCIÓN Calle 70A 4 41		No. TELÉFONO 7442200		
CIUDAD Bogotá, D.C.		CORREO ELECTRÓNICO notificaciones@bc.com.co		
PAÍS CO		No. RADICACIÓN O PROTOCOLO DE PODER GENERAL		
5	CONDICIONES PARA ACCEDER A ESTE PROCESO:			
5.1. DATOS DE PRIORIDAD ☒ SI ☐ NO				
(33) PAÍS DE ORIGEN 1. US 2. 3. 4.		CÓDIGO PAÍS US	(31) NÚMERO 14/511,912	(32) FECHA (AAAA/MM/DD) 2014/10/10
5.2. DATOS SOLICITUD INTERNACIONAL PCT ☒ SI ☐ NO				
Solicitud Internacional No.		PCT/US2015/054682	Fecha	08.10.2015
Publicación Internacional No.		WO 2016/057788	Fecha	14.04.2016
El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO				
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS				
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:				
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:				
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO				
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO				
(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☐ SI ☒ NO				
(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad.				
7	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL <i>Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)</i>			



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NOTICE OF ALLOWANCE AND FEE(S) DUE

11753 7590 11/02/2016
DUANE MORRIS LLP (prev. Boca Raton)
IP DEPARTMENT
30 SOUTH 17TH STREET
PHILADELPHIA, PA 19103-4196

EXAMINER

FAN, LYNN Y

ART UNIT

PAPER NUMBER

1651

DATE MAILED: 11/02/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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14/511,912

10/10/2014

Joseph Charles SALAMONE

F6099-01601

5443

TITLE OF INVENTION: COMPOSITIONS AND KITS FOR ENZYMATIC DEBRIDEMENT AND METHODS OF USING THE SAME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	02/02/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/511,912	10/10/2014	Joseph Charles SALAMONE	F6099-01601	5443

TITLE OF INVENTION: COMPOSITIONS AND KITS FOR ENZYMATIC DEBRIDEMENT AND METHODS OF USING THE SAME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	02/02/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
FAN, LYNN Y	1651	424-094200

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached. ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.	2. For printing on the patent front page, list (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____ (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____ 3 _____
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3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

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Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

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☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
☐ Applicant asserting small entity status. See 37 CFR 1.27
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/511,912	10/10/2014	Joseph Charles SALAMONE	F6099-01601	5443

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PHILADELPHIA, PA 19103-4196

EXAMINER

FAN, LYNN Y

ART UNIT	PAPER NUMBER
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1651

DATE MAILED: 11/02/2016

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/511,912	Applicant(s) SALAMONE ET AL.	
	Examiner Lynn Y. Fan	Art Unit 1651	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to Applicant's amendment filed on 9/28/2016.
☒ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on 9/26/2016.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 1,4,6,8-25 and 28. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|--|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☒ Examiner's Amendment/Comment |
| 2. ☐ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date ____ | 6. ☒ Examiner's Statement of Reasons for Allowance |
| 3. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material | 7. ☐ Other ____. |
| 4. ☒ Interview Summary (PTO-413),
Paper No./Mail Date <u>10/31/2016</u> . | |

/Lynn Y Fan/
Examiner, Art Unit 1651

DETAILED ACTION

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1, 4, 6, 8-22 and 28 are directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(b), claims 23-25 are directed to the process of making or using the allowable product, previously withdrawn from consideration as a result of a restriction requirement, are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Because a claimed invention previously withdrawn from consideration under 37 CFR 1.142 has been rejoined, the restriction requirement between groups I and II as set forth in the Office action mailed on 3/10/2015 is hereby withdrawn. In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR

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1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Gregory M. Lefkowitz on October 27, 2016.

1. The application has been amended as follows:

Claim 1 has been deleted and replaced with:

A tissue debridement composition comprising:

0.1 to 99.5 wt % of an amylase component and optionally a proteolytic enzyme component, wherein a ratio of the amylase component to the proteolytic enzyme component is at least 10:1 by weight; and

at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt %, wherein the percentages are based on a total weight of the debridement composition,

wherein said amylase component comprises at least 80% by weight of α -amylase from a source selected from a group consisting of porcine pancreas and *Bacillus subtilis*, and wherein the debridement composition is able to debride devitalized or necrotic tissue.

In claim 4, “The composition according to claim 3” on line 1 has been changed to “The composition according to claim 1”.

In claim 9, “in an amount of at least” on line 2 has been changed to “in an amount ranging from”.

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In claims 13, 14, 17, and 18, “selected from the group” on line 2 has been changed to “selected from a group”.

In claim 15, “in an amount of at least 0.01 weight %, and” on line 2 has been changed to “in an amount ranging from 0.01 weight % to 1.0 weight % based on the weight of the debridement composition.

In claims 21-23, “tissue” has been inserted before “debridement composition” on line 2.

Claims 2, 7 and 26, canceled.

REASONS FOR ALLOWANCE

2. The following is an examiner's statement of reasons for allowance: The art teaches debridement compositions comprising a substantially higher amount of a proteolytic enzyme component than an amylase component. However, the claimed debridement composition comprising a substantially higher amount of an amylase component than a proteolytic enzyme component, wherein the claimed debridement composition effectively debrides devitalized or necrotic tissue.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

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Contact Information

3. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lynn Y. Fan whose telephone number is (571)270-3541. The examiner can normally be reached on M-Th 8am-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Renee Claytor can be reached on (571)272-8394. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ Lynn Y. Fan /
Examiner, Art Unit 1651

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently amended) A debridement composition comprising:
0.1 to 99.5 wt % of an amylase component, and
at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt-%, wherein the percentages are based on a total weight of the debridement composition,
wherein, ~~if a proteolytic enzyme component is present,~~ a ratio of the amylase component to ~~a the~~ proteolytic enzyme component is at least ~~[[4]]~~ 10:1, and
wherein said amylase component comprises at least 80 % by weight of α -amylase.
2. (Previously presented) The composition according to claim 1, wherein the amylase component comprises an amylase selected from a group consisting of humans, animals, bacteria, plants, fungi and recombinant amylase.
3. (Cancelled)
4. (Previously presented) The composition according to claim 3, wherein said amylase component comprises up to 20 % by weight of an amylase selected from a group consisting of β -amylase, γ -amylase, and combinations thereof.
5. (Cancelled)
6. (Previously presented) The composition according to claim 1, further comprising up to 20 % by weight of hydrolytic enzymes selected from a group consisting of proteases, chondroitinases, hyaluronidases, lipases, glycosidases, heparanases, dermatanases, pullulanases,

N-acetylglucosaminidase, lactases, phospholipases, transglycosylases, esterases, thioester hydrolyases, sulfatases, escharases, nucleases, phosphatases, phosphodiesterases, mannanases, mannosidases, isoamylases, lyases, inulinases, keratinases, tannases, pentosanases, glucanases, arabinosidases, pectinases, cellulases, chitinases, xylanases, cutinases, pectate lyases, hemicellulases, and combinations thereof, wherein the percentage is based on the total weight of the amylase component and the hydrolytic enzymes.

7. (Previously presented) The composition according to claim 1, comprising α -amylase selected from a group consisting of human pancreas, animal pancreas, fungi, and bacteria.

8. (Previously presented) The composition according to claim 1, further comprising up to 20 % by weight of other enzymes selected from a group consisting of oxidases, peroxidases, glucose oxidases, catalases, oxidoreductases, phenoloxidases, laccases, lipoxigenases, isomerases, and ligninases, wherein the percentage is based on the total weight of the amylase component and the other enzymes.

9. (Previously presented) The composition according to claim 1, further comprising at least one polymeric biguanide in an amount of at least 0.01 weight percent to 1.0 weight percent based on the weight of the debridement composition.

10. (Previously presented) The composition according to claim 9, wherein said polymeric biguanide comprises poly(hexamethylene biguanide) and its salts.

11. (Previously presented) The composition according to claim 1, further comprising a water-soluble polymer at a concentration of from 0.01 weight % to 50 weight % based on the weight of the debridement composition.

12. (Previously presented) The composition according to claim 1, further comprising a chelating agent at a concentration from 0.01 weight % to 1 weight % based on a weight of the debridement composition.

13. (Original) The composition according to claim 1, further comprising a monoalkyl glycol selected from the group consisting of 1,2-propanediol (propylene glycol), 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-heptanediol, 1,2-octanediol (caprylyl glycol), 1,2-nonanediol, 1,2-decanediol, 1,2-undecanediol, 1,2-dodecanediol, 1,2-tridecanediol, 1,2-tetradecanediol, 1,2-pentadecanediol, 1,2-hexadecanediol, 1,2-heptadecanediol, 1,2-octadecanediol, 2-methyl-2,4-pentanediol, 1,3-butanediol, diethylene glycol, triethylene glycol, glycol bis(hydroxyethyl) ether, and combinations thereof.

14. (Original) The composition according to claim 1, further comprising a glycerol alkyl ether selected from the group consisting of 1-O-heptylglycerol, 1-O-octylglycerol, 1-O-nonylglycerol, 1-O-decylglycerol, 1-O-undecylglycerol, 1-O-dodecylglycerol, 1-O-tridecylglycerol, 1-O-tetradecylglycerol, 1-O-pentadecylglycerol, 1-O-hexadecylglycerol (chimyol alcohol), 1-O-heptadecylglycerol, 1-O-octadecylglycerol (batyl alcohol), 1-O-octadec-9-enyl glycerol (selachyl alcohol), glycerol 1-(2-ethylhexyl) ether, 2-ethylhexyl glycerin, glycerol 1-heptyl ether, glycerol 1-octyl ether, glycerol 1-decyl ether, and glycerol 1-dodecyl ether, glycerol 1-tridecyl ether, glycerol 1-tetradecyl ether, glycerol 1-pentadecyl ether, glycerol 1-hexadecyl ether, glycerol 1-octadecyl ether, and combinations thereof.

15. (Previously presented) The composition according to claim 1, further comprising at least one polymeric biguanide in an amount of at least 0.01 weight %, and a vicinal diol component selected from a group consisting of a monoalkyl glycol and a monoalkyl glycerol at a concentration of 0.05 weight % to 4 weight %, based on a weight of the

debridement composition.

16. (Original) The composition according to claim 1, further comprising at least one analgesic agent, anesthetic agent, neuropathic pain agent, or a combination thereof.

17. (Original) The composition according to claim 16, wherein the at least one analgesic agent, anesthetic agent, or neuropathic pain agent is selected from the group consisting of lidocaine, capsaicin, benzocaine, tetracaine, prilocaine, bupivacaine, levobupivacaine, procaine, carbocaine, etidocaine, mepivacaine, nortriptylene, amitriptyline, pregabalin, diclofenac, fentanyl, gabapentin, non-steroidal anti-inflammatory agents, salicylates, and combinations thereof.

18. (Original) The composition according to claim 1, wherein the debridement composition has a form selected from the group consisting of a powder, aqueous solution, organic liquid solution, silicone, gel, cream, film, latex, aerosol, slurry, paste, ointment and foam.

19. (Original) The composition according to claim 1, wherein said debridement composition is adsorbed on or in a dressing or a natural or synthetic fiber, mesh, hydrocolloid, alginate, hydrogel, semipermeable film, permeable film, or a natural or synthetic polymer.

20. (Original) The composition according to claim 1, further comprising a buffering agent.

21. (Previously presented) A kit comprising:
a debridement composition according to claim 1; and
instructions for debridement of devitalized tissue.

22. (Original) The kit according to claim 21, wherein said instructions comprise contacting the debridement composition with an area of skin in need of debridement.

23. (Withdrawn) A method of debridement of devitalized tissue comprising contacting a debridement composition according to claim 1 with an area of skin in need of debridement.

24. (Withdrawn) The method of claim 23, wherein said contacting step is repeated at least once a day.

25. (Withdrawn) The method of claim 23, further comprising removing debrided tissue from said area of skin.

26. (Previously presented) The composition according to claim 3, wherein said α -amylase is selected from the group consisting of *Bacillus* spp. and *Aspergillus* spp.

27. (Cancelled)

28. (Previously presented) The composition according to claim 1, wherein the amylase component is present in an amount ranging from 2 to 99.5 wt-% based on the total weight of the debridement composition.

REMARKS

Review and reconsideration of the rejections of the application found in the final Office Action mailed April 26, 2016 (the "Office Action"), is respectfully requested in view of the preceding amendments and the following remarks. The Commissioner is hereby authorized to charge the \$200 fee for a two month extension of time to Deposit Account No. 04-1679. Although no additional fees are believed due, the Commissioner is hereby authorized to charge any deficiency or credit any surplus to Deposit Account No. 04-1679.

In the Office Action, claims 1-4, 6-22, and 26-28 were drawn to an elected invention, with all elected claims being rejected under 35 U.S.C. §103(a). By this Amendment, claim 1 is amended and claims 3 and 27 are cancelled. Support for the subject matter of the amendments can be found throughout the specification. *See, e.g.*, Specification, Page 7-8, bridging paragraph.

The amendments presented herein have been made solely to expedite prosecution of the instant application to allowance and should not be construed as an indication of Applicant's agreement with or acquiescence to the Examiner's position. Accordingly, Applicant expressly maintains the right to pursue broader subject matter through subsequent amendments, continuation or divisional applications, reexamination or reissue proceedings, and all other available means. The rejections and responses thereto are set forth fully below.

Interview Summary

Applicant wishes to thank Examiner Fan for extending the courtesy of a telephonic interview held August 31, 2016, with Gregory M. Lefkowitz and Dr. Joseph Salamone. The pending rejections and potential amendments and arguments for overcoming the pending rejections were discussed. Applicant explained that proceeding contrary to the conventional wisdom in the field is evidence of non-obviousness and that the claimed subject matter is the result of just such an approach. Applicant submits herewith a declaration from Dr. Salamone providing additional details why this is so. Examiner Fan agreed to grant additional consideration upon submission of a formal response.

Claim Rejections under 35 U.S.C. §103

In the Office Action, claims 1, 2, 18, and 20 are rejected under 35 U.S.C. §103 being anticipated by U.S. Patent No. 3,681,197 by Smith (“Smith”). As amended, claim 1 includes the subject matter of claim 3, which is not rejected based on Smith. Thus, claim 1 and all claims dependent thereon, including claims 2, 18, and 20, are patentable over Smith. Accordingly, Applicant respectfully requests that the rejection of claims 1, 2, 18, and 20 as being anticipated by Smith be withdrawn.

In the Office Action, claims 1-4, 6, 7, 11-13, 16, 18-22, and 26-28 were rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Pre-Grant Publication No. 2013/0202581 by Fallon, et al. (“Fallon”) in view of U.S. Patent No. 3,003,917 by Beiler, *et al.* (“Beiler”) and Souze, et al. (Brazilian Journal of Microbiology, Vol. 41, Pages 850-861 (2010)) (“Souza”). As amended, claim 1 is drawn to:

1. (Currently amended) A debridement composition comprising:
0.1 to 99.5 wt % of an amylase component, and
at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt-%, wherein the percentages are based on a total weight of the debridement composition,
wherein, ~~if a proteolytic enzyme component is present,~~ a ratio of the amylase component to a the proteolytic enzyme component is at least [[4]]
10:1, and
wherein said amylase component comprises at least 80 % by weight of α -amylase.

The claimed debridement composition includes an amylase component and at least one pharmaceutically or cosmetically acceptable carrier or excipient. In addition, a ratio of the amylase component to the proteolytic enzyme component is at least 10:1, and said amylase component comprises at least 80 % by weight of α -amylase.

In order to place the claim amendment in context with the claimed ratios and the unexpected ability of amylase to enhance debridement, Applicant submits herewith the Safety

Data Sheet for α -Amylase #6, as well as, the Certificate of Analysis for α -Amylase #3 and α -Amylase #2. These documents demonstrate that the ratio of amylase to protease for α -Amylase #6, α -Amylase #3, and α -Amylase #2, is 10:1, 940:1, and 240:1, respectively. For instance, (1) the SDS for α -Amylase #6 (from Dyadic) shows that for each gram of powder, there are 0.32 grams of amylase and 0.03 grams of protease (10.67 :1), (2) the certificate of analysis for α -Amylase #3 shows that for each milligram of the powder, there are 0.47 milligrams of amylase and 0.0005 milligrams of protease (940:1), and (3) the certificate of analysis for α -Amylase #2 shows that for each milligram of the powder, there are 0.48 milligrams of amylase and 0.002 milligrams of protease (240:1). The efficacy of these compositions is discussed thoroughly in the specification. See Specification, “Examples” on Pages 28-37.

Thus, although proteolytic enzymes are known for digesting proteins and amylases are known for digesting starches, the claimed debridement composition is formulated to include a substantially higher amount of the amylase component than the proteolytic enzyme component, which may or may not be present. This is directly contrary to the expectations of those in the art, as evident from Fallon and Beiler.

As explained in Chapter 2145.X.D.3. of the MPEP, “***proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. In re Hedges***, 783 F.2d 1038 (Fed. Cir. 1986) (Applicant’s claimed process for sulfonating diphenyl sulfone at a temperature above 127°C was contrary to accepted wisdom because the prior art as a whole suggested using lower temperatures for optimum results as evidenced by charring, decomposition, or reduced yields at higher temperatures.).”

For example, in *Arkie Lures v. Gene Larew Tackle*, 119 F.3d 953, 9578-58 (Fed. Cir. 1997), the Federal Circuit held that whether some plastics manufacturers knew how to mix salt and plastisol, as was argued to the district court, *did not make it obvious to proceed against the general view in the field of plastic fish lures*. FitzSimons' warning against adding plastic-insoluble ingredients to the plastisol was repeated by Dr. Carver and Mr. Harville, and Mr. Larew was so advised long before this litigation arose. Further, these artisans of the plastic lure believed that salt would affect the surface, texture, and strength of the lure. Larew's eventual

demonstration that the desired product could indeed be successfully made did not render it obvious, nor did the ready appreciation of its value render it unpatentable. From these cases, it is apparent that the proper analysis focuses on the wisdom in the art as a whole, including testimony from those skilled in the art, and is not limited to vague disclosures of a specific reference.

In support of this position, Applicant submits the Declaration of Dr. Joseph C. Salamone Under 37 C.F.R. 1.132 of (“Salamone Declaration”). Dr. Salamone explains that

Prior to the research that lead to this Application, the idea that maximizing amylase, which is known for digesting starches, and minimizing proteolytic enzymes, which are known for digesting proteins, in a composition for treating skin was completely counter-intuitive. This is because skin is formed of proteins and fats, which are digested by proteases and lipases, respectively, but skin does not contain starches, which are the target of amylase. Thus, an artisan in enzymatic skin care compositions would have considered protease concentration and lipase concentration as important, while amylase content was more or less irrelevant. Thus, while an artisan in enzymatic skin care compositions may have tolerated amylase contamination in the protease or lipase isolates being used in a formulation, there would have been no motivation to increase the amount of amylase. In addition, it would have been unthinkable to minimize or eliminate protease.

Salamone Declaration, Section 4.

Dr. Salamone indicated that it is “directly contrary to the expectations of those in the art” to have a skin care formulation “*formulated to include a substantially higher amount of the amylase component, and α -amylase in particular, than the proteolytic enzyme component, which may not even be present.*” *Id.*

Dr. Salamone goes on to explain that:

The inventors were quite surprised to learn that amylase was so effective for use debriding necrotic tissue. The inventors even went so far as to conduct experiments to demonstrate to themselves that the effect was not the result of contamination with proteolytic enzymes. These experiments are discussed with respect to the collagen gel digestion experiments in the specification (Specification, Page 29, ln. 15 – Page 30, ln. 31). A comparison of the rheological properties of the treated collagen demonstrated that (1) the

protease completely liquefied the collagen gel, and (2) that the collagen gel treated with α -amylase exhibited no digestion (Specification, Page 30, ln. 7-10). The complex modulus also confirmed these results (Specification, Page 30, ln. 14-16). This study established that the debridement effect of the amylase was not the result of contamination with proteolytic enzymes.

Having established that the amylase was not, in fact, contaminated with sufficient amounts of proteolytic enzyme to digest proteins, we can now review the digestion results from the specification (Specification, Page 30, ln. 32 – Page 35, ln. 11). These experiments used boiled pig skin as a proxy for devitalized (necrotic) tissue present during debridement. As can be seen in Tables 1-6, the amylase compositions were able to effectively digest the boiled pig skin (*Id.*). It should be noted that α -amylase #2, which includes a higher protease impurity (0.2 wt-% v. 0.05 wt-%) than amylase #3, actually performed slightly worse, which reinforces the conclusion that the amylase debridement activity is not the result of protease contamination (Specification, Page 31, ln. 22-26).

This was quite unexpected considering that *amylases catalyze the hydrolysis of starches into sugars*, whereas proteases are enzymes that begin protein catabolism by hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain. ***In fact, while it is conventional for proteolytic enzymes to be used for skin treatments, it was quite unexpected to discover that amylases can be effective for use in debridement compositions.***

Id.

The Office Action asserts that the claimed ranges are merely the result of optimization. However, Fallon clearly focuses on proteases and lipases, and does not consider amylase to be an important component of the preparations disclosed therein. Furthermore, Fallon focuses on compositions where the amount of protease is almost double the amount of amylase. Thus, there would be nothing in the cited references to disclose or suggest using high ratios of amylase relative to protease. To the contrary, the knowledge of those in the art would be to increase the amount of protease and minimize the amount of amylase (if it is present at all) in debridement compositions.

Turning to the cited references in more detail, Fallon is drawn to a wound healing composition that can include protease, lipase, and amylase. While Fallon discloses some incredibly broad ranges, a person skilled in the art would recognize that ***Fallon is based on a study conducted using various doses of pancreatin.***

Dr. Salamone provides the following analysis of Fallon:

The primary reference cited in the Office Action is Fallon, which is drawn to an enzyme composition for use for wound healing. Fallon includes exceptionally broad ranges of protease, lipase, and amylase. However, anywhere that specific amounts of protease, lipase, and amylase are disclosed, there is more protease present than lipase. *See, for example*, Fallon, Paragraphs [0176]-[0178]. In fact, this is the specific ratio present in pancreatin. Fallon, Paragraph [0093]. In addition, the examples are all based on a “high protease enzyme concentrate,” which illustrates that Fallon teaches protease is the important enzyme. Fallon, Paragraphs [0175].

Another noteworthy observation is that Fallon includes a teaching about useful ratios of protease to lipase (Fallon, Paragraph [0011]), but nothing about the useful ratios of protease to amylase or lipase to amylase.

Thus, an artisan in enzymatic skin care compositions would interpret Fallon as a disclosure that pancreatin is useful for facilitating wound healing and that protease is the most important component of the pancreatin blend, which also includes lipase and protease. ***There is nothing in Fallon that contradicts or changes the knowledge that amylase is a minor component in wound healing compositions.***

Salamone, Section 5.

Applicant provided a number of additional, details points regarding Fallon in the previous response. Those points are incorporated herein by reference.

Beiler discloses a wound healing composition containing a 5:1:1 ratio of relaxin, trypsin, and amylase (Beiler, Col. 1, ln. 46-52). As noted in Beiler, trypsin can be substituted with ***other proteolytic enzymes*** (Beiler, Col. 1, ln. 27-30 & Col. 2, ln. 1-22). Thus, the ratio of non-proteolytic to proteolytic enzymes is 1:1 in Beiler’s composition, which is less than the 4:1 ratio required by amended claim 1.

Dr. Salamone provides the following analysis of Beiler:

Beiler discloses a wound healing composition containing relaxin and approximately equal amounts of trypsin and amylase. Beiler, Col. 1, ln. 36-52 & Table I. Beiler demonstrates that, when combined, these components accelerate healing, but that none of them alone are significantly different from the control. Beiler, Table I. In addition, the preferred ratio of components is 5 parts relaxin, 1 part proteolytic enzyme, and 1 part amylolytic enzyme. Beiler, Col. 1, ln. 46-

52. Thus, there is nothing in Beiler that contradicts or changes the knowledge that amylase is a minor component in wound healing compositions.

Salamone Declaration, Section 6.

Furthermore, Beiler does not disclose or suggest the claimed debridement composition where ***a ratio of the amylase component to a proteolytic enzyme component is at least 4:1, and the amylase component is at least 80% α -amylase***, in combination with the other features. In fact, when Fallon and Beiler are combined, Fallon's examples suggest increasing the amount of proteolytic enzymes present (*i.e.*, 7:4 ratio of protease to amylase) and Beiler discloses one composition where the amount of protease and amylase is equal (1:1 ratio). Thus, the combination of Fallon and Beiler not only fails to disclose or suggest the claimed debridement composition, but teaches away from the claimed debridement composition, where a ratio of the amylase component to a proteolytic enzyme component is at least 4:1, in combination with the other features.

It should be noted that the teachings of Fallon and Beiler makes sense, because amylase is an enzyme that catalyzes the hydrolysis of starches into sugars, whereas proteases are enzymes that begin protein catabolism by hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain. Thus, it was quite unexpected when Applicant discovered that amylase was particularly helpful for removing necrotic tissue. In fact, Applicant conducted studies specifically to rule out the possibility that the debridement effect was the result of contamination by proteolytic enzymes (*See, e.g.*, Specification, Pages 29-30; Page 31, last paragraph; and Pages 35-37, "Digestion Results, Rat Skin"). While not necessary for practicing the invention, it is believed that something about the transformation from healthy tissue to necrotic tissue makes the necrotic tissue susceptible to cleavage by amylase. However, as noted above, this was quite unexpected.

Souza is used solely for the disclosure of sources of α -amylase and does not correct the deficiencies of Fallon and Beiler.

In summary, the combination of Fallon, Beiler, and Souza fails to disclose or suggest the claimed debridement composition containing 0.1 to 99.5 wt % of an amylase component based

on a total weight of the debridement composition, *where a ratio of the amylase component to a proteolytic enzyme component is at least 10:1, and at least 80% of the amylase component is α -amylase*. In fact, such a combination actually proceeds contrary to the knowledge of artisans in the first of skin care compositions. Thus, claim 1 and all claims dependent thereon are patentable over the combination of Fallon in view of Beiler and Souza. Accordingly, Applicant respectfully requests that the rejection of claims 1-4, 6, 7, 11-13, 16, 18-22, and 26-28, based on Fallon in view of Beiler and Souza be withdrawn.

In the Office Action, claim 8 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fallon in view of Beiler, Souza, and Loo, *et al.* (PLoS One, Vol. 7(11), e49215 (2012)) (“Loo”). Loo is used solely for the disclosure of glucose oxidase in wound healing, but does not correct the deficiencies of Fallon, Beiler, and Souza described above. Thus, claim 1 and all claims dependent thereon are patentable over the combination of Fallon, Beiler, Souza, and Loo. Accordingly, Applicant respectfully requests that the rejection of claim 8 based on Fallon, Beiler, Souza, and Loo be withdrawn.

In the Office Action, claims 1, 9, 10, 14, and 15 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fallon, Beiler, and Souza in view of U.S. Pre-Grant Publication No. 2007/0237812 by Patel, *et al.* (“Patel”) and International Publication WO 2013/016255 by Parks (“Parks”). Patel and Parks are used solely for the disclosure of the limitations of claims 9, 10, 14, and 15, but do not correct the deficiencies of Fallon, Beiler, and Souza described above. Thus, claim 1 and all claims dependent thereon are patentable over the combination of Fallon, Beiler, Souza, Patel, and Parks. Accordingly, Applicant respectfully requests that the rejection of claims 1, 9, 10, 14, and 15 based on Fallon, Beiler, Souza, Patel, and Parks be withdrawn.

In the Office Action, claims 1, 16, and 17 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fallon, Beiler, and Souza in view of U.S. Pre-Grant Publication No.

2004/0076671 by Tippet, *et al.* ("Tippet"). Tippet is used solely for the disclosure of PHMB, chelating agent, and analgesic, and do not correct the deficiencies of Fallon, Beiler, and Souza described above. Thus, claim 1 and all claims dependent thereon are patentable over the combination of Fallon, Beiler, Souza, and Tippet. Accordingly, Applicant respectfully requests that the rejection of claims 1, 16, and 17 based on Fallon, Beiler, Souza, and Tippet be withdrawn.

Conclusion

For at least the reasons set forth above, the independent claim is believed to be allowable. In addition, the dependent claims are believed to be allowable due to their dependence on an allowable base claim and for further features recited therein. The application is believed to be in condition for immediate allowance. If any issues remain outstanding, Applicant invites the Examiner to call the undersigned, Greg Lefkowitz (direct line: 561-962-2110) if it is believed that a telephone interview would expedite the prosecution of the application to an allowance.

Respectfully submitted,



Date: September 28, 2016

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Gregory M. Lefkowitz, Reg. No. 56,216
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α-amylase #2

CERTIFICATE OF ANALYSIS

α-Amylase Porcine Pancreas

GENERAL INFORMATION

Catalogue Number	122-10	Storage Temperature	-20°C
Lot Number	M60404	Storage Note	NA
CAS Number	9000-90-2	Shipping Note	Cold packs
EC Number	3.2.1.1	Received By	_____
Synonyms	Alpha Amylase, Amylase	Receipt Date	_____

PRODUCT INFORMATION

Source	Porcine pancreas
Form	Lyophilized from 150 mM sodium chloride and 3 mM calcium chloride
Appearance	Off-white powder
Activity	230 units/mg
Unit Definition	One unit will catalyze the hydrolysis of one micromole of 2-chloro-4-nitrophenyl-α-D-maltotriose to yield 2-chloro-4-nitrophenol per minute at 37°C.
Protein	0.48 mg protein/mg (Coomassie)
Specific Activity	480 units/mg protein
Contaminants	Lipase 0.1% Protease 0.2%
Ammonia	< 0.1 micromole/mg
Recertification	June 29, 2015

HANDLING INFORMATION

Advice	Reconstitute into provided container and centrifuge to ensure full recovery.
Storage	Aliquot and freeze at -20°C after reconstitution.
Handling	Handle as a potentially hazardous substance. Refer to MSDS on website.

CERTIFIED BY: Douglas Held

DATE: February 24, 2014

The animal source of this product was collected at a USDA licensed establishment. The animals received ante and post mortem health inspection at the abattoir by a US FHS inspector and they were apparently free from infectious and contagious diseases. USE OF PRODUCT IS DETERMINED BY END USER. This product is for in vitro diagnostic use only - NOT FOR HUMAN USE.



2924 MARY AVENUE, ST. LOUIS, MISSOURI, USA 63144 T: 314.998.1991 F: 314.998.0951 WWW.LEEBIO.COM

α-amylase #3

CERTIFICATE OF ANALYSIS

PRODUCT: α-Amylase

SOURCE: Porcine Pancreas

E.C.: 3.2.1.1 CAS: 9000-90-2

CAT NO: 122-11 LOT NO: P70442

FORM: Powder, lyophilized from 150 mM sodium chloride and 3 mM calcium chloride.

APPEARANCE: Off-white powder

UNIT DEFINITION: One unit will catalyze the hydrolysis of one micromole of 2-chloro-4-nitrophenyl-α-D-maltotrioside to yield 2-chloro-4-nitrophenol per minute at 37°C.

ACTIVITY: 210 U/mg

PROTEIN: 0.47 mg protein/mg (Coomassie)

SPECIFIC ACTIVITY: 450 U/mg protein

CONTAMINANTS: Lipase 0.3%
Protease 0.05%
Ammonia < 0.01 umole/mg

STORAGE: -20°C

EXPIRATION: May 5th, 2015

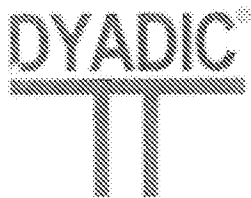
The animal source of this product was collected at a USDA licensed establishment. The animals received ante and post mortem health inspection at the abattoir by a US FSIS inspector and they were apparently free from infectious and contagious diseases. All donor animals were sourced from the United States. USE OF PRODUCT IS DETERMINED BY END USER

CERTIFIED BY:

Richard J. [Signature]

DATE:

6-2-2014



DYADIC INTERNATIONAL (USA), INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477
(561) 743-8333 tel.
(561) 743-8343 fax
<http://www.dyadic.com>

α - Amylase #6

SAFETY DATA SHEET

In accordance with Annex H to Regulation (EC) 1907/2006 as amended by Regulation 453/2010

SECTION 1: PRODUCT / COMPANY IDENTIFICATION

- 1.1. **Product Identifier:** ALPHASTAR CONC
Product Code: 208
REACH Pre-registration: exempt from REACH
CLP Notification No.: 02-2119789270-34-0000
- 1.2. **Relevant identified uses of the substance or mixture and uses advised against:** used as an industrial processing aid.
- 1.3. **Details of the supplier of the safety data sheet**
- Name of supplier/manufacture:
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter, Florida 33477
Richard Jundzil, Vice President of Operations
rjundzil@dyadic.com
TELEPHONE: (561) 743-8333
FACSIMILE: (561) 743-8343
 - Person responsible in EU Member State:
Dyadic Nederland BV
Nieuwe Kanaal 7-S
6709 PA Wageningen, The Netherlands
Dr. Ir. Wim van der Wilden, General Manager Dyadic Netherlands
wwilden@dyadic.com
TELEPHONE: +31 317 465454 or +31 6 20368609
- 1.4. **Emergency telephone number**
CHEMTREC: (800) 424-9300 [in US]
(703) 527-3887 (collect) [International]

SECTION 2: HAZARDS IDENTIFICATION

- 2.1. **Classification of the substance**
Category 1: Respiratory sensitizer and irritant
- 2.2. **Label elements**
Hazard statements:
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H315 Causes skin irritation
H319 Causes serious eye irritation
H335 May cause respiratory irritation

Signal word: DANGER

Pictograms: GHS08



Precautionary statements:

- P261 Avoid breathing vapor or dust.
P264 Wash hands thoroughly after handling.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P285 In case of inadequate ventilation wear respiratory protection.
P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P501 Disposal of contents/container in accordance national and international regulations.

2.3. Other hazards

May form combustible dust concentrations in the air.

Not classified as a PBT or vPvB.

SECTION 3: COMPOSITION / INFORMATION ON INGREDIENTS

3.1 Substances

The substance is a carbohydrase enzyme:

Component	CAS No.	EINECS/ ELINCS	IUB No.	Amount
Amylase	9000-90-2	232-565-6	3.2.1.1	32 %
Protease (subtilisin)	9014-01-1	232-752-2	3.4.21.62	3%

Other Components:

Remaining components of this product (water/inerts) are proprietary, non-hazardous and/or are present at concentrations below reportable limits.

SECTION 4: FIRST AID MEASURES

4.1. Description of first aid measures

- Inhalation
Remove from contaminated area to fresh air. Seek medical attention if allergic response exhibited.
- Skin
Wash skin with soap and water. Remove contaminated clothes and wash.
- Eye
Flush eyes with low pressure water for at least 15 minutes. Do not attempt to neutralize chemically. Seek medical attention immediately if irritation persists.

- Ingestion
Give large quantities of water. If unconscious or in convulsion, take immediately to hospital or physician.
- 4.2. **Most important symptoms and effects, both acute and delayed**
The substance is a respiratory sensitizer and overexposure by inhalation may cause sensitization and allergic responses including breathing difficulties in hypersensitive individuals.
- 4.3. **Indication of any immediate medical attention and special treatment needed**
If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If experiencing respiratory symptoms, call a POISON CENTER or doctor/physician.

SECTION 5: FIRE-FIGHTING MEASURES

- 5.1. **Extinguishing media**
Carbon dioxide, foam and/or ABC dry powder.
- 5.2. **Special hazards arising from the substance**
Oxides of carbon, oxides of nitrogen are hazardous decomposition materials under fire conditions.
- 5.3. **Advice for fire-fighters**
Fire-fighters should wear NIOSH/MSHA-approved self-contained breathing apparatus and full protective clothing. Cool containers exposed to fire with water.

SECTION 6: ACCIDENTAL RELEASE MEASURES

- 6.1. **Personal precautions, protective equipment and emergency procedures**
Contact emergency personnel and keep unnecessary personnel away. Use suitable protective equipment (Section 8). Provide sufficient ventilation and wash or dispose of contaminated clothing.
- 6.2. **Environmental precautions**
Removal by mechanical means (i.e. vacuuming with HEPA filters) is preferred. Solid may be placed into approved containers for disposal. Dilute remainder with plenty of water avoiding the formation of aerosols and flush to an approved drain.
- 6.3. **Methods and material for containment and cleaning up**
Removal by mechanical means (i.e. vacuuming with HEPA filters) is preferred. Solid may be placed into approved containers for disposal. Dilute remainder with plenty of water avoiding the formation of aerosols and flush to an approved drain.
Contaminated material is to be disposed of in accordance with State, local and Federal Regulations. Compliance includes, but is not restricted to, the Clean Water Act, the Clean Air Act, the Resource Conservation and Recovery Act, and complies with all applicable EU, national and local guidelines.

SECTION 7: HANDLING AND STORAGE

- 7.1. **Precautions for safe handling**
Segregate from acids, peroxides and all combustible organic or easily oxidizable materials. Keep from freezing. Protect against physical damage. Keep away from heat and flame.
- 7.2. **Conditions for safe storage, including any incompatibilities**
Keep away from heat and flame. Store in a cool, dry area in closed containers. Avoid accidental discharge into sewers and natural waters. Keep in original container. Empty container retains vapor and product residue. Observe all labelled safeguards until cleaned, reconditioned or destroyed.

- 7.3. Specific end use(s)
Not specified.

SECTION 8: EXPOSURE CONTROLS / PERSONAL PROTECTION

8.1 Control parameters

No national limit values established for amylase or protease. No OSHA PEL, TWA or STELs established. Relevant DNELs and PNECs have not yet been determined. Comply with all EU, national and local guidelines.

8.2 Exposure controls

8.2.1 Appropriate engineering controls

Efficient exhaust ventilation should always be provided to draw fumes and vapors away from workers to prevent routine inhalation. Ventilation should be adequate to maintain the ambient workplace atmosphere below the TLV of any items identified under section 2.

8.2.2 Individual protection measures, such as personal protective equipment

- (a) Eye/face protection: Goggles / Safety Glasses / Mono-goggles (EN 166)
- (b) Skin/hand protection: Impervious gloves, such as Neoprene or polyvinyl alcohol, should be worn when handling chemicals. (EN 374). Long Sleeve shirt / Rubber apron / Safety shoes
- (c) Respiratory protection: Normal. Wear a NIOSH/MSHA approved respirator whenever exposure to fumes or vapors exceeds the TLV of any items identified under hazardous components section. (EN 143)
- (d) Thermal hazards: not applicable

8.2.3 Environmental exposure controls

Avoid accidental discharge into sewers and natural waters. Spilled material should be removed immediately to avoid the formation of aerosols.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

- (a) Appearance: Light to dark brown powder; solid
- (b) Odour: Mild fermentation odor
- (c) Odour threshold: not established
- (d) pH: 5.0 - 7.5
- (e) Melting point / freezing point: pour point not established
- (f) Initial boiling point and boiling range: solid-not applicable
- (g) Flash point: data not available
- (h) Evaporation rate: solid-not applicable
- (i) Flammability (solid, gas): data not available
- (j) Upper/lower flammability or explosive limits: data not available
- (k) Vapor pressure: data not available
- (l) Vapor density: data not available
- (m) Relative density: solid-not applicable
- (n) Solubility(ies): completely miscible in water; soluble in other solvents
- (o) Partition coefficient: n-octanol/water; data not available
- (p) Auto-ignition temperature: data not available
- (q) Decomposition temperature: data not available
- (r) Viscosity: solid-not applicable
- (s) Explosive properties: data not available
- (t) Oxidising properties: data not available

- 9.2 Other information
May form combustible dust concentrations in the air.

SECTION 10: STABILITY AND REACTIVITY

- 10.1. Reactivity
None identified
- 10.2. Chemical stability
Stable
- 10.3. Possibility of hazardous reactions
None identified; hazardous polymerization does not occur
- 10.4. Conditions to avoid
None known
- 10.5. Incompatible materials
None known
- 10.6. Hazardous decomposition products
None

SECTION 11: TOXICOLOGICAL INFORMATION

- 11.1. Information on toxicological effects
- (a) Acute toxicity: ingestion of this material is not known to result in significant adverse effects. No specific data available.
 - (b) Skin corrosion/irritation: this material is known to be a skin irritant
 - (c) Serious eye damage/irritation: overexposure of the eye is characterized by irritation
 - (d) Respiratory or skin sensitization, respiratory sensitizer: overexposure by inhalation may cause sensitization and allergic response in hypersensitive individuals; not a skin sensitizer
 - (e) Germ cell mutagenicity: no data available
 - (f) Carcinogenicity: this product does not contain any substances that are considered by OSHA, NTP, IARC or ACGIH to be "probable" or "suspected" human carcinogens; no additional test data found for product
 - (g) Reproductive toxicity: no data available
 - (h) STOT-single exposure: may cause respiratory irritation
 - (i) STOT-repeated exposure: no data available
 - (j) Aspiration hazard: see respiratory sensitization

SECTION 12: ECOLOGICAL INFORMATION

- 12.1 Toxicity
No specific data available, however, enzymes are not known to be significantly ecotoxic.
- 12.2. Persistence and degradability
No specific data available, however, the substance is miscible in water and is not likely to be persistent. In addition, the substance is an enzyme and enzymes are known to be fully biodegradable.
- 12.3. Bioaccumulative potential
No specific data available, however, the substance is miscible in water and is not likely to be bioaccumulative. Also, enzymes are known to be fully biodegradable and therefore would not bioaccumulate.
- 12.4. Mobility in soil
No data available

12.5. Results of PBT and vPvB assessment

No specific data available, however, the substance is miscible in water and is not likely to be persistent or bioaccumulative. In addition, the substance is an enzyme and enzymes are known to be fully biodegradable and not bioaccumulative. The substance does not meet the criteria for characterization as either a PBT or vPvB.

12.6. Other adverse effects

No other adverse effects known.

SECTION 13: DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Waste Disposal Method: Chemical additions, processing or otherwise altering this material may make waste management information presented in this MSDS incomplete, inaccurate or otherwise inappropriate. Please be advised that state and local requirements for waste disposal may be more restrictive or otherwise different from Federal laws and regulations. Consult any regional local authority requirements regarding the proper disposal of this material.

Hazardous Waste: To present knowledge of the supplier, this product is not regarded as hazardous waste as defined by EU Directive 91/689/EC.

Container Handling and Disposal: Rinse containers before disposal.

SECTION 14: TRANSPORT INFORMATION

14.1. UN number

None assigned. The substance is not classified as hazardous for transport (UN, IMO, IATA, DOT, etc.)

14.2. UN proper shipping name

Not applicable

14.3. Transport hazard class(es)

Not applicable

14.4. Packing group

Not applicable

14.5. Environmental hazards

The substance is not classified as hazardous for transport (UN, IMO, IATA, DOT, etc.)

14.6. Special precautions for user

The substance is not classified as hazardous for transport (UN, IMO, IATA, DOT, etc.)

14.7. Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code

The substance is not classified as hazardous for transport (UN, IMO, IATA, DOT, etc.). See sections 7 and 8 for more handling information.

SECTION 15: REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance

Classification and labelling have been performed according to EU directives 67/548/EEC, 1999/45/EC, including amendments and the intended use – industrial processing aid.

National Paint and Coatings Hazardous Materials Identification System ("HMIS")

Hazard Ratings:

Health: 2

Flammability: 0

Reactivity: 0

Personal Protection: E

Irritating to eyes, respiratory system and skin.

May cause allergy or asthma symptoms or breathing difficulties if inhaled.

15.2. Chemical Safety Assessment

The relevant information in the chemical safety assessment is not yet available.

SECTION 16: OTHER INFORMATION

Safety Data Sheet First Issue: May 1, 2009

Refer to NFPA 654, Standard for the Prevention of Fire and Dust Explosions from the Manufacturing, Processing, and Handling of Combustible Particulate Solids, for safe handling.

Disclaimer: The information and recommendations in this data sheet are believed to be correct and reliable. However, the data offered for consideration and verification by the user and Dyadic International, Inc. offers no guarantee, warranty or representation as to the accuracy or completeness of the data.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/511,912	10/10/2014	Joseph Charles SALAMONE	F6099-01601	5443

11753 7590 04/26/2016
DUANE MORRIS LLP (prev. Boca Raton)
IP DEPARTMENT
30 SOUTH 17TH STREET
PHILADELPHIA, PA 19103-4196

EXAMINER

FAN, LYNN Y

ART UNIT	PAPER NUMBER
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1651

MAIL DATE	DELIVERY MODE
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04/26/2016

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 14/511,912	Applicant(s) SALAMONE ET AL.	
	Examiner Lynn Y. Fan	Art Unit 1651	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
 - If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
 - Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 4/11/2016.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 1-4 and 6-28 is/are pending in the application.
5a) Of the above claim(s) 23-25 is/are withdrawn from consideration.
- 6) ☐ Claim(s) _____ is/are allowed.
- 7) ☒ Claim(s) 1-4, 6-22 and 26-28 is/are rejected.
- 8) ☐ Claim(s) _____ is/are objected to.
- 9) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) ☐ Notice of References Cited (PTO-892) | 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date _____ | 4) ☐ Other: _____ |

DETAILED ACTION

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Applicant's amendment and response filed on 4/11/2016 have been received and entered into the case. Claim 5 has been canceled. Claims 1-4 and 6-28 are pending, claims 23-25 have been withdrawn, and claims 1-4, 6-22 and 26-28 have been considered on the merits, insofar as they read on the elected species of lipase (in claim 6) and oxidases (in claim 8). All arguments have been fully considered.

Withdrawn Objections

1. Objections are withdrawn in view of applicant's amendments.

Withdrawn Rejections

2. Rejections under pre-AIA 35 U.S.C. 102 are withdrawn in view of applicant's amendments.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

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The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102 of this title, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

3. Claims **1, 2, 18** and **20** are rejected under 35 U.S.C. 103 as being unpatentable over Smith (US 3,681,197 A; 8/1/1972).

The instant claims recite a debridement composition comprising: 0.1-99.5 wt % of an amylase component, and at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt %, wherein the percentages are based on a total weight of the debridement composition, wherein, if a proteolytic enzyme component is present, a ratio of the amylase component to the proteolytic enzyme component is at least 4:1.

Smith teaches a formula of solution comprising 1 gr of pancreatic amylase (0.09 wt % of an amylase component, from animals, col.6 line 71), 622 ml of water (pharmaceutically or cosmetically acceptable carrier in an amount of up to 99.9 wt % of the composition), and phosphate buffers (a buffering agent) (Example II), wherein the formulation is suitable and useful in debriding organic particles in an oral cavity (col.1 line 55-58).

Smith does not teach the claimed concentration of the amylase component (claim 1). However, Smith does teach the formula of solution comprising 0.09 wt % of an amylase component, which is close to the claimed concentration of 0.1 wt %. The reference does identify the instant component as an active ingredient in the formula of solution. Thus, the component achieves a recognized result and is therefore considered to be result effective variable. As such,

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before the effective filing date of the claimed invention, it is obvious to optimize the concentration of the amylase component as a matter of routine experimentation. Generally, differences in concentration will not support patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical. (MPEP 2144.05 II) Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited reference to optimize the concentration of the amylase component with a reasonable expectation for successfully obtaining a formula of solution.

4. Claims **1-4, 6, 7, 11-13, 16, 18-22** and **26-28** are rejected under 35 U.S.C. 103 as being unpatentable over Fallon et al (US 2013/0202581 A1; 8/8/2013) in view of Beiler et al (US 3,003,917; 10/10/1961) and Souza et al (Brazilian Journal of Microbiology. 2010;41:850-861).

The instant claims recite a debridement composition comprising: 0.1-99.5 wt % of an amylase component, and at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt %, wherein the percentages are based on a total weight of the debridement composition, wherein, if a proteolytic enzyme component is present, a ratio of the amylase component to the proteolytic enzyme component is at least 4:1.

Fallon teaches a composition comprising one or more amylases, proteases and lipases and with one or more pharmaceutically acceptable carriers (para 0066), wherein said one or more amylases having an activity in the range of about 200-300 USP units/mg, said proteases having an activity in the range of about 150-250 USP units/mg (para 0095), said carrier is in an amount of from about 0.1% to about 95% by weight of the composition (para 0130), and said enzymes (comprises an amylase) are derived from an animal source, a microbial source, a plant source, or

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a fungal source (para 0007). The composition further comprising polyethylene glycols (a water-soluble polymer) (para 0120), triethanolamine (a chelating agent) (para 0127), propylene glycol (a monoalkyl glycol) (para 0121), an analgesic substance (para 0142), and buffering agents (para 0029). The composition has a form of a powder, aqueous solution, gel, cream, or ointment, other formulations include dressing materials (para 0086).

Fallon does not teach the claimed concentrations of amylases, the proteolytic enzyme, lipases, the water-soluble polymer (polyethylene glycols), and the chelating agent (triethanolamine), as recited in claims 1, 6, 11, 12 and 28.

However, Fallon does teach the composition comprising an effective amount of enzymes including amylases, proteolytic enzymes and lipases, and polyethylene glycols (a water-soluble polymer) and triethanolamine (a chelating agent).

Thus, before the effective filing date of the claimed invention, it would have been within the purview of one of ordinary skill in the art to optimize the concentration of the instant components as a matter of routine experimentation, because the reference does identify each of the instant components as active ingredients in the composition. As such, each of the components achieves a recognized result and is therefore considered to be result effective variable. Generally, differences in concentration will not support patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical. (MPEP 2144.05 II) Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited reference to optimize the amount of the instant components with a reasonable expectation for successfully obtaining a composition.

Fallon does not teach the composition comprises α -amylase and β -amylase as well as the claimed concentrations of these amylases, as recited in claims 3-4, 7 and 26-27.

However, Fallon does teach the composition comprises an effective amount of one or more amylases (para 0095), where the composition is useful for wound healing (Title). Beiler teaches a composition useful for wound healing comprising mixtures of bacterial amylases, pancreatic or α -amylase and β -amylase (col.1 line 32-33, Table I). Before the effective filing date of the claimed invention, it was well-known in the art that α -amylase is mainly derived from the genus *Bacillus* or *Aspergillus* for commercial/industrial applications (p.855 col left – para 1, col right – para 3 of Souza).

Thus, before the effective filing date of the claimed invention, it would have been obvious to one of ordinary skill in the art to incorporate α -amylase (pancreatic amylase) and β -amylase since Fallon discloses a composition comprising amylases is useful for wound healing, and Beiler discloses that α -amylase (pancreatic amylase) and β -amylase help with wound healing (Table I). Since the references do identify each of the instant components as active ingredients in the composition effective for wound healing, each of the components achieves a recognized result and is therefore considered to be result effective variable. As such, it is obvious to optimize the concentration of the instant components as a matter of routine experimentation. Generally, differences in concentration will not support patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical. (MPEP 2144.05 II) Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited references to incorporate α - and

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β -amylases as well as to optimize their concentrations, with a reasonable expectation for successfully obtaining a composition.

The cited references above do not teach a kit comprising the claimed composition and the claimed instructions, as recited in claims 21-22. However, the combined references do teach a composition as claimed, wherein the composition is for topical administration (contacting the composition with an area of skin). It is noted that where the only difference between a prior art product and a claimed product is printed matter that is not functionally related to the product, the content of the printed matter will not distinguish the claimed product from the prior art. (MPEP 2112.01)

The limitation of the term “debridement” is regarded as intended use limitation. The claiming of a new use, function or unknown property does not necessarily make the composition patentable. The intended use of the claimed composition does not patentably distinguish the composition, per se, since such undisclosed use is inherent in the reference composition. In order to be limiting, the intended use must create a structural difference between the claimed composition and the composition of the prior art. In the instant case, the combined references do teach a composition as claimed. The intended use fails to create a structural difference, thus, the intended use is not limiting. Please note that when applicant claims a composition in terms of function, and the composition of the prior art appears to be the same, the Examiner may make rejections under both 35 U.S.C 102 and 103. (MPEP 2112)

5. Claim **8** is rejected under 35 U.S.C. 103 as being unpatentable over Fallon et al (US 2013/0202581 A1; 8/8/2013) in view of Beiler et al (US 3,003,917; 10/10/1961) and Souza et al (Brazilian Journal of Microbiology. 2010;41:850-861) as applied to claims 1-4, 6, 7, 11-13, 16, 18-22 and 26-28 above in view of Loo et al (PLoS One. 2012;7(11):e49215).

The references cited above do not teach the composition further comprising oxidases and its claimed concentration, as recited in **claim 8**.

However, Fallon does teach the composition is useful for wound healing (Title). Loo teaches that glucose oxidase is found to promote wound healing (p. e49215 col left – para 2).

Thus, before the effective filing date of the claimed invention, it would have been obvious to one of ordinary skill in the art to incorporate glucose oxidase into the composition of Fallon since Fallon discloses a wound healing composition, and Loo discloses that glucose oxidase promotes wound healing. Furthermore, before the effective filing date of the claimed invention, it is obvious to vary and/or optimize the amount of glucose oxidase provided in the composition, according to the guidance provided by the cited reference, to provide a composition having the desired properties such as the desired concentration of glucose oxidase for wound healing. It is noted that “[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.” Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited reference to incorporate an optimized amount of glucose oxidase with a reasonable expectation for successfully obtaining a composition.

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6. Claims **9, 10, 14** and **15** are rejected under 35 U.S.C. 103 as being unpatentable over Fallon et al (US 2013/0202581 A1; 8/8/2013) in view of Beiler et al (US 3,003,917; 10/10/1961) and Souza et al (Brazilian Journal of Microbiology. 2010;41:850-861) as applied to claims 1-4, 6, 7, 11-13, 16, 18-22 and 26-28 above in view of Patel et al (US 2007/0237812 A1; 10/11/2007) and Parks (WO 2013/016255 A1; 1/31/2013).

The references cited above do not teach the composition further comprising poly(hexamethylene biguanide) and its claimed concentration (**claims 9-10 and 15**), a glycerol alkyl ether (**claim 14**), and the claimed concentration of the monoalkyl glycol (**claim 15**).

However, Fallon does teach the composition further comprises propylene glycol (a monoalkyl glycol) (para 0121) and antimicrobials (para 0029), wherein the composition is a dressing material (para 0086). Patel teaches a wound dressing which is constructed to accelerate the wound-healing process (para 0028), comprising a suitable amount of antimicrobial agents such as polyhexamethylene biguanide (para 0052). In addition, Parks teaches a composition and wound dressing for treating a skin wound (Abstract), comprising polyhexamethylene biguanide and Sensiva SC 50 (glycerol 1-(2-ethylhexyl) ether, a glycerol alkyl ether) (Table 3).

Thus, before the effective filing date of the claimed invention, it would have been obvious to one of ordinary skill in the art to incorporate polyhexamethylene biguanide and Sensiva SC 50 into the composition of Fallon since Fallon, Patel and Parks all disclose a wound healing composition, Fallon discloses that the composition comprises antimicrobial agents, and Patel and Parks both disclose that the composition comprises polyhexamethylene biguanide as an antimicrobial agent accelerates the wound-healing process. Furthermore, before the effective filing date of the claimed invention, it is obvious to vary and/or optimize the amount of

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propylene glycol (a monoalkyl glycol) and antimicrobial agents such as polyhexamethylene biguanide provided in the composition, according to the guidance provided by the cited references, to provide a composition having the desired properties such as the desired concentrations of propylene glycol and polyhexamethylene biguanide for wound healing. It is noted that “[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.” Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited references to incorporate polyhexamethylene biguanide and Sensiva SC 50 as well as to optimize the amount of propylene glycol and polyhexamethylene biguanide, with a reasonable expectation for successfully obtaining a composition.

7. Claim **17** is rejected under 35 U.S.C. 103 as being unpatentable over Fallon et al (US 2013/0202581 A1; 8/8/2013) in view of Beiler et al (US 3,003,917; 10/10/1961) and Souza et al (Brazilian Journal of Microbiology. 2010;41:850-861) as applied to claims 1-4, 6, 7, 11-13, 16, 18-22 and 26-28 above in view of Tippet (US 2004/0076671 A1; 4/22/2004).

The references cited above do not teach the composition further comprising the claimed analgesic agent, as recited in **claim 17**.

However, Fallon does teach the composition further comprises an analgesic substance (para 0142). Tippet teaches a wound dressing which enhances healing of a wound comprising lidocaine (an analgesic agent) (para 0011).

Thus, before the effective filing date of the claimed invention, it would have been obvious to one of ordinary skill in the art to incorporate lidocaine (the claimed analgesic agent)

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into the composition of Fallon since Fallon and Tippet both disclose a wound healing composition, and Tippet discloses that the composition comprising lidocaine enhances healing of a wound. Moreover, before the effective filing date of the claimed invention, one of ordinary skill in the art would have been motivated by the cited reference to incorporate lidocaine with a reasonable expectation for successfully obtaining a composition.

Response to Arguments

8. Applicant argues that Smith does not anticipate amended claim 1, specifically, 0.1 to 99.5 wt % of an amylase component. However, these arguments are moot since those rejections are withdrawn in view of applicant's amendments.

Applicant argues that Fallon focuses on proteases and lipases, and does not consider amylase to be an important component of the preparations disclosed therein, that Fallon focuses on compositions where the amount of protease is almost double the amount of amylase, that Beiler does not teach the claimed amylase to protease ratio, that Fallon and Beiler teach away from the claimed amylase to protease ratio of at least 4:1, and that Souza does not correct the deficiencies of Fallon and Beiler.

These arguments are not found persuasive because preferred embodiments are not the only teaching of a reference. "The use of patents as references is not limited to what the patentees describe as their own inventions or to the problems with which they are concerned. They are part of the literature of the art, relevant for all they contain." *In re Heck*, 699 F.2d 1331, 1332-33, 216 USPQ 1038, 1039 (Fed. Cir. 1983) (quoting *In re Lemelson*, 397 F.2d 1006, 1009, 158 USPQ 275, 277 (CCPA 1968)). A reference may be relied upon for all that it would have

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reasonably suggested to one having ordinary skill in the art, including nonpreferred embodiments. *Merck & Co. v. Biocraft Laboratories*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989). In the instant case, Fallon clearly teaches a composition comprising amylases having an activity in the range of about 200-300 USP units/mg and proteases having an activity in the range of about 150-250 USP units/mg, and it may be useful to modify the amount of a particular enzymatic activity in the composition (para 0095). Generally, differences in concentration will not support patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical. (MPEP 2144.05 II) So far, Applicant has failed to show the criticality of the claimed amylase (or amylase/proteolytic enzyme) concentration. According to Applicant's own specification, amylase from different sources, or at different activity levels expressed in Units, showed different levels of *ex vivo* debridement activity, as demonstrated in Table 1, 0.1 wt % amylase from certain sources showed a high debridement efficacy while 0.1 wt % amylase from other sources showed no debridement efficacy (page 31-32). Furthermore, Table 8 demonstrated that amylolytic enzymes are comparable to proteolytic enzymes in their ability to digest rat skin (page 35-36). Unless Applicant shows evidence indicating the claimed amylase (or amylase/proteolytic enzyme) concentration is critical, differences in concentration of amylase (or amylase/proteolytic enzyme) will not support patentability of subject matter encompassed by the prior art. In addition, Beiler is relied upon to demonstrate that α -amylase and β -amylase can be included in a wound healing composition since α -amylase and β -amylase both help with wound healing. Souza is relied upon to demonstrate that α -amylase is mainly derived from the genus *Bacillus* or *Aspergillus* for commercial/industrial applications. Finally, "disclosed examples and preferred embodiments do

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not constitute a teaching away from a broader disclosure or non-preferred embodiments. *In re Susi*, 440 F.2d 442, 169 USPQ 423 (CCPA 1971).” (MPEP 2123) Further, “the prior art’s mere disclosure of more than one alternative does not constitute a teaching away from any of these alternatives because such disclosure does not criticize, discredit, or otherwise discourage the solution claimed....” *In re Fulton*, 391 F.3d 1195, 1201, 73 USPQ2d 1141, 1146 (Fed. Cir. 2004).” (MPEP 2141.02) In the instant case, Fallon and Beiler do not “criticize, discredit or otherwise discourage” using the claimed amylase (or amylase/proteolytic enzyme) concentration for healing wounds. Therefore, disclosed examples and preferred embodiments of Fallon and Beiler do not constitute a teaching away from the claimed amylase (or amylase/proteolytic enzyme) concentration.

Applicant argues that it was quite unexpected to discover that amylases can be effective for use in debridement compositions.

These arguments are not found persuasive because the claiming of a new use, function or unknown property does not necessarily make the composition patentable, since the prior art teaches a composition comprising an amylase component and at least one pharmaceutically or cosmetically acceptable carrier or excipient, as claimed. In addition, evidence relied upon should establish that the differences in results are in fact unexpected and unobvious and of both statistical and practical significance. Evidence of unexpected properties may be in the form of a direct or indirect comparison of the claimed invention with the closest prior art which is commensurate in scope with the claims. In the instant case, there was no comparison of the claimed invention (e.g., the claimed amylase (or amylase/proteolytic enzyme) concentration) with the closest prior art (e.g., the amylase (or amylase/proteolytic enzyme) concentrations

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disclosed in Fallon), and thereby lack of basis for judging the practical significance of data with regard to the disclosed unexpected results. Whether the unexpected results are the result of unexpectedly improved results or a property not taught by the prior art, the “objective evidence of nonobviousness must be commensurate in scope with the claims which the evidence is offered to support.” In other words, the showing of unexpected results must be reviewed to see if the results occur over the entire claimed range. (MPEP 716.02).

Applicant argues that Loo, Patel, Parks, and Tippet do not correct the deficiencies of Fallon.

These arguments are not found persuasive because Loo is relied upon to demonstrate oxidases, Patel is relied upon to demonstrate polyhexamethylene biguanide, Parks is relied upon to demonstrate glycerol alkyl ether, and Tippet is relied upon to demonstrate an analgesic agent. Fallon is described in the second paragraph under the section title Response to Arguments.

Conclusion

9. No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period

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will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Contact Information

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lynn Y. Fan whose telephone number is (571)270-3541. The examiner can normally be reached on M-Th 8am-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Renee Claytor can be reached on (571)272-8394. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ Lynn Y. Fan /
Examiner, Art Unit 1651



US009592280B2

(12) United States Patent
Salamone et al.**(10) Patent No.: US 9,592,280 B2**
(45) Date of Patent: Mar. 14, 2017**(54) COMPOSITIONS AND KITS FOR
ENZYMATIC DEBRIDEMENT AND
METHODS OF USING THE SAME****(71) Applicant: ROCHAL INDUSTRIES, LLP, San
Antonio, TX (US)****(72) Inventors: Joseph Charles Salamone, San
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TX (US)****(73) Assignee: ROCHAL INDUSTRIES LLC, San
Antonio, TX (US)****(*) Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.**(21) Appl. No.: 14/511,912****(22) Filed: Oct. 10, 2014****(65) Prior Publication Data**

US 2016/0101165 A1 Apr. 14, 2016

(51) Int. Cl.**A61K 38/54** (2006.01)
A61K 31/155 (2006.01)
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A61K 45/06 (2006.01)
A61L 26/00 (2006.01)
A61L 15/38 (2006.01)
A61L 15/44 (2006.01)**(52) U.S. Cl.**CPC **A61K 38/54** (2013.01); **A61K 31/155**
(2013.01); **A61K 31/785** (2013.01); **A61K**
38/47 (2013.01); **A61K 45/06** (2013.01); **A61L**
15/38 (2013.01); **A61L 15/44** (2013.01); **A61L**
26/0047 (2013.01); **A61L 26/0066** (2013.01);
C12Y 302/01001 (2013.01); **C12Y 302/01002**
(2013.01); **C12Y 302/01003** (2013.01); **C12Y**
304/22032 (2013.01); **C12Y 304/24007**
(2013.01); **A61L 2300/206** (2013.01); **A61L**
2300/254 (2013.01); **A61L 2300/402**
(2013.01); **A61L 2300/404** (2013.01); **A61L**
2300/45 (2013.01)**(58) Field of Classification Search**USPC 424/94.2
See application file for complete search history.**(56) References Cited**

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Primary Examiner — Lynn Y Fan

(74) Attorney, Agent, or Firm — Duane Morris LLP; J.
Rodman Steele, Jr.; Gregory M. Lefkowitz**(57) ABSTRACT**A debridement enzyme for necrotic tissue is described that
is not dependent upon proteolytic enzymatic activity but
instead utilizes the amylase family of enzymes. The amy-
lases (α -, β -, γ -amylase) are noted for the cleavage of the
 α -glycosidic bonds of polysaccharides, yielding lower
molecular weight carbohydrate/sugar fragments. It has now
been found that α -amylase is effective in the debridement of
devitalized tissue.**22 Claims, 3 Drawing Sheets**

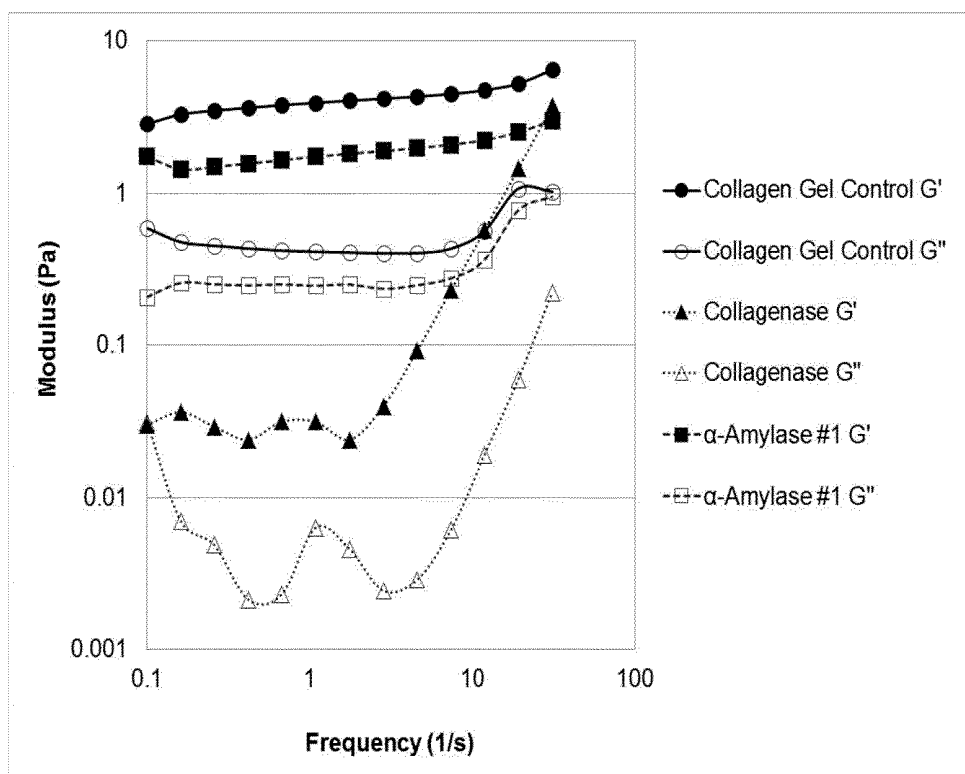


Figure 1

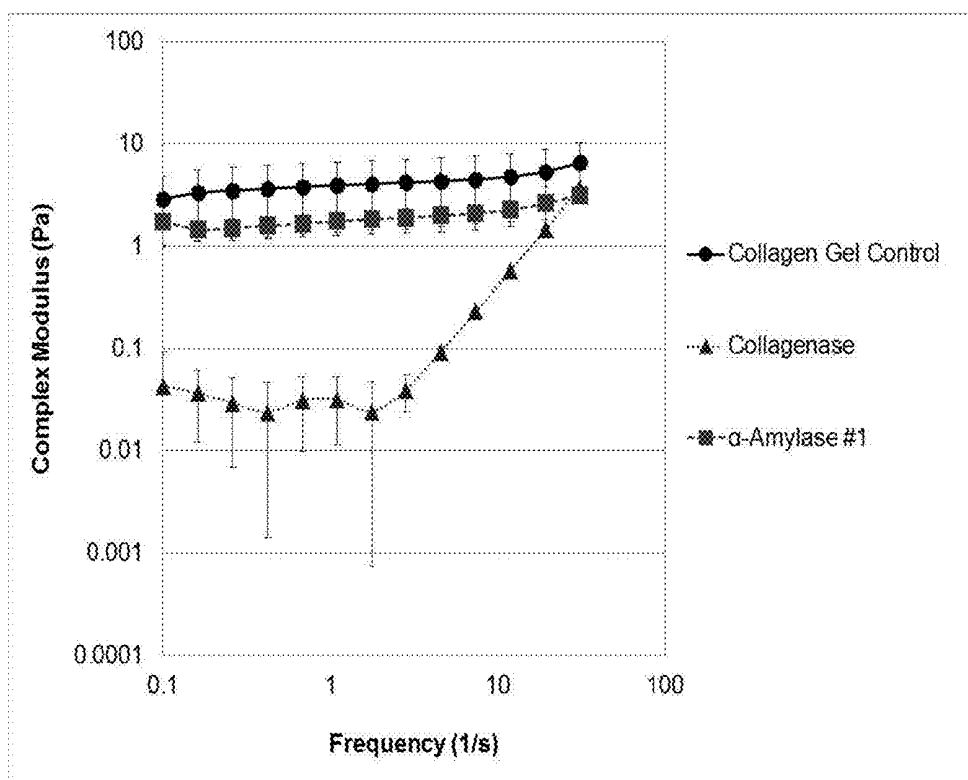
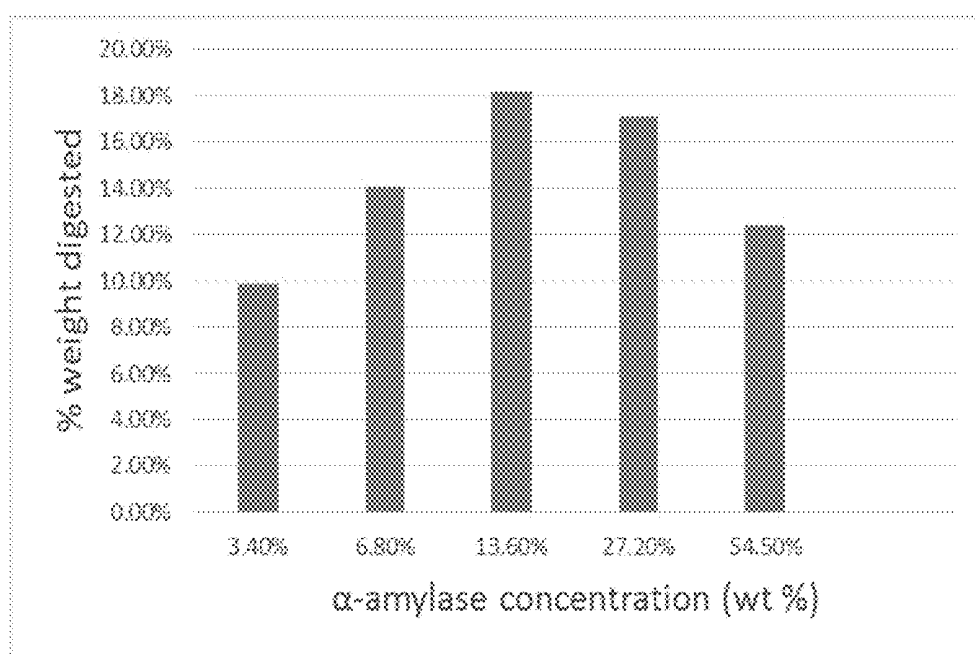


Figure 2

**Figure 3**

COMPOSITIONS AND KITS FOR ENZYMATIC DEBRIDEMENT AND METHODS OF USING THE SAME

FIELD OF INVENTION

Enzymatic debridement of necrotic tissue in wounds is often used when surgical debridement is unavailable or undesirable. This invention relates generally to the debridement of devitalized tissue by use of enzymes, specifically enzymes that cleave carbohydrate α -1,4-glycosidic linkages in polysaccharides, such as those of the amylase family, in contrast to the cleavage of peptide bonds in proteins.

BACKGROUND OF THE INVENTION

Wound healing is impaired by the presence of necrotic tissue, which contains dead cells and debris within second (deep partial thickness) and third degree (full thickness) burns (eschar) and chronic ulcers (e.g., diabetic ulcers). Necrotic tissue is devitalized tissue and its accumulation can result in a prolonged inflammatory response and mechanical obstruction of the wound contraction process with impeded reepithelialization, preventing wounds from healing and, if needed, hindering the wound bed from taking skin grafts. Necrotic tissue is susceptible to bacterial infection, which further impedes wound healing, and may induce sepsis in severe cases. When necrotic tissue is yellow or tan colored and a stringy mass in appearance, it is often termed slough, and when the tissue is desiccated and forms a thick and leathery texture, it is referred to as eschar. Debridement (removal) of devitalized tissue is needed to enhance wound closure. The debridement composition of this invention is thus useful in treating wounds.

Removing necrotic tissue helps to restore circulation at the wound site as adequate oxygen delivery to the wound is critical to healing. Methods to debride necrotic tissue include surgical debridement, enzymatic debridement, autolytic debridement, biological debridement, and mechanical debridement, with surgical and enzymatic debridement being most prevalent. Surgical debridement is the fastest and most efficient method for debridement and is performed by trained medical personnel/surgeons. However, viable tissue can be inadvertently removed because of a lack of clear demarcation between necrotic and viable tissue, resulting in an enlarged wound area and increased blood loss. Enzymatic debridement utilizes less training and it is generally performed by nursing/clinical staff, often requiring a longer duration of treatment for necrotic tissue removal. Enzymatic debridement involves the use of enzymes obtained from outside the body to remove non-living tissue. Debridement enzymes cleave (cut, digest, hydrolyze) large components of biological materials, particularly biomacromolecules into smaller molecules that can dissolve and be removed. Most debridement enzymes function as proteases by cleaving protein polymer chains, and a wide variety of proteases have been studied in this regard. Debridement enzymes are fast acting catalysts that produce slough of necrotic tissue. Fibrinolysin is a plasma enzyme which, after being activated, attacks fibroin components in blood clots and exudates. Deoxyribonuclease is a pancreatic enzyme that specifically attacks nucleoprotein components of purulent exudates. Trypsin and chymotrypsin are nonspecific pancreatic enzymes, but can sever protein backbones at specific amino acid residues. Enzymes from other animals include krillase, a protease derived from Antarctic krill. Tropical fruit-bearing plants provide a major source of

debridement enzymes. Bromelain is a group of proteolytic enzymes from the stem of pineapple plants, which include three cysteine proteases. Papain is a nonspecific cysteine protease from papaya latex that cleaves a wide variety of substances in necrotic tissues, including fibroin, collagen, and elastin. Ficin is a nonspecific cysteine protease of similar operating pH characteristics, and is derived from a plant latex of the ficus (fig) plant. Bacteria are also a source of debridement enzymes. Subtilisins, derived from *Bacillus subtilis*, are mixtures of nonspecific, water-soluble serine proteases that degrade necrotic tissues. Collagenases, which are metalloproteinases, are proteolytic enzymes that degrade collagen and are derived from *Clostridium histolyticum*. Vibriolysin is another collagen-attacking metalloproteinase, and it is derived from the bacteria *Vibrio proteolyticus*. Thermolysin is a bacterial debridement enzyme from *Bacillus thermoproteolyticus* that acts nonspecifically with outstanding productivity, even at high temperature. Streptokinase, a fibrinogen activating protease from *Streptococcus* spp. and streptodornase, a deoxyribonuclease from hemolytic streptococci, has also been used in debridement (US Patent Application Number 2008/0044459).

A number of non-FDA regulated topical proteolytic enzyme products were marketed prior to 2009 for enzymatic debridement but manufacturing and distribution were stopped by FDA because of adverse allergic events and/or lack of efficacy, including papain (papaya), trypsin and chymotrypsin (pancreatic enzymes), and *Bacillus subtilis* proteases. Currently, there are two proteolytic enzymatic debridement products available in the United States, FDA approved Santyl® Ointment (Smith & Nephew), a collagenase derived from *Clostridium histolyticum* that selectively digests triple helical collagen and utilizes 250 collagenase Units per gram of white petrolatum, and Debrase® Gel Dressing (NexoBrid), a mixture of bromelain enzymes derived from *Ananas comosus* (pineapple). Importantly, Santyl® Ointment cannot be used in conjunction with silver and iodine antimicrobials as they deactivate the collagenase. Debrase® (MediWound Ltd.) has recently received FDA orphan drug status for eschar debridement.

U.S. Pat. No. 4,668,228 discloses a debridement tape comprising an adhesive mass on a non-gel, non-bioerodable, biocompatible occlusive or semi-occlusive backing, where an effective amount of a debridement proteolytic enzyme in dry powdered form is situated on the adhesive surface. When the powder is brought into contact with wound exudates the entire load of enzymes is released immediately.

In U.S. Pat. No. 5,206,026 is disclosed a film for instantaneous delivery of a proteolytic enzyme to a wound. When exposed to aqueous liquid the film rapidly dissolves, thus releasing its contents of enzymes simultaneously.

In U.S. Patent Application 2002/0114798 is disclosed an enzymatic wound debrider that uses a combination of a proteolytic enzyme and an anhydrous hydrophilic poloxamer carrier.

In U.S. Pat. No. 5,120,656 there is provided a process for the debridement of harvested bone having its periosteum intact, which comprises contacting the periosteum with a solution of enzyme selected from the group consisting of proteolytic collagen-digesting enzyme and mixtures thereof under enzyme activity promoting conditions to loosen the periosteum from the underlying bone surface and removing the loosened periosteum from the bone.

In U.S. Pat. No. 7,368,128, a dressing for debridement of necrotic and non-viable tissue in a wound is described, wherein the dressing comprises an effective amount of one or more proteolytic enzymes incorporated in a degradable

polymeric material. The dressing of the invention provides effective debridement of necrotic wounds over a prolonged period of time, as the enzymes may be released over time.

In International Patent Publication Number WO 2012/155027, wound debridement compositions contain the proteolytic enzyme Seaprose (also known as Protease S, from the fungus *Aspergillus melleus*). The major enzyme in Seaprose is a semi-alkaline protease with a molecular weight around 31 kDa. It can also contain other enzymes such as amylase, a hydrolytic enzyme that breaks down carbohydrates.

In International Patent Publication Number WO1984/002846, a topical ointment for skin surface wounds is described comprising wound-healing amounts papain, bromelain, trypsin, chymotrypsin, pancreatin, lipase, amylase, aloe extract and an organic astringent agent formulated in a carrier mixture of penetrating and non-penetrating emollient oils and a polyhydric alcohol emollient, with a plurality of protease. The ointment is reported to reduce inflammation at the site of skin-surface wounds and acts to enhance the normal anti-inflammatory activities of the body.

In U.S. Pat. No. 6,548,556 it is reported that a proteolytic enzyme has in part or in total the capacity to hydrolyze peptide amide bonds and that such enzymes may also have some inherent lipolytic and/or amylolytic activity associated with the proteolytic activity, with the preferred proteolytic enzyme being papain. Other suitable proteolytic enzymes include trypsin, chymotrypsin, streptokinase, streptodornase, ficin, pepsin, carboxypeptidase, aminopeptidase, chymopapain, bromelain, as well as other suitable enzymes, such as pancreatin, trypsin, collagenase, keratinase, carboxylase, aminopeptidase, elastase, and aspergillopeptidase. Pancreatin contains a mixture of peptide hydrolases/proteases (trypsin, chymotrypsin, elastase, carboxypeptidase A, carboxypeptidase B), lipolytic enzymes (lipase, phospholipase A2, phospholipase B, cholinesterase, cholesterol esterase), glycosidases (α -amylase, glucosidase), and nucleases (deoxyribonuclease I, deoxyribonuclease II, ribonuclease).

Mixed debriding agent enzymes from *Bacillus subtilis* are described in U.S. Pat. No. 3,409,719. This enzyme product is reported to exhibit proteolytic activity against casein (phosphoprotein) and similar activity against hemoglobin (metalloprotein). It also exhibits amylolytic activity against gelatinized starch. It is capable of rapid lysis of fibrin, denatured collagen, elastin and exudate, which are reported to be the principal tissue protein components in the wound. The mixed enzyme compositions are shown in Table I of U.S. Pat. No. 3,409,719 to contain a minimum of amylase to proteases, ranging from 6.3% amylase and 93.7% proteases to 18.2% amylase and 81.8% proteases.

Severe burn wounds require surgical debridement in order to quickly apply antimicrobials and dressings to reduce the risk of infection, (e.g., *Pseudomonas aeruginosa* sepsis, U.S. Pat. No. 4,772,465) and to prepare the wound bed for healing or subsequent skin grafting. Because of the potential of enhanced bleeding from viable tissue removal, enzymatic debridement may be preferable for removal of necrotic tissue. Wound healing is also difficult for diabetics due to cardiovascular insufficiency and neuropathy; hence, enlarging the diabetic ulcer size by surgical debridement may not be desirable, and a critical need exists for improved debridement for diabetic ulcers.

In International Patent Publication Number WO1999046368 a method for treating wounds comprising the step of administering an effective amount of a carbohydrate-active enzyme is discussed, which is reported to have

broad-specificity for debriding burns and other wounds. Because of the high concentrations of glycosaminoglycans (GAGs) in skin, in burn patients enzymes that degrade glycosaminoglycans are considered to be useful adjuncts to burn wound debridement. Glycosaminoglycans are sugar chains consisting of repeating polymers of acidic polysaccharides, composed of building blocks of the following sugars in various combinations: galactose, glucose, N-acetylglucosamine, N-acetylgalactosamine, glucuronic acid, galacturonic acid and iduronic acid. It is known that carbohydrates have important roles in the functioning of living organisms. In addition to their metabolic roles, carbohydrates are structural components of the human body, being covalently attached to numerous other entities such as proteins (i.e., as glycoproteins). Since human skin is reported to contain 10% by weight of glycosaminoglycans (which include heparin, heparan sulfate, chondroitin sulfate, hyaluronic acid (hyaluronan), dermatan sulfate, and keratan sulfate, with chondroitin sulfate being the most prevalent glycosaminoglycan. Chondroitin sulfate also has β -1,3- and β -1,4-linkages between predominant monomeric units. The term "carbohydrate-active enzyme" as used to specifically encompass carbohydrate reducing enzymes, where examples of such enzymes include glycosaminoglycan reducing enzymes such as hyaluronidases, chondroitinases, dermatanases, heparanases, hepannases and keratanases, with preferred carbohydrate-active enzymes of chondroitinases and hyaluronidases.

Infection control is also a significant unaddressed need in debridement. Burn eschar is typically dry necrotic tissue not readily infected, but sepsis can occur with second and third degree burns; therefore, having antimicrobials in the eschar and wound bed during early stages of eschar (necrotic tissue) removal is highly desirable. In contrast to burn eschar, chronic wound (diabetic ulcer) necrotic tissue appears to promote bacterial colonization, supported by the presence of water and nutrient sources from dead cells and debris. Necrotic tissue in wounds may be associated with infection, while a majority of chronic wounds are infected with microorganism biofilm.

A difficulty in the use of proteolytic enzymes for debridement of necrotic tissue is their ability for auto-digestion in aqueous solution, in addition to their potential difficulty with adverse allergic events, hypersensitivity and/or lack of efficacy. It is thus desirable to identify a debridement composition, having a high debridement efficacy for necrotic tissue, that is clinically simple to use, exhibits a suitable shelf-life, and is non-allergenic.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of frequency versus modulus for collagen gels before and after treatment with different enzymes.

FIG. 2 is a graph of frequency versus complex modulus for collagen gels before and after treatment with different enzymes.

FIG. 3 is a graph of weight percent boiled pig skin digested versus α -amylase concentration after one hour at room temperature.

DETAILED DESCRIPTION OF THE INVENTION

In this investigation a novel composition and approach to enzymatic debridement is considered through the presumed cleavage of glycosidic bonds in and/or between gly-

cosaminoglycan polysaccharides and collagen fibrils, and not by cleavage of peptide bonds of collagen by proteases, as has been done with previous enzymatic debridement products. It was unexpectedly found that amylase, an enzyme noted for cleavage of α -1,4-glycosidic bonds, such as in the catalyzed hydrolysis of starch into low molecular weight sugars, is effective in the debridement of devitalized or necrotic tissue. These unique compositions provide high efficiency enzymatic debridement compositions, with a suitable shelf-life, without allergic reactions.

The debriding formulations described herein contain various types of the enzyme amylase. Amylases (α -, β -, γ -amylase) are a family of enzymes that preferentially hydrolyze the α -glycosidic bonds of polysaccharides, yielding lower molecular weight carbohydrate/sugar fragments. In some embodiments, α -amylase is used as the amylase. Amylase occurs naturally in humans and other mammals, and it is also found in plants, bacteria and fungi.

The physical behavior of skin tissue is determined primarily by an extensive extracellular matrix (ECM). The ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs). The GAGs are carbohydrate polymers and are usually attached to ECM proteins, forming proteoglycans. In skin, type I collagen is the main protein component of the ECM and the main proteoglycan components are decorin and versican. Presumably, these core proteins bind to the surface of type I collagen fibrils, which provide mechanical strength to skin. Proteoglycan binding is required for appropriate assembly of collagen fibrils in the ECM and inhibits cleavage of collagen fibrils by matrix metalloproteases. Proteoglycans are composed of a glycoprotein core to which one or several GAG chains are covalently bonded. Four different classes of glycosaminoglycans exist in vertebrates, chondroitin sulfate, dermatan sulfate, keratan sulfate, and heparan sulfate/heparin. Hyaluronan (hyaluronic acid) is one of the chief components of the extracellular matrix, and it contributes significantly to cell proliferation and migration. However, unlike the other glycosaminoglycans, hyaluronan does not attach to proteins to form proteoglycans but binds and retains water molecules and fills the gaps between collagen fibrils.

The GAGs are attached to a serine residue of the core protein by both glycosidic (β -1,4- (primarily by chondroitin sulfate, dermatan sulfate) and α -1,4-bonds (primarily by heparan sulfate/heparin), with the predominant GAG being chondroitin sulfate. For internal linkages, hyaluronan and chondroitin sulfate are predominantly composed of β -1,3- and β -1,4-linkages, dermatan sulfate has predominantly α -1,3- and β -1,4-linkages, and heparin/heparan sulfate have a mixture of (β -1,4- and α -1,4-linkages, wherein the primary repeating unit does not contain 3 or more α -1,4-linkages (Glycosaminoglycans and Proteoglycans, sigma.com/glycobiology), as required for cleavage by α -amylase.

A previous study discussed a method for treating wounds comprising the step of administering an effective amount of a carbohydrate-active enzyme, wherein such enzymes were preferentially chondroitinases, enzymes that catalyze the hydrolysis of the chondroitin chains on proteoglycans containing (1-4)- β -D- and (1-3)- β -D linkages, and hyaluronidases, enzymes that cleave hyaluronan, which contains β -1,4- and β -1,3-glycosidic bonds, with limited ability to degrade chondroitin and chondroitin sulfates (International Patent Publication Number WO1999046368).

The compositions and methods described herein pertain to the use of a non-protease hydrolytic enzyme for necrotic tissue debridement, where it was unexpectedly found that

the family of amylases, which include α -amylase, β -amylase, and γ -amylase, was able to digest and decompose a neurotic tissue analog: boiled pig skin (U.S. Pat. No. 8,119,124), as well as, freshly excised rat skin.

Amylase is a digestive enzyme that aids in the cleavage of bonds in sugar residues in polysaccharides. It is found in two primary types in the human body: salivary amylase and pancreatic amylase. In saliva, salivary amylase is responsible for the breakdown of starch and glycogen into glucose, maltose, and dextrin. Pancreatic amylase further degrades starches in the digestive system.

In some embodiments, the non-proteolytic component is greater than the proteolytic component of the debridement composition. In some embodiments, a ratio of non-proteolytic enzymes to proteolytic enzymes in the debridement composition is at least 4:1, at least 5:1, or at least 10:1, at least 20:1 at least 40:1, at least 60:1, at least 80:1, or at least 100:1. Where the amount of proteolytic enzymes is 0 and the amount of non-proteolytic enzyme is greater than 0, the ratio is ∞ :1. In some embodiments, the debridement composition comprises less than 0.01% by weight proteolytic enzymes, or less than 0.001% by weight of proteolytic enzymes, based on the total weight of the debridement composition. In some embodiments, the debridement composition comprises up to 20% by weight of proteolytic enzyme, or up to 15% by weight of proteolytic enzyme, or up to 10% by weight of proteolytic enzyme.

Relative to the three forms of amylase, α -amylase (also called 1,4- α -D-glucan glucanohydrolase) is an endoamylase that is found in all living organisms. It functions in a random manner by a multiple-attack mechanism on starch, glycogen and related polysaccharides and oligosaccharides with α -1,4-glycosidic linkages, ultimately yielding glucose and maltose, as well as, larger oligosaccharides, none of which are present in human skin. α -Amylase hydrolyzes 1,4- α -D-glucosidic linkages in polysaccharides that contain 3 or more 1,4- α -linked D-glucose units (Sigma Aldrich, <http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/carbohydrate-analysis.html>). However, α -amylase cannot hydrolyze α -1,6-bonds in glycogen and amylopectin.

β -Amylase (also called 1,4- α -D-glucan maltohydrolase) and γ -amylase (also called (amyloglucosidase, glucan 1,4- α -glucosidase, and glucoamylase) are exoamylases that are found exclusively in plants and microorganisms. Like α -amylase, β -amylase cannot hydrolyze 1,6- α -bonds. The β -amylase enzyme acts on the same substrates as α -amylase, but it removes successive maltose units from the non-reducing end. γ -Amylase releases β -D-glucose successively from the non-reducing end of the polysaccharide chains. Various forms of γ -amylase can hydrolyze 1,6- α -D-glucosidic bonds when the next bond in the sequence is a 1,4-bond, and some preparations can hydrolyze 1,6- and 1,3- α -D-glucosidic bonds in other polysaccharides. Combinations of the amylase enzymes are used in various preparations, such as food production, sweeteners, starch saccharification, brewing and distilling industries.

Calcium and chloride ions are essential for the activity of α -amylase. One Ca^{2+} is tightly bound by each enzyme molecule, facilitating the proper conformation for hydrolytic activity, and chloride ions have been regarded as natural activators of the enzyme. Excess calcium stabilizes α -amylase towards heat. Catalytic activity is optimum at a temperature range between 40° C. and 45° C. and a pH of 7.0-7.5.

In this investigation, a novel composition for and approach to enzymatic debridement by amylase is consid-

ered through the cleavage of glycosidic bonds in and/or between glycosaminoglycan polysaccharides and collagen fibrils, and not by cleavage of peptide bonds of collagen, as has been done with previous enzymatic debridement products. The debriding formulation contains various types of the enzyme amylase. All amylases (α -, β -, γ -amylase) are a family of enzymes that preferentially hydrolyze the α -glycosidic bonds of polysaccharides, yielding lower molecular weight carbohydrate/sugar fragments. α -Amylase randomly cleaves the 1,4- α -D-glycosidic linkages between the adjacent glucose units in the linear amylose chain of starch. A significant benefit of α -amylase, is that it occurs naturally in humans and other mammals, and it is also found in plants, bacteria and fungi. In some embodiments, the amylase comprises a microbial amylase. Plant and animal sources can contain more harmful material than microbial based amylases, including phenolic compounds (from plants), endogenous enzyme inhibitors, and proteases.

Since amylase is not proteolytic, it does not self-digest in water, and is more stable compared to proteolytic enzymes under similar aqueous conditions. The high stability of amylase facilitates its storage in a hydrophilic formulation, which can be easily removed from a necrotic wound post-debridement, unlike petrolatum-based proteolytic debriding ointments.

The presence of amylase in proteolytic enzyme debridement has been reported primarily at a much lower quantity than proteases in these procedures, and often is an impurity in pancreatic, proteolytic enzymes (U.S. Pat. Nos. 3,409, 719; 8,540,983). A carbohydrate-active enzyme formulation based upon chondroitinases and hyaluronidases has been reported to debride burns and other wounds (International Patent Publication Number WO1999046368). It is anticipated that these enzymes presumably would be active against the proteoglycans associated with collagen in skin, such as the predominant proteoglycan of chondroitin sulfate. No report has discussed the enzymatic debridement of necrotic tissue based upon amylase as the principal enzymatic debriding agent because amylase is presumed to degrade only α -1,4-glycosidic bonds such as in starch, a carbohydrate not involved in the extracellular matrix.

Starch molecules are glucose polymers linked together by the α -1,4- and α -1,6-glycosidic bonds, consisting of linear amylose and branched amylopectin components. In order to make use of the carbon and energy stored in starch, amylase, as part of the human digestive system, cleaves starch at multiple points, converting starch into smaller sugars, which are eventually converted to glucose units. Because of the existence of two types of linkages, the α -1,4- and the α -1,6-glycosidic bonds, different structures are possible for starch molecules. An unbranched, single chain polymer with only the α -1,4-glycosidic bonds is called amylose. On the other hand, the presence of α -1,6-glycosidic linkages results in the branched glucose polymer of amylopectin. Another closely related compound functioning as the glucose storage in human and animal cells is called glycogen. Glycogen has a structure similar to that of amylopectin, except that the branches in glycogen tend to be shorter and more numerous. Neither amylose, nor amylopectin, nor glycogen is believed to be present in human or animal skin as a component of stabilizing or interacting with collagen of the extracellular matrix.

The specificity of the bond attacked by α -amylase depends on the source of the enzyme. Currently, two major classes of α -amylase are commercially produced through microbial fermentation. Based on where the cleavage occurs in the glucose polymer chain, the initial step in random

depolymerization of starch is the splitting of large chains into various smaller sized segments. The breakdown of large segments drastically reduces the viscosity of the gelatinized starch solution, resulting in liquefaction because of the reduced viscosity of the solution. The final stage of the depolymerization is saccharification, which results predominantly in the formation of monosaccharides, disaccharides, and trisaccharides.

Because bacterial α -amylase randomly attacks only the α -1,4-bonds, it belongs to the liquefying category. On the other hand, the fungal α -amylase belongs to the saccharifying category and attacks the second linkage from the nonreducing terminals (i.e., C4 end) of the straight segment, resulting in the splitting off of two glucose units at a time, giving the disaccharide maltose. The bond breakage is thus more extensive in saccharifying enzymes than in liquefying enzymes. The starch chains are literally chopped into small bits and pieces. Finally, γ -amylase selectively attacks the last bond on the nonreducing terminals and can act on both the α -1,4- and the α -1,6-glycosidic linkages at a relative rate of 1:20, resulting in the splitting off of simple glucose units into the solution, α -Amylase and γ -amylase may be used together to convert starch to simple sugars.

Amylase has also been used in the cleaning of hard surfaces and fabrics, as described in International Patent Publication Number WO 2007/144856, which include all-purpose or "heavy-duty" washing agents, especially laundry detergents; liquid, gel or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid types; liquid fine-fabric detergents; hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type; machine dishwashing agents, including the various tablet, granular, liquid and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, laundry bars, mouthwashes, denture cleaners, car or carpet shampoos, bathroom cleaners; hair shampoos and hair-rinses; shower gels and foam baths and metal cleaners; as well as cleaning auxiliaries such as bleach additives and "stain-stick" or pre-treat types.

Amylases are one of the main enzymes used in industry. Amylases have potential application in a wide number of industrial processes such as food, fermentation and pharmaceutical industries. Although α -amylases can be obtained from plants, animals and microorganisms, enzymes from fungal and bacterial sources have dominated applications in industry, including microorganisms of *Bacillus* spp. and *Aspergillus* spp., with most commercial amylases being produced from bacterial sources such as *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, or *Bacillus stearothermophilus*.

The debridement compositions described herein can be formulated as a liquid, gel, powder, paste, ointment, lotion, emulsion, or microemulsion, and can be delivered to necrotic or devitalized tissue as a foam, spray, dressing, mesh, bandage, or film, wherein the latter may contain film-forming polymers, semi-permeable or permeable polymers, or a degradable or non-degradable substrate, such as a dressing, bandage, or foamed material. The debridement composition can include one or more pharmaceutically or cosmetically acceptable carrier that is compatible with the enzymatic debridement composition. Examples of pharmaceutically or cosmetically acceptable carriers include, but are not limited to, water, normal saline (isotonic saline), Dulbecco's phosphate buffered saline (DPBS), phosphate buffered saline (PBS), saline solutions containing added calcium chloride, Ringer's solution, glycerin, propylene

glycol, ethanol, isopropanol, butane-1,3-diol, liquid poly (alkylene glycol)s (e.g., poly(ethylene glycol), methyl ether-terminated poly(ethylene glycol), poly(ethylene glycol-block-propylene glycol-block-ethylene glycol)), and water-soluble liquid silicone polyethers, water-insoluble media,

such as, isopropyl myristate, isopropyl palmitate, mineral oil, dimethicone, and petrolatum. In some embodiments, excipients can be present in an amount ranging from 0% to 99.9 wt % based on the weight of the debridement composition.

The debridement composition can also include wetting agents, buffers, gelling agents, surfactants, chelating agents and emulsifiers. Other excipients could include various water-based buffers ranging in pH from 5.0-7.5, silicones, polyether copolymers, vegetable and plant fats and oils, essential oils, hydrophilic and hydrophobic alcohols, vitamins, monoglycerides, and penetration enhancement esters such as laurate esters, myristate esters, palmitate esters, and stearate esters. In some embodiments, the debridement composition can be in a form selected from liquid, gel, paste, cream, emulsion, and combinations thereof, and the like.

In some embodiments, the enzymatic debridement composition is lyophilized to a dry powder. The lyophilized enzymatic debridement composition may be used in powder form, or the powder may be further processed into solutions, creams, lotions, gels, sprays, foams, aerosols, films, or other formulations.

In some embodiments, surfactant emulsifiers can be used to form emulsions, which facilitate compatibilization with organic solvents. Examples of organic solvents include, but are not limited to, non-stinging solvents, such as volatile silicone solvents and volatile alkanes to form water-in-oil or oil-in-water emulsions, reverse emulsions, miniemulsions (nanoemulsions), microemulsions, and reverse microemulsions. Non-stinging volatile silicone solvents include, but are not limited to low molecular weight polydimethylsiloxanes, such as hexamethyldisiloxane or octamethyltrisiloxane; low molecular weight cyclic siloxanes, such as hexamethylcyclotrisiloxane or octamethylcyclotetrasiloxane; linear, branched or cyclic alkanes, such as propane, butane, and isobutane (aerosols under pressure), pentane, hexane, heptane, octane, isooctane, and isomers thereof, petroleum distillates, and cyclohexane; and chlorofluorocarbons, such as, trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane; fluorocarbons, such as tetrafluoroethane, heptafluoropropane, 1,1-difluoroethane, pentafluoropropane, perfluoroheptane, perfluoromethylcyclohexane; hydrofluoroalkanes, such as aerosols of 1,1,1,2-tetrafluoroethane and 1,1,1,2,3,3,3-heptafluoropropane, combinations thereof and the like; and volatile gases under pressure, such as air, nitrous oxide, and liquid carbon dioxide; or a mixture thereof. As will be understood, when stored under high pressure, carbon dioxide can be present in the form of a liquid at room temperature. In some embodiments, the volatile solvent can be hexamethyldisiloxane, isooctane, and mixtures thereof. In some embodiments, the volatile solvent can be hexamethyldisiloxane. In some embodiments, solvents can be present in an amount ranging from 0% to 99.9 wt % based on the weight of the debridement composition.

Water-soluble viscosity builders useful herein include, but are not limited to, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose, guar gum, hydroxyethylguar, hydroxypropylguar, hydroxypropylmethylguar, carboxymethylguar, carboxymethylchitosan, locust bean gum, carrageenan, xanthan gum, gellan gum, Aloe vera gel, sclerog-

lucan, schizophyllan, gum arabic, tamarind gum, poly(vinyl alcohol), poly(ethylene oxide), poly(ethylene glycol), poly(methyl vinyl ether), Carbomer and its salts, poly(acrylic acid) and its salts, poly(methacrylic acid) and its salts, sodium poly(2-acrylamido-2-methylpropanesulfonate), polyacrylamide, poly(N,N-dimethylacrylamide), poly(N-vinylacetamide), poly(N-vinylformamide), poly(2-hydroxyethyl methacrylate), poly(glyceryl methacrylate), poly(N-vinylpyrrolidone), poly(N-isopropylacrylamide) and poly(N-vinylcaprolactam), the latter two hydrated below their Lower Critical Solution Temperatures, and the like, and combinations thereof.

In some embodiments, water-soluble polymers that are neutral in charge and are not enzymatically degradable by amylase can be used as viscosity builders. Examples of such viscosity builders include, but are not limited to, poly(ethylene oxide), poly(ethylene glycol), poly(vinyl alcohol), and poly(N-vinylpyrrolidone). Other viscosity builders useful in the debridement compositions described herein include, but are not limited to neutral polysaccharides that have β -linkages between monosaccharide units, such as in methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose. Still other viscosity builders useful in the debridement compositions described herein include, but are not limited to, those that are anionic in charge, such as Carbomer and its salts, poly(acrylic acid) and its salts, and poly(methacrylic acid) and its salts. Still other viscosity builders are anionic polysaccharides that have β -linkages between monosaccharide units, such as in carboxymethylcellulose. Such viscosity builders may be employed in amounts ranging from about 0.01 to about 50.0 weight percent of the debridement composition for preparation of viscous gels or pastes. Viscosity builders can be present in amounts ranging from 0.1 to 45% by weight, from 0.5 to 25% by weight, or from 1.0 to 10.0% by weight.

Essential oils can also be added to the formulation as fragrance or aromatic agents, and/or as antimicrobial agents. Examples of essential oils useful in the debridement compositions described herein include, but are not limited to, thymol, menthol, sandalwood, camphor, cardamom, cinnamon, jasmine, lavender, geranium, juniper, menthol, pine, lemon, rose, eucalyptus, clove, orange, oregano, mint, linaleol, spearmint, peppermint, lemongrass, bergamot, citronella, cypress, nutmeg, spruce, tea tree, wintergreen (methyl salicylate), vanilla, and the like. In some embodiments, the essential oils can be selected from thymol, sandalwood oil, wintergreen oil, eucalyptol, pine oil, and combinations thereof. In some embodiments, essential oils can be present in an amount ranging from 0% to 5 wt % based on the weight of the debridement composition. In some embodiments, essential oils can be present in an amount of at least 0.1 wt %, or at least 0.25 wt %, or at least 0.5 wt % based on the weight of the debridement composition.

In some embodiments, chlorophyllin, a water-soluble semi-synthetic derivative of chlorophyll, may also be used to control wound odor and to provide anti-inflammatory properties. In some embodiments, chlorophyllin can be present in an amount ranging from 0% to 5 wt % based on the weight of the debridement composition. In some embodiments, chlorophyllin can be present in an amount of at least 0.1 wt %, or at least 0.25 wt %, or at least 0.5 wt % based on the weight of the debridement composition.

A keratolytic agent can also be added to the debridement composition to aid in digesting the eschar tissue. For example, the keratolytic agent can promote the softening and peeling of the epidermis. Keratolytic agents useful in the debridement compositions as described herein include, but

are not limited to, urea, salicylic acid and α -hydroxyacids, such as lactic acid, glycolic acid, and citric acid. For example, urea can be used to help remove dead tissue in necrotic wounds to help wound healing (U.S. Pat. No. 8,754,045). In some embodiments, the keratolytic agent can be present in an amount ranging from 0% to 15 wt % based on the weight of the debridement composition. In some embodiments, essential oils can be present in an amount of at least 0.1 wt %, or at least 0.25 wt %, or at least 0.5 wt % based on the weight of the debridement composition.

In some embodiments, the total amount of non-proteolytic enzyme in a debridement composition capable of debriding devitalized or necrotic tissue can be at least 0.001 wt % to 60 wt % based on the total weight of the debridement composition. In some embodiments, the total amount of non-proteolytic enzyme can be at least 1 wt % to 50 wt %, or at least 2 wt % to 40 wt %, or at least 5 wt % to 35 wt %, or at least 10 wt % to 30 wt %. In some embodiments, the non-proteolytic enzyme is an amylase. In some embodiments, the non-proteolytic enzyme is α -amylase.

In some embodiments, the amount of non-proteolytic enzymatic debridement component capable of debriding devitalized or necrotic tissue in the debridement composition can be 100 wt %, or at least 99.5 wt %, or at least 99 wt %, or at least 95 wt %, or at least 90 wt %, or at least 85 wt %, or at least 80 wt %. In some embodiments, the amount of non-proteolytic enzymatic debridement component capable of debriding devitalized or necrotic tissue in the debridement composition can be up to 100 wt %, or up to 99.5 wt %, or up to 99 wt %, or up to 95 wt %, or up to 90 wt %, or up to 85 wt %, or up to 80 wt %. In some embodiments, the amount of non-proteolytic enzymatic debridement component capable of debriding devitalized or necrotic tissue in the debridement composition can be at least 0.001 wt %, or at least 0.01 wt %, or at least 0.05 wt %, or at least 0.075 wt %, or at least 0.1 wt %, or at least 0.15 wt %.

In some embodiments, the amount of amylase in the debridement composition can be 100 wt %, at least 99.5 wt %, at least 99 wt %, at least 95 wt %, at least 90 wt %, at least 85 wt %, or at least 80 wt %. In some embodiments, the amount of amylase in the non-proteolytic enzymatic debridement component can be 100 wt %, at least 99.5 wt %, at least 99 wt %, at least 95 wt %, at least 90 wt %, at least 85 wt %, or at least 80 wt %, with the remainder of the non-proteolytic enzymatic debridement component being other non-proteolytic enzymes. The amount of α -amylase can be at least 10 wt %, at least 20 wt %, at least 30 wt %, at least 40 wt %, at least 50 wt %, at least 60 wt %, at least 70 wt %, at least 80 wt %, at least 90 wt %, or 100 wt % of the amylase content. In some embodiments, the remaining portion of the non-proteolytic enzymatic debridement component (20 wt % or less, 15 wt % or less, 10 wt % or less, 5 wt % or less, 1 wt % or less, 0.5 wt % or less) can be, but not limited to, hydrolytic, lytic, and oxidative/reductive enzymes including, but are not limited to, hydrolytic, lytic, and oxidative/reductive enzymes selected from the group consisting of lipases, hyaluronidases, chondroitinases, heparinases, heparinases, peroxidases, xylanases, nucleases, phospholipases, esterases, phosphatases, isoamylases, maltases, glycosylases, galactosidases, cutinases, lactases, inulases, pectinases, mannanases, glucosidases, invertases, pectate lyases, reductases, oxidases, phenoloxidases, lipoxigenases, ligninases, pullulanases, tannases, pentosanases, glucanases, arabinosidases, sulfatases, cellulases, hemicellulases, laccases, mixtures thereof, and the like.

Examples of proteolytic enzymes that can be present in the debridement composition include, but are not limited to, proteases and keratinases.

The debridement composition can include an aqueous media. In some embodiments, the aqueous media can have a pH in the range 3.0-10.0, or from 4.5-8.0, or from 5.5 to 7.5. In some embodiments, the debridement composition can have an osmolality of 10-340 mOsm/kg. Where the debridement composition is an aqueous-based solution, gel or paste, a water-soluble polymer can be added to increase solution viscosity and to prolong residence time of the enzymatic composition on the necrotic tissue, on the surface of a wound, or subcutaneously in a wound.

The enzymatic debridement composition can be applied to the devitalized tissue as needed to dissolve necrotic debris in and around a wound. For example, in some embodiments the enzymatic debridement composition can be in contact with the devitalized tissue for about 1 to 48 hours, 1 to 24 hours, 1 to 12 hours, 1 to 8 hours, 1 to 4 hours, or 1 to 2 hours before removal.

In some embodiments, the amylase formulation can be removed from a wound by wiping or by rinsing with saline or water. These steps may be repeated as needed. A wide variety of wounds can be treated with the debridement compositions described herein, including full and partial thickness burn wounds, diabetic ulcers, ulcerative lesions, principally pressure (decubitus) ulcers, venous ulcers, trophic ulcers, surgical wounds such as amputation, incisional, traumatic and pyogenic wounds, infected wounds by microorganisms, donor and receptor skin graft wounds, malignancy, cysts, radiation wounds, sunburn, and frostbite.

In some embodiments, the enzymatic debridement composition can be injected into the devitalized tissue. A penetration enhancer may also be utilized to enhance transdermal delivery of solutions, gels, creams, lotions, aerosols, and sprays. Penetration enhancers that can be used in the debridement compositions described herein include, but are not limited to, fatty acids such as branched and linear C_6 - C_{16} saturated acids, unsaturated acids, such as C_{14} to C_{22} , oleic acid, cis-9-octadecenoic acid, linoleic acid, linolenic acid, fatty alcohols, such as saturated C_8 - C_{18} terpenes, such as d-limonene, α -pinene, 3-carene, menthone, fenchone, pulegone, piperitone, eucalyptol, chenopodium oil, carvone, menthol, α -terpineol, terpinen-4-ol, carveol, limonene oxide, pinene oxide, cyclopentane oxide, triacetin, cyclohexane oxide, ascaridole, 7-oxabicyclo[2,2,1]heptane, 1,8-cineole, glycerol monoethers, glycerol monolaurate, glycerol monooleate, isostearyl isostearate, isopropyl myristate, isopropyl palmitate, isopropyl lanolate, pyrrolidones, such as N-methyl-2-pyrrolidone, 1-ethyl-2-pyrrolidone, 5-methyl-2-pyrrolidone, 1,5-dimethyl-2-pyrrolidone, 2-pyrrolidone-5-carboxylic acid, N-hexyl-2-pyrrolidone, N-lauryl-2-pyrrolidone, 1-dodecylazacycloheptan-2-one, 4-decyloxazolidin-2-one, N-dodecylcaprolactam, and 1-methyl-3-dodecyl-2-pyrrolidone. N-n-butyl-N-n-dodecylacetamide, N,N-di-n-dodecylacetamide, N-cycloheptyl-N-n-dodecylacetamide and N,N-di-n-propyldodecanamide, urea, 1-dodecylurea, 1,3-didodecylurea, 1,3-diphenyl urea, dimethyl sulfoxide, decylmethyl sulfoxide, tetradecylmethyl sulfoxide, cyclodextrins, and combinations thereof. Also effective penetration enhancers include 1-alkyl-2-piperidinones, 1-alkyl-2-azacycloheptanones, such as 1-dodecylazacycloheptan-2-one, 1,2,3-alkanetriols, such as 1,2,3-nonanetriol, 1,2-alkanediols, n-, 2-(1-alkyl)-2-methyl-1,3-dioxolanes, oxazolidinones, such as 4-decyloxazolidin-2-one, N,N-dimethylalkanamides, 1,2-dihydroxypropyl alkanoates, such as 1,2-dihydroxypropyl decanoate, 1,2-dihydroxypropyl

octanoate, sodium deoxycholate, trans-3-alken-1-ols, cis-3-alken-1-ols, and trans-hydroxyproline-N-alkanamide-C-ethylamide, and combinations thereof. In some embodiments, the penetration enhancers can include hydrophobic esters isopropyl myristate, isopropyl palmitate, or combinations thereof.

Because of the possibility of infection in devitalized necrotic tissue, the debridement composition can include a biological agent in an amount sufficient to hinder or eradicate microorganisms. Such biological agents include, but are not limited to, antibiotics, antiseptics, anti-infective agents, antimicrobial agents, antibacterial agents, antifungal agents, antiviral agents, antiprotozoal agents, sporicidal agents, antiparasitic agents. In some embodiments, the biological agent is biodegradable and non-cytotoxic to human and animal cells. Useful biocidal agents include, but are not limited to, biguanides, such as poly(hexamethylene biguanide hydrochloride) (PHMB), a low molecular weight synthetic polycation, chlorhexidine and its salts, such as chlorhexidine digluconate (CHG), and alexidine and its salts, where the latter two are bis(biguanides).

In some embodiments, the biguanide is PHMB because of its high biocidal activity against microorganisms, combined with its biodegradation and low cytotoxicity. PHMB is primarily active against Gram negative and Gram positive bacteria, fungi, and viruses, and also serves as a preservative. In contrast to antibiotics, which are considered regulated pharmaceutical drugs, to which bacterial resistance can occur, such resistance does not occur with PHMB. In general, an antimicrobial agent is defined as a substance that kills microorganisms or inhibits their growth or replication, while an anti-infective agent is defined as a substance that counteracts infection by killing infectious agents, such as microorganisms, or preventing them from spreading. Often, the two terms are used interchangeably. As used herein, PHMB is considered an antimicrobial agent.

In some embodiments, the debridement composition is an aqueous debridement composition. In some embodiments, aqueous debridement compositions described herein can include biocidal PHMB at a concentration ranging from 0.01 wt % (100 ppm) to 1 weight % (10,000 ppm), or ranging from 0.05 wt % (500 ppm) to 0.5 wt % (5,000 ppm), or ranging from 0.1 wt % (1,000 ppm) to 0.15 wt % (1,500 ppm) based on the total weight of the debridement composition. Bis(biguanides), such as alexidine and its salts and chlorhexidine and its salts, can also be added to the antimicrobial compositions in concentrations from 0.001 wt % (10 ppm) to 4.0 wt % (40,000 ppm).

The interaction of low molecular weight PHMB polycation (molecular weight 2,400 Daltons) with amylase, a negatively charged, high molecular weight amylase (molecular weight 55,000 Daltons) at physiological pH can generate a protein-polyelectrolyte complex of PHMB ionically interacted with amylase. In a wound, PHMB may be released from the protein-polyelectrolyte complex in a continuous release fashion as a function of the amount of low molecular weight cation in bodily fluids (such as sodium, potassium, calcium and magnesium ions) displacing the cationic PHMB ionic interaction with anionic amylase sites.

The dosage at which the therapeutic amylase compositions are administered is dependent upon the source of the amylase, the activity (i.e., the number of Units involved), the size of necrotic tissue, the age of the patient, the availability of clinical care, and the incidence of infection. The amount of therapeutic amylase that may be administered up to twice per day can range from application of a powder (at 100 wt %) to a dilute solution (of about 0.001 wt %). In some

embodiments, the activity of the amylase can range from 250 Units to 250,000 Units per gram of enzyme in 1 gram of the debridement composition.

The debridement composition on necrotic tissue can be performed by a clinician, such that the patient is not in any further medical difficulty. The amylase method of debridement can be performed in combination with other known debridement methods.

As used herein, "debridement" has its standard meaning and includes the removal of lacerated, devitalized, dead, damaged, infected or contaminated tissue, foreign bodies, and other debris from the wound bed in order to expose healthy tissue. Nonviable or necrotic tissue can either be eschar or slough.

As used herein, "necrotic tissue" has its standard meaning and includes dead tissue that contains dead cells and debris, as a consequence of the fragmentation of dying cells. The color of necrotic tissue changes from red to brown, or black or purple, as it becomes more dehydrated, finally resulting in a black, dry, thick, and leathery eschar structure, which can occur in a wide variety of wound types, including burns and all types of chronic wounds.

As used herein, "eschar" has its standard meaning and includes forms of necrotic tissue. It is the leather-like covering on the wound at the skin surface, and it can be either hard or pliable.

As used herein, "slough" has its standard meaning and includes moist necrotic tissue. This type of devitalized tissue is soft, moist and often stringy in consistency and is usually yellow, white or grey in color.

As used herein, "devitalized tissue" has its standard meaning and includes tissue devoid of vitality or life, that is, dead tissue.

As used herein, "proteolytic enzyme" has its standard meaning and includes enzymes that independently cleave (digest, break, hydrolyze) the long chainlike polymer molecules of proteins into shorter fragments of peptides and, eventually, into their basic components of amino acids.

As used herein, "excise" has its standard meaning and includes a surgical procedure requiring incision utilizing a scalpel or other sharp instrument through the deep dermis (including subcutaneous and deeper tissues).

As used herein, a covalent bond that is formed between a carbohydrate molecule and another molecule, particularly between two monosaccharide moieties, is a "glycosidic bond" or "glycosidic linkage."

As used herein, " α -1,4-glycosidic linkages" are bonds that are normally formed between the carbon-1 on one sugar and the carbon-4 on another sugar moiety in a polysaccharide. An α -glycosidic bond is formed when the —OH group on carbon-1 is below the plane of the glucose ring. On the other hand, a β -glycosidic bond is formed when it is above the plane. For example, cellulose is formed of glucose molecules linked by 1-4 β -glycosidic bonds, whereas starch is composed of 1-4 α -glycosidic bonds.

As used herein, " α -amylase" includes naturally occurring α -amylases as well as recombinant α -amylases, wherein recombinant α -amylase means an α -amylase in which the DNA genetic sequence encoding the naturally occurring α -amylase is modified to produce a mutant DNA sequence that encodes the substitution, insertion or deletion of one or more amino acids in the α -amylase sequence compared to the naturally occurring α -amylase.

As used herein, "amylolytic" is characterized by or capable of the enzymatic digestion of starch into dextrans and sugars, particularly by amylase.

As used herein, the amount of enzyme utilized is expressed in weight percent and its activity is given in Units of activity per gram, where a "Unit" is defined as the amount of enzyme that catalyzes the conversion of 1 micromole of substrate per minute.

As used herein, "surfactant" has its standard meaning and includes compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid and includes emulsifying agents, emulsifiers, detergents, wetting agents, and surface-active agents.

As used herein, "microemulsion" has its standard meaning and includes thermodynamically stable mixtures of oil, water (and/or hydrophilic compound) and surfactant. Microemulsions include three basic types: direct (oil dispersed in water, o/w), reverse (water dispersed in oil, w/o) and bicontinuous. Microemulsions are optically clear because the dispersed micelles have a diameter that is less than the wavelength of visible light (e.g., less than 380 nanometers, less than 200 nanometers, or less than 100 nanometers) in diameter. In the absence of opacifiers, microemulsions are optically clear, isotropic liquids.

As used herein, "reverse microemulsion" has its standard meaning and includes a microemulsion comprising a hydrophilic phase suspended in a continuous oil phase. A reverse microemulsion can include droplets of a hydrophilic phase (e.g., water, alcohol, or a mixture of both) stabilized in an oil phase by a reverse emulsion surfactant. In such instances, a hydrophilic active agent can be solubilized in the droplets. However, in other instances, the reverse microemulsion can be free of water and/or alcohol, and the hydrophilic active agent can be directly solubilized in the oil phase by the reverse emulsion surfactant.

As used herein, "hydrophilic" has its standard meaning and includes compounds that have an affinity to water and can be ionic or neutral or have polar groups in their structure that attract water. For example, hydrophilic compounds can be miscible, swellable or soluble in water.

As used herein, "aqueous" compositions refer to a spectrum of water-based solutions including, but not limited to, homogeneous solutions in water with solubilized components, emulsified solutions in water stabilized by surfactants or hydrophilic polymers, and viscous or gelled homogeneous or emulsified solutions in water.

As used herein, an enzyme is "soluble" or "solubilized" if the amount of enzyme present in the solvent system is dissolved in the solvent system without the enzyme forming a precipitate or visible, swollen gel particles in solution.

As used herein, "non-stinging" means that the formulation does not cause a sharp, irritatingly, burning or smarting pain as a result of contact with a biological surface.

As used herein, "volatile" has its standard meaning, that is, it can evaporate rapidly at normal temperatures and pressures. For example, a solvent is volatile if one drop (0.05 mL) of the solvent will evaporate completely between 20-25° C. within 5 minutes, or within 4 minutes, or within 3 minutes, or within 2 minutes, or within 1 minute, or within 30 seconds, or within 15 seconds.

As used herein, an "antimicrobial agent" is defined as a substance that kills microorganisms or inhibits their growth or replication, while an anti-infective agent is defined as a substance that counteracts infection by killing infectious agents, such as microorganisms, or preventing them from spreading. Often, the two terms are used interchangeably. Antibiotics are considered those substances that were originally produced by a microorganism or synthesized with active properties that can kill or prevent the growth of another microorganism. The term antibiotic is commonly

used to refer to almost any prescribed drug that attempts to eliminate infection. Antimicrobial agents do not cause biocidal resistance such as can occur with antibiotics, wherein antibiotic resistance to a drug can occur. Antimicrobial agents have a broad spectrum of activity against bacteria, fungi, viruses, protozoa and prions. Examples of antimicrobial agents include biguanides, such as poly(hexamethylene biguanide hydrochloride) (PHMB), chlorhexidine and its salts, alexidine and its salts, povidoniodine, cadexomer iodine, silver sulfadiazine, nanocrystalline silver, ionic silver, honey, dilute bleaching agents such as sodium hypochlorite and hypochlorous acid, hydrogen peroxide, organic peroxides such as benzoyl peroxide, alcohols such as ethanol and isopropanol, anilides such as triclocarban, bisphenols such as triclosan, chlorine compounds such as chlorine dioxide and N-chloramines, and quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetyltrimethylammonium chloride, cetylpyridinium chloride, and alkyltrimethylammonium bromides, as well as miconazole, clotrimazole, ketoconazole, fluconazole, crystal violet, amphotericin B, tee tree oil, and the like. Biguanides, such as PHMB, are useful in the debridement compositions described herein.

A polymeric biguanide useful in the debridement compositions described herein is poly(hexamethylene biguanide), commercially available from Arch Chemicals, Inc., Smyrna, Ga. under the trademark Cosmocil™ CQ. Poly(hexamethylene biguanide) polymers are also referred to as poly(hexamethylene biguanide) (PHMB), poly(hexamethylene bisbiguanide) (PHMB), poly(hexamethylene guanide) (PHMB), poly(aminopropyl biguanide) (PAPB), poly[aminopropyl bis(biguanide)](PAPB), polyhexanide and poly(iminoimidocarbonyl)imino-hexamethylene hydrochloride; however, PHMB is the preferred abbreviation. PHMB is a broad spectrum antimicrobial and has been used in contact lens multipurpose solutions, wound rinsing solutions, wound dressings, perioperative cleansing products, mouthwashes, surface disinfectants, food disinfectants, veterinary applications, cosmetic preservatives, paper preservatives, secondary oil recovery disinfectants, industrial water treatments, and in swimming pool cleaners. It is normally obtained commercially in the hydrochloride form in water. Other antimicrobial polymers can also be added, such as polyquaternium 1, polyquaternium 6, polyquaternium 10, cationic guar, and water-soluble derivatives of chitosan.

As used herein, an "antibiotic" is a medicine or drug, usually prescribed, such as penicillin, streptomycin, chloramphenicol, and tetracycline, usually produced by various microorganisms, that inhibits the growth of or destroys microorganisms, used primarily in the treatment of infectious disease. Antibiotics are considered those substances that were originally produced by a microorganism or synthesized with related active properties that can kill or prevent the growth of another microorganism. The term antibiotic is commonly used to refer to almost any prescribed drug that attempts to eliminate infection.

As used herein, "antibiotic resistance" is the ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once susceptible.

The debridement composition described herein can include a biocidal monoalkyl glycol, glycerol alkyl ether, and monoacyl glycerol at a combined concentration of from 0.05 wt % (500 ppm) to 4 wt % (4,000 ppm), or from 0.1 wt % (1,000 ppm) to 1 wt % (10,000 ppm), or from 0.4 wt % (4,000 ppm) to 0.6 wt % (6,000 ppm) based on the weight

of the debridement composition. The monoalkyl glycol, glycerol alkyl ether, and monoacyl glycerol can be hydrophobic.

Examples of monoalkyl glycols useful in the debridement compositions described herein include, but are not limited to, 1,2-propanediol (propylene glycol), 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-heptanediol, 1,2-octanediol (caprylyl glycol), 1,2-nonanediol, 1,2-decanediol, 1,2-undecanediol, 1,2-dodecanediol, 1,2-tridecanediol, 1,2-tetradecanediol, 1,2-pentadecanediol, 1,2-hexadecanediol, 1,2-heptadecanediol and 1,2-octadecanediol. Non-vicinal glycols can also be added to enhance biocidal activity. Exemplary, non-vicinal glycols include, but are not limited to, 2-methyl-2,4-pentanediol, 1,3-butanediol, diethylene glycol, triethylene glycol, and glycol bis(hydroxyethyl) ether.

Examples of glycerol alkyl ethers useful in the debridement compositions described herein include, but are not limited to, 1-O-heptylglycerol, 1-O-octylglycerol, 1-O-nonylglycerol, 1-O-decylglycerol, 1-O-undecylglycerol, 1-O-dodecylglycerol, 1-O-tridecylglycerol, 1-O-tetradecylglycerol, 1-O-pentadecylglycerol, 1-O-hexadecylglycerol (chimyl alcohol), 1-O-heptadecylglycerol, 1-O-octadecylglycerol (batyl alcohol), 1-O-octadec-9-enyl glycerol (se-lachyl alcohol), glycerol 1-(2-ethylhexyl) ether (also known as octoxyglycerin, 2-ethylhexyl glycerin, 3-(2-ethylhexyloxy)propane-1,2-diol, and Sensiva® SC 50), glycerol 1-heptyl ether, glycerol 1-octyl ether, glycerol 1-decyl ether, and glycerol 1-dodecyl ether, glycerol 1-tridecyl ether, glycerol 1-tetradecyl ether, glycerol 1-pentadecyl ether, glycerol 1-hexadecyl ether and glycerol 1-octadecyl ether.

Examples of monoacyl glycerols useful in the debridement compositions described herein include, but are not limited to, 1-O-decanoylglycerol (monocaprin), 1-O-undecanoylglycerol, 1-O-undecenoylglycerol, 1-O-dodecanoylglycerol (monolaurin, also called glycerol monolaurate and Lauricidin®), 1-O-tridecanoylglycerol, 1-O-tetradecanoylglycerol (monomyristin), 1-O-pentadecanoylglycerol, 1-O-hexadecanoylglycerol, 1-O-heptadecanoylglycerol, and 1-O-octanoylglycerol (monocaprylin). In general, glycerols substituted in the 1-O-position are more preferred than those substituted in the 2-O-position, or disubstituted in the 1-O and 2-O positions.

As used herein, "hydrophobic" refers to repelling water, being insoluble or relatively insoluble in water, and lacking an affinity for water. Hydrophobic compounds with hydrophilic substituents, such as vicinal diols, may form emulsions in water, with or without added surfactant.

As used herein, "amphoteric" refers to a mixture of cationic and anionic charges on a molecule or polymer in which overall charge is locally pH dependent, whereas "ampholytic" has an equal number of cationic and anionic charges over a broad pH range.

As used herein, an "excipient" is a usually inert substance that forms a vehicle, such as a liquid, fluid, or gel, that solubilizes or disperses an enzyme or other added ingredients. The amylase compositions can include one or more additional surfactants to enhance necrotic tissue removal. Suitable surfactants include, but are not limited to, cationic, anionic, nonionic, amphoteric and ampholytic surfactants. In some embodiments, the surfactants are nonionic and amphoteric surfactants. In some embodiments, the surfactant can be present in an amount ranging from 0% to 10 wt % based on the weight of the debridement composition. In some embodiments, the surfactant can be present in an amount of at least 0.01 wt %, or at least 0.1 wt %, or at least 0.25 wt %, or at least 0.5 wt %, or at least 1 wt %, based on the weight of the debridement composition. The surfactants can

have an HLB (hydrophilic-lipophilic balance) value of 18-30 in order to maintain the enzymes' catalytic structure in solution as well as not hindering the biocidal activity of any added antimicrobial agents, while facilitating a non-cytotoxic solution. The high values of the HLB represent surfactants that are more hydrophilic than those with lower HLB values.

Suitable nonionic surfactants include, but are not limited to, the ethylene oxide/propylene oxide block copolymers of poloxamers, reverse poloxamers, poloxamines, and reverse poloxamines. Poloxamers and poloxamines are preferred, and poloxamers are most preferred. Poloxamers and poloxamines are available from BASF Corp. under the respective trade names of Pluronic® and Tetronic®. Suitable Pluronic surfactants comprise but are not limited to Pluronic F38 having a HLB of 31, Pluronic F68 having a HLB of 29, Pluronic 68LF having a HLB of 26, Pluronic F77 having a HLB of 25, Pluronic F87 having a HLB of 24, Pluronic F88 having a HLB of 28, Pluronic F98 having a HLB of 28, Pluronic F108 having a HLB of 27, Pluronic F127 (also known as Poloxamer 407) having a HLB of 18-23, and Pluronic L35 having a HLB of 19. An exemplary poloxamine surfactant of this type is Tetronic 1107 (also known as Poloxamine 1107) having an HLB of 24.

In addition to the above, other neutral surfactants may be added, such as for example polyethylene glycol esters of fatty acids, e.g., coconut, polysorbate, polyoxyethylene or polyoxypropylene ethers of higher alkanes (C₁₂-C₁₈), polysorbate 20 available under the trademark Tween 20, polyoxyethylene (23) lauryl ether available under the trademark Brij 35, polyoxyethylene (40) stearate available under the trademark Myrj 52, and polyoxyethylene (25) propylene glycol stearate available under the trademark Atlas G 2612, all available by Akzo Nobel, Chicago, Ill. Other neutral surfactants include nonylphenol ethoxylates such as nonylphenol ethoxylates, Triton X-100, Brij surfactants of polyoxyethylene vegetable-based fatty ethers, Tween 80, decyl glucoside, and lauryl glucoside.

Amphoteric surfactants suitable for use in antimicrobial compositions according to the present invention include materials of the type offered commercially under the trademark Miranol (Rhodia). Another useful class of amphoteric surfactants is exemplified by betaines, including cocoamidopropyl betaine, undecylenamidoalkylbetaine, and lauramidoalkylbetaine and sodium cocoamphoacetate. Amphoteric surfactants are very mild and have excellent dermatological properties, making them particularly suited for use in personal care applications.

The debridement composition may further comprise a chelating agent at a concentration of from 0.01 weight % to 1 weight %. For example, the chelating agent can be present in an amount of at least 0.01 wt %, or at least 0.03 wt %, or at least 0.05 wt %, or at least 0.1 wt %, or at least 0.50 wt %, or at least 0.75 wt %, or at least 1.0 wt %. The chelating agent can be selected from the group that includes, but is not limited to, ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid, nitrilotripropionic acid, diethylenetriaminepentaacetic acid, 2-hydroxyethylethylenediaminetriacetic acid, 1,6-diaminohexamethylenetetraacetic acid, 1,2-diaminocyclohexanetetraacetic acid, O,O'-bis(2-aminoethyl) ethyleneglycoltetraacetic acid, 1,3-diaminopropanetetraacetic acid, N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid, ethylenediamine-N,N'-diacetic acid, ethylenediamine-N,N'-dipropionic acid, triethylenetetraaminehexaacetic acid, ethylenediamine-N,N'-bis(methyl-enephosphonic acid), iminodiacetic acid, monosodium-N-lauryl-β-iminodipropionic acid (sodium

lauriminodipropionate, Deriphat®160C), N,N-bis(2-hydroxyethyl)glycine, 1,3-diamino-2-hydroxypropanetetraacetic acid, 1,2-diaminopropanetetraacetic acid, ethylenediaminetetrakis(methylenephosphonic acid), N-(2-hydroxyethyl)iminodiacetic acid, biphosphonates, 5 editronate, and salts thereof.

The debridement compositions can also contain chlorophyllin and its water-soluble derivatives in order to reduce local inflammation, promote healing, and control odor. It has been reported that in treating wounds that require debridement, some of the end products of proteolysis are mucoproteins, which often produce an irritating action and other deleterious actions on the tissue in contact with said end products. In order to control such undesirable effects, the incorporation of a water-soluble chlorophyll derivative has been incorporated into a proteolytic debridement formulation, generally in an amount of 0.05 to 1% by weight of the total composition, and preferably in an amount of 0.1% to 0.5% (U.S. Pat. No. 2,917,433). The water-soluble chlorophylls which may be used for the aforementioned purpose are exemplified by those disclosed in U.S. Pat. No. 2,120, 667, including especially sodium or potassium copper chlorophyllin, sodium or potassium magnesium chlorophyllin and sodium or potassium iron chlorophyllin. The preferred water-soluble chlorophyll for this purpose was a mixture of sodium and potassium copper chlorophyllin, predominantly the potassium salt. 15

Other applications of chlorophyllin in wound treatment are exemplified by International Patent Publication Number WO 2008/1063229, wherein a protease enzyme debridement composition is a hydrophilic ointment that additionally comprises a supplemental agent that minimizes damage to healthy normal cells from compounds released by dying cells, wherein the agent is optionally sodium copper chlorophyllin, wherein the range of the stoichiometric ratio of moles of chlorophyllin administered to moles of debridement enzyme administered is optionally 0.1 to 10.0, 0.3 to 3.0, or 1.0. 25

Sodium copper chlorophyllin is also a food coloring agent and is known as natural green 3. Its addition to a debridement product also adds coloration, which is helpful in visualizing the removal of the debridement composition. 40

If pain reduction is required during debridement, the formulation can further comprise analgesic agents, anesthetic agents, and neuropathic pain agents, such as lidocaine, capsaicin, benzocaine, tetracaine, prilocaine, bupivacaine, levobupivacaine, procaine, carbocaine, etidocaine, mepivacaine, nortriptylene, amitriptyline, pregabalin, diclofenac, fentanyl, gabapentin, non-steroidal anti-inflammatory agents, salicylates, combinations thereof, and the like. 45

Whereas α -amylases catalyze the hydrolysis of internal α -(1-4)-linkages of glucose polymers as their main reaction, some α -amylases, particularly saccharifying amylases, however, catalyze transfer reactions in addition to hydrolysis (U.S. Pat. No. 8,486,664; International Patent Application Number WO 2012/013646). These α -amylases are capable of transferring glycoside residues to low molecular weight alcohols as well as to water, a property related to the transferase activity of the glycosidases. It is not known whether such a transglycosylation process is operative in the debridement of necrotic tissue as related to this invention. 50

In some embodiments, a kit that includes a container containing debridement composition according to any of the variations described herein, and instructions for using the debridement composition for debridement of devitalized tissue is described. The instructions can include contacting the debridement composition with an area of skin in need of 55

debridement. The instructions can include repeating the contacting step at regular intervals. The regular intervals can be at least once a day, or at least twice a day (every 12 hours), or at least three times a day (every 8 hours). The instructions can include mixing and or diluting the debridement composition in a solvent or other carrier liquid. The instructions can include removal of the necrotic tissue treated debridement composition by wiping and by solvent rinsing.

A method of debridement of devitalized tissue is also described. The method can include contacting a debridement composition according to any of the variations described herein with an area of skin in need of debridement, such as by a powder, liquid, gel, hydrogel, foam, paste, spray, or film. In some embodiments, the debridement composition is applied to a wound dressing, such as gauze, cloth, fiber, alginate, hydrocolloid, composite, or film. In some embodiments, the wound dressing is composed of natural or synthetic components, or combinations thereof. The method can also include abrading and/or removing the debridement composition after a given period of time. The method can include repeating the contacting step at regular intervals. In some embodiments, the regular intervals can be at least once a day, or at least twice a day (every 12 hours), or at least three times a day (every 8 hours). In some embodiments, the method also includes removing debrided tissue from the area of skin. 60

It is an object of the compositions, kits, and methods described herein to provide for enzymatic debridement of necrotic debris and eschar.

It is an object of the compositions, kits, and methods described herein to provide for enzymatic debridement treatment of chronic wounds, acute wounds and burn wounds. 65

It is a further object of the compositions, kits, and methods described herein to provide enzymatic debridement of necrotic tissue not based predominantly on peptide cleavage/hydrolysis by proteases.

It is a further object of the compositions, kits, and methods described herein to provide enzymes for debridement that cleave carbohydrate α -1,4-glycosidic linkages in polysaccharides or glycosidic linkages with proteins.

It is a further object of the compositions, kits, and methods described herein to provide carbohydrate hydrolytic enzymes for enzymatic debridement based on the amylase family of enzymes.

It is a further object of the compositions, kits, and methods described herein to treat necrotic wounds comprising the step of administering an effective amount of an amylase enzyme. 70

It is a further object of the compositions, kits, and methods described herein to provide carbohydrate hydrolytic enzymes for enzymatic debridement based upon α -amylase.

It is a further object of the compositions, kits, and methods described herein to provide carbohydrate hydrolytic enzymes for enzymatic debridement based upon β -amylase.

It is a further object of the compositions, kits, and methods described herein to provide carbohydrate hydrolytic enzymes for enzymatic debridement based upon γ -amylase in combination with α -amylase and with β -amylase. 75

It is a further object of the compositions, kits, and methods described herein to provide carbohydrate hydrolytic enzymes for enzymatic debridement based upon the

amylase family, selected from combinations of α -amylase, with β -amylase, and γ -amylase.

It is a further object of the compositions, kits, and methods described herein to provide for enzymatic tissue debridement based predominantly on α -amylase, with a minimum (20 wt % or less) of other families of enzymes.

It is a further object of the compositions, kits, and methods described herein to provide for enzymatic tissue debridement wherein families of hydrolytic cleavage enzymes other than amylases include 20 wt % or less of proteases, chondroitinases, hyaluronidases, lipases, glycosidases, heparanases, dermatanases, pullulanases, N-acetylglucosaminidase, lactases, phospholipases, transglycosylases, esterases, thioester hydrolyases, sulfatases, escharases, nucleases, phosphatases, phosphodiesterases, mannanases, mannosidases, isoamylases, lyases, inulinases, keratinases, tannases, pentosanases, glucanases, arabinosidases, pectinases, cellulases, chitinases, xylanases, cutinases, pectate lyases, hemicellulases, combinations thereof, and the like.

It is a further object of the compositions, kits, and methods described herein to provide for enzymatic tissue debridement wherein families of enzymes other than amylases include 20 wt % or less of oxidases, peroxidases, glucose oxidases, catalases, oxidoreductases, phenoloxidases, laccases, lipoxxygenases, isomerases, ligninases, combinations thereof, and the like.

It is a further object of the compositions, kits, and methods described herein to provide for debridement based upon α -amylase, wherein the α -amylase is administered as a powder, gel, paste, liquid, ointment, foam, bandage, mesh or dressing.

It is a further object of the compositions, kits, and methods described herein to provide for debridement based upon α -amylase that is administered topically or subcutaneously.

It is a further object of the compositions, kits, and methods described herein to provide for debridement based upon α -amylase, wherein the α -amylase is applied in a hydrophilic or aqueous medium.

It is a further object of the compositions, kits, and methods described herein to provide a pleasing fragrance to the debridement compositions.

It is a further object of the compositions, kits, and methods described herein to apply chlorophyllin to a wound to reduce local inflammation, promote healing, and control odor.

It is a further object of the compositions, kits, and methods described herein to provide a wound dressing for amylase treatment comprised of gauze, mesh, cloth, fiber, foam or film.

It is a further object of the compositions, kits, and methods described herein to provide for preserved formulations of amylase.

It is a further object of the compositions, kits, and methods described herein to provide for antimicrobial formulations of amylase.

It is a further object of the compositions, kits, and methods described herein to provide for antimicrobial formulations of amylase that reduce or eliminate Gram positive and Gram negative bacteria in wounds and biological surfaces.

It is a further object of the compositions, kits, and methods described herein to provide for antimicrobial formulations of amylase that reduce or eliminate yeast in wounds and biological surfaces.

It is a further object of the compositions, kits, and methods described herein to provide for antimicrobial amy-

lase formulations that incorporate antimicrobial essential oils to augment the antimicrobial activity of the compositions.

It is a further object of the compositions, kits, and methods described herein to provide for debridement based upon α -amylase by a surfactant capable of solubilizing, swelling, or hydrating devitalized tissue or eschar.

It is a further object of the compositions, kits, and methods described herein to provide for α -amylase-based debridement a hydrophilic polymer capable of increasing viscosity or causing gelation of the formulation.

It is a further object of the compositions, kits, and methods described herein to provide amylase in kit form for treatment of wounds and necrotic tissue.

EXAMPLES

The following ingredients and their abbreviations are used in this invention:

Enzymes

α -Amylase #1, porcine pancreas, 30 U/mg, Sigma Aldrich, A3176-500KU, lot SLBF3831V.

α -Amylase #2, porcine pancreas, (contains 0.2% protease), 230 U/mg, Lee BioSolutions, lot M60404.

α -Amylase #3, porcine pancreas, (contains 0.05% protease), 210 U/mg, Lee BioSolutions, lot P70442.

α -Amylase #4, human saliva, 117.5 U/mg, Sigma Aldrich, lot SLBB8953V.

α -Amylase #5, *Bacillus licheniformis*, 500-1500 U/mg, Sigma Aldrich, lot SLBG8595V.

α -Amylase #6, *Bacillus subtilis* spp., powder, 7278 U/mg, Dyadic International, lot ADY4001.

α -Amylase #7, *Bacillus subtilis* spp., solution, 1269 U/mg, Dyadic International, lot ASP3001.

β -Amylase, barley, 41.6 U/mg, Sigma Aldrich, lot SLBC2932V.

γ -Amylase #1, *Aspergillus niger*, 129.2 U/mg, Sigma Aldrich, lot BCB1453V.

γ -Amylase #2, *Rhizopus* spp., MyBiosource Inc., lot 22200303.

Bromelain, pineapple stem, 3-7 U/mg, Sigma Aldrich, lot SLBG2202V.

Collagenase, Type I, *Clostridium histolyticum*, 125 U/mg, Sigma Aldrich, C0130-100UG, lot SLBH5757V.

Lipase #1, porcine pancreas, 30-90 U/mg, Sigma Aldrich, lot SLBH6427V.

Lipase #2, porcine pancreas, (contains <0.05% protease), 360 U/mg, Lee BioSolutions, 400-10, lot R24160.

Other Ingredients

AC, Antimicrobial Composition, Water, 95.5 wt %, PHMB 0.1 wt %, EDTA 0.065 wt %, P407 2 wt %, HPMC, 2 wt %, SC50, 0.3 wt %, SC10, 0.1 wt %, pH 5.5.

CHG, Chlorhexidine gluconate, Spectrum Chemicals, lot ZQ1023.

Collagen, type I, rat tail, Corning Inc., 3542368, lot 3298599.

DC 193, PEG-12 Dimethicone, Dow Corning, lot 0002250697.

Dulbecco's Phosphate Buffered Saline, DPBS, pH 7.1, Sigma Aldrich, D8537, lot RNBC1143.

EDTA, Ethylenediaminetetraacetic acid di-, tri-sodium salts, Spectrum Chemicals, lots 1AE0430, YL0044.

Glycerin, Quality Choice, lot 519675.

Hydroxypropylmethylcellulose (HPMC), Amerchol Corp., lot WF15012N01.

Mineral Oil, CVS, lot 5BF0201.

PEG 400, Poly(ethylene glycol), 400 M_w, Sigma-Aldrich, lot MKBD2642V.

Petrolatum, Vaseline, lot 02011HU00.

PHMB, Poly(hexamethylene biguanide hydrochloride), Cosmocil™ CO, Arch Chemical, lot 11RC116995.

P407, Poloxamer 407, Pluronic F127, Spectrum Chemicals, lot 1AD0265.

Polymer JR-30M, Amerchol, lot XL2850GRXA.

SC10, Sensiva® SC 10, 1,2-Dihydroxyoctane), Schülke & Mayr, lot 1178933.

SC50, Sensiva® SC 50, Glycerol 1-(2-ethylhexyl) ether), Schülke & Mayr, lot 1179743.

Sodium Hydroxide, Puritan 50% NaOH, UN1824, lot 011043.

Urea, Sigma Aldrich, lot SLBF4607V.

Water, Deionized, adjusted to pH 7.

Collagen Gel Digestion

In order to determine if an α -amylase contained a protease and thus was able to cleave a collagen gel (i.e., a protein-based gel), collagen gel digestion was studied by rheology under varied frequency conditions using α -amylase and collagenase as potential digesting enzymes. If α -amylase had no digestion of the collagen gel, its debridement activity of tissue would not be based upon any contamination by a protease, and thus not by hydrolysis of peptide bonds of collagen polymer chains.

Collagen gels were prepared at 2.0 mg/mL using collagen type I. Gels were prepared by mixing 500 μ L collagen (~4.1 mg/mL), 500 μ L Dulbecco's phosphate buffered saline (DPBS), and 10 μ L 1 N NaOH (diluted from Puritan 50% NaOH). Solid collagen hydrogels were formed after 30 min in a 37° C. incubator. Gels were incubated at 37° C. for 24 hours with the following enzymes:

2 mg (250 U) collagenase

8 mg (250 U) α -amylase #1

Rheological testing was conducted on an Anton Paar MCR 302 rheometer using a 25 mm parallel plate (for solid gels) and 25 mm cone and plate (for completely liquefied gels, i.e., collagen gel treated with collagenase). All frequency sweeps were conducted at 37° C. and 1% strain (linear viscoelastic region as determined by a strain amplitude sweep). The data are shown in FIG. 1, for the storage and loss moduli vs. frequency, and in FIG. 2, for the complex moduli for untreated collagen gel, for amylase treated collagen gel and collagenase treated collagen gel.

In FIG. 1, storage (G') and loss (G'') moduli versus frequency are presented for untreated collagen gel controls, collagen gels treated with 250 Units collagenase, and collagen gels treated with 250 Units α -amylase #1. The storage modulus represents the solid-like nature and the loss modulus represents the liquid-like nature of the viscoelastic collagen gel. There is no significant difference between storage and loss moduli between the untreated collagen gel and the gel treated with α -amylase #1, demonstrating no collagen (protein) gel digestion by α -amylase. The protease collagenase completely liquefied the collagen gel, which is demonstrated by the significantly lower storage and loss moduli.

In FIG. 2, complex modulus (G*) is plotted versus frequency for untreated collagen gel controls, collagen gels treated with 250 Units collagenase, and collagen gels treated with 250 Units α -amylase #1. Complex modulus accounts for the storage modulus (solid-like behavior) and loss modulus (liquid-like behavior), which correlates with the gel's stiffness. α -Amylase #1 does not digest the collagen gels compared to the untreated collagen gel control (no statistically significant difference in complex moduli).

These Figures demonstrate that collagenase, a protease, completely liquefied the collagen gels within 24 hours, whereas α -amylase #1, a protein enzyme noted for cleavage of α -linked polysaccharides, such as those in starch and glycogen, did not digest the collagen gel compared to the untreated collagen gel control (no statistically significant difference in storage moduli, loss moduli, or complex moduli). From these Figures, moduli are observed to increase with frequency due to the viscoelastic nature of the polymer (collagen) tested. At high frequencies, the collagen polymer chains do not have time to relax, resulting in an observed stiffer viscoelastic behavior. The data for collagen gel treated with collagenase appears noisy due to the liquid-like nature of the resulting digestion of the collagen gel. Collagenase completely degraded and liquefied the collagen gel, and the resulting enzymatically degraded solution had to be evaluated using cone and plate geometry on the rheometer. While the gel was completely liquefied, the resulting solution was still viscoelastic due to the nature of the collagen and peptides remaining in the solution.

The rheology data supports the debridement activity on devitalized tissue by α -amylase as not being dependent on contamination by a protease.

Method for Evaluating Digestion Efficacy of Various Enzymes ex vivo

In U.S. Pat. No. 8,119,124, an in vivo (ex vivo) burn wound model was reported using the skin of young swine because of its similarity to that of humans. In our investigation, several samples of frozen pig skin were obtained from Culebra Meat Market, San Antonio, Tex., and used as a debridement analog. For each digestion experiment, the pig skin was boiled in water for 1 minute, and then cut into small square pieces. The boiled pig skin was weighed immediately prior to the application of a debridement formulation. It was then incubated with 1 gram of each debridement formulation for 16 hours at 34° C. in water at pH 7, and wiped gently with a piece of paper towel to remove any digested tissue. The remaining tissue was then weighed. As a control, boiled pig skin was treated without active enzyme by the same procedure. The percent digestion of pig skin was calculated using the following formulation:

$$\% \text{ digested pig skin} = \frac{W_2 \text{ enz}}{W_1 \text{ enz} \cdot \frac{W_2 \text{ veh}}{W_1 \text{ veh}}} * 100\%$$

where

W₁ enz: weight of pig skin prior to digestion in a formulation that contains one or more active enzymes.

W₁ veh: weight of pig skin prior to digestion in the same formulation except without any active enzymes.

W₂ enz: weight of pig skin after digestion in a formulation that contains one or more active enzymes.

W₂ veh: weight of pig skin after digestion in the same formulation except without any active enzymes.

Digestion Results, Pig Skin

Amylase from different sources, or at different activity levels expressed in Units (U), showed different levels of ex vivo debridement activity (Table 1). α -Amylase from porcine pancreas from both Sigma Aldrich (α -amylase #1) and Lee BioSolutions (α -amylase #2, α -amylase #3) showed high debridement efficacy, where 1 gram of formulation contains 250 U of enzyme. Surprisingly, the α -amylase (Lee BioSolutions, 0.2% protease) that contained a higher amount of protease impurity (α -amylase #2) had a slightly lower

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debridement efficacy than the α -amylase that contained a lower amount of protease impurity (Lee BioSolutions, 0.05% protease) (α -amylase #3), further supporting the conclusion that amylase debridement activity is not a result of protease contamination. α -Amylase from human saliva (α -amylase #4) was ineffective in digesting pig skin at the concentration studied. The activity of α -amylase derived from bacteria is highly variable depending on the bacterial strain and the vendor. The α -amylase from *Bacillus licheniformis* (α -amylase #5) showed very weak debridement efficacy, whereas the α -amylase from *Bacillus subtilis* spp. showed high debridement efficacy (α -amylase #6, α -amylase #7), particularly at an activity of 250,000 Units of enzyme in 1 gram of formulation. β -Amylase showed some activity in digesting pig skin based upon its ability to act upon the same substrates as α -amylase but with a different catalytic mechanism, whereas γ -amylase #1 and #2 were ineffective in this debridement model.

TABLE 1

Ex vivo debridement efficacy of amylases from various sources on boiled pig skin in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #1	99.2/0.8	1 g/250U	96.30%
H ₂ O/ α -amylase #2	99.891/0.109	1 g/250U	96.60%
H ₂ O/ α -amylase #3	99.88/0.12	1 g/250U	97.90%
H ₂ O/ α -amylase #4	99.8/0.2	1 g/250U	4.30%
H ₂ O/ α -amylase #5	99.95 g/0.05	1 g/250U	0%
H ₂ O/ α -amylase #5	99.9/0.1	1 g/500U	13.50%
H ₂ O/ α -amylase #6	99.9966/0.0033	1 g/250U	17.50%
H ₂ O/ α -amylase #6	99.966/0.034	1 g/2,500U	70.80%
H ₂ O/ α -amylase #6	96.6/3.4	1 g/250,000U	100%
H ₂ O/ α -amylase #7	99.9803/0.0197	1 g/250U	15.00%
H ₂ O/ α -amylase #7	99.803/0.197	1 g/2,500U	77.60%
H ₂ O/ α -amylase #7	80.3/19.7	1 g/250,000U	100%
H ₂ O/ β -amylase	99.4/0.6	1 g/250U	18.60%
H ₂ O/ β -amylase	94/6	1 g/2500U	38.90%
H ₂ O/ γ -amylase #1	99.8/0.2	1 g/250U	0%
H ₂ O/ γ -amylase #1	99/1	1 g/1,250U	0%
H ₂ O/ γ -amylase #1	98/2	1 g/2,500U	0%
H ₂ O/ γ -amylase #2	99.4/0.6	1 g/250U	0%

In Table 2 are listed the effects of various additives and excipients on the debridement efficacy of α -amylase #1 with boiled pig skin. Included is a DPBS buffered solution, a formulation in mineral oil, a formulation of a hydrophilic, a viscosity increasing polymer, HPMC, in DPBS, and a formulation with a viscosity increasing cationic polymer, Polymer JR (cationic hydroxyethylcellulose). The results are compared to α -amylase #1 in water at pH 7. Each formulation contained the same amount of α -amylase #1, namely 250 Units. It is seen that the formulation consistency affected the amylase debridement efficacy. The addition of DPBS buffer appeared to enhance the debridement efficacy compared to α -amylase in water, whereas mineral oil greatly decreased the debridement efficacy, presumably because of the lack of water for enzymatic solubility and activity; the added water-soluble polymers of neutral cellulose-based HPMC in DPBS and cationic cellulose-based Polymer JR in water somewhat decreased the debridement efficacy, possibly because of a lower content of water in their formulations compared to α -amylase #1 by itself in water.

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TABLE 2

Ex vivo debridement efficacy of α -amylase #1 in various formulations on boiled pig skin			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #1	99.2/0.8	1 g/250U	96.30%
DPBS/ α -amylase #1	99.2/0.8	1 g/250U	100%
Mineral oil/ α -amylase #1	99.2/0.8	1 g/250U	62.90%
HPMC/DPBS/ α -amylase #1	5/94.2 g/0.8	0.05 g/0.95 g/250U	80.10%
Polymer JR-30M/H ₂ O/ α -amylase #1	5/94.2 g/0.8	0.05 g/0.95 g/250U	84.30%

In Table 3 are described combinations of α -amylase and β -amylase using bacterial α -amylase from *Bacillus subtilis* (α -amylase #6) in conjunction with plant derived β -amylase. In this model, the greater amount of β -amylase appeared to hinder the overall digestion of the boiled pig skin.

TABLE 3

Ex vivo debridement efficacy of boiled pig skin by α -amylase #6 with β -amylase in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #6	99.66/0.34	1 g/250U	79.20%
H ₂ O/ α -amylase #6/ β -amylase	96.6/0.34/3.16	1 g/250U/131U	53.70%
H ₂ O/ α -amylase #6/ β -amylase	99.32/0.34/0.34	1 g/250U/14U	72.30%

In Table 4, the addition of another non-proteolytic hydrolytic enzyme, lipase, to α -amylase #3 on the digestion of boiled pig skin is given, using amylase contents of 100 wt %, 90 wt %, and 80 wt % to lipase. Lipase is noted for its ability to catalyze the hydrolysis of ester bonds in lipids (triglycerides, fats, and oils). There are multiple sources of lipase as it is produced by the pancreas, liver, intestine, tongue, stomach, and many other cells, as well as the seeds of plants. The addition of lipase to α -amylase could be a major adjunct in debridement wherein fatty tissue is involved. In the pig model studied, the % boiled pig skin digested was relatively constant for the three samples studied, using a total enzyme concentration of 0.2 wt %, even though the amount of amylase #3 decreased with increasing lipase content, indicating that both non-proteolytic enzymes contributed to the digestion of boiled pig skin.

TABLE 4

Ex vivo debridement efficacy of boiled pig skin by α -amylase #3 with lipase #2 in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #3	99.8/0.2	1 g/420U	83.90%
H ₂ O/ α -amylase #3/lipase #2	99.8/0.18/0.02	1 g/378U/72U	84.10%
H ₂ O/ α -amylase #3/lipase #2	99.8/0.16/0.04	1 g/336U/144U	81.20%

In Table 5 are given the results of pig skin digested by α -amylase #1 in water with the keratolytic agent urea, with chlorophyllin for reducing local inflammation, promoting healing, and controlling odor, and with a combination of urea and chlorophyllin. All solutions with chlorophyllin were green colored. It is seen that a different source of pig skin, compared to that of Table 1, done under similar conditions, had a reduced digestion level. The addition of urea appeared to increase the digestion efficacy by α -amy-

lase, while the addition of two concentrations of chlorophyllin appeared to decrease the amylase efficacy. A combination of the three ingredients gave a digestion efficacy greater than chlorophyllin and α -amylase, and less than that of urea and amylase, or α -amylase by itself.

TABLE 5

Ex vivo debridement efficacy of boiled pig skin by α -amylase #1 with urea and chlorophyllin in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #1	99.2/0.8	1 g/250U	78.60%
H ₂ O/urea/ α -amylase #1	89.2/10/0.8	1 g/250U	84.90%
H ₂ O/chlorophyllin/ α -amylase #1	98.7/0.5/0.8	1 g/250U	54.80%
H ₂ O/chlorophyllin/ α -amylase #1	98.2/1/0.8	1 g/250U	59.30%
H ₂ O/urea/chlorophyllin/ α -amylase #1	88.7/10/0.5/0.8	1 g/250U	67.20%

Necrotic tissue is susceptible to bacterial infection, which further impedes wound healing, and may induce sepsis in severe cases. Because of the possibility of infection in necrotic wounds and damaged and irritated tissue, addition of a biological agent that hinders or eradicates microorganisms is desired. In this regard, we investigated two antimicrobial biguanides used in wound care, poly(hexamethylene biguanide hydrochloride) (PHMB) and chlorhexidine digluconate (CHG). Table 6 is a comparison of three formulations of aqueous solutions of PHMB with α -amylase #6 and CHG with α -amylase #6, all at the same wt % of α -amylase #6. For the PHMB-based solutions, these activities were analogous or slightly greater than that of α -amylase by itself, although the higher PHMB level of 0.15 wt % (1500 ppm) may have slightly decreased the amylase activity. The use of CHG, however, appeared to decrease markedly the amylase digestion ability.

TABLE 6

Ex vivo debridement of pig skin by α -amylase #6 with PHMB and CHG in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #6	99.66/0.34	1 g/25,000U	83.90%
H ₂ O/PHMB/ α -amylase #6	99.51/0.15/0.34	1 g/25,000U	81.30%
H ₂ O/PHMB/ α -amylase #6	99.56/0.1/0.34	1 g/25,000U	84.90%
H ₂ O/PHMB/ α -amylase #6	99.61/0.05/0.34	1 g/25,000U	86.80%
H ₂ O/CHG/ α -amylase #6	97.66/2/0.34	1 g/25,000U	60.50%

Digestion Results, Rat Skin

Freshly excised rat skin tissue was obtained from two adult rats 9 and 11 months of age from the Laboratory Animal Resources Center at the University of Texas at San Antonio, San Antonio, Tex., under an IACUC approved protocol. A large piece of skin was retrieved from the back of each male Sprague-Dawley rat. Half of the skin was cut and boiled in water for 60 seconds. Both the boiled and unboiled halves of the skin were cut into smaller pieces and trimmed so that each small piece weighed from 0.23 g to 0.25 g. One small piece of skin was soaked in 1 gram of each debridement formulation and incubated at 34° C. to debride for 24 hours. After the 24-hour incubation, the skin was removed from the debridement formulation. The debrided skin (soft and mushy tissue) was gently wiped with paper towels, and the remaining non-debrided skin was weighed. The percent of debrided skin was then calculated based on the weight before and after the debridement procedure.

Tables 7 and 8 present the debridement results of freshly excised rat skin from that which was debrided under unboiled and boiled conditions, respectively, using the amylolytic enzymes α -amylase #1 and α -amylase #6 as well as the protease enzymes of bromelain and collagenase, all in water at pH 7. For the unboiled rat skin (Table 7), it is seen that the two protease enzymes were more effective than either amylase, with collagenase being the most effective in digestion of rat skin at 79%. However, for boiled rat skin (Table 8), which is more analogous to devitalized, necrotic tissue, the amylolytic enzymes were comparable to the proteolytic enzymes in their ability to digest rat skin.

TABLE 7

Ex vivo debridement of unboiled rat skin by α -amylase, bromelain, and collagenase in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #1	99.2/0.8	1 g/250U	31.80%
H ₂ O/ α -amylase #6	99.66/0.34	1 g/25,000U	29.80%
H ₂ O/bromelain	90/10	1 g/250U	51.90%
H ₂ O/collagenase	99.8/0.2	1 g/250U	79%

TABLE 8

Ex vivo debridement of boiled rat skin by α -amylase, bromelain, and collagenase in water			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
H ₂ O/ α -amylase #1	99.2/0.8	1 g/250U	86.80%
H ₂ O/ α -amylase #6	99.66/0.34	1 g/25,000U	90.40%
H ₂ O/bromelain	90/10	1 g/250U	92.90%
H ₂ O/collagenase	99.8/0.2	1 g/250U	86%

If necrotic tissue is infected with a microbial biofilm, potentially further covering the necrotic tissue with additional polysaccharides (a component of an extracellular polymeric substance, sometimes referred to as slime) emanating from the biofilm microorganisms, which may be potentially bacterial in origin, removal or reduction of the biofilm may expedite the removal of necrotic or devitalized tissue. Tables 9 and 10 give the results of using an aqueous antimicrobial composition (AC) at pH 5.5 that has demonstrated efficacy in biofilm reduction (U.S. Pat. No. 8,829, 053). The antimicrobial composition contains a polymeric biguanide antimicrobial agent of PHMB, EDTA as a chelating and pH stabilizing agent, HPMC as a viscosity increasing polymer, a neutral surfactant of P407 for cleansing, and a combination of 2-ethylhexyl glycerin and 1,2-octanediol for emollient and antimicrobial properties. With the rat skin in an unboiled condition in the antimicrobial composition (Table 9), neither α -amylase #1 nor α -amylase #6 was as effective in digestion relative to that displayed by the same enzymes for unboiled rat skin in water (Table 7). Although bromelain in the antimicrobial composition was more effective than either amylase with the unboiled rat skin (Table 9), its digestion effect is less than that demonstrated for the digestion of the rat skin in water (Table 7). While collagenase was very effective for both unboiled and boiled rat skin digestion in water (Tables 7 and 8), for the unboiled rat skin in the presence of the aqueous antimicrobial composition, its effect in digestion was negligible (Table 9). This effect also occurred for collagenase with the boiled rat skin in the antimicrobial composition (Table 10), while α -amylase #1,

α -amylase #6, and bromelain were highly effective (Table 10). Since the antimicrobial composition contains the metal ion chelating agent EDTA, this may have inactivated collagenase, which is a metalloproteinase (zinc endopeptidase). The calcium complex of amylase appears not to be affected by EDTA chelation, and the antimicrobial compositions of α -amylase would be an effective non-proteolytic treatment of necrotic tissue infected with microbial biofilm. The low debridement efficacy of α -amylase #1 and α -amylase #6 on unboiled, freshly excised rat skin in the antimicrobial composition (Table 9) also supports the amylolytic debridement composition being highly specific to devitalized tissue and not toward the surrounding living tissue.

TABLE 9

Ex vivo debridement of unboiled rat skin by α -amylase, bromelain, and collagenase in an antimicrobial composition			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
AC/ α -amylase #1	99.2/0.8	1 g/250U	6.00%
AC/ α -amylase #6	99.66/0.34	1 g/250U	0%
AC/Bromelain	90/10	1 g/250U	18.20%
AC/collagenase	99.8/0.2	1 g/250U	4.70%

TABLE 10

Ex vivo debridement of boiled rat skin by α -amylase, bromelain, and collagenase in an antimicrobial composition			
formulation	formulation ratio (wt %)	formulation (Units)	% digested
AC/ α -amylase #1	99.2/0.8	1 g/250U	96.50%
AC/ α -amylase #6	99.66/0.34	1 g/250U	100%
AC/bromelain	90/10	1 g/250U	100%
AC/collagenase	99.8/0.2	1 g/250U	0%

Excipients

In addition to water and buffered solutions (Table 2), as well as being utilized in powder form, the amylases can be mixed with several excipients, including hydrophobic hydrocarbons of petrolatum and mineral oil, hydrophilic —OH containing alcohols of glycerin and PEG 400, and the amphiphilic liquid PEG-12 Dimethicone, a silicone polyether. In each case, α -amylase #1 could be dispersed in the excipient (Table 11). Other excipients could include various water-based buffers ranging in pH from 5.0-7.5, surfactants, silicones, polyether copolymers, polyoxyethylene ethers, vegetable and plant fats and oils, essential oils, hydrophilic and hydrophobic alcohols, vitamins, monoglycerides, laurate esters, myristate esters, palmitate esters, and stearate esters, preferably in liquid, gel, or paste form, combinations thereof, and the like. In some embodiments, excipients can be present in an amount ranging from 0% to 99.9 wt % based on the weight of the debridement composition.

TABLE 11

Excipients for α -amylase #1		
formulation	formulation ratio (wt %)	formulation (Units)
petrolatum/ α -amylase #1	99.2/0.8	1 g/250U
mineral oil/ α -amylase #1	99.2/0.8	1 g/250U
glycerin/ α -amylase #1	99.2/0.8	1 g/250U

TABLE 11-continued

Excipients for α -amylase #1		
formulation	formulation ratio (wt %)	formulation (Units)
PEG 400/ α -amylase #1	99.2/0.8	1 g/250U
DC 193/ α -amylase #1	99.2/0.8	1 g/250U

Effective α -Amylase Concentration

To determine the amount of α -amylase needed for debridement of necrotic tissue, a study was conducted to measure debridement efficacy (e.g., wt. % digested) versus α -amylase concentration. The results in FIG. 3 shows the concentration of α -amylase #6 versus the percent by weight of boiled pig skin digested in one hour at room temperature. The bell-shaped curve shows that debridement activity occurs at a maximum amylase concentration in the range of between 13.6 to 27.2 wt %, though the efficacy is 10 wt % or greater over the entire range from 3.4% to 54.5% α -amylase.

While the above specification contains many specifics, these should not be construed as limitations on the scope of the invention, but rather as examples of preferred embodiments thereof. Many other variations are possible. Accordingly, the scope of the invention should be determined not by the embodiments illustrated, but by the appended claims and their legal equivalents.

The invention claimed is:

1. A tissue debridement composition comprising:

0.1 to 99.5 wt % of an amylase component and optionally a proteolytic enzyme component, wherein a ratio of the amylase component to the proteolytic enzyme component is at least 10:1 by weight; and

at least one pharmaceutically or cosmetically acceptable carrier or excipient in an amount of up to 99.9 wt %, wherein the percentages are based on a total weight of the debridement composition,

wherein said amylase component comprises at least 80% by weight of α -amylase from a source selected from a group consisting of porcine pancreas and *Bacillus subtilis*, and wherein the debridement composition is able to debride devitalized or necrotic tissue.

2. The composition according to claim 1, wherein said amylase component comprises up to 20% by weight of an amylase selected from a group consisting of β -amylase, γ -amylase, and combinations thereof.

3. The composition according to claim 1, further comprising up to 20% by weight of hydrolytic enzymes selected from a group consisting of proteases, chondroitinases, hyaluronidases, lipases, glycosidases, heparanases, dermatanases, pullulanases, N-acetylglucosaminidase, lactases, phospholipases, transglycosylases, esterases, thioester hydrolyases, sulfatases, escharases, nucleases, phosphatases, phosphodiesterases, mannanases, mannosidases, isoamylases, lyases, inulinases, keratinases, tannases, pentosanases, glucanases, arabinosidases, pectinases, cellulases, chitinases, xylanases, cutinases, pectate lyases, hemicellulases, and combinations thereof, wherein the percentage is based on the total weight of the amylase component and the hydrolytic enzymes.

4. The composition according to claim 1, further comprising up to 20% by weight of other enzymes selected from a group consisting of oxidases, peroxidases, glucose oxidases, catalases, oxidoreductases, phenoloxidases, laccases,

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lipoxigenases, isomerases, and ligninases, wherein the percentage is based on the total weight of the amylase component and the other enzymes.

5. The composition according to claim 1, further comprising at least one polymeric biguanide in an amount ranging from 0.01 weight percent to 1.0 weight percent based on the weight of the debridement composition.

6. The composition according to claim 5, wherein said polymeric biguanide comprises poly(hexamethylene biguanide) and its salts.

7. The composition according to claim 1, further comprising a water-soluble polymer at a concentration from 0.01 weight % to 50 weight % based on the weight of the debridement composition.

8. The composition according to claim 1, further comprising a chelating agent at a concentration from 0.01 weight % to 1 weight % based on a weight of the debridement composition.

9. The composition according to claim 1, further comprising a monoalkyl glycol selected from a group consisting of 1,2-propanediol (propylene glycol), 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-heptanediol, 1,2-octanediol (caprylyl glycol), 1,2-nonanediol, 1,2-decanediol, 1,2-undecanediol, 1,2-dodecanediol, 1,2-tridecanediol, 1,2-tetradecanediol, 1,2-pentadecanediol, 1,2-hexadecanediol, 1,2-heptadecanediol, 1,2-octadecanediol, 2-methyl-2,4-pentanediol, 1,3-butanediol, diethylene glycol, triethylene glycol, glycol bis(hydroxyethyl) ether, and combinations thereof.

10. The composition according to claim 1, further comprising a glycerol alkyl ether selected from a group consisting of 1-O-heptylglycerol, 1-O-octylglycerol, 1-O-nonylglycerol, 1-O-decylglycerol, 1-O-undecylglycerol, 1-O-dodecylglycerol, 1-O-tridecylglycerol, 1-O-tetradecylglycerol, 1-O-pentadecylglycerol, 1-O-hexadecylglycerol (chimyol alcohol), 1-O-heptadecylglycerol, 1-O-octadecylglycerol (batyl alcohol), 1-O-octadec-9-enyl glycerol (selachyl alcohol), glycerol 1-(2-ethylhexyl) ether, 2-ethylhexyl glycerin, glycerol 1-heptyl ether, glycerol 1-octyl ether, glycerol 1-decyl ether, and glycerol 1-dodecyl ether, glycerol 1-tridecyl ether, glycerol 1-tetradecyl ether, glycerol 1-pentadecyl ether, glycerol 1-hexadecyl ether, glycerol 1-octadecyl ether, and combinations thereof.

11. The composition according to claim 1, further comprising

at least one polymeric biguanide in an amount ranging from 0.01 weight % to 1.0 weight % based on the weight of the debridement composition,

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a vicinal diol component selected from a group consisting of a monoalkyl glycol and a monoalkyl glycerol at a concentration of 0.05 weight % to 4 weight %, based on a weight of the debridement composition.

12. The composition according to claim 1, further comprising at least one analgesic agent, anesthetic agent, neuropathic pain agent, or a combination thereof.

13. The composition according to claim 12, wherein the at least one analgesic agent, anesthetic agent, or neuropathic pain agent is selected from a group consisting of lidocaine, capsaicin, benzocaine, tetracaine, prilocaine, bupivacaine, levobupivacaine, procaine, carbocaine, etidocaine, mepivacaine, nortriptylene, amitriptyline, pregabalin, diclofenac, fentanyl, gabapentin, non-steroidal anti-inflammatory agents, salicylates, and combinations thereof.

14. The composition according to claim 1, wherein the debridement composition has a form selected from a group consisting of a powder, aqueous solution, organic liquid solution, silicone, gel, cream, film, latex, aerosol, slurry, paste, ointment and foam.

15. The composition according to claim 1, wherein said debridement composition is adsorbed on or in a dressing or a natural or synthetic fiber, mesh, hydrocolloid, alginate, hydrogel, semipermeable film, permeable film, or a natural or synthetic polymer.

16. The composition according to claim 1, further comprising a buffering agent.

17. A kit comprising:

a tissue debridement composition according to claim 1; and instructions for debridement of devitalized tissue.

18. The kit according to claim 17, wherein said instructions comprise contacting the tissue debridement composition with an area of skin in need of debridement.

19. A method of debridement of devitalized tissue comprising contacting a tissue debridement composition according to claim 1 with an area of skin in need of debridement.

20. The method of claim 19, wherein said contacting step is repeated at least once a day.

21. The method of claim 19, further comprising removing debrided tissue from said area of skin.

22. The composition according to claim 1, wherein the amylase component is present in an amount ranging from 2 to 99.5 wt-% based on the total weight of the debridement composition.

* * * * *

TABLA DE CORRESPONDENCIA ENTRE LAS REIVINDICACIONES CONCEDIDAS POR LA USPTO Y LAS REIVINDICACIONES PENDIENTES ANTE LA SIC

REIVINDICACIONES CONCEDIDAS USPTO	REIVINDICACIONES PENDIENTES SIC	CORRESPONDENCIA
1. Una composición de desbridación de tejido que comprende: 0.1 a 99.5% en peso de un componente de amilasa, y opcionalmente un componente de enzima proteolítica, en donde, una proporción de componente de amilasa al componente de enzima proteolítica es al menos 10:1 en peso; y al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente en una cantidad de hasta el 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación, en donde dicho componente de amilasa comprende al menos 80% en peso de α-amilasa de una fuente seleccionada de un grupo que consiste de páncreas porcino y <i>Bacillus subtilis</i>, y en donde la composición de desbridación es capaz de debridar tejido desvitalizado o necrótico.	1. Una composición de desbridación de tejido que comprende: 0.1 a 99.5% en peso de un componente de amilasa, y opcionalmente un componente de enzima proteolítica, en donde, una proporción de componente de amilasa al componente de enzima proteolítica es al menos 10:1 en peso; y al menos un transportador o excipiente aceptable farmacéuticamente o cosméticamente en una cantidad de hasta el 99.9% en peso, en donde los porcentajes están basados en un peso total de la composición de desbridación, en donde dicho componente de amilasa comprende al menos 80% en peso de α-amilasa de una fuente seleccionada de un grupo que consiste de páncreas porcino y <i>Bacillus subtilis</i>, y en donde la composición de desbridación es capaz de debridar tejido desvitalizado o necrótico.	Las reivindicaciones actualmente pendientes ante la SIC tienen un alcance igual a las concedidas por la USPTO.
2. La composición de acuerdo con la reivindicación 1, en donde dicha amilasa comprende hasta 20% en peso de una amilasa seleccionada de un grupo que consiste de β-amilasa, γ-amilasa y combinaciones de las mismas.	2. La composición de acuerdo con la reivindicación 1, en donde dicha amilasa comprende hasta 20% en peso de una amilasa seleccionada de un grupo que consiste de β-amilasa, γ-amilasa y combinaciones de las mismas.	

3.	La composición de acuerdo con la reivindicación 1, que además comprende hasta 20% en peso de enzimas hidrolíticas seleccionadas de un grupo que consiste de proteasas, condroitinasas, hialuronidasas, lipasas, glucosidasas, heparanasas, dermatanasas, pululanasa, N-acetilglucosaminidasa, lactasas, fosfolipasas, transglucosilasas, ésterasas, tioéster-hidrolisas, sulfatasas, escarasas, nucleasas, fosfatasas, fosfodiésterasas, mananasas, manosidasas, isoamilasas, liasas, inulinasas, queratinasas, tanasas, pentosanasas, glucanasas, arabinosidasas, pectinasas, celulasas, quitinasas, xilanasas, cutinasas, pectato liasas, hemicelulasas y combinaciones de estas, en donde el porcentaje se basa en el peso total del componente de amilasa y las enzimas hidrolíticas.	3.	La composición de acuerdo con la reivindicación 1, que además comprende hasta 20% en peso de enzimas hidrolíticas seleccionadas de un grupo que consiste de proteasas, condroitinasas, hialuronidasas, lipasas, glucosidasas, heparanasas, dermatanasas, pululanasa, N-acetilglucosaminidasa, lactasas, fosfolipasas, transglucosilasas, ésterasas, tioéster-hidrolisas, sulfatasas, escarasas, nucleasas, fosfatasas, fosfodiésterasas, mananasas, manosidasas, isoamilasas, liasas, inulinasas, queratinasas, tanasas, pentosanasas, glucanasas, arabinosidasas, pectinasas, celulasas, quitinasas, xilanasas, cutinasas, pectato liasas, hemicelulasas y combinaciones de estas, en donde el porcentaje se basa en el peso total del componente de amilasa y las enzimas hidrolíticas.	
4.	La composición de acuerdo con la reivindicación 1, que además comprende hasta el 20% en peso de otras enzimas seleccionadas de un grupo que consiste de oxidasas, peroxidasas, glucosa oxidasas, catalasas, oxidorreductasas, fenoloxidasas, laccasas, lipoxigenasas, isomerasas y ligninasas, en donde el porcentaje está basado en el peso total del componente de amilasa y las otras enzimas.	4.	La composición de acuerdo con la reivindicación 1, que además comprende hasta el 20% en peso de otras enzimas seleccionadas de un grupo que consiste de oxidasas, peroxidasas, glucosa oxidasas, catalasas, oxidorreductasas, fenoloxidasas, laccasas, lipoxigenasas, isomerasas y ligninasas, en donde el porcentaje está basado en el peso total del componente de amilasa y las otras enzimas.	
5.	La composición de acuerdo con la reivindicación 1, que además comprende por lo menos una biguanida polimérica en una cantidad en el rango de 0,01 por ciento en peso hasta 1,0 por ciento en peso basado en el peso de la composición de desbridación.	5.	La composición de acuerdo con la reivindicación 1, que además comprende por lo menos una biguanida polimérica en una cantidad en el rango de 0,01 por ciento en peso hasta 1,0 por ciento en peso basado en el peso de la composición de desbridación.	

6.	La composición de acuerdo con la reivindicación 5, en la que dicha biguanida polimérica comprende poli (hexametileno biguanida) y sus sales.	6.	La composición de acuerdo con la reivindicación 5, en la que dicha biguanida polimérica comprende poli (hexametileno biguanida) y sus sales.	
7.	La composición de acuerdo con la reivindicación 1, que además comprende un polímero soluble en agua en una concentración de 0.01% en peso a 50% en peso basado en el peso de la composición de desbridación.	7.	La composición de acuerdo con la reivindicación 1, que además comprende un polímero soluble en agua en una concentración de 0.01% en peso a 50% en peso basado en el peso de la composición de desbridación.	
8.	La composición de acuerdo con la reivindicación 1, que además comprende un agente quelante en una concentración de 0.01% en peso a 1% en peso basado en un peso de la composición de desbridación.	8.	La composición de acuerdo con la reivindicación 1, que además comprende un agente quelante en una concentración de 0.01% en peso a 1% en peso basado en un peso de la composición de desbridación.	
9.	La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un monoalquilglicol seleccionado del grupo que consiste de 1,2-propanediol (propilen glicol), 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-heptanediol, 1,2-octanediol (caprilil glicol), 1,2-nonanediol, 1,2-decanediol, 1,2-undecanediol, 1,2-dodecanediol, 1,2-tridecanediol, 1,2-tetradecanediol, 1,2-pentadecanediol, 1,2-hexadecanediol, 1,2-heptadecanediol, 1,2-octadecanediol, 2-metil-2,4-pentanediol, 1,3- butanediol, dietilene glicol, trietileno glicol, glicol bis(hidroxietil) éter y combinaciones de los mismos.	9.	La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un monoalquilglicol seleccionado del grupo que consiste de 1,2-propanediol (propilen glicol), 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol, 1,2-heptanediol, 1,2-octanediol (caprilil glicol), 1,2-nonanediol, 1,2-decanediol, 1,2-undecanediol, 1,2-dodecanediol, 1,2-tridecanediol, 1,2-tetradecanediol, 1,2-pentadecanediol, 1,2-hexadecanediol, 1,2-heptadecanediol, 1,2-octadecanediol, 2-metil-2,4-pentanediol, 1,3- butanediol, dietilene glicol, trietileno glicol, glicol bis(hidroxietil) éter y combinaciones de los mismos.	

10. La composición de acuerdo la reivindicación 1, que además comprende un alquil éter de glicerol seleccionado del grupo que consiste de 1-O-heptilglicerol, 1-O-octilg1icerol, 1-O-nonilglicerol, 1-O-decilglicerol, 1-O-undecilglicerol, 1-O-dodecilglicerol, 1-O-tridecilglicerol, 1-O-tetradecilglicerol, 1-O-pentadecilglicerol, 1-O-hexadecilglicerol (quimil alcohol), 1-O-heptadecilglicerol, 1-O-octadecilglicerol (batil alcohol), 1-O-octadec-9-enil glicerol (selaquil alcohol), glicerol 1-(2-etilhexil) éter, 2-etilhexil glicerol, glicerol 1-heptil éter, glicerol 1-octil éter, glicerol 1-decil éter, and glicerol 1 -dodecil éter, glicerol 1-tridecil éter, glicerol 1-tetradecil éter, glicerol 1-pentadecil éter, glicerol 1-hexadecil éter, glicerol 1-octadecil éter y combinaciones de los mismos.	10. La composición de acuerdo la reivindicación 1, que además comprende un alquil éter de glicerol seleccionado del grupo que consiste de 1-O-heptilglicerol, 1-O-octilg1icerol, 1-O-nonilglicerol, 1-O-decilglicerol, 1-O-undecilglicerol, 1-O-dodecilglicerol, 1-O-tridecilglicerol, 1-O-tetradecilglicerol, 1-O-pentadecilglicerol, 1-O-hexadecilglicerol (quimil alcohol), 1-O-heptadecilglicerol, 1-O-octadecilglicerol (batil alcohol), 1-O-octadec-9-enil glicerol (selaquil alcohol), glicerol 1-(2-etilhexil) éter, 2-etilhexil glicerol, glicerol 1-heptil éter, glicerol 1-octil éter, glicerol 1-decil éter, and glicerol 1 -dodecil éter, glicerol 1-tridecil éter, glicerol 1-tetradecil éter, glicerol 1-pentadecil éter, glicerol 1-hexadecil éter, glicerol 1-octadecil éter y combinaciones de los mismos.	
11. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos una biguanida polimérica en una cantidad en el rango de 0,01% en peso a 1.0% en peso basado en el peso de la composición de desbridación, un componente diol vecino seleccionado de un grupo que consiste de un monoalquilglicol y un monoalquilglicerol en una concentración de 0,05% en peso a 4% en peso, basado en el peso de la composición de desbridación.	11. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos una biguanida polimérica en una cantidad en el rango de 0,01% en peso a 1.0% en peso basado en el peso de la composición de desbridación, un componente diol vecino seleccionado de un grupo que consiste de un monoalquilglicol y un monoalquilglicerol en una concentración de 0,05% en peso a 4% en peso, basado en el peso de la composición de desbridación.	

12. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos un agente analgésico, agente anestésico, agente de dolor neuropático, o una combinación de los mismos.	12. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente por lo menos un agente analgésico, agente anestésico, agente de dolor neuropático, o una combinación de los mismos.	
13. La composición de acuerdo con la reivindicación 12, en el que el por lo menos un agente analgésico, agente anestésico, o agente de dolor neuropático se selecciona del grupo que consiste de lidocaína, capsaicina, benzocaína, tetracaína, prilocaína, bupivacaína, levobupivacaína, procaína, carbocaína, etidocaína, mepivacaína, nortripileno, amitriptilina, pregabalina, diclofenaco, fentanilo, gabapentina, agentes antiinflamatorios no esteroideos, salicilatos y sus combinaciones.	13. La composición de acuerdo con la reivindicación 12, en el que el por lo menos un agente analgésico, agente anestésico, o agente de dolor neuropático se selecciona del grupo que consiste de lidocaína, capsaicina, benzocaína, tetracaína, prilocaína, bupivacaína, levobupivacaína, procaína, carbocaína, etidocaína, mepivacaína, nortripileno, amitriptilina, pregabalina, diclofenaco, fentanilo, gabapentina, agentes antiinflamatorios no esteroideos, salicilatos y sus combinaciones.	
14. La composición de acuerdo con la reivindicación 1, en el que la composición de desbridación tiene una forma seleccionada del grupo que consiste de un polvo, solución acuosa, solución líquida orgánica, silicona, gel, crema, película, látex, aerosol, suspensión, pasta, ungüento y espuma.	14. La composición de acuerdo con la reivindicación 1, en el que la composición de desbridación tiene una forma seleccionada del grupo que consiste de un polvo, solución acuosa, solución líquida orgánica, silicona, gel, crema, película, látex, aerosol, suspensión, pasta, ungüento y espuma.	
15. La composición de acuerdo con la reivindicación 1, en el que dicha composición de desbridación se adsorbe en un apósito o una fibra natural o sintética, malla, hidrocoloide, alginato, hidrogel, película semipermeable, película permeable o un polímero natural o sintético.	15. La composición de acuerdo con la reivindicación 1, en el que dicha composición de desbridación se adsorbe en un apósito o una fibra natural o sintética, malla, hidrocoloide, alginato, hidrogel, película semipermeable, película permeable o un polímero natural o sintético.	

16. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un agente buffer.	16. La composición de acuerdo con la reivindicación 1, que comprende adicionalmente un agente buffer.	
17. Un kit que comprende: una composición de desbridación de acuerdo con la reivindicación 1; e instrucciones para la desbridación de tejido desvitalizado.	17. Un kit que comprende: una composición de desbridación de acuerdo con la reivindicación 1; e instrucciones para la desbridación de tejido desvitalizado.	
18. El kit de acuerdo con la reivindicación 17, en el que dichas instrucciones comprenden poner en contacto la composición de desbridación con un área de piel en necesidad desbridación.	18. El kit de acuerdo con la reivindicación 17, en el que dichas instrucciones comprenden poner en contacto la composición de desbridación con un área de piel en necesidad desbridación.	
19. Un método de desbridación de tejido desvitalizado que comprende poner en contacto una composición de desbridación de acuerdo con la reivindicación 1 con un área de la piel en necesidad de desbridación.	19. Un método de desbridación de tejido desvitalizado que comprende poner en contacto una composición de desbridación de acuerdo con la reivindicación 1 con un área de la piel en necesidad de desbridación.	
20. El método de la reivindicación 19, en el que dicha etapa de contacto se repite al menos una vez un día.	20. El método de la reivindicación 19, en el que dicha etapa de contacto se repite al menos una vez un día.	
21. El método de la reivindicación 19, que además comprende remover el tejido desbridado de dicha área de la piel.	21. El método de la reivindicación 19, que además comprende remover el tejido desbridado de dicha área de la piel.	
22. La composición de acuerdo con la reivindicación 1, en el que el componente de amilasa se presenta en una cantidad en el rango de 2 a 99.5% en peso basado en el peso total de la composición de desbridación.	22. La composición de acuerdo con la reivindicación 1, en el que el componente de amilasa se presenta en una cantidad en el rango de 2 a 99.5% en peso basado en el peso total de la composición de desbridación.	