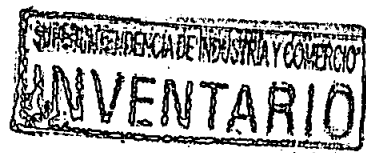


SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



DELEGATURA PROPIEDAD INDUSTRIAL

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCION

ENTRADA EN FASE NACIONAL

08-70809

21. EXPEDIENTE No.

54. TITULO

MÉTODOS PARA MODULAR RECEPTORES DE LA SUPERFICIE CELULAR

PARA PREVENIR O REDUCIR LA INFLAMACIÓN

51. CLASIFICACION INTERNACIONAL

71. SOLICITANTE

COLGATE - PALMOLIVE COMPANY

DOMICILIO

NUEVA YORK, NY, ESTADOS UNIDOS DE AMÉRICA

74. APODERADO

JUAN PABLO CADENA SARMIENTO

22. SANTAFE DE BOGOTA, D.C.

PETITORIO

AS: 107.720



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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FORMULARIO ÚNICO DE SOLICITUD DE PATENTE PCT ENTRADA EN FASE NACIONAL

1

SOLICITUD DE:



Patente de Invención.



Patente de Modelo de Utilidad.



Capítulo I.



Capítulo II.

2

**SOLICITANTE
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Nacionalidad o Domicilio: Estados Unidos de América
Lugar de Constitución: Estados Unidos de América
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C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Cual _____
Número _____

3

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IDENTIFICACIÓN

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C.E. ☐ Otro ☐

Cual _____
Número 80.410.337 TP 57492

4

**INVENTOR (ES)
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Dirección: 45 WESTBROOK CIRCLE, Westfield, NJ 07090, Estados Unidos de América.

Nacionalidad o Domicilio: Estados Unidos de América.

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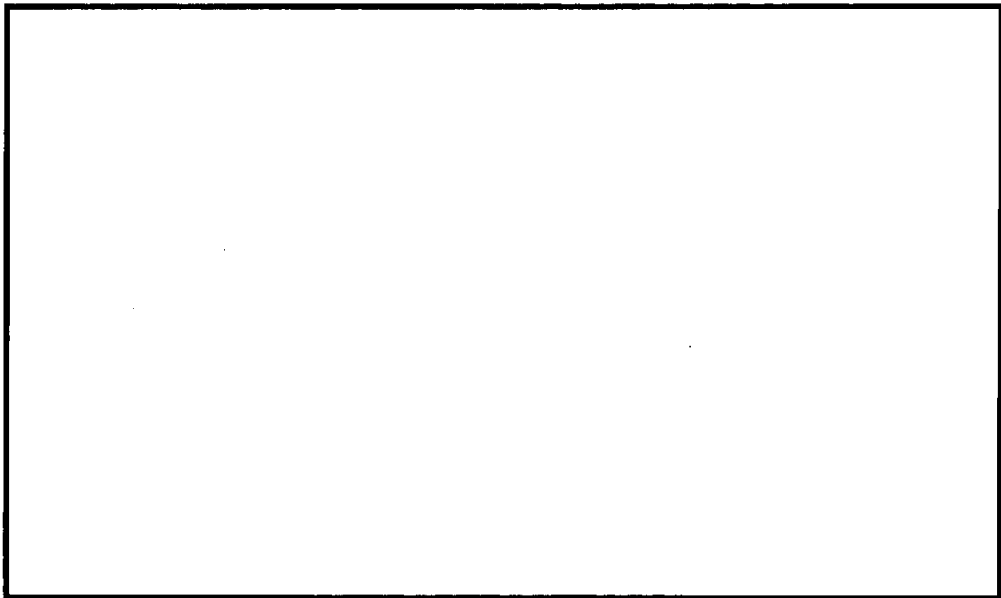
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ANEXOS

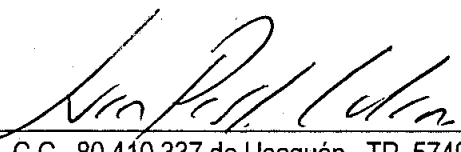
- ☒ Comprobante de pago de la tasa de presentación de la solicitud. \$ 501.000
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso. **Fotocopia autenticada**
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☐ Copia del examen preliminar efectuado por la IPEA.
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- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☒ Otros: Tarjeta de Propietarios
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11 FIGURA CARACTERÍSTICA:



12 Solicito la concesión de la patente,

NOMBRE: JUAN PABLO CADENA SARMIENTO

FIRMA: 
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3

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
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***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	501,000.00
		TOTAL :	\$ 501,000.00

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RESPONSABLE : _____

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4

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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PATENTE ☒ PCT/US2007/060222 MODELO DE UTILIDAD ☐ DISEÑO INDUSTRIAL ☐

SOLICITANTE/S. RAZON SOCIAL O APELLIDOS Y NOMBRES COLGATE-PALMOLIVE COMPANY

DOMICILIO NUEVA YORK, NY, ESTADOS UNIDOS DE AMERICA

APODERADO JUAN PABLO CADENA SARMIENTO

TITULO METODOS PARA MODULAR RECEPTORES DE LA SUPERFICIE CELULAR PARA PREVENIR O REDUCIR
LA INFLAMACION

CLASIFICACION

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CERTIFICADO No. _____ FECHA DE CONCESION _____ VIGENCIA DESDE _____ HASTA _____

PRORROGA _____ CAMBIO DE NOMBRE _____

TRASPASO A _____

INVENTOR (ES) PREM SREENIVASAN.

OTROS _____

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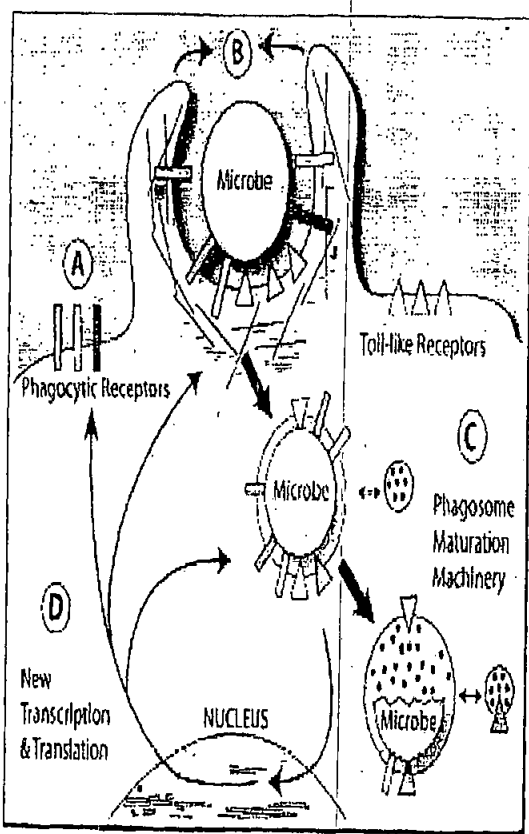
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[Continued on next page]

(54) Title: METHODS OF MODULATING CELL SURFACE RECEPTORS TO PREVENT OR REDUCE INFLAMMATION



(57) Abstract: The invention includes a method of distinguishing among oral bacteria species to determine whether a species is orally deleterious. Such method includes contacting at least one bacterium or portion of a bacterium of a species of oral bacteria a gingival cell; and detecting the presence of an indicator compound. The substantial absence of an indicator material signifies that the species of bacteria is not a deleterious species. Also included within the scope of the invention are methods for determining the anti-inflammatory effect of an agent. Such methods include contacting the cell with the agent in the presence of a deleterious bacterium or portion of such bacterium and detecting the presence of an indicator compound. The substantial absence of an indicator material signifies that agent is an anti-inflammatory agent.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

Methods of Modulating Cell Surface Receptors to Prevent or Reduce Inflammation

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Patent Application Serial No. 60/757,751 filed January 10, 2006, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] The soft tissues of the mammalian oral cavity are known to exhibit the earliest indications of inflammation. This generalized low level of inflammation may lead to gingivitis and/or periodontitis. Such inflammation is generally believed to be the result, at least in part, by the bacteria present in the oral cavity. Further, oral tissue inflammation can be caused by surgery, localized injury, trauma, necrosis, improper oral hygiene or various systemic origins.

[0003] It is generally believed that the cellular components implicated by these diseases and conditions include epithelial tissue, gingival fibroblasts, and circulating leukocytes, all of which contribute to the host response to pathogenic factors generated by the bacteria. Some bacterial pathogens implicated in these oral infections are known although many may remain unknown or uncharacterized. Although the infection by certain types of bacteria is often the etiological event in many of these oral diseases, the pathogenesis of the disease state or condition is mediated by the host response. Use of antibacterial agents reduces the bacterial population of a given oral cavity and may result in a reduction of inflammation. However, this approach is disadvantageous as such killing is accomplished indiscriminately (both beneficial oral bacteria and deleterious oral bacteria may perish) and it is dose and time sensitive.

[0004] Bacterial infection of the oral tissue stimulates the host's immune response and diminishes the healing process by up-regulating inflammatory mediators that cause significant tissue damage. These metabolites have been implicated as the prime mediators in gingivitis, periodontitis, osteomyelitis and other inflammatory diseases.

[0005] It has been reported that one mechanism of inflammation is mediated through certain transmembrane receptors of mammalian cells. For example, toll-like receptors ("TLRs"), are glycosylated transmembrane proteins and once activated by ligand-induced oligomerization initiate an immune response within the cell, ultimately resulting in the expression of cytokines, interleukins, and other molecules that mediate the state of inflammation.

[0006] There is a need in the art for agents and techniques useful in the diagnosis, treatment, and prevention of such inflammatory effects.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1 is a schematic diagram showing a cell's surface in contact with a microbe.

[0008] Figure 2 shows the signaling pathways of toll-like receptors (TLR) 1, 2, 3, 4, 5, 6, 7 and 9, as well as the various molecules generated by the modulation of each TLR.

BRIEF SUMMARY OF THE INVENTION

[0009] The invention includes a method of distinguishing among oral bacteria species to determine whether a species is orally deleterious. Such method includes contacting at least one bacterium or portion of a bacterium of a species of oral bacteria a gingival cell; and detecting the presence of an indicator compound. The substantial absence of an indicator material signifies that the species of bacteria is not a deleterious species.

[0010] Also included within the scope of the invention are methods for determining the anti-inflammatory effect of an agent. Such methods include contacting the cell with the agent in the presence of a deleterious bacterium or portion of such bacterium and detecting the presence of an indicator compound. The substantial absence of an indicator material signifies that agent is an anti-inflammatory agent.

DETAILED DESCRIPTION OF THE INVENTION

[0011] The invention provides methods for (1) distinguishing among deleterious bacterial species and beneficial oral bacteria and (2) methods for determining the anti-

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inflammatory effect of a specific agent. Also provided are methods of preventing the modulation of a toll-like receptor in a cell using triclosan.

[0012] "Inflammation" as used herein refers to the localized protective response elicited by injury or destruction of tissues that serves to destroy, dilute, or sequester both the injurious agent and the injured tissue. In the acute form, it may be characterized by pain, heat, redness, and swelling. Histologically, inflammation involves a complex series of events, including dilation of arterioles, capillaries, and venules, with increased permeability and blood flow; exudation of fluids, including plasma proteins, and leukocytic migration into the inflammatory locus. Inflammation corresponds to enhanced levels of pro-inflammatory cellular mediators, or substances that are released for example, as the result of the interaction of an antigen with an antibody or by the action of antigen with a sensitized lymphocyte. By extension, an anti-inflammatory agent is any that reduces the quality and/or quantity of these histological effects in a cell, relative to a like cell not treated with the agent.

[0013] The invention provides, in part, a method of evaluating a specific species or strain of bacteria in order to determine whether such species or strain is deleterious in the oral cavity. By "deleterious" it is meant pathogenic bacteria the presence of which results in generation of inflammatory mediators by the affected cells. Some strains/species of bacterial have been reported to be deleterious, including those of Table I, below:

Table 1: Oral Bacteria Reported to Be Deleterious

<i>A. gerencseriae</i>
<i>A. naeshundii</i> 1
<i>A. naeshundii</i> 2
<i>V. parvula</i>
<i>S. mitis</i>
<i>S. oralis</i>
<i>C. gingivalis</i>
<i>C. sputigena</i>
<i>F. nucleatum</i> ss <i>nucleatum</i>
<i>F. nucleatum</i> ss <i>polymorphum</i>
<i>F. periodonticum</i>
<i>P. intermedia</i>

<i>T. forsythia</i>
<i>L. buccalis</i>
<i>N. mucosa</i>
<i>P. acnes</i>
<i>P. melaninogenica</i>
<i>S. noxia</i>

[0014] The method includes contacting at least one bacterium of the species or strain of oral bacteria to be evaluated with at least one gingival cell. Alternatively, the gingival cell can be contacted with a portion of the bacterium, the cell membrane, the cytoplasmic contents, the bacterium's metabolic, end or by-products and/or virulence factors.

[0015] This step may occur *in vitro*, for example, by maintaining culture of bacteria or bacterial parts and of gingival cells and combining such cultures. Alternatively, the contact may occur *in vivo*, by application of the bacterial culture in an appropriate delivery vehicle to the oral cavity.

[0016] After such contact, one or more indicator material may be detected. Such indicator materials include any that are known or to be discovered that are generated by in the ordinary course of a host's immune response including intermediate compounds, enzymes, proteins, RNAs, DNAs, and other molecules that are involved in the cellular production of cytokines and other pro-inflammatory mediators. Preferred are indicator materials that are generated by the modulation of any of the toll-like receptors (*e.g.*, as shown in Figure 2), including, for example, TLRs 2, 3, 4, 5, 7, and 9; molecules of the NFκ-B pathway, cytokines, interleukins (*e.g.*, 1, 6, 8, 12), tumor necrotic factor (TNF), peptidases, and mRNAs coding for the interleukin or TNF subunits and/or interleukins, chemokines (*e.g.*, CCL5, CCL4, CCL3, and CC10), and matrix metalloproteinases.

[0017] The detection of the selected indicator material may be carried out by in any manner known or to be developed in the art and may be a relative or an absolute measurement. Detection may be accomplished by direct measurement of the quantity of selected indicator material(s). Alternatively, it may be accomplished by indirect

measurement using additional detection systems, such as radioactive and/or fluorescent markers, antibodies, alterations in the level of gene expression of specific genes, mRNA analysis, microarray analysis, and/or changes in antioxidant status. For example, if the indicator material is an enzyme, one may subject the sample to an appropriate substrate and measure the end product of an enzyme-catalyzed reaction to determine the presence or absence of the enzyme indicator material. In the practice of this aspect of the invention, the substantial absence of the selected indicator material demonstrates that the species/strain of bacteria is not deleterious – *i.e.*, does not affect the cell in such a manner as to initiate the production of any pro-inflammatory mediators.

[0018] The invention also includes a method for evaluating the anti-inflammatory capacity or effect of a specific agent, *i.e.*, the capability of the agent to reduce or eliminate inflammation in a tissue exposed to a pathogenic bacterium. The method includes selection of an agent to be evaluated. Such agent may be a protein, peptide, organic molecule, inorganic molecule or conjugate of any of the same.

[0019] Under some circumstances, it may be desirable that the selected agent to be evaluated does not exhibit significant bactericidal effects in the oral context. For example, the agent may be selected from those that exhibit a Minimal Inhibitory Concentration (MIC) of 0, less than about 5%, less than about 10%, less than about 20% and less than about 30%.

[0020] MIC studies general include preparation of a solution of the active agent in an appropriate solvent and subsequent serial dilution of the solubilized active. A standard suspension of the selected bacteria (these could be a range of bacteria each of which requires its own specialized protocol for growth and handling) is added to each concentration of the diluted active. The bacteria + active are incubated under appropriate conditions at 37°C and bacterial growth monitored typically after 48 hours of incubation.

[0021] Controls for the MIC studies include a growth control that monitors bacterial growth in the absence of any added solvents or excipients. Additional controls

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monitor the sterility of the media used for tests. The effects of the solvent(s) used to solubilize the active agents on bacteria are the final series of controls included for these studies.

[0022] Following incubation, the plates are read in a microplate spectrophotometer at 610 nm. Results are interpreted as the lowest concentration of the active agent that inhibits the bacterial growth. At high active concentrations, bacteria would not grow (shows as a low optical density reading) and at low active concentrations bacteria would proliferate (shows as a high optical density reading). The lowest active concentration to stop bacterial growth is defined as the MIC. The controls should come out as follows:

[0023] Sterility of media - no bacterial growth

[0024] Monitoring bacterial growth - the cultures show demonstrate luxurious growth

[0025] The solvents used to solubilize active agents should not inhibit bacteria (as they must be essentially innocuous).

[0026] The method of evaluating the anti-inflammatory capacity or effect of a specific agent includes the step of contacting a cell with a pathogenic or deleterious bacterium. The cell to be contacted may be *in vitro* or *in vivo*, prokaryotic or eukaryotic, and may be obtained from a cell culture line or a clinical sample.

[0027] Suitable pathogenic or deleterious bacteria for use in the method include any know or to be discovered in the art that affect the selected cell in such a way as to elicit an immune response. Such bacteria may include, for example, those listed in Table I, above.

[0028] The method of evaluating the anti-inflammatory capacity or effect of a specific agent includes the step of detecting the presence or absence of one or more indicator compound(s). Indicator compounds and detection methods and systems may be any of those described above.

[0029] Also included in the invention are oral formulations containing the agents found to exhibit an anti-inflammatory effect by the assay described above, as

well as methods of preventing the modulation of toll-like receptors on oral tissue cells by contacting such cell with triclosan.

[0030] The invention also provides methods of reducing or preventing inflammation of an oral tissue by application of a compound, such as triclosan, to the tissue at sub-MIC levels. By sub-MIC levels.

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CLAIMS

1. A method of distinguishing among oral bacteria species, the method comprising:
 - (a) contacting at least one bacterium or portion of a bacterium of a species of oral bacteria a gingival cell; and
 - (b) detecting the presence of an indicator compoundwherein the substantial absence of an indicator material signifies that the species of bacteria is not a deleterious species.
2. The method of claim 1, wherein the gingival cell is *in vitro*.
3. The method of claim 1, wherein the gingival cell is *in situ* in a mammalian oral cavity.
4. The method of claim 1, wherein the indicator material is selected from the group consisting of molecules generated by the modulation of a toll-like receptor.
5. The method of claim 1, the indicator material is selected from the group consisting of molecules generated by the modulation of TLR 2, TLR 3, and TLR 4.
6. The method of claim 1, the indicator material is selected from the group consisting of molecules generated by the modulation of TLR 5, TLR 7, and TLR 9.
7. The method of claim 1, wherein the indicator material is selected from the group consisting of molecules of the NF- κ B pathway.
8. The method of claim 1, wherein the indicator material is selected from the group consisting of cytokines and interleukins.

9. The method of claim 1, wherein the indicator material is selected from the group consisting of IL-1, IL-6, IL-8, IL-12, and TNF- α .
10. The method of claim 1, wherein the indicator molecule is detected directly.
11. The method of claim 1, wherein the indicator material is detected indirectly.
12. The method of claim 1, wherein the at least one bacterium is obtained from a clinical sample.
13. A method for determining the anti-inflammatory effect of an agent comprising contacting the cell with the agent in the presence of a deleterious bacterium or portion of such bacterium and detecting the presence of an indicator compound, wherein the substantial absence of an indicator material signifies that agent is an anti-inflammatory agent.
14. The method of claim 13, wherein the cell is a eukaryotic cell.
15. The method of claim 13, wherein the cell is a prokaryotic cell.
16. The method of claim 13, wherein the indicator material is selected from the group consisting of molecules generated by the modulation of a toll-like receptor.
17. The method of claim 13, wherein the indicator material is selected from the group consisting of molecules generated by the modulation of TLR 2, TLR 3, TLR 4, TLR 5, TLR 7, and TLR 9.

18. The method of claim 13, wherein the indicator material is selected from the group consisting of molecules of the NF- κ B pathway.

19. The method of claim 13, wherein the indicator material is selected from the group consisting of cytokines and interleukins.

20. The method of claim 13, wherein the indicator material is selected from the group consisting of IL-1, IL-6, IL-8, IL-12, and TNF- α .

21. The method of claim 13, wherein the indicator molecule is detected directly.

22. The method of claim 13, wherein the agent is selected from agents exhibiting an MIC of less than about 20%.

23. A method of preventing the modulation of a toll-like receptor in a cell of the mammalian oral cavity comprising contacting the receptor with triclosan.

24. The method of claim 23, wherein the cell is a gingival cell.

25. The method of claim 23, wherein the toll-like receptor is selected from TLR 2, TLR 3, TLR 4, TLR 5, TLR 7, and TLR 9.

26. A method of reducing or preventing the inflammation of an oral tissue, the method comprising applying to the oral tissue a composition comprising triclosan in an concentration that results in application of the triclosan to the tissue at sub-MIC levels.

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Replacement Sheet

1/2

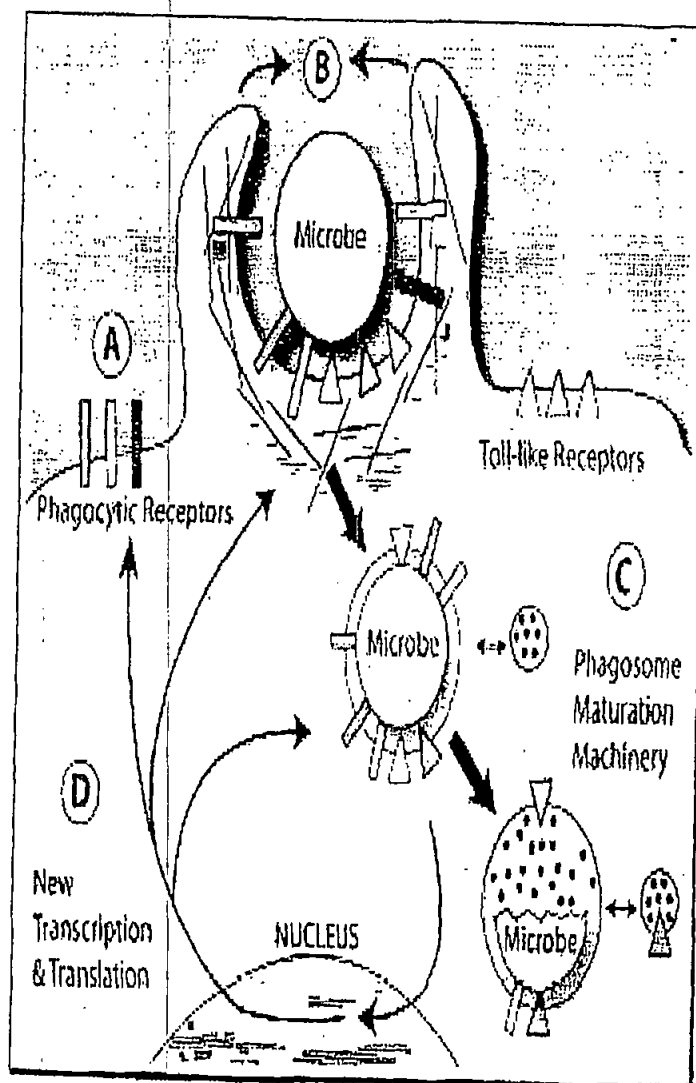


FIGURE 1

SUBSTITUTE SHEET (RULE 26)

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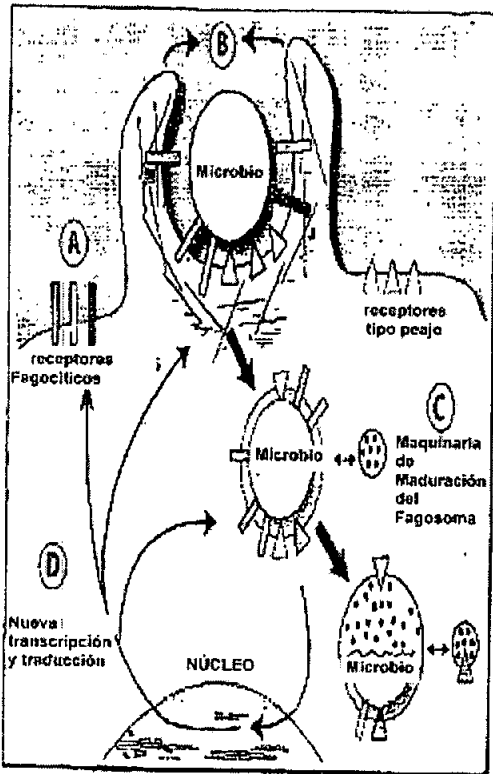
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- (71) Solicitante (para todos los Estados designados menos los Estados Unidos): COLGATE-PALMOLIVE COMPANY [EU/EU]; 300 Park Avenue, New York, New York 10022 (EU).
- (72) Inventores; e
- (75) Inventores/Solicitantes (solo para los Estados Unidos): SREENIVASAN, Prem [EU/EU]; 45 WESTBROOK CIRCLE, Westfield, NJ 07090 (EU).
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- (81) Estados Designados (a menos que se indique lo contrario, para todo tipo de protección nacional disponible): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Estados Designados (a menos que se indique lo contrario, para todo tipo de protección regional disponible): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Publicado:
— sin el informe de búsqueda internacional y a ser publicado nuevamente al recibir dicho informe

Para códigos de dos letras y otras abreviaciones, referirse a las "Notas de guía sobre Códigos y Abreviaturas" que aparecen al comienzo de cada edición de la Gaceta del PCT.

(54) Título: MÉTODOS PARA MODULAR RECEPTORES DE LA SUPERFICIE CELULAR PARA PREVENIR O REDUCIR LA INFLAMACIÓN



(57) Resumen: La invención incluye un método para distinguir especies de bacterias orales a fin de determinar si una especie es oralmente deletérea. Dicho método incluye poner en contacto al menos una bacteria o una porción de una bacteria de una especie de bacteria oral con una célula gingival; y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que la especie de bacteria no es deletérea. También están incluidos en el alcance de la invención métodos para determinar el efecto antiinflamatorio de un agente. Dichos métodos incluyen poner en contacto la célula con el agente en presencia de una bacteria deletérea o una porción de dicha bacteria y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que el agente es antiinflamatorio.

MÉTODOS PARA MODULAR RECEPTORES DE LA SUPERFICIE CELULAR PARA PREVENIR O REDUCIR LA INFLAMACIÓN

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ANTECEDENTES DE LA INVENCION

Se sabe que los tejidos blandos de la cavidad oral de los mamíferos son los primeros en exhibir signos de inflamación. Este bajo nivel generalizado de inflamación puede conducir a la gingivitis y/o periodontitis. En general se cree que dicha inflamación es el resultado, al menos en parte, de la presencia de bacterias en la cavidad oral. Asimismo, la inflamación del tejido oral puede ser causada por una cirugía, una herida local, una lesión, necrosis, higiene oral indebida o diversos orígenes sistémicos.

En general se cree que los componentes celulares implicados en estas enfermedades y estados incluyen tejido epitelial, fibroblastos gingivales y leucocitos circulantes, todos los cuales contribuyen a la respuesta del huésped a los factores patógenos generados por las bacterias. Algunos patógenos bacterianos implicados en estas infecciones orales son conocidos aunque muchos siguen siendo desconocidos o no están caracterizados. Si bien la infección causada por determinados tipos de bacterias suele ser el evento etiológico en muchas de estas enfermedades orales, la patogénesis del estado de enfermedad es mediada por la respuesta del huésped. El empleo de agentes antibacterianos reduce la población bacteriana de una cavidad oral dada y puede contribuir a reducir la inflamación. Sin embargo, este enfoque no es ventajoso ya que dicha eliminación se logra de manera indiscriminada (pueden perecer tanto las bacterias beneficiosas como las deletéreas) y es sensible a la dosis y el tiempo.

La infección bacteriana del tejido oral estimula la respuesta inmune del huésped y disminuye el proceso de regeneración aumentando los mediadores inflamatorios que causan daño tisular significativo. Estos metabolitos han estado implicados como los principales mediadores en la gingivitis, periodontitis, osteomielitis y otras enfermedades inflamatorias.

Se sabe que un mecanismo de inflamación es mediado a través de determinados receptores transmembrana de células mamíferas. Por ejemplo, los receptores tipo peaje (TLR) son proteínas transmembrana glicosiladas y una vez activadas por oligomerización inducida por ligando inician una respuesta inmune dentro de la célula, lo cual en última instancia da como resultado la expresión de citoquinas, interleuquinas y otras moléculas que median el estado de inflamación.

Existe la necesidad en el arte de contar con agentes y técnicas útiles para el diagnóstico, el tratamiento y la prevención de dichos efectos inflamatorios.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

La Figura 1 es un diagrama esquemático que muestra la superficie de una célula en contacto con un microbio.

La Figura 2 muestra las vías de señalización de los receptores tipo peaje (TLR) 1, 2, 3,

4, 5, 6, 7 y 9, como también las diversas moléculas generadas por la modulación de cada TLR.

BREVE DESCRIPCIÓN DE LA INVENCION

Esta invención incluye un método para distinguir diversas especies de bacterias orales a fin de determinar si una especie es oralmente deletérea. Dicho método incluye poner en contacto al menos una bacteria o porción de una bacteria de una especie de bacteria oral con una célula gingival; y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que la especie de bacteria no es deletérea.

También se encuentran dentro del alcance de la invención métodos para determinar el efecto antiinflamatorio de un agente. Dichos métodos incluyen poner en contacto la célula con el agente en presencia de una bacteria deletérea o una porción de esa bacteria y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que el agente es antiinflamatorio.

DESCRIPCIÓN DETALLADA DE LA INVENCION

La invención proporciona métodos para (1) distinguir especies de bacterias deletéreas de bacterias orales beneficiosas y (2) métodos para determinar el efecto antiinflamatorio de un agente específico. También se proporcionan métodos para prevenir la modulación de un receptor tipo peaje en una célula utilizando triclosan.

En la presente, "inflamación" se utiliza para referirse a la respuesta protectora localizada producida por una lesión o destrucción de tejidos que sirve para destruir, diluir o apartar tanto el agente perjudicial como el tejido dañado. En forma aguda, puede estar caracterizada por dolor, calor, enrojecimiento e hinchazón. Histológicamente, la inflamación implica una compleja serie de eventos, incluyendo la dilatación de arteriolas, capilares y vénulas, con aumento de permeabilidad y flujo sanguíneo; exudación de fluidos, incluyendo proteínas del plasma, y la migración leucocítica en el locus inflamatorio. La inflamación corresponde a niveles elevados de mediadores celulares proinflamatorios, o sustancias que se liberan, por ejemplo, como resultado de la interacción de un antígeno con un anticuerpo o por medio de la acción de un antígeno con un linfocito sensibilizado. Por extensión, un agente antiinflamatorio es aquel que reduce la calidad y/o cantidad de estos efectos histológicos en una célula, con relación a una célula similar que no es tratada con el agente.

En parte, la invención proporciona un método para evaluar una especie específica o cepa de bacteria a fin de determinar si dicha especie o cepa es deletérea en la cavidad oral. Por "deletérea" se entiende bacteria patógena cuya presencia tiene como resultado la generación de mediadores inflamatorios por parte de las células afectadas. Se sabe que algunas cepas/especies de bacterias son deletéreas, incluyendo las de la Tabla 1 a continuación:

Tabla 1: Bacterias orales conocidas como deletéreas

<i>A. gerencseriae</i>
<i>A. naeslundii</i> 1

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A. naeslundii 2
V. parvula
S. mitis
S. oralis
C. gingivalis
C. sputigena
F. nucleatum ss nucleatum
F. nucleatum ss polymorphum
F. periodonticum
P. intermedia
T. forsythia
L. buccalis
N. mucosa
P. acnes
P. melaninogenica
S. noxia

El método incluye poner en contacto al menos una bacteria de la especie o cepa de bacterias orales que se evaluarán con al menos una célula gingival. De manera alternativa, la célula gingival puede ponerse en contacto con una porción de la bacteria, la membrana celular, los contenidos citoplasmáticos, los subproductos, productos finales o metabólicos de la bacteria y/o factores de virulencia.

Este paso puede llevarse a cabo *in vitro*, por ejemplo, manteniendo un cultivo de la bacteria o partes de la bacteria y de las células gingivales y combinando dichos cultivos. De manera alternativa, el contacto puede tener lugar *in vivo*, aplicando el cultivo bacteriano en un vehículo de administración adecuado a la cavidad oral.

Después de dicho contacto, puede detectarse uno o más materiales indicadores. Dichos materiales indicadores incluyen cualquiera conocido o por descubrirse generados en el transcurso normal de la respuesta inmune de un huésped incluyendo compuestos intermedios, enzimas, proteínas, ARN, ADN y otras moléculas involucradas en la producción celular de citoquinas y otros mediadores proinflamatorios. Se prefieren los materiales indicadores generados por la modulación de cualquiera de los receptores tipo peaje (por ejemplo, como se muestra en la Figura 2), incluyendo, por ejemplo, TLR 2, 3, 4, 5, 7 y 9; moléculas de la vía NFκ-B, citoquinas, interleuquinas (por ejemplo, 1, 6, 8, 12), factor de necrosis tumoral (FNT), peptidasas y codificación de ARNm para las subunidades de FNT o interleuquina y/o interleuquinas, quimioquinas (por ejemplo, CCL5, CCL4, CCL3 y CCL10) y metaloproteinasas de matriz.

La detección del material indicador seleccionado puede llevarse a cabo de cualquier modo conocido o por desarrollarse en el arte y puede ser una medición relativa o absoluta. La detección puede lograrse por medición directa de la cantidad de material indicador seleccionado. De manera alternativa, puede lograrse por medición indirecta utilizando sistemas de detección adicionales, tales como marcadores radiactivos y/o fluorescentes, anticuerpos,

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alteraciones del nivel de expresión genética de genes específicos, análisis de ARNm, análisis de microensayo y/o cambios en el estado antioxidante. Por ejemplo, si el material indicador es una enzima, la muestra puede someterse a un sustrato adecuado y medirse el producto final de una reacción catalizada por enzima con el propósito de determinar la presencia o ausencia del material indicador de la enzima. En la práctica de este aspecto de la invención, la ausencia sustancial del material indicador seleccionado demuestra que la especie/cepa de bacteria no es deletérea, es decir, no afecta la célula de modo tal que se inicie la producción de mediadores proinflamatorios.

La invención también incluye un método para evaluar la capacidad o efecto antiinflamatorio de un agente específico, es decir, la capacidad del agente de reducir o eliminar la inflamación en un tejido expuesto a una bacteria patógena. El método incluye seleccionar un agente que será evaluado. Dicho agente puede ser una proteína, péptido, molécula orgánica, molécula inorgánica o conjugado de cualquiera de los mismos.

En algunas circunstancias, puede ser conveniente que el agente seleccionado que se evaluará no exhiba efectos bactericidas significativos en el contexto oral. Por ejemplo, el agente puede seleccionarse entre los que exhiben una Concentración Inhibidora Mínima (CIM) de 0, menos que alrededor de 5%, menos que alrededor de 10%, menos que alrededor de 20% y menos que alrededor de 30%.

Los estudios de CIM generalmente incluyen la preparación de una solución del agente activo en un solvente adecuado y la posterior dilución en serie del activo solubilizado. Se agrega una suspensión estándar de la bacteria seleccionada (puede ser una serie de bacterias, cada una de las cuales requiere su propio protocolo especializado para el crecimiento y manejo) a cada concentración del activo diluido. Las bacterias más el activo se incuban en condiciones adecuadas a 37 °C y el crecimiento bacteriano se monitorea típicamente después de 48 horas de incubación.

Los controles para los estudios de CIM incluyen un control de crecimiento que monitorea el crecimiento bacteriano en ausencia de cualquier solvente o excipiente. Otros controles monitorean la esterilidad de los medios utilizados para las pruebas. Los efectos del solvente(s) utilizado para solubilizar los agentes activos en las bacterias son la serie final de controles incluidos en estos estudios.

Después de la incubación, las placas se leen en un espectrofotómetro de microplacas a 610 nm. Los resultados se interpretan como la concentración inferior del agente activo que inhibe el crecimiento bacteriano. En concentraciones altas de activo, las bacterias no crecen (se muestra como una lectura de baja densidad óptica) y en concentraciones bajas de activo las bacterias proliferan (se muestra como una lectura de alta densidad óptica). La concentración más baja de activo para detener el crecimiento bacteriano se define como la CIM. Los controles deben dar los siguientes resultados:

Esterilidad de los medios – sin crecimiento bacteriano.

Monitoreo del crecimiento bacteriano – los cultivos deben demostrar crecimiento abundante.

Los solventes utilizados para solubilizar agentes activos no deben inhibir bacterias (ya que deben ser esencialmente inocuos).

El método para evaluar la capacidad o el efecto antiinflamatorio de un agente específico incluye el paso de poner en contacto una célula con una bacteria patógena o deletérea. La célula que se pondrá en contacto puede estar *in vitro* o *in vivo*, ser procariota o eucariota, y puede obtenerse de una línea de cultivo celular o una muestra clínica.

Las bacterias patógenas o deletéreas adecuadas para ser utilizadas en el método incluyen cualquiera conocida o por descubrirse en el arte que afectan la célula seleccionada de modo tal que provocan una respuesta inmune. Dichas bacterias pueden incluir, por ejemplo, las enumeradas en la Tabla 1, arriba.

El método para evaluar la capacidad o el efecto antiinflamatorio de un agente específico incluye el paso de detectar la presencia o ausencia de uno o más compuestos indicadores. Los compuestos indicadores y los métodos y sistemas de detección pueden ser cualquiera de los descritos arriba.

También están incluidas en la invención formulaciones orales que contienen los agentes que exhiben un efecto antiinflamatorio según el ensayo descrito antes, como también métodos para impedir la modulación de receptores tipo peaje en células del tejido oral poniendo en contacto dicha célula con triclosan.

La invención también proporciona métodos para reducir o prevenir la inflamación de un tejido oral mediante la aplicación de un compuesto, tal como triclosan, al tejido en niveles sub-CIM.

REIVINDICACIONES

1. Un método para distinguir especies de bacterias orales, caracterizado porque comprende:
 - (a) poner en contacto al menos una bacteria o porción de una bacteria de una especie de bacteria oral con una célula gingival; y
 - (b) detectar la presencia de un compuesto indicadordonde la ausencia sustancial de un material indicador significa que la especie de bacteria no es deletérea.
2. El método de la reivindicación 1, caracterizado porque la célula gingival está *in vitro*.
3. El método de la reivindicación 1, caracterizado porque la célula gingival está *in situ* en la cavidad oral de un mamífero.
4. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas generadas por la modulación de un receptor tipo peaje.
5. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas generadas por la modulación de TLR 2, TLR 3 y TLR 4.
6. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas generadas por la modulación de TLR 5, TLR 7 y TLR 9.
7. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas de la vía de NFκ-B.
8. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de citoquinas e interleuquinas.
9. El método de la reivindicación 1, caracterizado porque el material indicador se selecciona del grupo que consiste de IL-1, IL-6, IL-8, IL-12 y FNT-α.
10. El método de la reivindicación 1, caracterizado porque la molécula indicadora se detecta directamente.
11. El método de la reivindicación 1, caracterizado porque el material indicador se detecta indirectamente.
12. El método de la reivindicación 1, caracterizado porque al menos una bacteria se obtiene de una muestra clínica.
13. Un método para determinar el efecto antiinflamatorio de un agente caracterizado porque comprende poner en contacto la célula con el agente en presencia de una bacteria deletérea o una porción de dicha bacteria y detectar la presencia de un compuesto indicador, donde la ausencia sustancial de un material indicador significa que el agente es antiinflamatorio.
14. El método de la reivindicación 13, caracterizado porque la célula es una célula

eucariota.

15. El método de la reivindicación 13, caracterizado porque la célula es una célula procariota.
16. El método de la reivindicación 13, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas generadas por la modulación de un receptor tipo peaje.
17. El método de la reivindicación 13, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas generadas por la modulación de TLR 2, TLR 3, TLR 4, TLR 5, TLR 7 y TLR 9.
18. El método de la reivindicación 13, caracterizado porque el material indicador se selecciona del grupo que consiste de moléculas de la vía NFκ-B.
19. El método de la reivindicación 13, caracterizado porque el material indicador se selecciona del grupo que consiste de citoquinas e interleuquinas.
20. El método de la reivindicación 13, caracterizado porque el material indicador se selecciona del grupo que consiste de IL-1, IL-6, IL-8, IL-12 y FNT-α.
21. El método de la reivindicación 13, caracterizado porque la molécula indicadora se detecta directamente.
22. El método de la reivindicación 13, caracterizado porque el agente se selecciona entre agentes que exhiben una CIM menor que alrededor de 20%.
23. Un método para impedir la modulación de un receptor tipo peaje en una célula de la cavidad oral de un mamífero caracterizado porque comprende poner en contacto el receptor con triclosan.
24. El método de la reivindicación 23, caracterizado porque la célula es una célula gingival.
25. El método de la reivindicación 23, caracterizado porque el receptor tipo peaje se selecciona de TLR 2, TLR 3, TLR 4, TLR 5, TLR 7 y TLR 9.
26. Un método para reducir o prevenir la inflamación de un tejido oral, caracterizado porque comprende aplicar al tejido oral una composición que comprende triclosan en una concentración que tiene como resultado la aplicación de triclosan al tejido a niveles sub-CIM.

RESUMEN

MÉTODOS PARA MODULAR RECEPTORES DE LA SUPERFICIE CELULAR PARA PREVENIR O REDUCIR LA INFLAMACIÓN

Un método para distinguir especies de bacterias orales a fin de determinar si una especie es oralmente deletérea. Dicho método incluye poner en contacto al menos una bacteria o una porción de una bacteria de una especie de bacteria oral con una célula gingival; y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que la especie de bacteria no es deletérea. También están incluidos en el alcance de la invención métodos para determinar el efecto antiinflamatorio de un agente.

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Dichos métodos incluyen poner en contacto la célula con el agente en presencia de una bacteria deletérea o una porción de dicha bacteria y detectar la presencia de un compuesto indicador. La ausencia sustancial de un material indicador significa que el agente es antiinflamatorio.

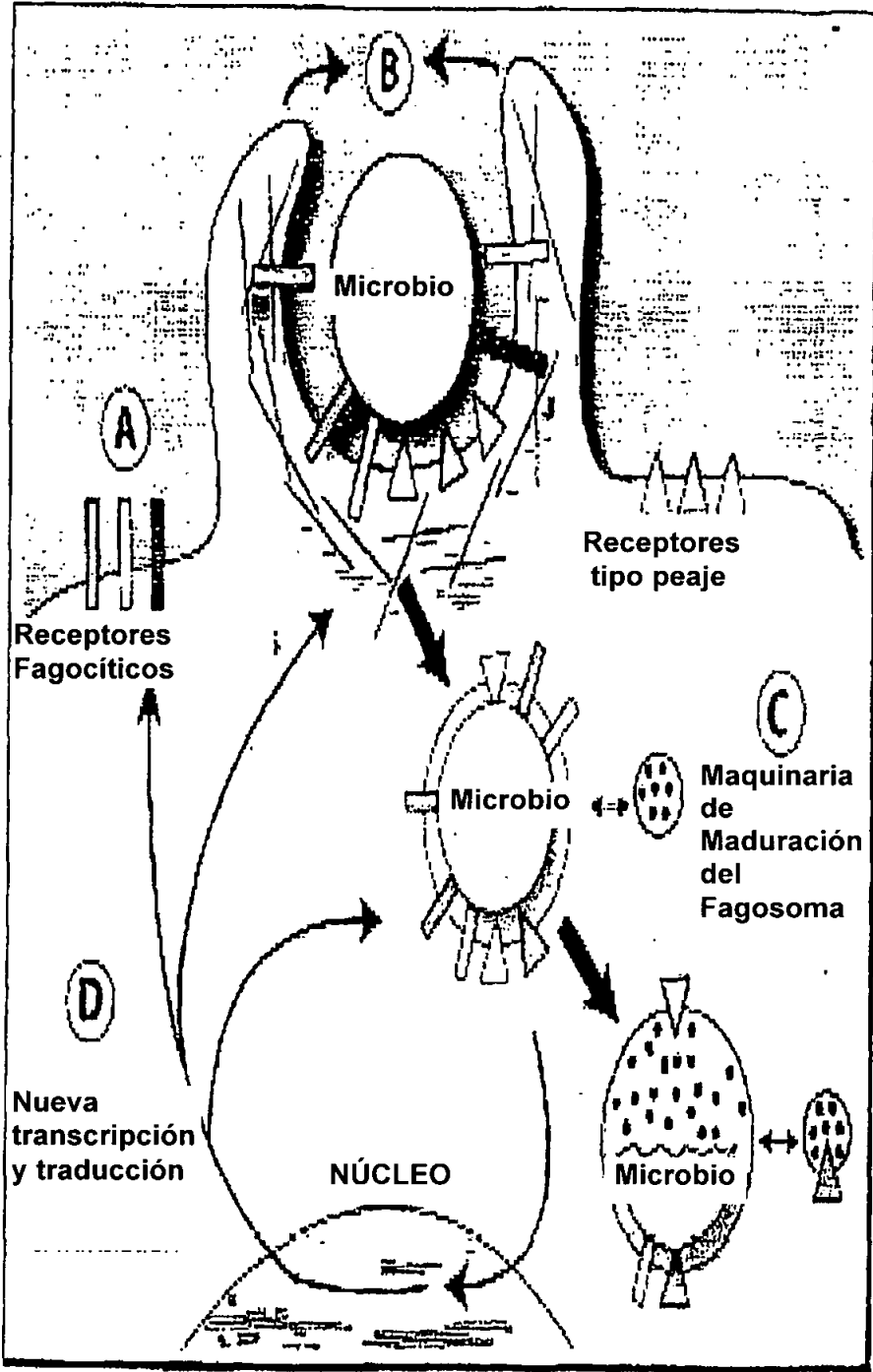


Figura 1

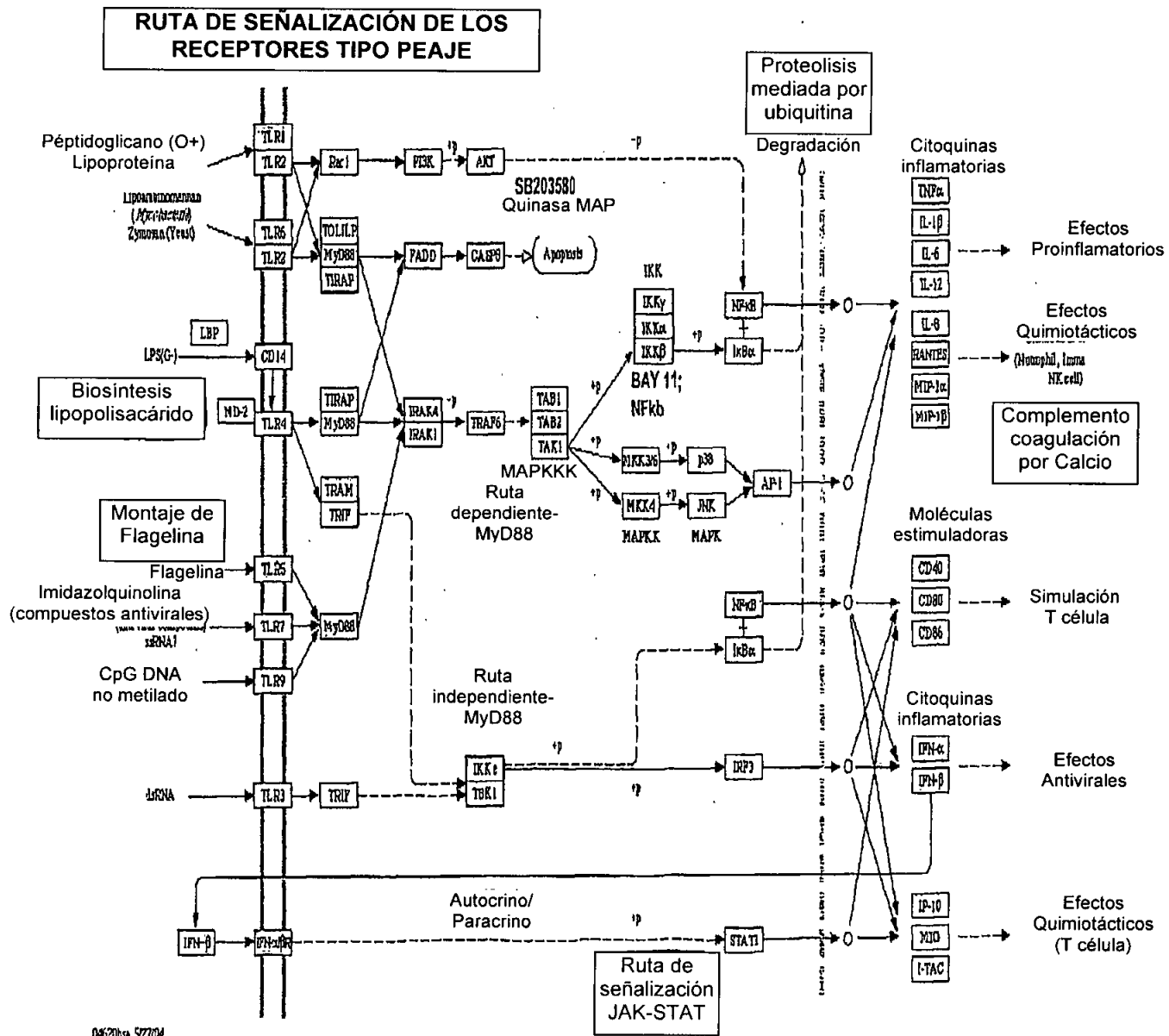


Figura 2

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 8073-00-WO	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2007/060222	International filing date (day/month/year) 08/01/2007	(Earliest) Priority Date (day/month/year) 10/01/2006
Applicant SREENIVASAN, Prem		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 8 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (See Box No. II)

3. ☒ Unity of invention is lacking (see Box No. III)

4. With regard to the title,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. 1
☒ as suggested by the applicant
☐ as selected by this Authority, because the applicant failed to suggest a figure
☐ as selected by this Authority, because this figure better characterizes the invention
- b. ☐ none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

national application No

PCT/US2007/060222

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/569 G01N33/50 C12Q1/04 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, INSPEC, FSTA, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/13094 A (SQUIBB BRISTOL MYERS CO [US]) 18 May 1995 (1995-05-18) page 13, line 33 - page 14, line 32 claims 11-21 page 5, line 10 - page 6, line 36 page 10, line 24 - page 11, line 33 page 18, lines 1-24	13-25
X	WO 2005/057222 A (WHITEHEAD BIOMEDICAL INST [US]; HUG CHRISTOPHER [US]; LODISH HARVEY F) 23 June 2005 (2005-06-23) page 24, line 1 - page 25, line 18 example 5 claims 1,14	13-25

-/--



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

25 July 2007

Date of mailing of the international search report

02/08/2007

Name and mailing address of the ISA/

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Authorized officer

Boiangiu, Clara

INTERNATIONAL SEARCH REPORT

national application No
PCT/US2007/060222

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/55386 A (GLAXOSMITHKLINE [GB]; LEWIS ALAN PETER [GB]; RAY KEITH PAUL [GB]) 2 August 2001 (2001-08-02) page 2, lines 15-27 page 4, line 11 - page 5, line 21 page 8, line 11 - page 10, line 2 page 19, line 18 - page 20, line 28 page 23, lines 4-19 claims 18-28	13-25
X	US 5 578 295 A (FRANCIS MARION D [US] ET AL) 26 November 1996 (1996-11-26) column 3; line 40 - column 5, line 24 claim 22; examples I-III	26
X	EP 0 528 468 A1 (UNILEVER NV [NL]; UNILEVER PLC [GB]) 24 February 1993 (1993-02-24) the whole document	26
X	HOLT R ET AL: "Dental damage, sequelae, and prevention." THE WESTERN JOURNAL OF MEDICINE APR 2001, vol. 174, no. 4, April 2001 (2001-04), pages 288-290, XP002443683 ISSN: 0093-0415 abstract	26
Y	UEHARA A ET AL: "Priming of human oral epithelial cells by interferon-gamma to secrete cytokines in response to lipopolysaccharides, lipoteichoic acids and peptidoglycans." JOURNAL OF MEDICAL MICROBIOLOGY AUG 2002, vol. 51, no. 8, August 2002 (2002-08), pages 626-634, XP002434615 ISSN: 0022-2615 abstract page 626, left-hand column, paragraph 1 - page 627, left-hand column, paragraph 2 page 628, right-hand column, paragraph 3 - page 629, left-hand column, paragraph 1; figures 1,2 page 629, right-hand column, paragraph 2 - page 631, left-hand column, paragraph 1 figures 3-7 page 632	1-12

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INTERNATIONAL SEARCH REPORT

national application No
PCT/US2007/060222

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEINBERG A ET AL: "EPITHELIAL ANTIMICROBIAL PEPTIDES: REVIEW AND SIGNIFICANCE FOR ORALAPPLICATIONS" CRITICAL REVIEWS IN ORAL BIOLOGY AND MEDICINE, CRC PRESS, BOCA RATON, FL, US, vol. 9, no. 4, November 1998 (1998-11), pages 399-414, XP000972953 ISSN: 1045-4411 abstract page 408, right-hand column, paragraph 2 - page 410, right-hand column, paragraph 3 figures 6,7,9	1-12
Y	MADIANOS P N ET AL: "Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva." JOURNAL OF CLINICAL PERIODONTOLOGY 2005, vol. 32 Suppl 6, 2005, pages 57-71, XP002435073 ISSN: 0303-6979 abstract page 58, left-hand column, paragraph 1 page 58, middle column, paragraph 1 - page 59, right-hand column, paragraph 1 page 59 page 59, right-hand column, paragraph 2 page 60, left-hand column, paragraph 3 page 60, middle column, paragraphs 2,3 page 60, right-hand column, paragraph 3 - page 61, left-hand column, paragraph 1 page 63, right-hand column, paragraph 2 - left-hand column, paragraph 1 figures 1,2 page 65, right-hand column, paragraph 2 figure 3	1-12
Y	UEHARA A ET AL: "CONTRASTING RESPONSES OF HUMAN GINGIVAL AND COLONIC EPITHELIAL CELLS TO LIPOPOLYSACCHARIDES, LIPOTEICHOIC ACIDS AND PEPTIDOGLYCANS IN THE PRESENCE OF SOLUBLE CD14" MEDICAL MICROBIOLOGY AND IMMUNOLOGY, BERLIN, DE, vol. 189, no. 4, September 2001 (2001-09), pages 185-192, XP009078055 abstract	1-12
Y	WO 01/48481 A1 (BAXTER INT [US]; PATEL MEHUL [US]; POOLE STEPHEN [GB] BAXTER INT [US];) 5 July 2001 (2001-07-05) abstract page 18, lines 11-15; example 6 claims 1,8,9,23,24	1-12

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/060222

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SUGAWARA SHUNJI ET AL: "INNATE IMMUNE RESPONSES IN ORAL MUCOSA" JOURNAL OF ENDOTOXIN RESEARCH, CHURCHILL LIVINGSTONE, EDINBURGH, GB, vol. 8, no. 6, 2002, pages 465-468, XP009078070 ISSN: 0968-0519 abstract. page 465, paragraph 3 - page 466, left-hand column; figure 1 page 466, right-hand column, paragraph 3 - page 467, right-hand column, paragraph 1	1-12
Y	OGAWA TOMOHIKO ET AL: "Cell activation by Porphyromonas gingivalis lipid A molecule through Toll-like receptor 4- and myeloid differentiation factor 88-dependent signaling pathway." INTERNATIONAL IMMUNOLOGY NOV 2002, vol. 14, no. 11, November 2002 (2002-11), pages 1325-1332, XP002434613 ISSN: 0953-8178 abstract	1-12
Y	WANG P-L ET AL: "Porphyromonas gingivalis lipopolysaccharide signaling in gingival fibroblasts-CD14 and Toll-like receptors." CRITICAL REVIEWS IN ORAL BIOLOGY AND MEDICINE: AN OFFICIAL PUBLICATION OF THE AMERICAN ASSOCIATION OF ORAL BIOLOGISTS 2002, vol. 13, no. 2, 2002, pages 132-142, XP002434614 ISSN: 1045-4411 abstract	1-12

INTERNATIONAL SEARCH REPORT

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 26 partially
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

- 1. claims: 1-12
method of distinguishing among oral bacteria

- 2. claims: 13-25
method for determining the the anti-inflammatory effect of an agent.

- 3. claim: 26
method of reducing or preventing the inflammation of an oral tissue.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/060222

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9513094	A	18-05-1995	CA 2176237 A1 EP 0728017 A1 JP 9511220 T US 5840302 A	18-05-1995 28-08-1996 11-11-1997 24-11-1998
WO 2005057222	A	23-06-2005	AU 2004297914 A1 CA 2546126 A1 EP 1701978 A2	23-06-2005 23-06-2005 20-09-2006
WO 0155386	A	02-08-2001	AU 2866501 A EP 1250436 A1 US 2003022302 A1	07-08-2001 23-10-2002 30-01-2003
US 5578295	A	26-11-1996	NONE	
EP 0528468	A1	24-02-1993	AT 135569 T AU 2081592 A CA 2075551 A1 DE 69209190 D1 DE 69209190 T2 JP 5194203 A US 5240696 A	15-04-1996 11-02-1993 09-02-1993 25-04-1996 08-08-1996 03-08-1993 31-08-1993
WO 0148481	A1	05-07-2001	AU 4903801 A EP 1234181 A1 JP 2003518627 T	09-07-2001 28-08-2002 10-06-2003

From the
INTERNATIONAL SEARCHING AUTHORITY

To: see form PCT/ISA/220	PCT WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)
Date of mailing (day/month/year) see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	FOR FURTHER ACTION See paragraph 2 below
International application No. PCT/US2007/060222	International filing date (day/month/year) 08.01.2007
Priority date (day/month/year) 10.01.2006	
International Patent Classification (IPC) or both national classification and IPC INV. G01N33/569 G01N33/50 C12Q1/04 G01N33/68	
Applicant SREENIVASAN, Prem	

1. This opinion contains indications relating to the following items:
- ☒ Box No. I Basis of the opinion
 - ☐ Box No. II Priority
 - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☒ Box No. IV Lack of unity of invention
 - ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - ☐ Box No. VI Certain documents cited
 - ☐ Box No. VII Certain defects in the international application
 - ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Date of completion of this opinion see form PCT/ISA/210	Authorized Officer Boiangiu, Clara Telephone No. +31 70 340-2085
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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

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International application No.
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Box No. 1 Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in electronic form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No. 41
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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 26 partially

because:

☒ the said international application, or the said claims Nos. 26 partially relate to the following subject matter which does not require an international search (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for the whole application or for said claims Nos. 26 partially

☐ a meaningful opinion could not be formed without the sequence listing: the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings: the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:
- ☒ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☐ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☒ all parts.
 - ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes: Claims 1-12
No: Claims 13-26

Inventive step (IS)

Yes: Claims
No: Claims 1-26

Industrial applicability (IA)

Yes: Claims 1-26
No: Claims

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)

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Re Item III.

1. In view of the lack of unity (see item IV) and since all the inventions have been paid an opinion will be given on the searched subject-matter; therefore, the subject-matter of claims 1-25 fully and 26 partially (see also below; point 2.) (Rule 66.1(e) PCT).
2. For the assessment of the present claim 26 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV.

1. The separate inventions/groups of inventions are:

claims 1-12: method of distinguishing among oral bacteria
claims 13-25: method for determining the anti-inflammatory effect of an agent.
claim 26: method of reducing or preventing the inflammation of an oral tissue.
2. They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:
3. Reference is made to the following documents:

D4: SUGAWARA SHUNJI ET AL: JOURNAL OF ENDOTOXIN RESEARCH,
CHURCHILL LIVINGSTONE, EDINBURGH, GB, vol. 8, no. 6, 2002, pages 465-
468, XP009078070 ISSN: 0968-0519
D5: UEHARA A ET AL: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, BERLIN,
DE, vol. 189, no. 4, September 2001 (2001-09), pages 185-192, XP009078055
4. The problems to be solved in the present application relate to "Distinguishing among

oral bacteria" and "Determining the anti-inflammatory effect of an agent". The single general concept which can be identified a priori as linking the various claimed inventions and which forms a solution to the above-mentioned problem relates to "The notion that after contacting a cell with a bacterium or portion of it an indicator compound can be detected".

5. However, D4 as well as D5 disclose such a notion. Thus, D4 (as well as D5) discloses a method (an assay) comprising contacting cells (gingival or not) with a portion of bacteria (a bacterial cell surface) and then detecting (measuring) the presence of an indicator compound (such as cytokine)(D4: page 466, left-hand column paragraph 4 - right-hand column, paragraph 1; page 466, right-hand column, paragraph 4 - page 467, left-hand column, paragraph 3; figures 1 and 2; D5: page 187, left-hand column, paragraphs 2 and 3; figures 3-7). In the light of D4 and D5, each document taken alone, the above identified single general concept is not novel and inventive and can thus not be the single general inventive concept as required by Rule 13.1 PCT.
6. The present application is therefore considered not to fulfill the requirement of unity as laid down in Rule 13.1 PCT.
7. Moreover, no special technical features could be found in common between the subject-matters of claim 1 and claim 26. Thus claim 1 discloses a method of distinguishing among oral bacteria species, while claim 26 discloses a method of reducing the inflammation in oral tissue, but none of the technical feature of claim 1 could be found in claim 26. Therefore, there is no single general concept that links the method of claim 1 to the claimed compounds of claim 26.
8. Consequently the groups of inventions are split up as follows:
 - 1) claims 1-12: method of distinguishing among oral bacteria
 - 2) claims 13-25: method for determining the anti-inflammatory effect of an agent.
 - 3) claims 26: method of reducing or preventing the inflammation of an oral tissue.
9. No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as special

technical feature within the meaning of Rule 13.2 PCT.

10. The search for subjects 2)-3) represent a major extra search burden. In consequence the applicant was invited to pay 2 additional search fees and accepted to do so.

Invention 1: claims 1-12

Re Item III.

1. Reference is made to the following documents:

- D1: UEHARA A ET AL: JOURNAL OF MEDICAL MICROBIOLOGY AUG 2002, vol. 51, no. 8, August 2002 (2002-08), pages 626-634, XP002434615 ISSN: 0022-2615
- D2: WEINBERG A ET AL: CRITICAL REVIEWS IN ORAL BIOLOGY AND MEDICINE, CRC PRESS, BOCA RATON, FL, US, vol. 9, no. 4, November 1998 (1998-11), pages 399-414, XP000972953 ISSN: 1045-4411
- D3: MADIANOS P N ET AL: JOURNAL OF CLINICAL PERIODONTOLOGY 2005, vol. 32 Suppl 6, 2005, pages 57-71, XP002435073 ISSN: 0303-6979
- D4: SUGAWARA SHUNJI ET AL: JOURNAL OF ENDOTOXIN RESEARCH, CHURCHILL LIVINGSTONE, EDINBURGH, GB, vol. 8, no. 6, 2002, pages 465-468, XP009078070 ISSN: 0968-0519
- D5: UEHARA A ET AL: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, BERLIN, DE, vol. 189, no. 4, September 2001 (2001-09), pages 185-192, XP009078055
- D6: WO 01/48481 A1 (BAXTER INT [US]; PATEL MEHUL [US]; POOLE STEPHEN [GB] BAXTER INT [US];) 5 July 2001 (2001-07-05)

2. Novelty

2.1 Claim 1

- 2.1.1 The document D1 (as well as D2) is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (see references of the search report):

A method of determining the human gingival cells production of cytokines in response to different bacterial species. The method of D1 **comprising** contacting the gingival cells with portions of a bacterium (such as, e.g. LPS, LTA) and detecting the presence of an indicator compound (such as TLRs, cytokines,...for D1, or hBD-1, hBD-2, IL-8...for D2) (D1: abstract; page 626, right-hand column, paragraph 1 - page 627, left-hand column, paragraph 1; page 628, left-hand column, paragraph 2 - right-hand column, paragraph 1; D2: page 408, right-hand column, paragraph 2 - page 410, right-hand column, paragraph 3; figures 6,7,9).

D1 further teaches, that different oral bacterial species have different potentials in activating gingival host cells (D1: figures 1; D2: figures 6,7,9).

2.1.2 The subject-matter of claim 1 therefore differs from this known D1 (or D2) in that:

That in claim 1 the method is used to distinguish among bacterial species.

2.1.3 Therefore, the subject-matter of claim 1 is new (Article 33(2) PCT).

3. Inventive step

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-12 does not involve an inventive step in the sense of Article 33(3) PCT.

3.2 Claim 1

3.2.1 The technical effect deriving from the above-mentioned difference (item 2.1.2) is distinguishing between deleterious and non deleterious oral bacterial species.

The problem to be solved by the present invention may therefore be regarded as providing a method allowing distinguishing deleterious and non deleterious oral bacterial species.

The solution to this problem can be found in detecting an indicator compound after contacting oral bacteria with gingival cells.

3.2.2 As mentioned before D1 as well as D2 already clearly teaches that wall fragments (the

bacterial virulence factors) from different oral bacteria species induce the production of different indicators and show considerable variability in the degree to which they activate those indicators compounds (such as the inflammatory mediators). For example D2 teaches that *Fusobacterium nucleatum* activates IL-8 in gingival epithelial cells, while *Porphyromonas gingivalis* inhibits gingival epithelial cell IL-8 production.

3.2.3 Moreover, it is a general common knowledge (see D3) that, due to the variation of the bacterial virulence factors, **different bacterial species** have **different potentials** in activating gingival host cells (D3: abstract; page 58, left-hand column, paragraph 1; page 58, middle column, paragraph 1 - page 59, right-hand column, paragraph 1; page 59, right-hand column, paragraph 2; page 60, left-hand column, paragraph 3; page 60, middle column, paragraphs 2,3; page 60, right-hand column, paragraph 3 - page 61, left-hand column, paragraph 1; page 63, right-hand column, paragraph 2 - left-hand column, paragraph 1; figures 1,2; page 65, right-hand column, paragraph 2; figure 3). Moreover, D3 also clearly teaches that Several hundred different bacterial species colonize the sulcus and the periodontal pockets. However, some of these bacteria are protective for the host cell, and only a part of these bacteria may be pathogenic and stimulate host cells to activate a wide range of inflammatory responses.

3.2.4 Therefore, implicitly D3 distinguishes the deleterious from the non deleterious oral bacteria by the fact that the first type of bacterium (the deleterious bacteria) induces an inflammatory response and the second type (the non deleterious bacteria) does not, but even they can have a protective role for the host cells.

3.2.5 Because document D3 clearly suggests that the additional technical feature solves the problem posed combining it with the method or assay of document D1 (or D2) is obvious for a person skilled in the art, therefore the subject-matter of claim 1 does not involve an inventive step in the sense of Article 56 EPC.

3.3 Claims 2-12

3.3.1 Dependent claims 2-12 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT) for the following reasons:

3.3.2 The features of dependent claims 2-12 are either known from D1-D6 or fall within the knowledge and the ability of a person skilled in the art.

4. Industrial applicability

The subject-matter of claims 1-12 is considered as industrially applicable, because it can be used or made in the medical or pharmaceutical industry or research (Article 33(4) PCT).

Re Item VIII.

1. It is clear from the description on page 4 that the following feature is essential to the definition of the invention:

(1) indicator materials (or compounds) that are generated by in the ordinary course of a host's immune response.

Since independent claim 1 does not contain this feature, it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

Invention 2: claims 13-25

Re Item III.

1. Reference is made to the following documents:

D7: WO 95/13094 A (SQUIBB BRISTOL MYERS CO [US]) 18 May 1995 (1995-05-18)

D8: WO 2005/057222 A (WHITEHEAD BIOMEDICAL INST [US]; HUG CHRISTOPHER [US]; LODISH HARVEY F) 23 June 2005 (2005-06-23)

D9: WO 01/55386 A (GLAXOSMITHKLINE [GB]; LEWIS ALAN PETER [GB]; RAY

KEITH PAUL [GB]) 2 August 2001 (2001-08-02)

D10: US-A-5 578 295 (FRANCIS MARION D [US] ET AL) 26 November 1996 (1996-11-26)

D11: EP-A1-0 528 468 (UNILEVER NV [NL]; UNILEVER PLC [GB]) 24 February 1993 (1993-02-24)

D12: HOLT R ET AL: THE WESTERN JOURNAL OF MEDICINE APR 2001, vol. 174, no. 4, April 2001 (2001-04), pages 288-290, XP002443683 ISSN: 0093-0415

2. Novelty

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 13 is not new in the sense of Article 33(2) PCT.

3.2 Document D7 (as well as document D8) discloses (see references in the Search Report):

A method for determining the anti-inflammatory effect of an agent (therefore, determining the ability of the agent for preventing/treating/modulating) the chronic inflammatory diseases associated with infections by such bacteria, e.g. periodontitis, gingivitis) comprising contacting the cell (endothelial cells) with the agent in the presence of a bacterium (e.g. *P. gingivalis* or *H. pylori*) or portion of such bacterium and detecting the presence of an indicator compound (selectin) (D7:

page 13, line 33 - page 14, line 32; claims 11-21; page 5, line 10 - page 6, line 36; page 10, line 24 - page 11, line 33; page 18, lines 1-24).

3.3 The subject-matter of claim 13 is therefore not new (Article 33(2) PCT).

4. Inventive step

4.1 Dependent claims 14-25 do not contain any features which, in combination with the features of claim 1, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT) for the following reasons:

4.2 The features of dependent claims 14-25 are either known from D1-D12 or fall within the knowledge and the ability of a person skilled in the art.

5. Industrial applicability

The subject-matter of claims 13-25 is considered as industrially applicable, because it can be used in the medical or pharmaceutical industry (Article 33(4) PCT).

Invention 3: claims 26

1. Reference is made to the following documents:

D10: US-A-5 578 295 (FRANCIS MARION D [US] ET AL) 26 November 1996
(1996-11-26)

D11: EP-A1-0 528 468 (UNILEVER NV [NL]; UNILEVER PLC [GB]) 24 February
1993 (1993-02-24)

D12: HOLT R ET AL: THE WESTERN JOURNAL OF MEDICINE APR 2001, vol.
174, no. 4, April 2001 (2001-04), pages 288-290, XP002443683 ISSN: 0093-
0415.

2. Novelty

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 26 is not new in the sense of Article 33(2) PCT.

3.2 Document D10 (as well as document D11 or D12) discloses (see references in the Search Report):

A method of reducing or preventing the inflammation of an oral tissue by applying to the oral tissue a composition comprising triclosan in a concentration that results in application of the triclosan at sub-MIC levels (D10: column 3, line 40-column 5, line 24; claim 22; examples I-III).

3.3 The subject-matter of claim 26 is therefore not new (Article 33(2) PCT).

3. Industrial applicability

The subject-matter of claim 26 is considered as industrially applicable, because it

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2007/060222

can be used in the medical or pharmaceutical industry (Article 33(4) PCT).

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: **United States of America**

This public document

2. has been signed by **Norman Goodman**

3. acting in the capacity of **County Clerk**

4. bears the seal/stamp of the county of **New York**

Certified

5. At New York, New York 6. the 30th day of October 2001

7. by Special Deputy Secretary of State, State of New York

8. No. NYC-9786540B

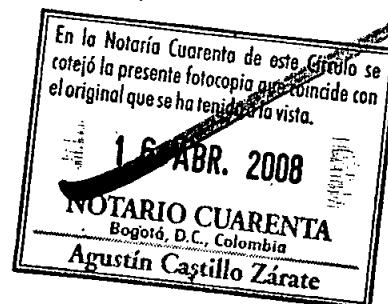
9. Seal/Stamp

10. Signature

Aqil M. Qureshi

Aqil M. Qureshi

Special Deputy Secretary of State



FROM : BRIGARD Y CASTRO

PHONE NO. : 3802700

OCT. 29 2001 01:09PM P2

Brigard & Castro

SANTA FE DE BOGOTA - COLOMBIA

PODER

POWER OF ATTORNEY

Yo (nosotros)

I (We)

domiciliado(s) en

COLGATE-PAULMOLIVE COMPANY, a Delaware corporation, of 300 Park Avenue, New York, New York 10022, U. S. A.

Nombro(amos) a: RAMIRO CASTRO DUQUE y/o JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO S.

Hereby appoint RAMIRO CASTRO DUQUE and/or JUAN PABLO CADENA SARMIENTO and/or BEATRIZ JARAMILLO S.

de Santafé de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñanzas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

of Santafé de Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names and emblems, registration of copyright and contracts related thereto, certificates of obtainers of vegetables varieties, as well as extensions, renewals and recordal of assignments and changes of name and domicile related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or exploitation of licenses, action of unfair competition; penal demands, actions of indemnities for damages; claims or observations on patent applications, models, industrial designs and utility models; in the exercise of preventive measures; and in other actions or similar proceedings.

A este efecto, lo(s) faculta(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeren conducentes al resguardo de mis (nuestros intereses), con todas las demás facultades legales necesarias.

To that effect they are hereby empowered to file applications, formulate descriptions, amendments, protests, declarations, oppositions, cancellations, nullities, appeals and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights granted or in the process of granting; to receive all kind of documents and valuables, giving the respective release of receipt, and, in general, to take before said authorities the necessary steps to such effect, and any measures that they may deem pertinent to the safeguard of my (our) interest(s) with all the other necessary legal powers.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer, y la de sustituirlo en todo o en parte y revocar sustituciones. Asimismo nombro(amos) a: RAMIRO CASTRO DUQUE y/o JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO S. domiciliado(s) en la Carrera 7 No. 16-56 Piso 9, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales.

This power includes the faculty to ratify or confirm desist, settle and compromise on my (our) behalf, and to substitute this power in whole or in part and to revoke substitutions. At the same time I (We) hereby appoint RAMIRO CASTRO DUQUE and/or JUAN PABLO CADENA SARMIENTO and/or BEATRIZ JARAMILLO S. domiciled in Carrera 7 No. 16-56, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

Otorgado y firmado en

Granted and signed in New York, N.Y.

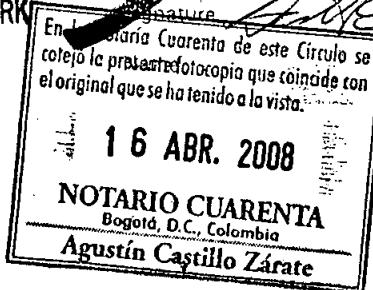
hoy de

de 2001
VICE PRESIDENT &
ASSOCIATE GENERAL COUNSEL
TRADEMARK

this OCT 29 2001 day of 2001

Firma

Nombre



SCOTT E THOMPSON

CERTIFICADO NOTARIAL (SOCIEDAD)

NOTARIAL CERTIFICATE (CORPORATION)

Hoy de de
personalmente compareció ante mí

a quien conozco, y de quien me consta que firmó el anterior documento, debidamente juramentado declaró y dijo que él es

de

Sociedad legalmente constituida para un período de duración que aún no ha terminado; que el declarante está autorizado por dicha compañía para conferir este poder; que el sello estampado por él al pie de tal poder, en nombre y representación de la expresada compañía, es el sello oficial de ella; y que el objeto para el cual se confiere este instrumento está comprendido dentro del de la sociedad.

El suscrito Notario hace constar que tuvo a la vista las pruebas de la existencia de la sociedad.

Estado de
con fecha

y que quien confiere el presente poder es su representante.

On this OCT 29 2001 day of of
personally appeared before me

to me known, and known to me to be the person who signed the foregoing instrument who being by me duly sworn did depose and say that he (she) is the

VICE PRESIDENT &
ASSOCIATE GENERAL COUNSEL
TRADEMARK
COLGATE-PALMOLIVE COMPANY

Which is legally constituted for a term not yet expired, that deponent is authorized by said Company to confer this Power of Attorney: that the seal affixed hereunto by the deponent in the name and on behalf of said Corporation is the corporate seal thereof; and that the purpose for which this instrument is granted is included within the corporate purpose of company.

The undersigned Notary Public attests that he has seen the documents that prove the existence of the company.

State of Delaware
under date of May 13, 1997

and that he (she) who grants the present power of attorney is its Representative.

Notario Público (sello)

Rosie S. Dizon
Notary Public (Seal)

CERTIFICADO NOTARIAL
PERSONA NATURAL

ROSIE S. DIZON
Notary Public, State of New York
No. 31-4941117
Qualified in New York County
Commission Expires Aug. 15, 2002

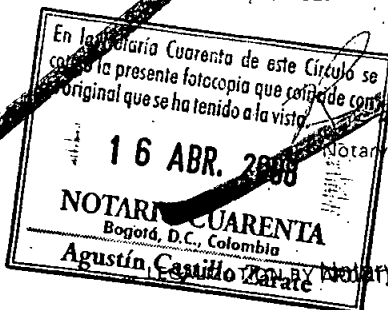
Hoy de de , compareció(eron)
ante mí

a quien (es) conozco y me consta que es(son) la(s) persona(s) firmante(s) del documento que precede y quien(es) declara(n) que otorgan el mismo para los fines y propósitos que en él se expresan.

Notario Público (sello)

On this OCT 29 2001 day of of
personally appeared before me

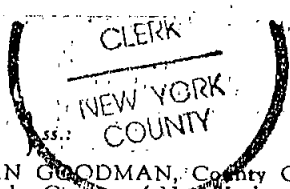
SCOTT E THOMPSON
who is known to me and who I attest executed the foregoing document and he (they) declared he (they) grant(s) the document for the uses and purposes therein expressed.



ROSIE S. DIZON
Notary Public, State of New York
No. 31-4941117
Qualified in New York County
Commission Expires Aug. 15, 2002

LEGALIZACION POR APOSTILLA ES REQUERIDA.

State of New York
County of New York



252373

Form 1

I, NORMAN GOODMAN, County Clerk and Clerk of the Supreme Court of the State of New York, in and for the County of New York, a Court of Record, having by law a seal, DO HEREBY CERTIFY pursuant to the Executive Law of the State of New York, that

Rosie S. Dizon

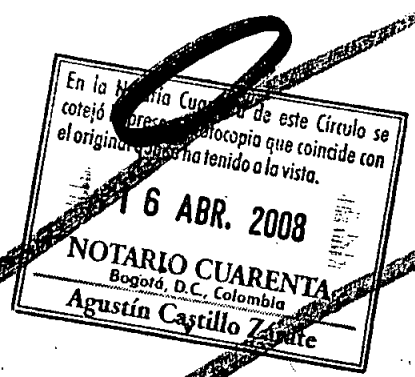
whose name is subscribed to the annexed affidavit, deposition, certificate of acknowledgment or proof, was at the time of taking the same a NOTARY PUBLIC in and for the State of New York duly commissioned, sworn and qualified to act as such; that pursuant to law, a commission or a certificate of his official character, with his autograph signature has been filed in my office; that at the time of taking such proof, acknowledgment or oath, he was duly authorized to take the same; that I am well acquainted with the handwriting of such NOTARY PUBLIC or have compared the signature on the annexed instrument with his autograph signature deposited in my office, and I believe that such signature is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand affixed my official seal this

OCT 30 2001

FEE PAID \$3.00

Norman Goodman
County Clerk and Clerk of the Supreme Court, New York County



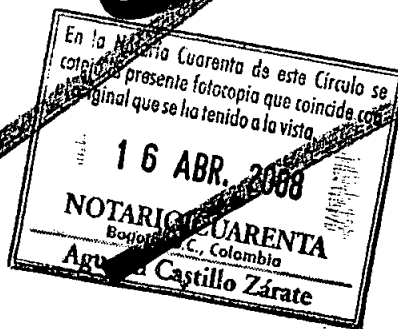
APOSTILLE

(Convención de La Haya del 5 de Octubre de 1961)

1. País: ESTADOS UNIDOS DE AMERICA
Este documento público
2. ha sido firmado por **Norman Goodman**
3. actuando en capacidad de **Oficial del Condado**
4. lleva el sello/timbre del condado de **New York**

CERTIFICADO

5. en New York, New York
6. el 30 de Octubre de 2001
7. por Secretario de Estado Encargado Especial, Estado de New York
8. No. NYC-9786540B
9. Sello/Timbre
10. Firma
(Firma)
Aquil M. Qureshi
Secretario de Estado Encargado Especial



TRADUCCIÓN SIMPLE AL ESPAÑOL del Certificado Notarial y el la apostilla que vienen junto con el poder dado por **Colgate-Palmolive Company**, a Delaware Corporation, domiciliada en 300 Park Avenue, New York, New York, Estados Unidos de América a **Ramiro Castro Duque y/o Juan Pablo Cadena Sarmiento y/o Beatriz Jaramillo S.** domiciliados en la Carrera 7 # 16-56 P. 9, Bogotá.

Certificado Notarial

Yo, NORMAN GOODMAN, Oficial del Condado y Oficial de la Corte Suprema del Estado de New York, en y para el Condado de New York, un Tribunal de Registros, que tiene un sello por ley.

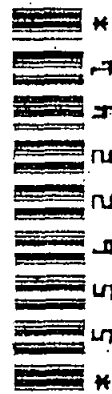


GAVIRI A

ORGANIZACION ELECTORAL
REGISTRADURÍA NACIONAL DEL ESTADO CIVIL

REGISTRO CIVIL DE DEFUNCIÓN

Indicativo
Serial 5562241



Datos de la oficina de registro							
Clase de oficina:	Registraduría	Notaría	X	Consulado	Corregimiento	Insp. de Policía	Código 9795
País - Departamento - Municipio - Corregimiento a/o Inspección de Policía							
COLOMBIA CUNDINAMARCA BOGOTA D.C.				NOTARIA 32.			

Datos del inscrito	
Apellidos y nombres completos	
CASTRO DUQUE RAMIRO	
Documento de identificación (Clase y número)	Sexo (en Letras)
C.C.No. 170322 DE BOGOTA	MASCULINO

Datos de la defunción			
Lugar de la Defunción: País - Departamento - Municipio - Corregimiento a/o Inspección de Policía			
COLOMBIA CUNDINAMARCA BOGOTA D.C.			
Fecha de la defunción		Hora	Número de certificado de defunción
Año 2004	Mes 10	Día 30 04:10 PM	A- 2094375.
Presunción de muerte			
Juzgado que profiere la sentencia		Fecha de la sentencia	
-----		Año Mes Día	
Documento presentado		Nombre y cargo del funcionario	
Autorización judicial	Certificado Médico X	ANDRES DIAZ CAMPOS.- R.M. No. 79982662	

Datos del denunciante	
Apellidos y nombres completos	
EDGAR DAVID REGINO H.	
Documentos de identificación (Clase y número)	Firma
C.C.No. 15.030.154 DE LORICA/ CORDOBA	

Primer testigo	
Apellidos y nombres completos	

Documentos de identificación (Clase y número)	Firma
-----	-----

Segundo testigo	
Apellidos y nombres completos	

Documentos de identificación (Clase y número)	Firma
-----	-----

Fecha de inscripción		Nombre y firma del funcionario que autoriza	
Año 2004	Mes 11	Día 02	BLANCA LUCIA RESTREPO

PADRES: ALFON SO CASTRO		ESPACIO PARA NOTAS
MERCEDES DUQUE.		

ORIGINAL PARA LA OFICINA DE REGISTRO

NOTARIA 32

DEL CIRCULO DE SANTA FE DE BOGOTA, D.C.

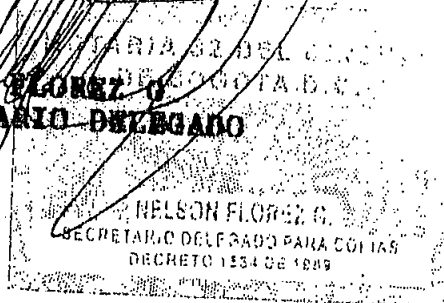
Germán Eleázar Moreno L.
Notario

COMO SECRETARIO DELEGADO DE LA NOTARIA TREINTA Y DOS (32) DEL
CIRCULO DE BOGOTA

CERTIFICO QUE:

La presente acta es fiel copia del original tomada del registro
civil de defunciones serial indicativo número: 5562241
que expido en la ciudad de Bogotá D.C. hoy: 05 NOV. 2004

NELSON FLORES G.
SECRETARIO DELEGADO





No. 08-070809-00000-0000

Fecha: 2008-07-09 16:57:02 Dep. 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 57



Industria y Comercio
SUPERINTENDENCIA

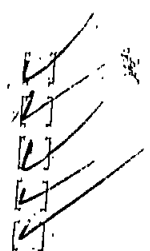
REQUISITOS MINIMOS PARA ADMISION A TRAMITE PCT

PATENTE DE INVENCION



MODELO DE UTILIDAD []

- ◆ Indicación de que se solicita la concesión de una patente
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ◆ Descripción de la invención
- ◆ Dibujos de ser estos pertinentes
- ◆ Comprobante de pago de las tasas establecidas

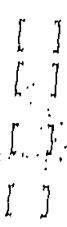


COMPLETA []

INCOMPLETA []

DISEÑO INDUSTRIAL []

- ◆ Indicación de que se solicita el registro de Diseño Industrial
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ◆ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño
- ◆ Comprobante de pago de las tasas establecidas

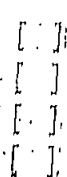


COMPLETA []

INCOMPLETA []

ESQUEMA DE TRAZADO []

- ◆ Indicación de que se solicita el registro de un Esquema de Trazado
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ◆ Representación gráfica del esquema trazado
- ◆ Comprobante de pago de las tasas establecidas



COMPLETA []

INCOMPLETA []

Marta B...

Firma Responsable

Fecha

2 de 12 / 08

Carrera 13 No. 27-00 Piso 5, 7 y 10
Commutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@slc.gov.co
www.slc.gov.co
Bogotá, D.C., Colombia

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
CADENA SARMIENTO JUAN PABLO
Apoderado(a) y/o Representante de
COLGATE - PALMOLIVE COMPANY

REFERENCIA	Radicación No.	8 70809
	Solicitud internacional	PCT/US2007/060222
	Fecha inicio fase nacional	10/08/2008
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	12145

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante, conforme a lo establecido por el artículo 26 literal k) de la Decisión 486 de la Comisión de la Comunidad Andina. Cuando se trate de documentos otorgados en el extranjero, estos deberán legalizarse de conformidad con lo dispuesto por los artículos 259 y 260 del Código de Procedimiento Civil. Cuando se trate de las declaraciones a que hace referencia la Regla 4.17 ii) del Tratado de Cooperación en Materia de Patentes PCT., el alcance de las tales declaraciones se determinará según indiquen claramente una cesión de derechos del inventor al solicitante o su causante, de conformidad con lo dispuesto en el numeral 5.2.15 del Capítulo Quinto, Título X de la Circular Única de Superintendencia de Industria y Comercio.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

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

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de
fecha 26 SET. 2008
adpall