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(54) **Title**: PROTEIC ACID POLYMER, PRODUCTION PROCESSES, USE OF PROTEIC ACID POLYMER, PHARMACEUTICAL COMPOSITION AND METHOD OF TREATMENT

(57) **Abstract**: The present invention relates to proteic acid polymers (pLNs) comprising specific properties to reduce tissue damage and improve functional recovery after injury, and the production process of said proteic acid polymers. Those pLNs are preferably obtained using the protein laminin diluted in an acidic pH in the presence of divalent cation. The use of said proteic acid polymers for the production of a drug, a pharmaceutical composition containing such pLNs and a method of treatment of animals affected by traumatic, degenerative or inflammatory tissue injuries in nervous tissue, muscle, epithelial and conjunctive are also objects of the present invention.

# Description

## PROTEIC ACID POLYMER, PRODUCTION PROCESSES, USE OF PROTEIC ACID POLYMER, PHARMACEUTICAL COMPOSITION AND METHOD OF TREATMENT

## Field of the Invention

The present invention relates to proteic acid polymer, the process of polymerization of a protein in acid medium and the use of said proteic polymer. Specifically, the polymerised protein described here is the polymerised laminin, extremely effective as promoter of tissue regeneration in humans or not humans mammals animals, due to its extraordinary anti-inflammatory effect.

The invention is also related to a pharmaceutical composition containing a proteic acid polymer, toward the treatment of humans or non-human mammals, affected by traumatic degenerative or inflammatory tissue injuries.

The use of such proteic acid polymer for the production of a drug intended mainly to the treatment of neurological, spinal cord, muscular dystrophy treatment and heart disease injuries is also an objective of this invention.

The use of such proteic acid polymer for the production of a drug intended mainly to the treatment of inflammation in nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue, epithelium-connective tissue in general, also is an objective of this invention.

The invention also refers to a method of treatment of in humans or not humans mammals animals, affected by traumatic, degenerative or inflammatory injuries in the group of tissues including nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue, epithelium-connective tissue in general, based on administration of a drug containing a proteic acid polymer in humans or non-human mammals, injured to the nervous system.

Finally, the invention refers to a method of treatment for inflammatory diseases based on the administration of a drug containing a proteic acid polymer to a humans or non-human mammals.

## Background of the Invention

Laminin is an extracellular matrix protein originally described in the 70s and has a trimeric structure, consisting of a longer chain, the alpha chain and two shorter chains, called beta and gamma. These three are associated through interactions "coiled-coil" resulting a protein of approximately 800 KDa in the cross shape. This protein was originally purified from an extract of the tumor mass from a tumor in mice called EHS, which produces an excess of a laminar structure known as basal membrane (basal lamina). Later it was discovered that the protein was first described in an isoform of a family or a group of related proteins, which now has 15 members. The isolated protein from EHS is now known as laminin-1 (LN-1) and more recently as LN111. The LN-1 is primary expressed in embryonic tissues, but is also present in adult animals, but not in the nervous tissue itself. The expression of laminin in adult nervous tissue was studied in detail in 1989. The increase of its expression is significantly related to regenerative processes in invertebrates, in regions in the mammalian brain where axonal growth occurs during adulthood.

The American patent U.S. 4,829,000, assigned to The United States of America represented by the Secretary of the Department of Health and Human Service and entitled "Reconstituted basement membrane complex with biological activity," describes the production and use of an extract of basal membrane of mice, called Matrigel, where laminin can be extracted.

The American patent U.S. 6,632,790, assigned to University of Medicine and Dentistry of New Jersey and entitled "Laminin 2 and methods for its use", describes the production and possible uses of recombinant human laminin 2, also called LN-2, and more recently LN-211. A point to be emphasized here is that the use of protein for the treatment of spinal cord injury was not anticipated in that patent. Moreover, there is no suggestion that the human recombinant

laminin 2 as well as any other isoform of protein could be polymerized in acid pH.

The American patent U.S. 5,019,087, assigned to American Biomaterials Corporation and entitled "Nerve regeneration conduit", describes tubular prostheses for regeneration of peripheral nerves. The prostheses are made of collagen I or collagen I in addition to laminin. Interestingly, as the solubilization of collagen I requires acid pH, when laminin is added, the pH is already acid. This is not done so purposeful, only circumstantial.

The International application WO 03/035675, entitled "Biologically active peptides and their use for repairing injured nerves" describes small peptides derived from the sequence of laminin-1, containing the KDI tripeptide, which can promote the recovery of movement after a spinal cord transection. This patent describes the use of only a small fragment of the protein.

In 2000, Freire and Coelho-Sampaio showed that in the temperature of 35°C, LN-1 at low concentrations (between 5nM to 60nM) is able to self-polymerize provided that the solution is acid, and more effectively, with a pH of 4.0. In this experiment was essential to have the pre-treatment of the quartz cuvette, in which the laminin was contained, with non-adherent material such as, silane. This treatment with silane was essential to maintain the laminin polymerized in its soluble state and not adhered to the quartz cuvette walls.

In 2002, Freire et al, showed that laminin polymers produced from adsorption on a glass surface in acidic pH (acid laminin matrix) played a role in migration and differentiation of embryonic neurons in cell culture or in cortical tissue explants, i.e. in vitro. This paper presents no test *in vivo* and makes no disclosure of other possible roles of such laminin polymers, such as for example, neural regeneration.

Thus, this invention seeks to fill all the gaps left in the state of the art, describing a new process, more efficient for polymerized laminin production in acid medium and employment of this polymerized protein in the treatment of spinal cord injury in animal model.

**Summary of the Invention**

It is an object of the invention, a proteic acid polymer that has anti-inflammatory and regenerative properties of traumatic, degenerative or inflammatory injuries of a tissue selected from the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue, epithelium-connective tissue in general, which in the presence of acid pH and a divalent cation polymerizes regardless of the presence of the cell membrane and extracellular medium.

It is an additional object of this invention a process of production of proteic polymers in a solution containing acid pH, from a protein concentration of from 80 nM to 1 μM, performed at temperatures from 10 to 35 °C in acid pH in the presence of a divalent cation, for a maximum time of 12 hours and occurring within containers produced with inert material, contaminant-free and without pre-treatment with anti-adherent substances.

Another object of the present invention concerns a pharmaceutical composition containing a pharmaceutically effective amount of a proteic acid polymer and non-active components, which are pharmaceutically acceptable.

It is still an object of this invention, the use of such proteic acid polymer for the production of a useful drug in the treatment of traumatic, degenerative or inflammatory injuries of a tissue selected from the group of tissues comprising nervous tissue, muscle tissue, smooth muscle, cardiac muscle, epithelium of coating, adipose tissue, epithelium-connective tissue in general, such as the treatment of spinal cord and pulmonary injuries, muscular dystrophy and heart disease in general, based on the application of a therapeutically effective amount of proteic polymers, on the injured region of an injured human or non-human mammal.

It is still an object of this invention, the use of such proteic acid polymer for production of a drug useful in the treatment of inflammation of a tissue selected from the group of tissues comprising nervous tissue, skeletal muscle tissue, smooth muscle, cardiac muscle, epithelium lining, adipose tissue, epithelium conjunctive in general, based on the administration of a

therapeutically effective amount of proteic polymer in an injured human or non-human mammal.

Another object of this invention is a treatment method of traumatic, degenerative or inflammatory injuries of a tissue selected from the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general based on the administration of a drug comprising a proteic acid polymer to a human or non-human mammal, with a nervous system injury.

The last object of this invention is a treatment method of inflammation of a tissue selected from the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, based on administration of a drug containing a proteic acid polymer to a human or non-human mammal.

**Description of the Drawings**

Figure 1 shows a graph of BBB score versus weeks after injury, demonstrating the effectiveness of the laminin acid polymer in the open field locomotion test performance improvement (BBB) after spinal cord injury by compression. Functional recovery profiles observed after treatment with pLN (■), compared to control: laminin diluted in pH 7.0 (●), acid buffer without protein (▼), neutral buffer without protein (▲).

Figure 2 shows the recovery of the nerve tissue where the spinal cord injury by compression was induced, after application of the laminin acid polymer. In A - control treated with acid buffer only; in B - animal treated with laminin acid polymer; Both animals tested 8 weeks after injury.

Figure 3 shows the effect of inhibition of GFAP protein promoted by treatment with laminin acid polymer. In A can be observed the area of cystic cavity formed 8 weeks after injury in control animal treated with acid buffer only. There is also a high expression of GFAP. In B there is a decrease of cavity area in treated animals with the laminin acid polymer and the decrease of GFAP expression. Both animals tested 8 weeks after injury.

Figure 4 shows the neuronal regeneration indicator protein expression, GAP-43, 8 weeks after injury. In A, control animals treated with acid buffer only; in B animals treated with laminin acid polymer.

Figure 5 shows the marking for macrophages (ED1 antibody), 8 weeks after injury. In A, control animals treated with acid buffer only, in B, animals treated with the laminin acid polymer. The result indicates that the effect of treatment with the laminin acid polymer includes an inflammation reduction by macrophages infiltration inhibition in the injury region.

Figure 6 shows the serum levels of C-reactive protein a week after injury. The analysis of the values obtained in each condition shows that treatment with laminin acid polymer promotes a reduction in systemic inflammation, supporting the hypothesis that the functional improvement observed in treated animals involves an anti-inflammatory effect of the polymer.

Figure 7 shows the light scattering spectra of acid polymer samples formed by recombinant human LN-2 (continuous line) or control at pH 7 (dashed line).

Figure 8 shows the BBB score versus weeks after transection injury. Observed functional recovery profiles are also shown: animals treated with PLN-1 (▲),laminin polymers purified from human placenta (■) and PLN-2 (●). Control shows the recovery achieved in the absence of laminin (▼).

Figure 9 shows the general anti-inflammatory effect of pLN in the body, giving the total number of cells in the bronchoalveolar wash of mice subjected to inhalation of LPS. A: LPS + Buffer; B: LPS + Acid Laminin; C: LPS; D: Control.

## Detailed Description of the Invention

This invention describes proteic acid polymer with anti-inflammatory and regenerative activity in traumatic, degenerative or inflammatory tissue injuries selected from the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, being formed by polymerization of a particular protein in the present of acid pH, a divalent cation

and suitable temperature, being independent of the presence of cell membrane, the basal membrane and the extracellular medium, being formed *in vitro*, in inert containers produced in any material, free of contaminants and without pre-treatment with anti-adherent substances.

The proteic acid polymers object of this invention are formed mainly by the interaction between the short arms of each molecule of laminin, which occurs without the cross linking between long and short arms of the laminin molecule.

The expression "injured region" should be understood as any animal organ formed by nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, including organs such as brain, spinal cord marrow, muscles, heart, lungs and glands.

In nature, it is also verified the existence of laminin polymers, which form a proteic matrix. The formation of this matrix occurs without any cross interactions between long and short arms of laminin. The hindrance of such interactions is achieved in natural conditions when the long arms of laminin molecules interact with the specific receptor of cell membrane. Therefore, it is essential that naturally occurs the anchoring of the laminin long arms to the receptor located in the cell membrane in order to avoid the cross interaction between long and short arms of laminin adjacent molecules.

In the case of laminin polymers object of this invention that are formed *in vitro*, inhibition of cross reactions between long and short chains of molecules of adjacent laminin is due to medium acidification in which these polymers were formed. Thus the acidification of the medium mimics the action of these receptors of the cell membrane, which are absent in the object of this invention.

In tests performed, it was verified that the tissue regenerative activity mediated by proteic acid polymers developed by this invention, is increased when the application of these proteic acid polymers on the injured area in a short period of time after injury occurs.

The greater effectiveness of the proteic acid polymers, when applied onto the injury in a short period of time after the event of traumatic, degenerative or inflammatory injury is due to anti-inflammatory capacity promoted by proteic acid polymers of this invention. This anti-inflammatory ability occurs, because such proteic acid polymers act in the maintenance of basal serum levels of C-reactive protein, and in promoting the macrophages mobilization reduction to the injured area. In tissues such as nerve tissue, this preservation is perceived by a reduction in the cystic cavity formation, a decrease in GFAP expression and the number of activated astrocytes in the damaged region. Another determinant factor of this tissue preservation is the reduction of natural inflammatory process that occurs at site of injury, demonstrated by the macrophages infiltrate reduction and maintenance of basal serum levels of C-reactive protein. The latter effects induced by proteic acid polymers of this invention, which makes the site of injury more suitable for regeneration, e.g., the axonal and bronchoalveolar regeneration.

Besides, it was found that the proteic acid polymers of this invention, are able to promote regeneration, in the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, due to activation of protein production cell routes associated with growth-43 (GAP43). It is well known that, in the nervous tissue this protein plays an important role in neurites formation, regeneration and plasticity.

Due to the mechanisms of action played by proteic acid polymer target of this invention, such polymers have an effective role, never before shown in promoting traumatic, degenerative or inflammatory injuries tissue regeneration in humans and non-human mammals.

The proteic acid polymer target of this invention can be useful in treatment of traumatic, degenerative or inflammatory injuries in a tissue selected from the group comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general . Preferably, the use of said proteic acid polymer is not

restricted to spinal cord injury and pulmonary inflammation treatments. Due to the fact that such polymers induce neuroprotection and regeneration of nerve fibers, they can be used in the treatment of traumatic or degenerative injuries of the central and peripheral nervous system, where loss of nervous tissue may occur, and in treatment of muscular dystrophy, heart disease.

One advantage of proteic acid polymer described here is its ability to self-polymerize if involved in a medium with the appropriate acid pH. Moreover, it was demonstrated that the proteic acid polymer formed due to its anti-inflammatory and regenerative capacity, is able to restore neuronal plasticity lost during development to the cerebral cortex explants of born animals, and promote the morphologic regeneration of nervous tissue, and the functional recovery of spinal injured mammals.

It was also shown that the anti-inflammatory ability of such proteic acid polymer of this invention is systemic and not merely local, because it occurs in different body tissues, such as nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, epithelium lining, adipose tissue, epithelium conjunctive in general.

The proteic acid polymer object of this invention are preferably, the most effective therapeutic agents described to date to prevent degeneration and induce regeneration of spinal cord tissue and therefore allow the recovery of locomotion and sensitivity of treated animals.

Still preferably, these proteic acid polymers of this invention are effective therapeutic agents to combat respiratory tract and nervous system inflammatory injuries.

Preferably the proteic acid polymer object of this invention are laminin polymers and LN-1 from the EHS murine tumor, the recombinant human LN-2 or human LN extracted from placenta can be used for production of these laminin polymers.

Henceforth, the proteic acid polymer will simply be called pLN, however, it should be said that this abbreviation should not mean in any case the limitation of the nature of these polymers only to the class of laminin proteins.

The production process of pLNs described here, beginning with the addition of a high concentration solution of a particular protein to be polymerized in a medium containing acid pH and a divalent cation, and should be done at temperature between 10 °C and 35 °C, not requiring the pre-treatment of the container where the polymerization will occurs with anti-adherent substances.

Besides the advantage to dispense the container pre-treatment with anti-adherent substances such as silane, where the production of pLNs will occur, the production process of such pLNs object of this invention is effective in promoting the polymerization of a high protein concentration that after being polymerized, will produce the pLN. The protein concentration used in this process vary from 80 nM to 1 μM preferably, the process is able to provide the polymerization of a protein in concentration from 90nM to 900nM; more preferably, from 95nM to 300 Nm.

Another factor that differentiates the pLNs production process object of this invention, with other protein polymerization processes in acid medium described above, is the fact that it occurs at room temperature, i.e. approximately 25°C, and is effective in pH from 3.0 and 6.0, occurring preferably at pH from 4.5 to 5.5. The acid solution used in this process, is any acid solution usually used in biochemistry, cell biology, cell culture or tissue culture or in animals *in vivo*.

The divalent cation required for the polymerization is preferably calcium, and all process of polymerization occurs at a maximum period of 12 hours, preferably the time required for the polymerization to be complete is, at maximum, 2 hours. More preferably, the process occurs in a maximum of 10 minutes.

The container required for the polymerization process object of this invention is an inert material container, free of contaminants and without pre-treatment with anti-adherents substances, such as silane. The container can be produced in any shape, among the shapes normally employed in the production

of chemical, medical and pharmaceutical containers, with the material preferably employed being plastic or glass.

The pharmaceutical composition target object of this invention contains a pharmaceutically effective amount of pLN, object of this invention, and non-active agents, such as adjuvants, stabilizers, solvents and lubricants. Preferably, the pharmaceutical composition contais a pharmaceutically effective amount of pLN, described by this invention.

In this invention, the term pharmaceutically effective amount is the amount of pNLs able to provoke a desirable effect on a human or non-human mammal.

Therefore, the pLNs target of this invention can be used in the production of a drug toward the treatment of traumatic, degenerative or inflammatory tissue injury, such tissue being selected from the group comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, preferably, among the injuries that can be treated by a drug containing the pLNs, are spinal cord injury, muscular dystrophy, heart disease and lung injuries in humans or non-human mammals.

The pNLs can also be used in drug production aimed mainly to the treatment of inflammation in nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general.

The drugs described in this invention can be prepared in any known pharmaceutical forms, provided that is allows the product application directly on the injured area.

The method of treatment subject of this invention can be used in the treatment of traumatic, degenerative or inflammatory tissue injury such tissue being selected from the group comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, lining epithelium, adipose tissue, epithelium conjunctive in general, preferably, among the injuries that can be treated by a drug containing the pLNs, are spinal cord injury, muscular dystrophy, heart disease and lung injuries in humans or non-human mammals.

According to the treatment method of this invention, from 0.1 ng/kg to 1 µg/kg of a pLN should be applied directly on the site of spinal cord injury in a period of time less than 30 days after the lesion occurance.

Preferably, from 0.5 to 500ng/kg of a pLN should be applied directly on the injured region of a mammalian animal, and even more preferably, the treatment method of this invention requires the application directly on the injured region in mammals carry a traumatic, degenerative or inflammatory injury in a amount from 1 to 250 ng/kg of a pLN, in a period of time lower than 15 days in the event of injury.

The following examples are related to tests conducted to prove the efficiency of the pLNs described, the production process of pLNs of this invention, as well as therapeutic clinical utility of such pLNs, being merely illustrative and should not be used in limiting the scope of the invention.

The tests were divided into three steps, as described below, during which were used two different types of pLNs: the PLN-1 corresponding to polymer obtained by the polymerization of protein extracted from a murine tumor EHS, and PLN-2 - corresponding to the polymer obtained from recombinant human laminin expressed in mammalian cells.

Example 1: production of pLNs

The first step of the description of the invention is the polymerization of laminin in acidic pH. The protein used can be the LN-1 extracted from the EHS murine tumor or recombinant human LN-2. The polymerization was made by diluting the protein in Tris-acetate buffer at concentration of 20 mM, pH about 4, containing 1 mM of calcium chloride, being calcium essential to the polymerization process. The protein previously partitioned in volumes sufficient for animal treatment is removed from freezer at -20 °C, and kept on ice until the moment of injection in animals, which occurs after injury, preferably between 20 and 60 minutes after injury. The acetate buffer, maintained at room temperature (25 °C), is added to the partition of laminin present in a plastic tube (Eppendorf type), and homogenized with the pipette tip. The laminin final concentration

varies between 50 to 200 μg/ml. About 5 to 10 μL of this suspension is injected into the animal preferably between 20 and 60 minutes after injury. In one assay was used the LN-1 at 100 μg/ml, buffer 4.5 pH, injected into the marrow of the animal 30 minutes after the injury, and in another assay was used the recombinant LN-2 at 120 μg/ml, buffer 4.5 pH, being injected 10 μL suspension.

Example 2: Assays

In the example reported here an injury by compression between the eighth and ninth thoracic vertebrae (T8-T9), generated when a catheter placed between the cord and vertebra is inflated was used. The catheter was a Fogarty 2F and the volume of saline solution used to inflate the catheter was 15 μL. In this case, the untreated animal loses the proper functioning of the fore-feet, which is partially recovered over 8 weeks, but remains a cystic lesion within the cord, even after the animal has reached its best ability to move at the end of the eighth week (BBB = 18). Surgery for catheter introduction was performed with the animal sedated with a cocktail of xilasine, acepromazine and ketamine for a laminectomy at the seventh thoracic vertebra (T7). After injury by compression the injections of LN-1 acid (pLN-1), pLN-2 acid (pLN-2), vehicles (acetate buffer pH 4 or tris buffer pH 7) were made. The injections were made locally (intramedullar injections) as described above.

After treatment, 10 ml of Ringer's solution was injected for hidric and ionic restoration. Subsequently, the animals were treated with antibiotic (gentamicin sulfate), to reduce the chance of infections, mainly urinary, and analgesics, to minimize the sensation of pain due to surgery.

The monitoring of locomotor function of these animals through BBB scale (Basso, Beatie and Bresnahan) revealed that the animals treated with pLN had a functional recovery much faster than those treated with vehicle. Figure 1 show that in 6 weeks the treated animals received a grade of BBB of 20, which corresponds to an almost normal locomotion, while the untreated animals had a maximum score of 17 after 8 weeks. Morphological analysis of tissue recovery shows that while the control animals (not treated with pLN) had a large cystic

cavity at the site of injury, treated animals had a tissue morphologically more organized, as seen in Figure 2.

The results documented in this invention were obtained with LN-1 extracted and purified from EHS murine tumor. As the use of a protein obtained from animal source can lead to the transfer and adaptation of animal viruses, it was proposed that human laminin should be used in therapies to human patients. Similar results to those presented here were obtained with recombinant human LN-2 and are shown in Figure 8.

Example 3: application of laminin

The application of laminin was made through a manually controlled injection so that approximately 1 μL penetrate the tissue every minute. The site was exactly the site of compression or in the proximal area of spinal cord in relation to the injury, in the case of trans-section. The injection syringe used was a Hamilton 80330 for 10 μL.

This invention is a solution for the treatment of spinal cord injuries being innovative mainly because therapeutic strategies for this type of injury does not exist. The conventional treatment currently available aims to stop the progression of initial damage, i.e., try to reduce the inflammation reaction and secondary tissue damage. This treatment promotes surgical stabilization of spine and treatment with methylprednisolone and does not result in consistent benefit for the patient. The polymerized laminin in acid pH proposed here can induce the regeneration of nerve tissue injured in a compression, contusion or trans-section of the spinal cord.

## Claims

# PROTEIC ACID POLYMER, PRODUCTION PROCESSES, USE OF PROTEIC ACID POLYMER, PHARMACEUTICAL COMPOSITION AND METHOD OF TREATMENT

1. Proteic acid polymers comprising a regenerative and an anti-inflammatory activity, being formed by polymerization of a protein in the presence of acidic pH medium and a divalent cation.
2. Polymers according to claim 1 wherein said polymerized protein is preferably a laminin.
3. Polymers according to claim 2 wherein the polymerized laminin may comprise: the LN-1 from EHS murine tumor, recombinant human LN-2, laminin extracted from human placenta, or a combination thereof.
4. Polymers according to claim 1, which have anti-inflammatory and regenerative properties in traumatic, degenerative or inflammatory injuries in a tissue selected from the group comprising the nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue, epithelium-conjunctive in general and combination thereof.
5. Process for the production of proteic acid polymers comprising the step of adding a high concentration of a particular protein to be polymerized in a acidic pH medium containing a divalent cation, performed at temperature between 10 °C and 35 °C for a maximum time of 12 hours.
6. Process according to claim 5 in which the temperature of reaction is approximately 25 °C, and the acidic medium has a pH between 3.0 and 6.0.
7. Process according to claim 5 in which calcium is the divalent cation.
8. Process according to claim 5 in which polymerization occurs to a maximum of 2 hours.
9. Process according to claim 6 comprising a pH in the range between 4.0 and 5.5.

10. Process according to claim 5 comprising the ability to polymerize proteins in a concentration between 80 nM to 1000 μM.
11. Process according to claim 10 comprising the ability to polymerize proteins in a concentration between 90 nM to 500 μM.
12. Process according to claim 11 comprising the ability to polymerize proteins in a concentration between 95 nM to 300 μM.
13. Pharmaceutical composition comprising an pharmaceutically effective amount of a proteic acid polymers and non-active components.
14. Composition according to claim 13 comprising a pharmaceutically acceptable amount of a laminin proteic acid polymers.
15. Method for treatment of traumatic, degenerative or inflammatory tissue injuries in the group of tissues comprising nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue and epithelium-conjunctive and combinations thereof comprising the application of a therapeutically effective amount of a proteic acid polymers in an mammal.
16. Method of treatment according to claim 15 comprising administering of an amount between 0.1 μg/Kg to 1 ng/Kg of a proteic acid polymers on traumatic, inflammatory or degenerative tissue injuries, such as spinal cord injury, muscular dystrophy, heart and lung injuries of a human or non human mammals.
17. Method of treatment according to claim 16 comprising administering of an amount between 0.5 μg/Kg to 500 μg/Kg of a proteic acid polymers directly on the injured area.
18. Method of treatment according to claim 17 comprising administering of an amount between 1 to 250 μg/Kg of a proteic acid polymers on traumatic, degenerative or inflammatory tissue injuries in a human or non human mammal.
19. Use of proteic acid polymers in the manufacture of a drug for the treatment of traumatic, degenerative or inflammatory injuries in the group of tissues selected from the group of nervous tissue, skeletal muscle, smooth

muscle, cardiac muscle, the lining epithelium, adipose tissue and epithelium-conjunctive and combinations thereof.

20. Use of proteic acid polymers in the manufacture of a drug for the treatment of inflammation in nervous tissue, skeletal muscle, smooth muscle, cardiac muscle, the lining epithelium, adipose tissue, epithelium-conjunctive in general and combinations thereof.

**Figure 1**

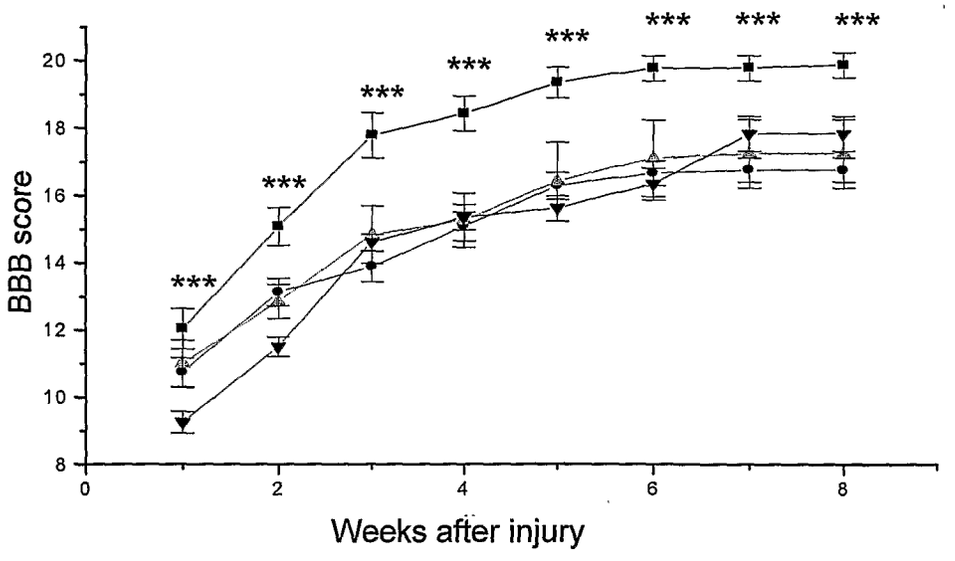



**Figure 2**

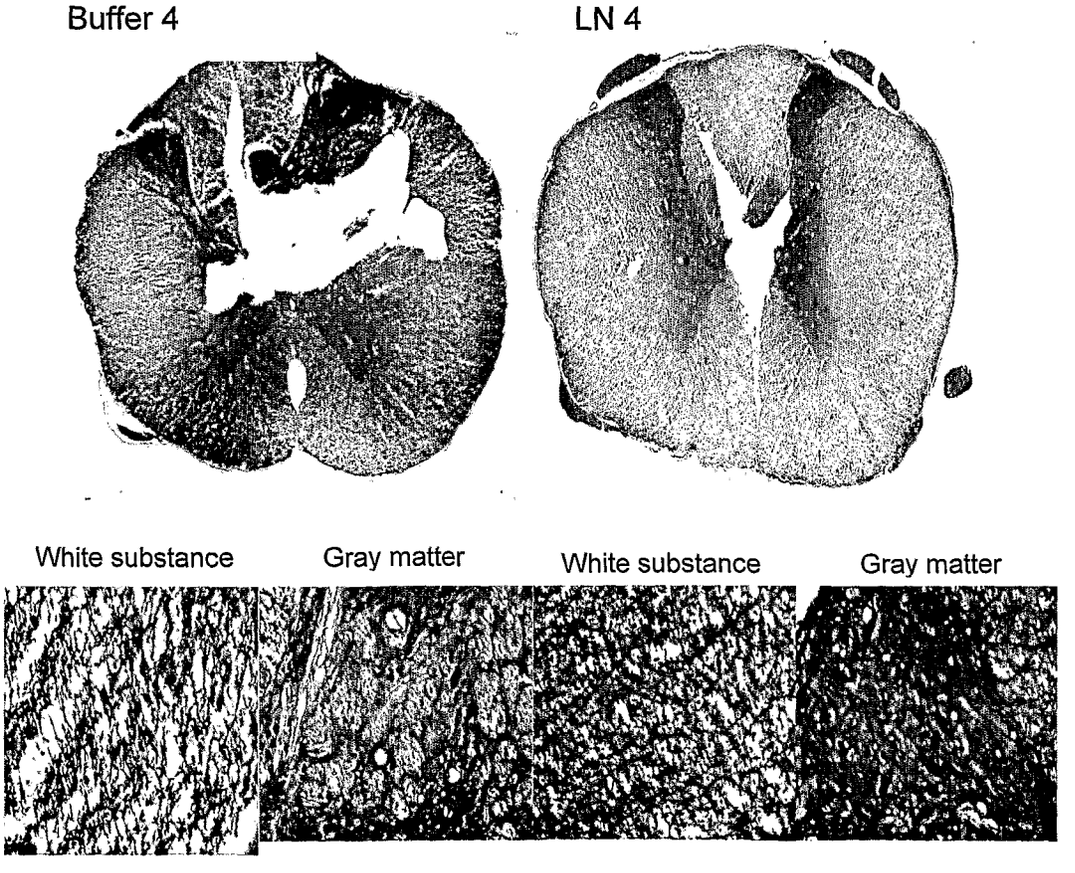


**Figure 3**

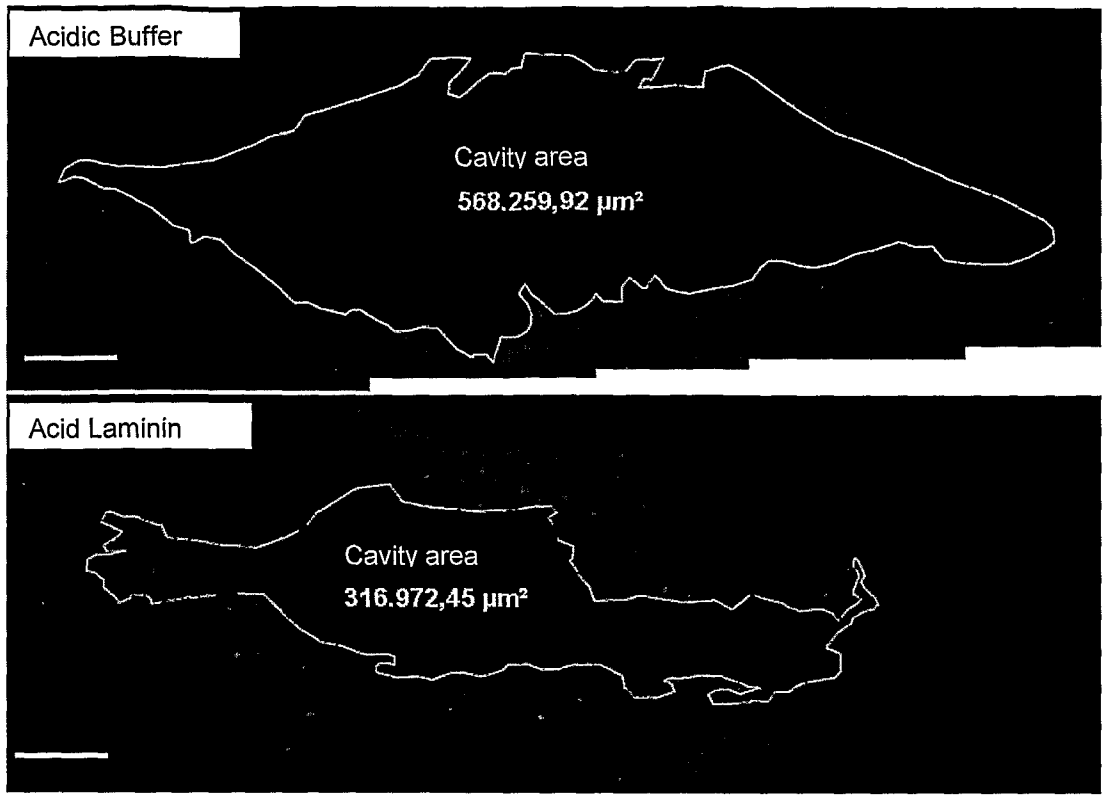



**Figure 4**

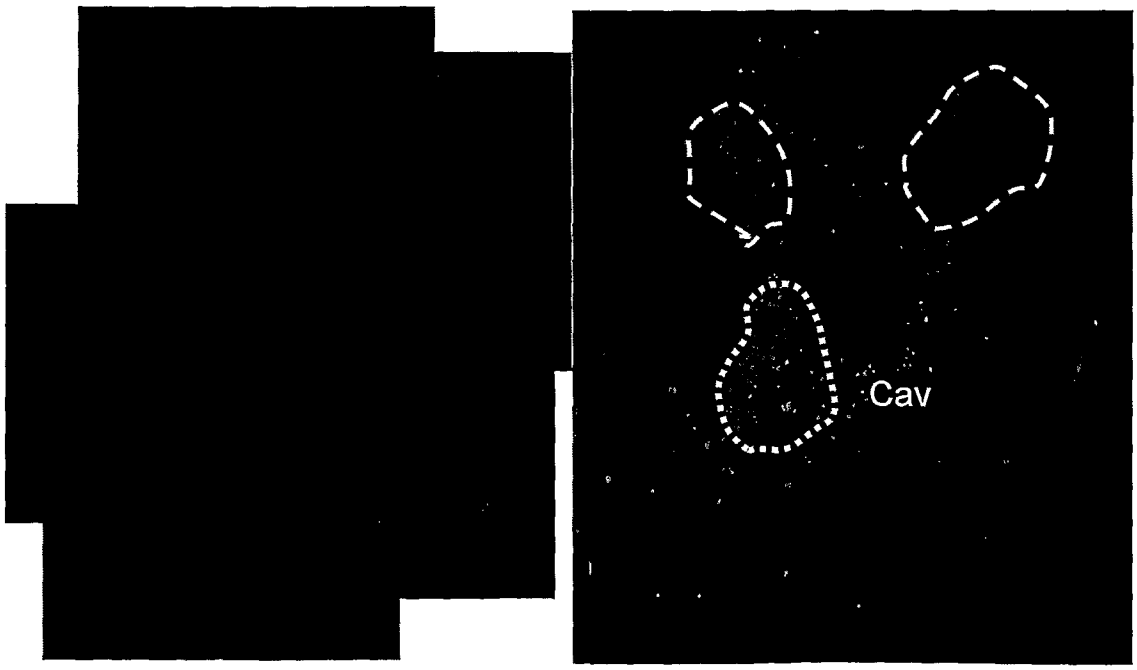


**Figure 5**

**Figure 6**

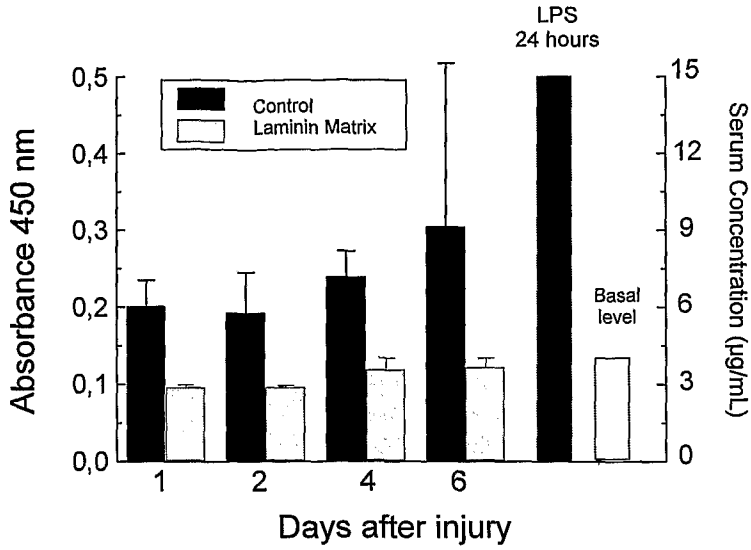



**Figure 7**

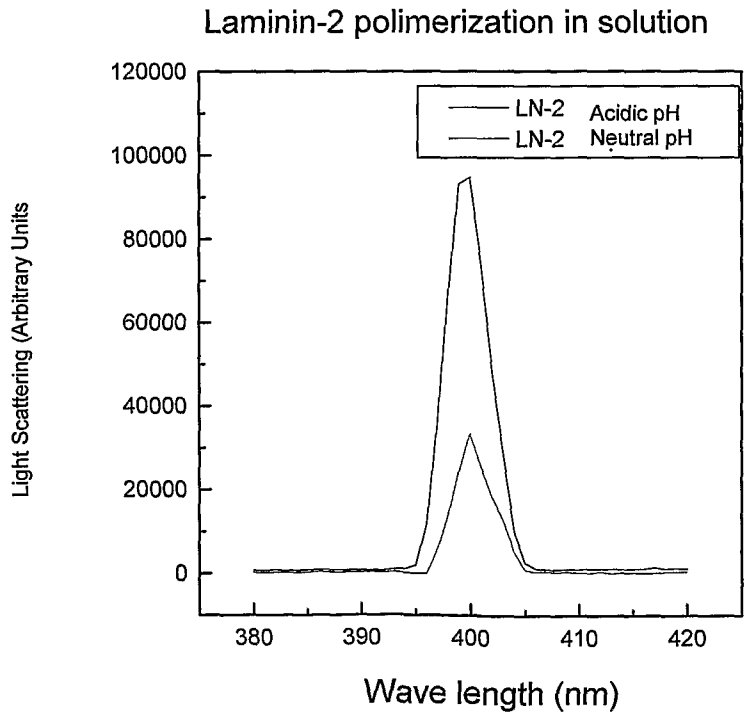


Figure 8

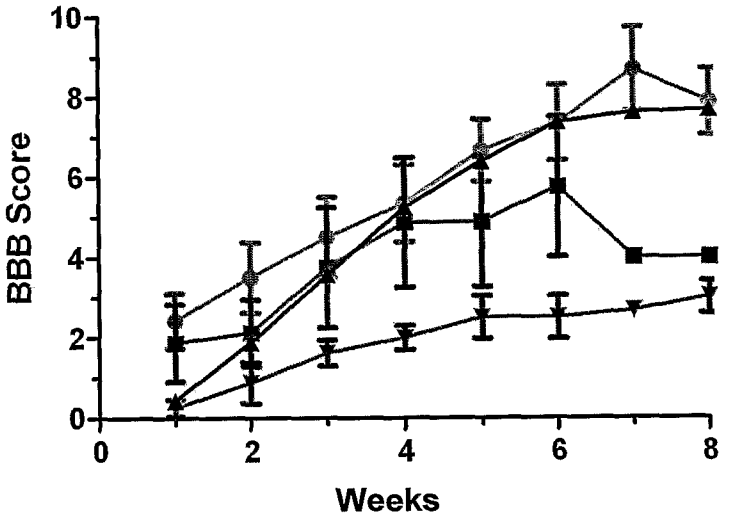
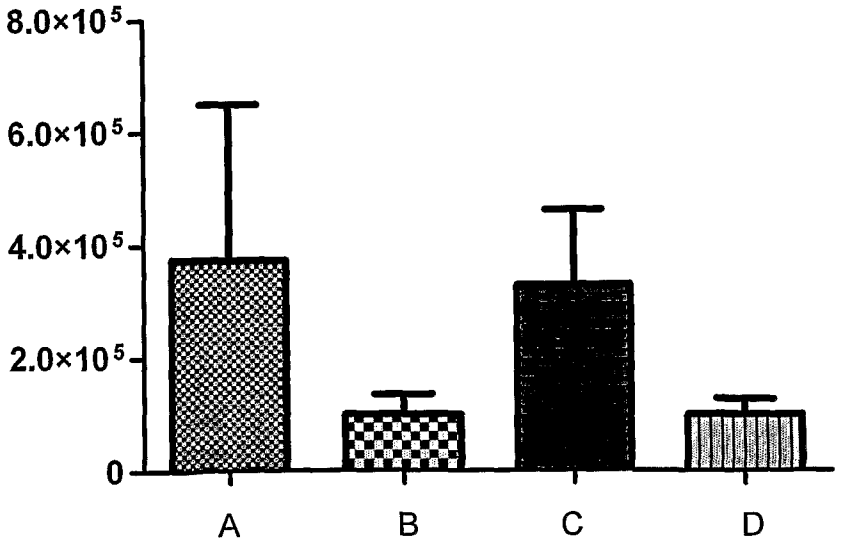



Figure 9

Number of cells in bronchoalveolar lavage



**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/BR 2009/000051

A. CLASSIFICATION OF SUBJECT MATTER
IPC[8]: ***C07K 14/78*** (2006.01); *A61K 38/39* (2006.01)
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)
IPC[8]: C07K, A61K

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 practicable, search terms used)
Wpi, Epodoc, Pubmed, Embase, Medline, Internet, NPL, Xprd,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | Barroso MM, Freire E, Limaverde GS, Rocha GM, Batista EJ, Weissmüller G, Andrade LR, Coelho-Sampaio T. "Artificial laminin polymers assembled in acidic pH mimic basement membrane organization." J Biol Chem. 2008 Apr 25;283(17):11714-20. Epub 2008 Feb 13. **Page 11714 right column top paragraph, page 11715 right column last half of first paragraph, page 11719 rigth column** | 1-9,13-20 |
| | -- | |
| X | Freire E, Gomes FC, Linden R, Neto VM, Coelho-Sampaio T. "Structure of laminin substrate modulates cellular signaling for neuritogenesis." J Cell Sci. 2002 Dec 15;115(Pt 24):4867-76. **Abstract, Figures 1-8, page 4868 right column top paragraph, page 4875 last paragraph** | 1-9,13-20 |
| | -- | |

☒ 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 application or patent 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 | Date of mailing of the international search report |
|---|---|
| 24 June 2009 (24.06.2009) | 23 July 2009 (23.07.2009) |
| Name and mailing address of the ISA/ AT<br>**Austrian Patent Office**<br>Dresdner Straße 87, A-1200 Vienna<br>Facsimile No. +43 / 1 / 534 24 / 535 | Authorized officer<br>GÖRNER W.<br>Telephone No. +43 / 1 / 534 24 / 558 |

## Continuation of first sheet

## Continuation No. 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:

Claims Nos.: 15-18 because they relate to subject matter not required to be searched by this Authority, namely:

Although the subject matters of claims 15-18 are directed to treatments of the human or animal body and therefore may be excluded in accordance with PCT rule 39 iv), the search as well as the establishment of novelty, inventive step and industrial applicability has been carried out based on the alleged effects of the therapeutic agent or pharmaceutical composition.

Claims Nos.: 10-12 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:

Claims 10-12 are unclear due to the term "comprising the ability to polymerize proteins in a concentration between ..." which is an effect of the process according to claim 5 and dependent claims but not a distinguishing technical feature - independently of the named protein concentration.

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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. |
|---|---|---|
| X | Freire E, Coelho-Sampaio T. "Self-assembly of laminin induced by acidic pH." J Biol Chem. 2000 Jan 14;275(2):817-22. **Figures** | 1-9,13-20 |
| X | WO2000/066732 A2 (BIOSTRATUM INC et al.) 9 November 2000 (09.11.2000) **pages 25 lines 17 ff - page 26 line 10** | 13-20 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/BR 2009/000051

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO A 2000066732 | | none | |