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(71) Applicant *(for all designated States except US)*: **SPHERIC NANOHEALTH, S.L.** [ES/ES]; Vía Augusta 13-15 - despacho 603, E-08006 Barcelona (ES).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **MARTINEZ ESCOBAR, Sergio** [ES/ES]; Vía Augusta 13-15 - despacho 603, E-08006 Barcelona (ES).

(74) Agent: **CARPINTERO LOPEZ, francisco**; C/ Alcala, 35, E-28014 Madrid (ES).

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(54) **Title**: ANTISEPTIC MICROSPHERES

(57) **Abstract**: The present invention describes new microspheres which comprise: (i) a layer or core of a polyanion, such as alginate; (ii) a polycation coating, such as poly-L-lysine in contact with the outer part of the polyanion layer or core; and (iii) a silver coating in contact with the outer part of the polycation coating. The silver is initially found as silver (0) and can oxidize to silver (+1) to be active against infectious processes. The invention also describes pharmaceutical compositions comprising these microspheres, particularly, compositions for use in the treatment and/or prophylaxis of infectious processes.

ANTISEPTIC MICROSPHERES

Field of the Invention

The present invention relates to a new pharmaceutical composition for the treatment and/or prevention of infectious processes, comprising antimicrobial microspheres comprising a polyanion layer, a polycation layer and a silver nanoparticle coating which can be activated immediately before administration.

Background of the Invention

It is well known that in sites of the organism in which a transformation occurs due to harmful processes changes the microenvironment/environment and an environment that is favorable for infection and with poor antibiotic diffusion capacity is generated. These harmful processes include, for example, lesions caused by knives, bull horns, as well as surgical treatments in the area of dirty or dirty-contaminated surgeries, and those having a high risk of suffering infectious complications due to post-surgical complications (a number of surgical times, very long surgeries, high risk of suture dehiscence, risk of bacterial translocation, etc...).

With regard to surgical methods in the prior art, one of the preventive measures against nosocomial surgical site infection (SSI) is the justified prophylactic administration of antibiotics as it is a clean-contaminated surgery. In other invasive processes, said antibiotic treatment is further justified, if that is possible, because they are abdominal dirty or dirty-contaminated surgeries, for example, in the case of fecal peritonitis, or in the case of patients with the tendency to suffer infections, such as transplant patients, immunosuppressed patients, pluri-pathological patients, neoplastic patients, or patients subjected to peritoneal dialysis. An antibiotic treatment can also be indicated in morbid processes the possible progression of which involves an infectious process of transudates-exudates, whether or not they are side effects of surgical or medical treatment.

A problematic aspect of antibiotic treatments in general, and particularly antibiotic treatments administered in infectious processes in natural cavities, in surgical processes (before/during/after), or harmful processes of another type, is the poor diffusion of the antibiotic treatment administered intravenously or orally to areas where its concentration must be maximum due to the change in the microenvironment/environment

mentioned above. In this sense, any antibiotic available at the systemic level presents diffusion problems, especially when associated with a hypoperfusion process (hemodynamic instability), perilesional inflammation or abscessing, as well as the presence of devitalized tissues.

At the level of local treatment, the use of antibiotics has not proven to be advantageous with respect to their exclusively systemic use given that the half-life of the antibiotic in its *in situ* disposition is not high enough to control and eradicate an infectious process. (This is discussed only in cases of peritonitis in patients in peritoneal dialysis in which antibiotics can be used in continuous perfusion or in multiple doses and are rapidly absorbed in the peritoneum after administration [Piraino B *et al.* Peritoneal dialysis-related infections recommendations: 2005 update. Perit Dial Int 2005 Mar-Apr; 25/2): 107-31]). Furthermore, local level administration of some antibiotics has the additional drawback of causing irritation processes in the administration area.

The difficulty indicated for diffusing antibiotic to sites in the organism in which microbial activity is required, both for those the anatomical disposition of which is typically complex upon establishing antibiotic therapy (for example the peritoneum), and for those which are transformed by harmful processes, complicates and even hinders the efficacy therapeutic/prophylactic antibiotic treatment.

In addition to the foregoing, antibiotic treatments have other important additional drawbacks, such as the narrow spectrum of antibiotics which sometimes makes it necessary to combine at least two different antibiotics in order to broaden said spectrum or the cost of a sequential antibiotic treatment, for example in continuous irrigation at the peritoneal level or in discontinuous multiple doses. The risk inherent to handling the antibiotic solution can also increase the risk of infections. In some surgeries (for example in colorectal surgery), the SSI can occur during the first week after surgery, so it is typical to maintain antibiotic treatment during this time to help reduce the morbidity-mortality of an infectious processes if one exists, withdrawing it once the time of risk of a complication has been surpassed. In these cases, in addition to the unwanted side effects of the antibiotic, added side effects derived from its prolonged use can occur.

In the prior art, on the other hand, the microbicidal or antimicrobial capacity of silver, which is used in many different applications, such as deodorants, gels, creams and even in tissues, is known. The use of the silver

as an antibacterial agent in topical antibacterial treatment of healed wounds with an infectious process by means of using dressings comprising a support, for example of alginate, which further acts as a carrier of silver aggregates is also known. There are also commercially available dressings with different combinations having silver, such as for example the combination of activated charcoal cloth with silver (Actisorb Plus 25®). These dressings have the drawback that their use is solely limited to the topical and local level when wounds are superficial.

In view of the foregoing, there is a need in the prior art to provide an effective new antibiotic treatment and a new alternative antimicrobial and/or microbicidal pharmaceutical composition, preferably with a broad spectrum of action against bacteria and fungi which can be used in any part or area of the organism, and particularly in sites of the organism in which antimicrobial activity is necessary which have an anatomical disposition that is typically complex upon establishing antibiotic therapy or those which are transformed by harmful processes, reducing or eliminating the need to use conventional antibiotic treatments entailing a number of the drawbacks mentioned above.

In this sense, the present invention proposes a new antibacterial and antifungal treatment, as well as a new silver-coated microsphere for use in a new pharmaceutical composition as it is defined below.

Brief Description of the Drawings

Figure 1 shows a schematic depiction of a microsphere.

Figure 2 shows an electron micrograph of the surface of the microspheres

Description of the Invention

In one aspect, the invention relates to a microsphere which comprises:

(i) a polyanion layer;

(ii) a polycation coating in contact with the outer part of the polyanion layer;

(iii) a silver coating in contact with the outer part of the polycation coating.

This microsphere, hereinafter microsphere of the invention, has antimicrobial properties, therefore being useful in the treatment and/or prophylaxis of an infectious or microbial process in an animal, including a human, in need of said treatment. Due to this application as a medicinal product, the materials forming the microspheres are preferably biocompatible

and biodegradable. Antimicrobial must be understood as the capacity of killing or inhibiting the growth of microbes, such as bacteria, fungi, parasites or viruses.

In this sense, the polyanion can be, in principle, any conventional polyanion that is biocompatible and biodegradable. In a particular embodiment of the invention, the polyanion is selected from the group consisting of alginate, polyglycolic acid, polyglycolic acid and lactic acid copolymer, agarose, polyacrylates, carrageenans and mixtures thereof. In a particular embodiment the polyanion is alginate.

The alginate that can be used in the present invention can be of any origin, bacterial and/or of brown algae, and can be commercially obtained as a mixture of sodium, potassium, calcium and magnesium salts. They are heterogeneous macromolecular structures with structures that are not regularly repeated, comprising αL-glucuronic (G) acid monomers and βD-mannuronic (M) acid monomers, giving rise to blocks within the structure referred to as G-blocks, M-blocks, and GM-blocks having different flexibilities. In this sense, the inventors have observed that the bonds forming the structures of the αL-glucuronic acid dimers allow better adhesion of the polycation. In alginate, the G and M fractions (fm and fg) vary depending on origin and on the subsequent treatment thereof. In a preferred embodiment, the alginate that is used has at least 70% of fraction G.

The carrageenans useful for putting the invention into practice are commercially available and can, in principle, be of any type, origin and structure, such as κ-carrageenan for example.

The polycation can, in principle, be any conventional polycation which is biocompatible and biodegradable. In a particular embodiment of the invention, the polycation is selected from the group consisting of poly-L-lysine, heparin, polyethylene glycol, chitosan, poly-L-ornithine, conventional synthetic polymers, such as for example poly-methylene-co-guanidine and poly-ethylene-amine, and mixtures thereof. In a particular embodiment, the polycation is a mixture of heparin and poly-L-lysine. The use of heparin combined with poly-L-lysine can reduce fibrotic response. In another particular embodiment, the polycation is poly-L-lysine.

The microsphere of the invention has a variable size within wide margins. Its size can generally be comprised between 5 microns and 5000 microns, preferably between 50 and 1000 microns and more preferably

between 100 and 500 microns. The thicknesses of the polyanion and polycation layers and of the silver coating can also range between wide margins, depending on the number of nanolayers making up the layers. Generally, the thickness of the polycation coating is comprised between 1 and 100 nm, particularly between 5 and 75 nm, and more particularly between 15-50 nm.

In a particular embodiment, the polyanion layer is deposited and in contact with a core having variable size and composition. The microspheres of the invention can therefore have different structures and compositions without this altering their antibacterial and/or antifungal capacity.

In a preferred embodiment, the polyanion layer (i) is the core of the microsphere as shown in Figure 1 and its size is also variable within wide margins.

In another preferred embodiment, the microsphere comprises an alginate core, a poly-L-lysine coating on said core and silver nanoparticles on the of poly-L-lysine coating.

The silver of the coating is found as generally polydispersed Ag nanoparticles consisting of aggregates of silver atoms with a variable size, typically comprised between 1 and 100 nm. As is described below, the silver can be neutral (as Ag(0)), i.e., inactive, oxidized, as Ag^+ i.e., active, or as mixtures of Ag(0) and Ag^+.

The microspheres of the invention can be produced by means of a method which comprises coating microspheres comprising a polyanion layer and a polycation layer with silver nanoparticles on the polyanion layer. The microspheres which can be coated with Ag(0) can have different structures as mentioned above. In this sense, these microspheres can contain a variable core, a polyanion layer thereon, and on the latter a polycation layer or a polyanion core and on the latter a polycation layer.

The microspheres can be produced, in principle, according to several conventional methods of the prior art which will vary depending on their structure. In a particular embodiment, microspheres are produced comprising a polyanion core and a polycation layer according to the method described below and which comprises the following stages:

a) preparing a solution of a polyanion in distilled water; a solution of a polycation in distilled water and a solution comprising at least one divalent cation;

b) producing polyanion microspheres by contacting the polyanion and divalent cation solution;

c) contacting the polyanion microspheres produced in stage b) and the polycation solution; and

d) recovering the microspheres (polyanion-polycation microspheres).

A polyanion solution is prepared in stage a) with a concentration typically between 1-4% by weight of a polyanion in distilled water; the polycation solution is prepared with a concentration typically between 0.1-1% by weight in distilled water and the solution comprising at least one divalent cation can comprise, for example, Ca, Sr, Ba, or mixtures thereof typically with a concentration comprised between 20-100 nM in distilled water.

The polyanion microspheres are produced in stage b) by generating a microdroplet of the polyanion solution which is subjected to a potential. The microdroplet fragments and falls into the solution containing the divalent cation. The microspheres which are produced are formed by a polyanion stabilized by ionic bonds.

These microspheres are contacted with the polycation solution, the polyanion-polycation microspheres being produced by electrostatic attraction.

In a particular embodiment of this method, the polyanion solution microdroplets are formed by passing the solution through a nozzle by extracting it from a container which contains it by means of the action of a peristaltic pump. The solution is passed through an extrusion needle (unbeveled cone-shaped) in a perfusion typically of 4 ml/hour. The microdroplet which is generated in the tip of the needle is subjected to a generally 10,000 V potential in a device in which the needle is the positive pole and a copper ring is the negative pole, the distance between the tip of the needle and the ring being 6 cm. The microdroplet subjected to the potential fragments and falls into the solution containing the cation and is maintained under stirring like the polyanion solution. The divalent ions diffuse into the polyanion mixture, forming microspheres formed by a polymer stabilized by ionic bonds. The microspheres are kept for a time in the solution, washed with abundant distilled water and then introduced in the polycation suspension. The resulting solution is typically maintained for 2 hours, the polycation layer being formed by electrostatic attraction.

The formed polyanion-polycation microspheres act like a semipermeable membrane, in which the mechanical stability confers to it the

polyanion-polycation complex, the electropositivity is determined by the polycation and this allows the subsequent surface anchoring of the Ag(0) nanoparticles.

The preceding method provides microspheres with variable diameter sizes ranging between approximately 5 and 5000 microns, depending on the following control parameters: a) the concentration of polyanion in the solution; in general, the lower the concentration the smaller the size; b) the diameter of the extrusion needle (the smaller the diameter, the smaller the size); and c) the voltage and potential difference between the needle and the copper ring (the larger the potential difference, the smaller the size).

The silver nanoparticles can be produced by means of methods well known by a person skilled in the art. In a particular embodiment they are produced in the form of a silver nanoparticle solution stabilized with sodium citrate by means of a well known method which comprises: (i) dissolving a precursor silver salt, such as $AgNO_3$ for example, in water; (ii) taking the solution to the boiling point in the presence of sodium citrate; and (iii) leaving it to cool at room temperature. A yellow solution showing the presence of silver nanoparticles is produced by means of this method.

The polyanion-polycation microspheres previously produced according to any of the conventional methods of the art are then added to the silver nanoparticle solution, the microspheres with Ag(0) of the invention being produced.

The following must be pointed out among the alternative methods for preparing the polyanion-polycation microspheres:

The method in which the microspheres are produced by means of extruding through a needle, controlling the diameter. According to the inventors, this method does not allow suitable control of the diameters of the microspheres. Inner diameters (of the needle) less than 1 mm are required for sizes less than 500 microns.

The method in which the microspheres are produced by means of using coaxial air (Venturi effect), which allows generating small microspheres of less than 500 micrometers, although the size distribution is not very homogenous. Coaxial air is used to push the drops from the tip of the needle to the electrolyte bath.

The method in which the microspheres are produced by means of using a vibrating system in a pressure chamber.

The method in which the microspheres are produced by means of using a rotary cutting system. The gel is cut into cylindrical segments, the microspheres being formed as a result of the surface tension. It is thus difficult to control the size of the microspheres, which tend to coalesce into large masses.

The microspheres of the invention can be stored in the inactive condition in any container, for example made of glass or plastic, with distilled water. Advantageously, they do not undergo photodegradation and do not require cold for preservation. Nevertheless, it is unadvisable to store them at temperatures close to freezing temperature or at temperatures greater than 80ºC. The inventors have observed that at physiological temperature and at room temperature they maintain their antibacterial capacity intact.

In another aspect the invention relates to a pharmaceutical composition comprising the microspheres of the present invention. Said pharmaceutical composition is suitable for use in the treatment and/or prevention of an infectious process. An infectious process must be understood as any infectious or microbial process caused by a pathogenic microorganism, for example a bacteria, a fungus, a virus or a parasite in an animal, including man. In general, the concentration or amount of microspheres in the composition can range between wide margins. In a particular embodiment the pharmaceutical composition of the invention contains a therapeutically effective amount of microspheres. Typically, the amount is comprised between 10^2 and 10^{12} spheres dispersed in a volume ranging from 1 to 10 cm^3. In the context of the present invention, therapeutically effective amount is understood as that amount which is sufficient for and capable of providing a therapeutic and/or prophylactic effect.

However the microspheres with Ag(0) are not active from the antimicrobial point of view. This is because said activity of the silver, which ultimately leads to the death of the microorganism and/or the detention of its proliferation, is due to the Ag+ cation which produces a chemical wound in the microorganism. The type of chemical wound can be different and in some cases the mechanisms are not clear or are currently being studied. In general, it is well known that Ag^+ produces a chemical wound at the level of the bacterial wall creating pores, and subsequently fixes to DNA. In the case of parasites and viruses, their antiparasitic or virucidal activity is in principle

due to the direct wound in the DNA and possibly the virus capsid, but these mechanisms are currently not clear.

To reach this active condition, the transformation of microspheres having inactive Ag(0) into microspheres referred to as activated microspheres having Ag^+ at least in part by means of an oxidizing agent is necessary. This oxidation is preferably performed in the moment before administering a pharmaceutical composition containing the microspheres of the invention in which Ag(0) has begun to oxidize. Nevertheless, and alternatively or consecutively, the oxidation can take place at the site of applying the microspheres in the animal body, including man. An example of a medium capable of activating Ag in a body is a medium rich in chloride anions, such as the intercellular space or the internal medium of cavities for example, such as the peritoneal cavity.

In this sense, the method of preparing the previously described microspheres can further comprise a stage referred to as activation stage, which comprises contacting microspheres with Ag(0) with an oxidizing composition to prepare microspheres comprising active Ag^+. Therefore, an additional object of the present invention relates to a method for activating the microspheres with Ag(0) of the present invention which comprises contacting them with an oxidizing agent to produce microspheres comprising Ag^+.

Therefore, the pharmaceutical composition of the invention can comprise microspheres in which the silver can be as Ag(0), or they can be as oxidized silver (Ag^+), or the can be as a mixture in any proportion of Ag(0) and (+1).

In a particular embodiment the pharmaceutical composition comprising microspheres is a suspension thereof. In a particular embodiment, it is a suspension in distilled water, which optionally can further contain one or more pharmaceutically acceptable excipients. In a particular embodiment, the aforementioned oxidizing composition is a solution comprising any pharmaceutically acceptable oxidizing agent. In a preferred embodiment, the oxidizing agent is H_2O_2, more preferably in distilled water. This oxidizing composition can additionally contain one or more pharmaceutically acceptable excipients. To prepare the pharmaceutical composition of the invention as well as the oxidizing composition, any other pharmaceutically acceptable medium other than distilled water, or mixtures of said medium

with distilled water in any proportion, can be used. The optional excipients for these compositions, such as buffers for controlling the pH, surfactants, etc., can be easily selected by the person skilled in the art.

The amount of microspheres in the pharmaceutical composition can range between wide margins as has been mentioned above. The concentration of oxidizing agent in the oxidizing composition can also range between wide margins. In another particular embodiment, the oxidizing composition contains an amount of oxidizing agent capable of initiating oxidation of the Ag(0) present in a pharmaceutical composition according to the invention. It typically has between 0.1% and 50% of H_2O_2 at a 90% concentration in aqueous solution. Said aqueous solution can be distilled water, saline, glucose saline, or lactated Ringer's solution.

Another additional object of the present invention relates to a kit comprising the pharmaceutical composition comprising microspheres having Ag(0) and an oxidizing composition. Both the pharmaceutical composition and the oxidizing composition must both be sterile and pharmaceutically acceptable, and they can be prepared according to standard methods described or referred to in Spanish or US pharmacopoeias or in similar texts.

In a particular embodiment of the kit of the present invention, the pharmaceutical composition consists of 10 ml of a suspension of microspheres in distilled water (produced according to Example 1) and the oxidizing composition consists of 0.1 ml of H_2O_2 at 90% concentration, in aqueous solution, the necessary amount for activating those 10 ml.

The pharmaceutical composition containing the microspheres can be administered in a therapeutically effective amount by any suitable administration route, such as for example topical, local, local-regional, intraperitoneal, intrapleural, intrathecal, intramedullary or intravesical administration, by means of direct deposition -surgery-, by means of pleural or intraperitoneal puncture of an animal, including humans, in need of a therapeutic and/or prophylactic treatment of an infectious process. In the context of the invention, therapeutically effective amount is understood as that amount which is sufficient and capable of providing a therapeutic or prophylactic effect in a treated animal, including man.

In a particular embodiment, the pharmaceutical composition comprising the microspheres with Ag(0) is activated by contact with an oxidizing composition and is then administered to an animal, including man.

In another particular embodiment, the pharmaceutical composition that is administered can contain microspheres with Ag(0) and the silver can be activated *in situ*, i.e., at the administration site, by the presence of an oxidizing agent. This is the case, for example, of chloride anions existing in an internal medium for example. In another particular embodiment, the activation of the silver can take place both before its administration by contacting it with an oxidizing composition, and by oxidation after its administration in an internal medium.

The pharmaceutical composition can be administered, in principle, in any anatomical area of an animal, including man. In a particular embodiment, and due to the advantages inherent to the microspheres, said area is an area in which a conventional antibiotic treatment by intravenous or oral administration, or its local use, is not effective enough due to its characteristics. Said area can be, for example, a natural cavity of the organism, such as the peritoneum, or a wound surface of solid organs or the cutaneous tegument. The peritoneum is an especially interesting area because due to its anatomical disposition it is characteristically complex to establish antibiotic therapy. Other interesting areas are those which have harmful processes modifying the microenvironment/environment of the area, resulting in an environment that is favorable for infection and with poor antibiotic diffusion capacity.

In a particular embodiment, the pharmaceutical composition is for use at the local or local-regional level of natural cavities such as the chest, abdomen, pelvis etc., more particularly for use in patients in whom dirty or dirty-contaminated surgery, such as fecal peritonitis, deterioration of intestinal loops, mesenteric necrosis, suture dehiscences, washing of the retroperitoneal region in contexts such as acute severe hemorrhagic necrotizing pancreatitis, abscess drainages, etc.

In another particular embodiment, the pharmaceutical composition is for use in the prevention or prophylaxis of a severe septic-type infectious process, particularly in a patient with a hypoproteinemia process or who has a poor nutritional state prior to surgery, in interventions on devitalized tissues, re-interventions, or in palliative surgeries on neoplastic processes, etc., the complicated progress of which can be suture dehiscence or bacterial translocation with the subsequent septic process.

In another particular embodiment, the pharmaceutical composition is

for use in the treatment and/or prophylaxis of an infectious process in a morbid process, the possible progress of which involves an infectious process of transudates-exudates, whether or not they are side effects of surgical or medical treatment (e.g. bacterial peritonitis in cirrhosis patients, accumulation of peritoneal liquid in the context of chronic hepatic cirrhosis, neoplastic ascites, portal hypertension, hypoalbuminemia or hypoproteinemia of any origin, etc.).

In another particular embodiment, the pharmaceutical composition is for use in the treatment and/or prophylaxis of an infectious processes in relation to a surgical or invasive process, particularly in patients with a greater tendency to suffer infections, such as transplant patients, the immunosuppressed patients (whether therapeutically or morbidly), pluri-pathological patients, neoplastic patients, patients subjected to peritoneal dialysis, etc.

In another particular embodiment, said pharmaceutical composition is for use in the coadjuvant treatment and/or prophylaxis to a conventional treatment and/or prophylaxis of an infectious process. In the context of the present invention, coadjuvant treatment and/or prophylaxis means that said treatment and/or prophylaxis of an infectious process comprising the administration of the pharmaceutical composition of the invention is performed jointly with a conventional treatment and/or prophylaxis of an infectious process. The term jointly within the context of the present invention must be broadly interpreted in the sense that both treatments and/or prophylaxis can be carried out in a patient one after the other in time, or simultaneously, and in any administration regimen, type of administration route, administration dose, etc, which is suited to the needs of a patient.

The amount of pharmaceutical composition which is used for the treatment and/or prophylaxis in a patient can be variable depending on, among other factors, if it is a therapeutic treatment, a prophylactic treatment, or on if it is a coadjuvant treatment as mentioned above. The amount of pharmaceutical composition which is used will also depend on the severity and extent of the infectious process, of the condition of the patient, sex, weight, etc. In a particular embodiment, a sufficient amount for covering the abdominal cavity in a diffuse process is used. Said amount is typically 10 ml of a suspension of microspheres (in which there are about 200,000 spheres 500 microns in diameter or about 127,000,000,000 spheres 50 microns in

diameter) (after activation with an oxidizing composition).

The microspheres of the present invention have numerous advantages, including the high diffusion capacity of the silver ions at the local level, both because of their intrinsic features and their direct administration/disposition at the local level, i.e., at the site of an infection for example. Their spherical configuration and small size (of the order of 5 to 5000 microns) confer a high surface of exposure and allow minimal amounts of microspheres to generate microbicidal activity in large distribution volumes. Furthermore, the duration of their activity is maintained locally even for weeks, or months, this therefore being much greater than the half-life of locally applied antibiotics (hours). This duration in time is on one hand due to the fact that the antimicrobial activity of silver is has been known for a long time, such that as long as there are silver nanoparticles in the environment releasing silver ions, said activity exists and on the hand is due to the fact that silver is not absorbed at the tissue level, so its local action remains.

Since they are biocompatible and biodegradable, it is not necessary to withdraw them, which is an important advantage in comparison with therapies involving the use of devices to irrigate an antibiotic solution, changes during treatment and handling, which increase the risk of infections and are a connecting port of a cavity with the exterior (septic).

In comparison, for example, with the treatment with local antibiotherapy in dialysis patients through the peritoneum with bacterial peritonitis, the microspheres have an additional advantage due to the duration of the effect, not requiring continuous perfusion of antibiotics or discontinuous repeated doses according to levels of bioavailability of the drug in blood.

In a final additional aspect, the invention relates to the use of the microspheres of the invention in preparing a pharmaceutical composition for the treatment and/or prophylaxis of an infectious process. In a particular embodiment, said pharmaceutical composition is for the treatment and/or prophylaxis coadjuvant to a treatment and/or prophylaxis of a conventional infectious process.

Illustrative examples of the invention which are set forth to better understand the same and in no case should be considered as a limitation of the scope thereof are presented below.

EXAMPLES

Example 1: Alginate-poly-L-lysine and Ag(0) microspheres, their production and activity assays

Device: the device used consisted of:

- Peristaltic pump (for producing continuous perfusion flows)
- Unbeveled cone-shaped extrusion needle with an inner diameter less than 1 mm.
- Nozzles.
- High voltage source (1-20 kV). Insulated connections for copper ring and needle. The potential difference facilitating the extrusion of the alginate microdroplet through the needle is established between the tip of the needle and the copper ring, the microdroplet adopting a spherical shape during its fall.
- Bases for securing the elements such as the nozzle, needle and copper ring.
- Conventional copper ring having a diameter of 10 cm.

Stages:

The following were prepared: an alginate solution (intended for clinical use – assuring 70% or more of Fg) in distilled water at 4%; a poly-L-lysine solution in distilled water at 0.1%; and a solution of calcium chloride at 1.5%.

The solution of alginate maintained under magnetic stirring was passed through a nozzle which extracts the alginate from the container which contains it by means of the action of a peristaltic pump. The solution was passed through an extrusion needle (unbeveled cone-shaped) at a perfusion of 4 ml/hour. The microdroplet generated at the tip of the needle was subjected to a potential of 10,000 V in a device in which the needle is the positive pole and a copper ring is the negative pole, the distance between the tip of the needle and the ring being 6 cm. The fragmented microdroplet fell into the $ClCa_2$ solution maintained under magnetic stirring. The suspension produced was maintained for 5 minutes. The spheres formed were washed with abundant distilled water and were introduced in the poly-L-lysine suspension for 2 hours, the alginate-poly-L-lysine microspheres being formed.

A silver nanoparticle solution stabilized with sodium citrate was prepared separately. To that end, 9 mg of silver nitrate were dissolved in 50 mL of water in a container; this solution was taken to the boiling point (100ºC)

adding 1 mL of 1% sodium citrate for two hours. It was subsequently left to cool at room temperature and a yellow solution was produced which showed the presence of silver nanoparticles. The spectrum of said solution showed a typical maximum peak at 437 nm, indicative of the presence of silver nanoparticles.

The nanoparticle solution and the alginate-poly-L-lysine microsphere solution were contacted with one another, the Ag0 nanoparticle coating being produced.

The alginate microgel coating functionalized with a positive charge (poly L Lys) becomes negative with the silver nanoparticle coating. This is demonstrated by means of measuring the Z-potential of this solution using a Zetasizer Nano-Z. The result of this measurement was -31.6 mV, corresponding to the surface charges of the microspheres.

The microspheres produced were stored in a glass or plastic container with distilled water at room temperature, the amount of microspheres being 20,000 spheres/cc 500 microns in diameter.

Characterization

The microspheres of the invention with Ag(0) have been characterized by means of electron micrograph (SEM) (Figure 2) in a Hitachi Scanning Electron Microscope ® model S-3500-N. Application of 10 kV in vacuum conditions with a magnification range of (40-10,000)x. Bal-Tec sputtering machine technology for providing coverage of a thin layer of graphite, 5 nm to optimize the electron conduction of the samples.

Activation of the microspheres

The microspheres were activated from 10 ml of the microsphere suspension produced above with 0.1 ml of 3% hydrogen peroxide.

Activity assay in vitro

The strains of multi-sensitive E. coli were isolated. These strains were obtained from samples for culture extracted from patients with an infectious pathology admitted in the Internal Medicine Department of the Torrecárdenas Hospital (Almería). After isolating the strains of E. coli, the sample was seeded with a sterile loop in Petri dishes with common agar. The inoculated plates were subjected to exposure for testing the bactericidal activity of rings (filter type) impregnated with 870-125 mg of amoxicillin-clavulanic acid (experimental control arm) versus the use of rings of microspheres (about 10 microspheres in each ring) (experimental intervention arm). The spheres

were activated before their use with 2 ml of H_2O_2 (for a suspension of 10 ml of microspheres). The results after 48 hours of incubation in a CO_2 stove with a wet sleeve were inhibition halos without the presence of resistance in both groups with a statistically significant size difference (t-Student for contrasting means; $p<0.05$//considering the mean diameters of the halos of both groups); control plates diameter 12±7 mm vs. intervention plates 19±4 mm.

CLAIMS

1.- Microsphere which comprises:

(i) a polyanion layer;

(ii) a polycation coating in contact with the outer part of the polyanion layer;

(iii) a silver coating in contact with the outer part of the polycation coating.

2.- Microsphere according to claim 1, wherein the polyanion layer (i) is the core of the microsphere.

3.- Microsphere according to claim 1 or 2, wherein the polyanion is selected from alginate, polyglycolic acid, polyglycolic acid and lactic acid copolymer, agarose, polyacrylates, carrageenan and mixtures thereof, preferably alginate.

4.- Microsphere according to any one of claims 1 to 3, wherein the polycation is selected from poly-L-lysine, heparin, polyethylene glycol, chitosan, poly-L-ornithine, poly-methylene-co-guanidine, poly-ethylene-amine and mixtures thereof, preferably poly-L-lysine, mixture of heparin and poly-L-lysine, more preferably poly-L-lysine.

5.- Microsphere according to any one of claims 1 to 4, comprising a size between 5 microns and 5000 microns, preferably between 50 and 1000 microns and more preferably between 100 and 500 microns.

6.- Microsphere according to any one of claims 1 to 5, comprising an alginate core or layer, a poly-L-lysine coating and silver nanoparticles.

7.- Microsphere according to any one of claims 1 to 6, wherein the silver is in condition (0), oxidized (+1) or as a mixture of both.

8.- Pharmaceutical composition comprising microspheres, according to any one of the previous claims.

9.- Pharmaceutical composition according to claim 8, for use in the treatment and/or prophylaxis of an infectious process.

10.- Pharmaceutical composition according to claim 9, wherein said use is coadjuvant to a treatment and/or prophylaxis of an infectious process.

11.- Pharmaceutical composition according to any one of claims 8 to 10, for topical, local, local-regional, intraperitoneal, intrapleural, intrathecal, intramedullary or intravesical administration, by means of direct deposition -surgery-, by means of pleural or intraperitoneal puncture.

12.- Method for producing a microsphere according to any one of claims 1 to 7, which comprises:

1) producing microspheres which comprise:

(i) a polyanion layer or core;

(ii) a coating in contact with the outer part of said polyanion layer or core; and

2) contacting them with silver nanoparticles (0).

13.- Method for activating the microspheres any one of claims 1 to 7, having a silver coating (0) which comprises contacting said microspheres with an oxidizing composition.

14.- Method according to claim 13, wherein the oxidizing composition comprises H_2O_2 or chloride anion.

15.- A kit comprising a pharmaceutical composition according to any one of claims 8 to 11, wherein the microspheres have silver in condition (cero) and an oxidizing composition.

16.- A kit according to claim 15, wherein the oxidizing composition is an aqueous solution comprising H_2O_2.

17.- Use of a microsphere according to any one of claims 1 to 7, in preparing a medicinal product for the treatment and/or prophylaxis of an infectious process.

18.- Use according to claim 17, wherein said treatment and/or prophylaxis is coadjuvant to a treatment and/or prophylaxis of an infectious process.

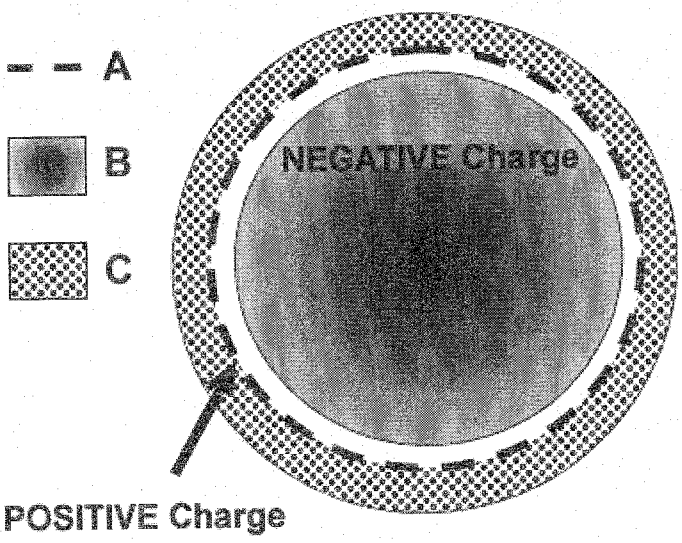

FIG. 1

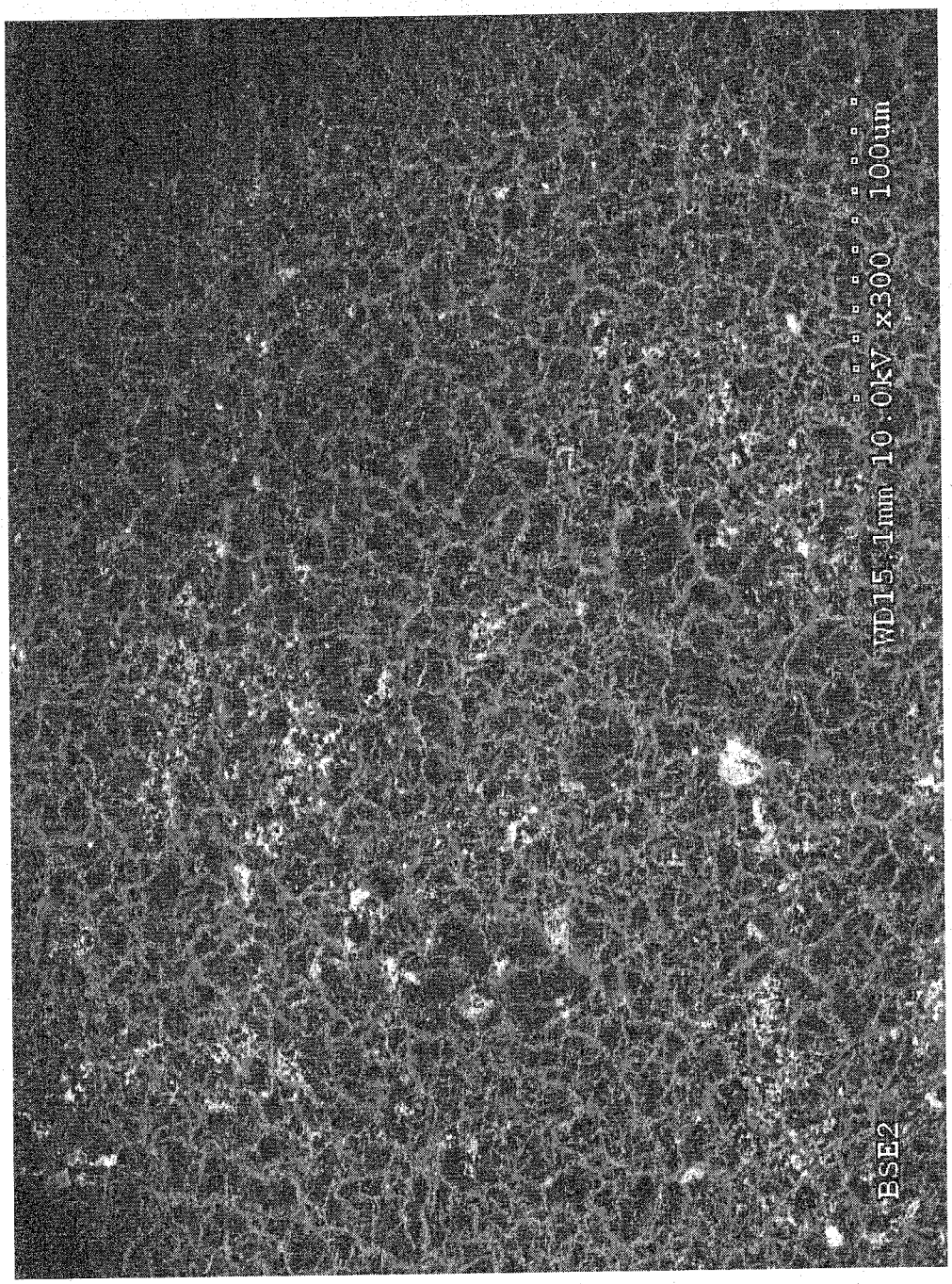

FIG. 2A

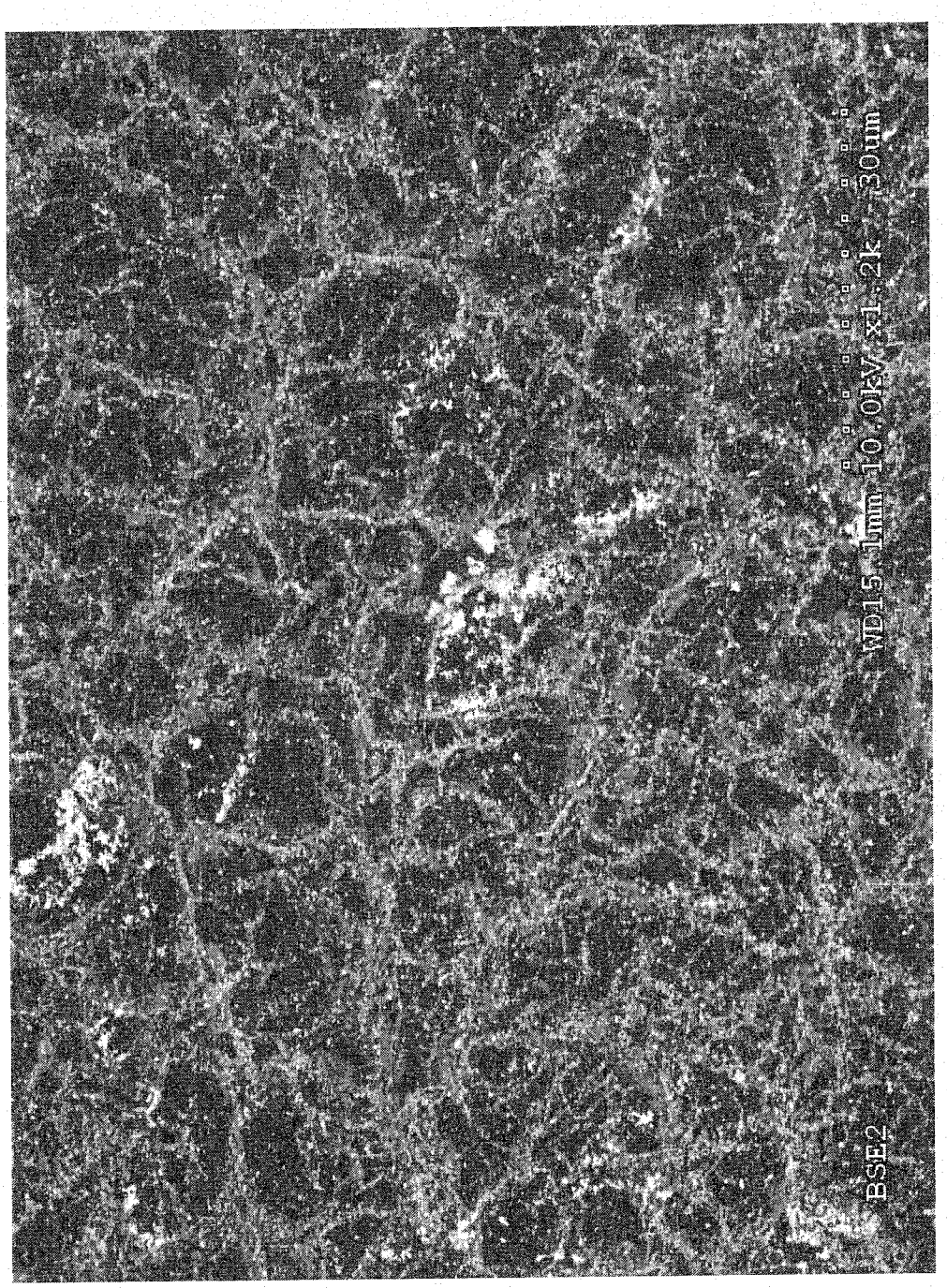

FIG. 2B

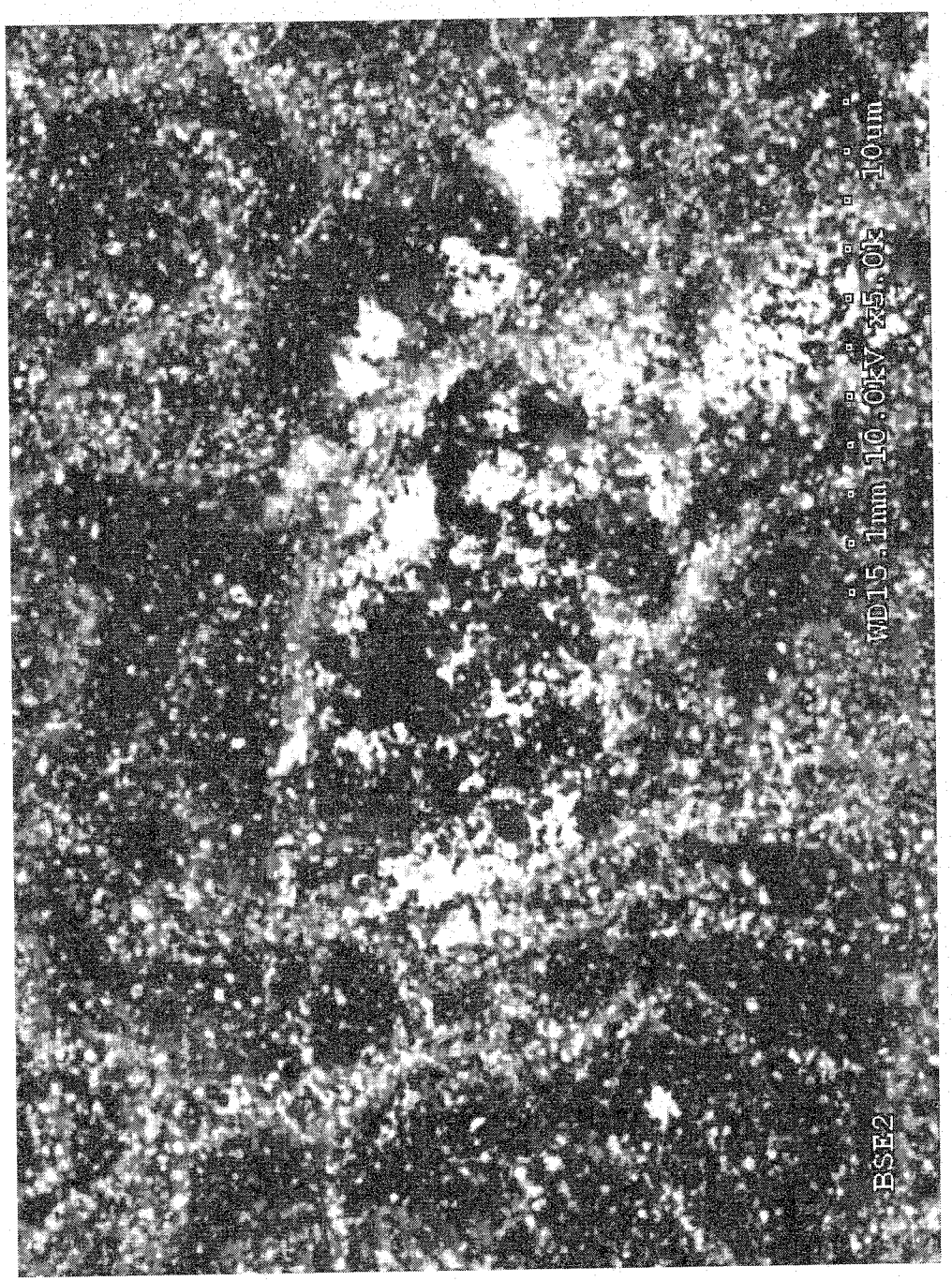

FIG. 2C

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/050593

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/16 A61K33/38
ADD.

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)
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 practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEE DAEYEON ET AL: "Antibacterial properties of Ag nanoparticle loaded multilayers and formation of magnetically directed antibacterial microparticles.", LANGMUIR : THE ACS JOURNAL OF SURFACES AND COLLOIDS 11 OCT 2005 LNKD-PUBMED:16207049, vol. 21, no. 21, 11 October 2005 (2005-10-11), pages 9651-9659, XP002631810, ISSN: 0743-7463 the whole document -----	1-18
X	EP 1 593 374 A1 (MAX PLANCK GESELLSCHAFT [DE]) 9 November 2005 (2005-11-09) the whole document -----	1-18
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 April 2011	21/04/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sproll, Susanne

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/050593

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	UENG STEVE W N ET AL: "In vivo study of biodegradable alginate antibiotic beads in rabbits.", JOURNAL OF ORTHOPAEDIC RESEARCH : OFFICIAL PUBLICATION OF THE ORTHOPAEDIC RESEARCH SOCIETY MAY 2004 LNKD- PUBMED:15099640, vol. 22, no. 3, May 2004 (2004-05), pages 592-599, XP002631811, ISSN: 0736-0266 the whole document	1-18
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/050593

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