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(71) Applicant: **INTERALIA S.R.L.** [IT/IT]; Via Monte Cengio, 28/1, I-35138 Padova (IT).

(72) Inventors: **COMELLI, Maria Cristina**; Via dei Mille, 24, I-35141 Padova (IT). **CALDERINI, Gabriella**; Via IV Novembre 61, I-35020 Due Carrare (Padova) (IT).

(74) Agent: **NOTARBARTOLO & GERVASI S.P.A.**; Corso di Porta Vittoria, 9, I-20122 Milan (IT).

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(54) Title: COMPOSITIONS COMPRISING A SILVER SALT OF SUCROSE OCTASULFATE AND A POTASSIUM SALT OF SUCROSE OCTASULFATE FOR PROTECTION OF MUCOSAL EPITHELIA

(57) Abstract: It is herein described compositions comprising a silver salt of sucrose octasulfate and a potassium salt sucrose octasulfate and the use thereof for prevention and treatment of mucosal epithelial infections.

Compositions comprising a silver salt of sucrose octasulfate and a potassium salt of sucrose octasulfate for protection of mucosal epithelia

Field of the invention

The present invention relates to compositions based on the association of a sucrose octasulfate silver salt [$(SOS)^-(Ag)^+$] and of a sucrose octasulfate potassium salt [$(SOS)^-(K)^+$] providing a synergistic antimicrobial and anti-microbial adhesion activity and to the use thereof for the achievement of an efficient protection of mucosal epithelia against infections. The compositions can be particularly useful for the prevention and treatment of mucosal affections where biofilm-former microbial strains are involved.

Background of the invention

Mucosal membranes are sheets of epithelial tissues covering the internal cavities of an organism; such cavities are always somehow in contact with the external environment and thus exposed to microbial contamination. Under optimal conditions, mucosal epithelia are naturally provided with efficient protective physico-chemical systems consisting mainly of cilia, fluids, mucous secretions, proteolytic enzymes and bacteriocins, beside the immune response. However, in spite of these defense mechanisms, they are frequently affected by more or less threatening microbial infections in the course of a human life.

To colonize a tissue, microorganisms need as an essential first step, to “stick” to the surface of the target cell in order to resist the natural defensive mechanisms of the host (Pieters RJ, *Intervention with bacterial adhesion by multivalent carbohydrates,* Med. Res. Rev., 2007;27:796-816). Therefore, adherence is the “key” event in a microbial infection; it allows pathogens to get a better access to nutritional sources, facilitates the delivery of toxins into the cells of the host, favours deep penetration of microbial cells into the tissue and enhances the chance for microorganisms to organize the colony in a biofilm. Biofilms are aggregates of microorganisms embedded within a self-produced polymeric matrix that protects microbial cells from the environment; this is the reason why often they are resistant to antibiotic treatment. When the microbial biofilm is mature,

dispersion of "planktonic" cells allows colonization of new surfaces with spreading or recurrence of infections.

Disruption or inhibition of pathogen attachment to host cells by anti-adherence agents is considered an innovative and interesting approach to prevention and treatment of microbial infections (Ofek I et al., *Antiadhesion therapy of bacterial diseases: prospects and problems*. FEMS Immunology and Medical Microbiology 2003;38:181-191). Saccharides are considered among the promising molecules for a safe anti-adhesion approach since they are neither toxic nor immunogenic (Sharon N and Ofek I., *Safe as mother's milk: carbohydrates as future anti-adhesion drugs for bacterial diseases*. Glycoconjugate J., 2000;17:659-664).

Sucrose octasulfate is a disaccharide well-known for its aluminum salt (e.g. sucralfate). In fact, sucralfate is widely used for the treatment of gastro-intestinal ulcers. In acidic conditions, like those encountered in the stomach, this insoluble salt forms viscous gels and, then, adheres to the gastro-intestinal mucosal surface, promoting in this manner the protection thereof. In addition, sucrose octasulfate as such has been shown to potentiate the Fibroblast Growth Factor (FGF) pathway not only by binding and stabilizing FGF but also by promoting FGF receptor dimerization. In physiological conditions this event requires the presence of heparin or heparan sulfate proteoglycans to occur (Yeh BK et al., *Structural basis for activation of Fibroblast Growth Factor signalling by sucrose octasulfate*. Mol. Cell. Biol., 2002;22:7184-7192). Therefore, sucrose octasulfate can also contribute to mucosal wound repair, beside physical barrier protection.

Despite being devoid of anticoagulant activity, sucrose octasulfate could be considered a chemical analog of heparin, thus, potentially competing for the binding to the same cellular receptors. In an experimental study drawn to assess the inhibition of pathogenic bacterial adhesion of a vegetal-derived acidic polysaccharide, comparative data on Low Molecular Heparin (LMWH) and the sodium salt of sucrose octasulfate are reported. In trypsinized erythrocytes the sodium salt of sucrose octasulfate, can effectively inhibit *in vitro* only the hemagglutination induced by *P. gingivalis* (Lee JH et al., *Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea* (*Camellia sinensis*). J. Agric. Food Chem., 2006;54:8717-8723).

Besides as sucralfate, sucrose octasulfate is currently available as ammonium, potassium or sodium salts for research purpose only.

Additionally, a sucrose octasulfate silver salt [$(SOS)^-(Ag)^+$] as antimicrobial compound is disclosed (EP1458733). In the patent the anti-microbial activity of the [$(SOS)^-(Ag)^+$] on *Staphylococcus aureus* in comparison with a silver colloidal product (Katoxyn®) and silver sulphadiazine and the methods of preparation thereof are described.

Before antibiotics advent, in fact, silver has long been recognized for its broad-spectrum antimicrobial activity and widely used in a large number of different pathological conditions to prevent and/or cure microbial infections. Currently, 0.5% silver nitrate or 1% silver sulfadiazine are commonly applied to fight cutaneous Gram-negative infections not effectively controlled by conventional antibiotics. Other silver based compounds, mainly ionisable salts, are currently available for clinical use, such as silver citrate, silver lactate or silver picrate. Recently silver citrate has been approved as preservative for cosmetic preparations and is currently used in various products. Colloid forms of silver are also available on the market; their antimicrobial activity depends not only on the total silver content, but on the amount of provided silver ions.

Therefore, the silver salt of sucrose octasulfate can be a useful tool to meet the need of prevention and treatment of infections, and beyond acting as an antimicrobial agent, the silver sucrose octasulfate could potentially be endowed with a tissue repair promotion property as well.

Besides, for preventing and efficiently treating mucosal infections it is necessary not only to intervene on the growth of the microbial pathogens but also to interfere with the spreading of infection through inhibition of microbial cells adhesion. This is particularly important in presence of biofilm-forming microorganisms.

Summary of the invention

It is a purpose of the present invention to provide a composition suitable to be used for protecting mucosal epithelia by inhibition of both microbial adhesion and pathogen microbial proliferation.

The inventors have now found that the potassium salt of the disaccharide sucrose octasulfate is an effective inhibitor of bacterial adhesion to mammalian fibroblasts, i.e. to cells that are structural components of mammalian tissues in their native form, and whose surface is not modified by any enzymatic treatment, differently from the previous state of the art focusing on circulating cells enzymatically treated.

This discover opens to a new way for the development of useful anti-bacterial compositions to be effectively used to protect mucosal epithelia from microbial colonization through a double mechanism: inhibition of adhesion to host cells by the potassium salt of the disaccharide octasulfate and antimicrobial activity by the silver salt of sucrose octasulfate.

In particular, the Inventors found that compositions based on the association of silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] result in a stronger synergistic protection from microbial colonization of mammalian tissues, than the one exerted by each component when used alone. The stronger protection comes from the synergy between the antimicrobial activity of [$(SOS)^-(Ag)^+$] and the inhibition of microbial adhesion to mammalian cells by [$(SOS)^-(K)^+$]. From a clinical point of view this is particularly relevant for mucosal infections and in particular in case of infections sustained by biofilm-former micro-organisms, which have the potential to be the most difficult to eradicate. In addition, these microorganisms are often reported to colonize indwelling medical devices and, as it is well-known, this aspect is of great concern in the clinical practice.

Furthermore, the association of the two compounds could also act synergically in the promotion of tissue repair and this biological property can be a further advantage in the clinical management of infected wounds of mucosal epithelia.

Therefore, in a first aspect the present invention relates to a composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$], said composition having a combined and synergistic antimicrobial and anti-microbial adhesion biological activity against infections of mucosal epithelia, and in particular including those sustained by biofilm-forming strains.

The compositions object of the invention provide a safe, combined biological property against infection of mucosal epithelia, which results superior to the biological properties exerted by each compound of the association when used alone, thus making the association of $[(SOS)^-(Ag)^+]$ and $[(SOS)^-(K)^+]$ suitable for use in paraphysiological and pathological conditions of mucosa epithelia, as per nasal, genital, ocular and oral mucosae.

Therefore, in a further aspect the invention relates to an use of a silver salt of sucrose octasulfate and a potassium salt of sucrose octasulfate for the preparation of compositions for prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof, or to a method of prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof comprising the administration to a subject in a need thereof of a composition comprising a silver salt and a potassium salt of sucrose octasulfate.

The mucosal infections for which the compositions of the invention can be used are infections sustained by bacteria, yeasts and fungi occurring in nasal, genital, ocular and oral (mouth) mucosae and the paraphysiological and pathological conditions associated thereof are symptomatological mucosal inflammation/congestion states, prone to the development and recurrence of infections. The prevention and treatment of symptomatological mucosal inflammation/congestion states can be achieved facilitating the tissue repair from inflammation, thus recovering from subjective complaints and objective measures of altered functionality, and regaining health related quality of life (HRQoL).

In another aspect, compositions comprising $[(SOS)^-(Ag)^+]$ and $[(SOS)^-(K)^+]$ could be used for providing a surface antimicrobial coating of medical devices.

Brief description of the figures

Figure 1 shows phase contrast microscopy images obtained with a Leica DM-Il IMC microscope at 100x magnification: (a) control fibroblasts treated with 2% $[(SOS)^-(K)^+]$ indicated as KSOS; (b) fibroblasts + *S. aureus*; (c) fibroblasts + *S. aureus* treated with 2% $[(SOS)^-(K)^+]$ indicated as KSOS.

Figure 2 shows the evaluation of barrier effects towards microbial strains colonization in *S. aureus* cell cultures. (a) *S. aureus* control cultures; (b) *S. aureus* cultures treated with the gel comprising $[(SOS)^-(K)^+]$ 1.0% and $[(SOS)^-(Ag)^+]$ 0.010% expressed as silver ions. Arrows indicate some of the bacterial colonies identified, after a 2 day incubation at 37°C, in the bottom solid agar of the control cultures, arising from the top soft agar inoculated with a massive amount of *Staphylococcus aureus*. Such colonization is not observed when a thin layer of gel is interposed within the two agar layers.

Detailed description of the invention

The present invention relates to a composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$, having a combined and synergistic antimicrobial and anti-microbial adhesion biological activity against infections of mucosal epithelia, including those produced by biofilm-forming strains.

For the purpose of the invention, the composition comprises the silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and the potassium salt sucrose octasulfate $[(SOS)^-(K)^+]$ in an amount comprised in a range of: $[(SOS)^-(Ag)^+]$ expressed in ppm of Ag^+ from 10 ppm (corresponding to 0.001% by weight) to 200 ppm (corresponding to 0.020% by weight) and $[(SOS)^-(K)^+]$ from 0.5% to 5% by weight (expressed in g/100g).

The preferred amount ranges are for $[(SOS)^-(Ag)^+]$, expressed in ppm of Ag^+, from 25 ppm (corresponding to 0.0025% by weight) to 100 ppm (corresponding to 0.01% by weight) and for $[(SOS)^-(K)^+]$ from 1.0% to 2.5 % by weight. The most preferred amounts of $[(SOS)^-(Ag)^+]$ and $[(SOS)^-(K)^+]$ are respectively 0.01% expressed as Ag^+ by weight and 1.0% by weight and 0.0025% expressed as Ag^+ by weight and 1.5% by weight

Therefore, according to the different intended applications, in a first embodiment compositions according to the invention can comprise $[(SOS)^-(Ag)^+]$, expressed as Ag^+, in an amount in the range from 0.001% to 0.02 % by weight and $[(SOS)^-(K)^+]$ in an amount in the range from 0.5% to 5% by weight.

Hence, compositions comprising [$(SOS)^-(Ag)^+$], expressed as Ag^+, in an amount of 0.001% by weight and in an amount [$(SOS)^-(K)^+$] of 0.5% by weight and compositions comprising [$(SOS)^-(Ag)^+$], expressed as Ag^+, in an amount of 0.02% by weight and in an amount [$(SOS)^-(K)^+$] of 5% by weight are embodiments of the present invention.

It is yet an embodiment of the invention compositions comprising [$(SOS)^-(Ag)^+$], expressed as Ag^+, in an amount in the range from 0.0025% to 0.01% by weight and [$(SOS)^-(K)^+$] in an amount in the range from of 1.0% to 2.5% by weight.

Further embodiments of the invention are a composition comprising [$(SOS)^-(Ag)^+$], expressed as Ag^+, in an amount of 0.01% by weight and [$(SOS)^-(K)^+$] of 1.0% by weight and a composition comprising [$(SOS)^-(Ag)^+$], expressed as Ag^+, in an amount of 0.0025% by weight and [$(SOS)^-(K)^+$] of 1.5% by weight.

The compositions of the invention further comprise excipients and/or diluents compatible with silver ions selected from polysaccharides, non-ionic surfactants, weak organic acids, highly purified vegetable extracts and amino acids.

According to the intended uses of the final products, the compositions can be added with ingredients chemically compatible with silver, such as glycerol, propylene glycol, carbopol, non-ionic surfactants, acidic polysaccharides, lactates, citrates, purified vegetable extracts and amino acids, as an example. The preferred polysaccharide is hyaluronic acid, being this polysaccharide highly biocompatible and having as known healing property.

Due to the high solubility in water of the two compounds, aqueous compositions in form of hydrogels and solutions, can be preferred. Solutions at different ionic strength and pH can be prepared for nasal, ocular and oral application, while hydrogel with different viscosity and pH can be prepared for nasal, oral, and vaginal treatment. The pH of the composition can be adjusted using sodium hydroxide, triethanolamine, EDTA or mild organic acid as lactic or citric acid, dependent upon the intended application on specific mucosae.

However, the composition according to the invention can be more various; the composition can in fact contain, according to the different and final body district of application, the two sucrose octasulfate silver and potassium salts as a such or encapsulated in nanospheres and/or microspheres based on natural,

semisynthetic, synthetic polymers or entrapped in cyclodextrins to obtain a slow release delivery system. The compositions can be in form of liquids, semi-solids or solids, containing excipients and/or diluents of pharmaceutical or cosmetic grade (for example solutions and aqueous, non-aqueous, hydro-alcoholic suspensions, drops, gels, emulsions, creams, ovules, powder sprays, sprays with or without propellants, foams). Furthermore, the $[(SOS)^{-}(K)^{+}]$ and $[(SOS)^{-}(Ag)^{+}]$, as a such or encapsulated in nanospheres and/or microspheres based on natural, semisynthetic, synthetic polymers or entrapped in cyclodextrins, can be loaded upon inert biocompatible systems such as films, membranes, patches and dressings acting as slow release delivery systems, or can be incorporated into biomaterials, such as for example hydrogels, membranes, sponges, or into materials dissolving rapidly in aqueous environments. They can also be applied as protective antimicrobial coating on various kinds of medical devices.

For example, in the finished products the compositions of the invention can be in form of spray, aerosol, drops, sachets, capsules, vaginal douches, mouth washes, plain solutions, any kind of multiple or single dose and any other suitable form of delivery available for the market for the application pursued.

As proved by the results obtained with $[(SOS)^{-}(K)^{+}]$ and $[(SOS)^{-}(Ag)^{+}]$ alone or combined hereinafter reported in detail, the compositions of the invention can be used in the prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof and in particular in mucosal infections sustained by biofilm-forming strains.

The mucosal infections for which the compositions of the invention can be used are infections sustained by bacteria, yeasts and fungi occurring in nasal, genital, ocular and oral (mouth) mucosae and the paraphysiological and pathological conditions associated thereof are symptomatological mucosal inflammation/congestion states, prone to the development and recurrence of infections. The prevention and treatment of symptomatological mucosal inflammation/ congestion states can be further achieved also facilitating the tissue repair from inflammation, thus recovering from subjective complaints and objective measures of altered functionality, and regaining health related quality of life (HRQoL).

In particular, the compositions comprising [$(SOS)^-(K)^+$] and [$(SOS)^-(Ag)^+$] of the invention can be usefully employed for prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof selected from sinonasal diseases of different etiology, such as common seasonal affections of obstruction and congestion of the upper respiratory tract, acute, acute-post viral, recurrent and chronic rhinosinusitis, otitis, oral mucosal ulcerations and periodontitis, ocular dryness as well as other ocular conditions such as corneal abrasions or ocular infections, cold mucosal sore and infections, and vaginal bacterial vaginosis and vaginitis, including those sustained or superinfected with *Candida* spp.

Preferred uses of the composition of the invention are for preventing and treating vaginal infections and recurrences thereof, including those sustained by *Candida* spp, vaginal biofilm formers difficult to eradicate and responsible of fastidious vaginitis symptoms. Another preferred use is prevention and treatment of obstruction and congestion of the upper respiratory tract, with or without rhinosinusitis and/or nasal polyps, irrespective whether acute, acute post-viral, recurrent or chronic, and characterized by a persistent obstructive symptomatology since at least 10 days.

With reference to sinonasal diseases of different etiology, these can be of moderate severity as per VAS>5 for one of the primary symptoms nasal congestion, nasal obstruction and rhinorrea, or a VAS>5 for one of the primary symptoms and at least one of the secondary symptoms facial pain/pressure, reduction or loss of smell, headache.

The treatment with the compositions object of the invention of said obstructive rhinopathy can facilitate the recovery of health related quality of life and nasal patency with consequent improved respiration.

Hereinafter the results obtained on the characterisation of the anti-adhesive and antimicrobial properties of the association of the two sucrose-octasulfate salts [$(SOS)^-(K)^+$] and [$(SOS)^-(Ag)^+$] are reported in detail.

Furthermore, examples of compositions containing the two sucrose-octasulfate salts [$(SOS)^-(K)^+$] and [$(SOS)^-(Ag)^+$] are shown for exemplificative and not-limiting

purposes. The antimicrobial and anti-adhesive activities and the tolerability and efficacy of compositions prepared are also reported.

A. Characterisation of the anti-adhesive and antimicrobial activities of the association $[(SOS)^-(K)^+]$ and $[(SOS)^-(Ag)^+]$

The properties of $[(SOS)^-(K)^+]$ and of the association of $[(SOS)^-(K)^+]$ and $[(SOS)^-(Ag)^+]$ were studied in comparison to $[(SOS)^-(Ag)^+]$ alone.

$[(SOS)^-(K)^+]$ was preliminary dose-dependently tested for its capability to inhibit the adhesion of *Staphylococcus aureus* to mammalian tissue cells and to interfere on mammalian cells vitality. Table 1 summarizes the results of the dose-dependent inhibitory effect of $[(SOS)^-(K)^+]$ on *Staphylococcus aureus* adherence to murine fibroblast 3T3, with no toxic effect on plated mammalian cells vitality.

Briefly, murine fibroblasts 3T3, cultivated in MEM medium supplemented with 10% FBS and antibiotics, were plated at the concentration of $1x10^4$ cells/well in 96-well plates. Before the test, the culture medium was replaced in each well with 100 µl of fresh medium without antibiotics and then a 100 µl of a fresh *Staphylococcus aureus* suspension, pretreated for 30 minutes with different amount (0, 0.5, 1.0, 2%) of $[(SOS)^-(K)^+]$, were added. The samples were incubated at 37°C in presence of 5% CO_2 for 1 hour and 45 minutes to allow bacteria to adhere to the cell surface. After removal of the surnatant, wells were washed with pre-warmed PBS to remove non-adherent bacteria. The mammalian cells were then lysed with 0.1% Triton-X 100 and the lysates properly diluted to a concentration of $1x10^7$ before inclusion in Plate Count Agar (PCA). The plates were then incubated at 37°C for 48 hours and at the end, the number of adherent bacteria were calculated as the mean CFU corrected by the dilution factor.

Table 1. Inhibitory activity of $[(SOS)^-(K)^+]$ on *Staphylococcus aureus* adherence to murine fibroblast 3T3

Staphylococcus aureus				
	TVC before incubation CFU/ml*	*TVC* after incubation CFU/ml*	*Bacterial adhesion %*	*Adhesion inhibition %*
$[(SOS)^-(K)^+]$ 0% (control)	1.5×10^5	3.2×10^4	21.3	-

$[(SOS)^-(K)^+]$ 2%	4.2×10^5	2.4×10^4	5.7	73.2
$[(SOS)^-(K)^+]$ 1%	3.6×10^5	2.7×10^4	7.5	64.8
$[(SOS)^-(K)^+]$ 0.5%	3.1×10^5	3.6×10^4	11.6	45.5

**TVC=Total vital count.*

Figure 1 shows the images obtained by phase contrast microscopy relative to the adhesion of *Staphylococcus aureus*, a microorganism known for its capability to form biofilm, to mammalian tissue cells, e.g. fibroblasts, after exposure to 2% $[(SOS)^-(K)^+]$.

Then, an aqueous solution formed by the association of $[(SOS)^-(Ag)^+]$ and $[(SOS)^-(K)^+]$ was tested to assess whether the association could result in a more efficient antimicrobial profile than that of the sole $[(SOS)^-(Ag)^+]$.

Table 2 reports the microbiological analysis at 24 (bacteria) and 48 hours (yeast), of $[(SOS)^-(Ag)^+]$ alone in aqueous solutions at several dosages, demonstrating its bacteriostatic and bactericidal activity against various kind of pathogens, with the only exclusion of *Staphylococcus aureus* on which $[(SOS)^-(Ag)^+]$ acted as a bacteriostatic agent only. As regards fungi and yeasts, for all of the various microorganisms tested only a fungistatic effect was observed and therefore only the Minimal Inhibitory Concentration (MIC) could be determined. The data relative to the yeast *Candida albicans* only are reported in Table 2, indicating that concentration up to 139 ppm Ag^+ were not fungicidal.

Table 2. Antimicrobial properties of $[(SOS)^-(Ag)^+]$

	MIC	*MMC*
Micro-organisms	$[(SOS)^-(Ag)^+]$ (as ppm Ag^+)	$[(SOS)^-(Ag)^+]$ (as ppm Ag^+)
Haemophilus influenzae	4.3	4.3-9.3
Streptococcus pneumoniae	17.4	17.4
Moraxella osloensis	8.7	8.7-17.4
Staphylococcus aureus	17.4	277.7
Pseudomonas aeruginosa	8.7-17.4	8.7-17.4

Candida albicans	1.1	>139

MIC: Minimum Inhibitory Concentration; MMC: Minimum Microbiocidal Concentration

Differently from data reported in Table 2, the association of, e.g., 1.5% $[(SOS)^-(K)^+]$ and $[(SOS)^-(Ag)^+]$ as 25 ppm Ag^+, exerted a clear bactericidal and fungicidal activity as early as 48 hours post-treatment, when challenged against *Staphylococcus aureus* and *Candida albicans* (Table 3), thus demonstrating a synergistic antimicrobial profile of the composition. Worth noting that *Staphylococcus aureus* and *Candida albicans* have been selected among those microbial strains known to be prone to form colonies organized in biofilms.

Table 3. Bactericidal and fungicidal activity of the association

Total microbial count	*Staphylococcus aureus* *CFU/G*	*Candida albicans* *CFU/G*
T_0 = inoculum	1.0×10^6	1.6×10^5
T = 2 days	< 10	< 10

B. Examples of compositions comprising the association $[(SOS)^-(K)^+]$ and $[(SOS)^-(Ag)^+]$ and tolerability, efficacy and anti-adhesive studies

The two sucrose octasulfate silver and potassium salts $[(SOS)^-(Ag)^+]$ and $[(SOS)^-(K)^+]$ were used to prepare compositions according to the invention with other appropriate excipients and diluents. Due to high solubility in water of the two compounds, aqueous compositions were preferentially prepared, that is hydrogels and solutions. Different concentrations of the two active compounds were incorporated within the formulations.

Examples of final products comprising the composition of the present invention and excipients selected and added in amounts complying with the physiology of the anatomical district to be treated are reported below.

Example 1. Mild hypertonic water solution for nasal application - osmolality between 380-420 mOsm/kg

Component	*Quantity g/100 g*
$[(SOS)^-(K)^+]$	1.5

$[(SOS)^-(Ag)^+]$ (expressed as silver ions)	0.0025
Glycerol	3.1
sodium hydroxide q.s. to pH 6.0-7.0	
Water q.s. to	100.

Example 2. Hydrogel for vaginal application - Viscosity (G21/V1.0 rpm = 15000-25000 cPs)

Component	*Quantity g/100 g*
$[(SOS)^-(K)^+]$	1.0
$[(SOS)^-(Ag)^+]$ (expressed as silver ions)	0.010
Propylene glycol	10.0
Sodium hyaluronate	0.2
Carbopol® Ultrez 10	0.765
Sodium hydroxide q.s. to pH 5.0-5.5	
Water q.s. to	100.

Example 3. Mild hypertonic water solution for mouth washing - osmolality between 380-420 mOsm/kg

Component	*Quantity g/100 g*
$[(SOS)^-(K)^+]$	2.0
$[(SOS)^-(Ag)^+]$ (expressed as silver ions)	0.005
Glycerol	2.9
sodium hydroxide q.s. to pH 6.5-7.4	
Water q.s. to	100.

Example 4. Hydrogel for external genital application - Viscosity (G21/V1.0 rpm = 15000-25000 cPs)

Component	*Quantity g/100 g*
$[(SOS)^-(K)^+]$	2.5
$[(SOS)^-(Ag)^+]$ (expressed as silver ions)	0.010
Propylene glycol	10.0
Sodium hyaluronate	0.2
Carbopol® Ultrez 10	0.9
Sodium hydroxide q.s. to pH 5.0-5.5	
Water q.s. to	100.

Example 5. Viscous water solution for ocular application

Component	*Quantity g/100 g*
$[(SOS)^-(K)^+]$	1.0
$[(SOS)^-(Ag)^+]$ (expressed as silver ions)	0.002
Sodium hyaluronate	0.2
Glycerol	2.35
Sodium hydroxide q.s. to pH 7.0	
Water for injection q.s. to	100.

Besides these exemplificative examples several other compositions comprising $[(SOS)^-(Ag)^+]$ in concentrations ranging from 10-200 ppm of Ag^+ and $[(SOS)^-(K)^+]$ in concentrations from 0.5% to 5% can be prepared without any further burden by one skilled in the art.

The compositions of examples 1 and 2 were tested *in vitro* for the assessment of the cytotoxicity on cells.

Example 6. *In vitro* cytotoxicity assay on the composition of example 1

The solutions as per example 1 were tested in tolerability studies *in vitro* on murine fibroblasts (Balb/c 3T3, clone A31-1-1) according to the rule UNI EN ISO 10993-5 for Medical Devices, demonstrating the safety of the composition.

Cells were seeded in 96 well plates (10000 cells/well) and allowed to grow for 24h at 37°C and 5% CO_2. The second day fresh medium is added, supplemented with scalar dilutions of the product ranging from 50 to 0.06 mg/ml. After an incubation of 24h, the medium is removed and the cells are incubated for 2 h with 1mg/ml MTT {3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide} solution. The purple crystals of Formazan formed by the activity of viable cell mitochondrial dehydrogenases are dissolved with isopropanol and the resulting purple solution is measured spectrophotometrically. Any change in cell viability will modify the amount of Formazan formed, indicating the degree of cytotoxicity of the test material.

Table 4 reports the *in vitro* data relative to the cytotoxicity tests of the solution. No significant cell mortality was observed at any concentration.

Table 4. Cytotoxicity of a solution as per example 1

Dose (mg/ml)	*0.06*	*0.12*	*0.25*	*0.50*	*1.0*	*2.0*	*10.0*	*50.0*
% viable cells *vs* negative control	107.8	110.6	110.2	110.9	105.9	105.0	99.8	87.2

% cell viability [OD test product/OD negative control] x 100; OD = optical density.

Example 7. *In vitro* cytotoxicity assay on the composition of example 2

The composition formulated as per example 2 with excipients selected to comply with the physiology of the vaginal environment and in form of hydrogel, was tested at different dosages on Transformed Human Keratinocytes (HaCaT cells), in cytotoxicity studies. HaCaT cells were exposed for 24h to scalar amounts of the gel composition, ranging from 5 to 0.15 mg/ml. Cells were next tested for viability by MTT {3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide} incubation; the absorbance of the newly formed Formazan was then measured spectrophotometrically at 550nm. An increase or a decrease in cell number results in a corresponding change in the amount of Formazan formed, indicating the degree of cytotoxicity caused by the gel composition. Data collected on the vaginal gels are reported in Table 5, confirming the optimal safety of the composition.

Table 5. Cytotoxicity of an hydrogel as per example 2

Dose (mg/ml)	*0.15*	*0.3*	*0.6*	*1.25*	*2.5*	*5.0*
% viable cell vs negative control	102.8	99.2	98.0	100.9	103.0	102.6

% cell viability [OD test product/OD negative control] x 100; OD = optical density.

Example 8. Anti-adhesive property of the composition of example 2

The anti-adhesive, protective effects of the composition of the present invention against microbial colonization was found when a thin film of the composition was applied between two layers of agar forming a sandwich-like structure, with the bottom layer being sterile and the top soft agar containing a massive microbial inoculum of biofilm-forming pathogenic strains (e.g. *S. aureus*).

Briefly, Petri dishes of 90 mm Ø were prepared with 10 ml of Plate Count Agar (PCA) on top of which the sample was uniformly spread at the following amounts:
130 mg (corresponding to 2 mg/cm^2),
65 mg (corresponding to 1 mg/cm^2),
13 mg (corresponding to 0.2 mg/cm^2).
Next, on the top of these layers, 5 ml of soft agar (that is a medium containing a low percentage of agar), previously inoculated with various microorganisms were poured without damaging the film of applied gel. The dishes were incubated at 37°C for 2 days (bacteria) or 4 days (yeast) and then a part of the soft agar was gently removed from the Petri dish to investigate the presence of colonies in the bottom solid agar. No film of gel was applied between the two agar layers in the control plates. The results were visualized through stereomicroscopic observations with a magnification of 40 X and digital images taken with a Nikon Colplix 4600. Results are shown in Figure 2. Differently from what occurs in the control plates, no colonization of the bottom solid agar was observed in presence of the composition as per example 2, and the effect was dose-dependent.
In the same experimental model, similar results were obtained also against the colonization by other pathogenic strains, such as *Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Nesseriae gonorrhoeae*, suggesting that the composition of the present invention, i.e. the association of [$(SOS)^-(Ag)^+$] and [$(SOS)^-(K)^+$], particularly in hydrogels, provides an efficient barrier against microbial colonization.

Example 9. Mucosal protection property of the composition of example 2

Besides antimicrobial and anti-adhesive properties, the composition of the present invention can also protect mucosal epithelia from the damaging effects induced by irritant molecules.
Briefly, the assay was performed using tridimensional epithelial units reconstituted by airlifted cultures of transformed human keratinocytes, kept for 5 days in chemically defined medium on inert polycarbonate filters; 30 mg of the gel of example 2 was applied on the mucosal surface 2 h before a 15' exposure to 5% Sodium Lauryl Sulfate (SLS). At the end of the treatment with SLS, the epithelial units were washed with PBS and a further amount of sample was applied.

Exposure to the gel was carried out for 2 and 24h at 37°C and 5% CO_2. At the end of the exposure period, the product was removed, the tissue was washed with PBS and the MTT assay was performed to evaluate cell survival. Beside, an Elisa assay was performed on the culture medium 6 h and 24 h after the irritant stress to evaluate the amount of IL-1α released by the cells. Mucosal epithelia not treated with the gel were used as controls.

When a tridimensional (3D) reconstructed mucosa was challenged *in vitro* with 5% sodium lauryl sulfate (SLS), the composition in the form of a hydrogel as per example 2 warranted a reduced cell mortality and a decreased release into the medium of Interleukin 1 (IL-1), a well-known pro-inflammatory cytokine, suggesting that *in vivo* the composition could exert a soothing effect (Table 6). On the other hand the hydrogel by itself does not induce a significant IL-1 α release.

Table 6. Inhibition of SLS induced IL-1 release in a reconstructed 3D mucosal epithelium

	IL-1 α (pg/ml)	*% inhibition/protection*
Control	29.9	-
5% SLS	419.3	-
composition as per ex. 2 (30 mg)	40.5	-
5% SLS + composition as per ex. 2 (30 mg)	204.05	55%

Example 10. *In vivo* tolerability and efficacy of the composition of example 1

The solution prepared as a nasal spray of example 1 underwent a clinical trial aimed to assess tolerability and efficacy over a 20 days exposure period on N= 25 outpatients of both genders, aged 18-70, affected by obstructive rhinopathy of different etiology, with or without rhino-sinusitis, lasting since 10 days in advance of enrollment and therefore suggestive of ongoing bacterial super-infection. To better assess the stand-alone properties of the composition in sinonasal affections, neither concomitant nor previous treatment (within one week from enrollment) with local and/or systemic corticosteroid, antibiotic, decongestants and nasal saline washes, was allowed. Nasal obstruction as well as treatment efficacy

was assessed by means of: a) Patient Related Outcome Measure (PROM), administered in the form of the validated linear 0-10 cm Visual Analog Score (VAS) and the Sino-Nasal Outcome Test 22 (SNOT-22) questionnaire, a disease-related symptoms scoring method validated for chronic rhino-sinusitis, with and without polyps; and b) Anterior Active Rhinomanometry (AAR) to measure the reduced nasal patency, expressed as nasal resistance to airflow in Pascal/ml/sec (Pa/ml/s). Enrolled patients should have been scored for moderate disease severity due to either a VAS > 5 for two of the primary symptoms: nasal congestion, nasal obstruction and Rhinorrea, or a VAS > 5 for one of the primary symptoms above + a moderate score for at least one of the secondary symptoms: facial pain/pressure, reduction or loss of smell. A VAS > 5 does impact on health related quality of life (HRQoL; ref: EPOS 2012), and the validated 22-items Sino-Nasal Outcome Tests (SNOT-22), ranging from 0 (absence of symptoms) to 5 (the highest severity degree) is commonly applied to assess treatment efficacy and surgical outcome. Clinical data indicates that the composition of the present invention, i.e. the association of $[(SOS)^{-}(Ag)^{+}]$ and $[(SOS)^{-}(K)^{+}]$, formulated as per example 1 with excipients selected to specifically comply with the physiology of the sinonasal cavities, successfully relieved patients' symptomatology (Table 7), and reduced nasal resistance, i.e. improved respiration (Table 8). After 20 days of treatment with the composition as per example 1, all the items in SNOT-22 relevant to nasal obstruction/congestion were improved, demonstrating its efficacy on the recovery of subjective HRQoL and objective sinonasal functional parameters.

Table 7. Efficacy of the composition of example 1 on the Patient Related Outcome Measure: SNOT-22

ITEMS	*T = 0* *Mean ± SD*	*T = 20* *Mean ± SD*
Nasal obstruction congestion	4.64 ±0.5	2.96 ± 0.9**
Posterior nasal discharge	1.68 ± 1.5	0.60 ±0.8*
Thick nasal discharge	1.28 ± 1.1	0.56 ± 0.8*
Ear fullness	1.40 ± 1.0	0.92 ±0.5*
SNOT-22 Total Score	30.00 ± 9.7	20.8 ± 8.7**

*Wilcoxon test * p<0.05 ; **P< 0.01*

Table 8 shows that the composition as per example 1 decreased the objectively measured airflow resistance, indicating its capability to support mucosal functional repair from nasal obstruction and/or mucosal congestion. These data support the composition as per example 1 as a suitable method to treat sinonasal affections of different etiology, having an obstructive component.

Table 8. Effect of the mild hypertonic solution on Nasal Resistance

Time	*Total nasal Resistance (Pa/ml/s)*	
	Inspiratory flow	Expiratory flow
T = 0	0.660 ± 0.601	0.662 ± 0.579
T = 20	0.420 ± 0.218	0.382 ± 0.205*

The decrease in total nasal resistance further supports the concept that the composition-induced recovery from altered airflows (respiration) related to nasal obstruction and mucosal congestion, may be due to a decongestant, anti-inflammatory activity of the composition.

All together these data indicate that compositions based on the specific association of $[(SOS)^{-}(Ag)^{+}]$ and $[(SOS)^{-}(K)^{+}]$ are devoid of toxicity and can be beneficially used as a preventive or therapeutic method to protect mucosal epithelia in many paraphysiological and pathological conditions of, and not limited to, obstruction and congestion of the upper respiratory pathways (e.g. common seasonal affections, acute, acute non-viral, recurrent, and chronic rhinosinusitis), vaginal bacterial vaginosis and vaginitis, otitis, oral mucosal ulcerations and periodontitis, ocular dryness as well as other ocular conditions, cold sore and skin infections. Finally, they could be used as protective antimicrobial coating on the surface of medical devices.

Claims

1. A composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$ having combined and synergistic antimicrobial and anti-microbial adhesion biological activities against infections of mucosal epithelia.

2. The composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$ according to claim 1, wherein said composition comprises the silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ in an amount comprised in a range expressed in ppm of Ag^+ from 10 ppm (corresponding to 0.001% by weight) to 200 ppm (corresponding to 0.020% by weight) and the potassium salt sucrose octasulfate $[(SOS)^-(K)^+]$ in an amount comprised in a range from 0.5% to 5% by weight.

3. The composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$ according to claim 1, wherein said composition comprises the silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ in an amount comprised in a range expressed in ppm of Ag^+ from 25 ppm (corresponding to 0.0025% by weight) to 100 ppm (corresponding to 0.010% by weight) and the potassium salt sucrose octasulfate $[(SOS)^-(K)^+]$ in an amount comprised in a range from 1.0% to 2.5% by weight.

4. The composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$ according to claim 1, wherein said composition comprises the silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ in an amount expressed in ppm of Ag^+ of 100 ppm (corresponding to 0.01% by weight) and the potassium salt sucrose octasulfate $[(SOS)^-(K)^+]$ in an amount of 1.0% by weight.

5. The composition comprising a silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ and a potassium salt of sucrose octasulfate $[(SOS)^-(K)^+]$ according to claim 1, wherein said composition comprises the silver salt of sucrose octasulfate $[(SOS)^-(Ag)^+]$ in an amount expressed in ppm of Ag^+ of 25 ppm

(corresponding to 0.0025% by weight) and the potassium salt sucrose octasulfate [$(SOS)^-(K)^+$] in an amount of 1.5% by weight.

6. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to one of the claims 1 to 5, further comprising excipients and/or diluents compatible with silver ions selected from polysaccharides, non-ionic surfactants, weak organic acids, highly purified vegetable extracts and amino acids.

7. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 6, wherein the polysaccharide is hyaluronic acid.

8. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 1, for use in prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof.

9. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 8, wherein said of mucosal infections are sustained by bacteria, yeasts and fungi of nasal, genital, ocular and oral mucosae.

10. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 8, wherein said mucosal infections are sustained by biofilm-forming strains.

11. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 8, wherein the paraphysiological and pathological conditions associated with the mucosal infections are symptomatological mucosal inflammation/congestion states, prone to the development and recurrence of infections.

12. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 8, wherein said mucosal infections and paraphysiological and pathological conditions associated thereof are selected from sinonasal disorders, otitis, oral mucosal ulcerations and periodontitis, ocular

conditions of ocular dryness, corneal abrasions and ocular infections, cold mucosal sore and infections, and vaginal bacterial vaginosis and vaginitis.

13. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 12, wherein the mucosal infections and paraphysiological and pathological conditions associated thereof are selected from obstruction and congestion of the upper respiratory tract and vaginal bacterial vaginosis and vaginitis.

14. The composition comprising a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to one of the claims 1 to 7, for use as surface antimicrobial coating to medical devices.

15. Use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] for the preparation of compositions according to one of the claims 1 to 7 for prevention and treatment of mucosal infections and paraphysiological and pathological conditions associated thereof.

16. The use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 15, wherein said of mucosal infections are sustained by bacteria, yeasts and fungi and are located on nasal, genital, ocular and oral mucosae.

17. The use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 15, wherein said mucosal infections are sustained by biofilm-forming strains.

18. The use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 15, wherein the paraphysiological and pathological conditions associated with the mucosal infections are symptomatological mucosal inflammation/congestion states, prone to the development and recurrence of infections.

19. The use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 15,

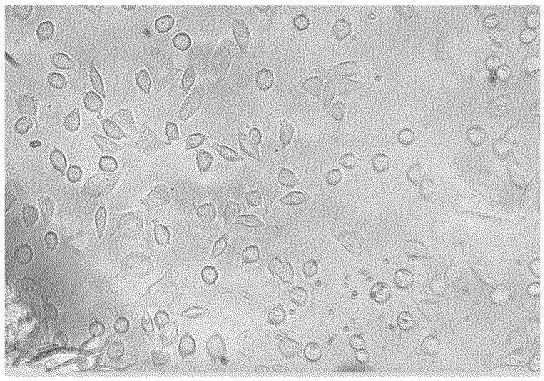
wherein said mucosal infections and paraphysiological and pathological conditions associated thereof are selected from sinonasal disorders, otitis, oral mucosal ulcerations and periodontitis, ocular conditions of ocular dryness, corneal abrasions and ocular infections, cold mucosal sore and infections, and vaginal bacterial vaginosis and vaginitis.

20. The use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] according to claim 19, wherein the mucosal infections and paraphysiological and pathological conditions associated thereof are selected from obstruction and congestion of the upper respiratory tract and vaginal bacterial vaginosis and vaginitis.

21. Use of a silver salt of sucrose octasulfate [$(SOS)^-(Ag)^+$] and a potassium salt of sucrose octasulfate [$(SOS)^-(K)^+$] for the preparation of compositions according to one of claims 1 to 7 for providing a surface antimicrobial coating to medical devices.

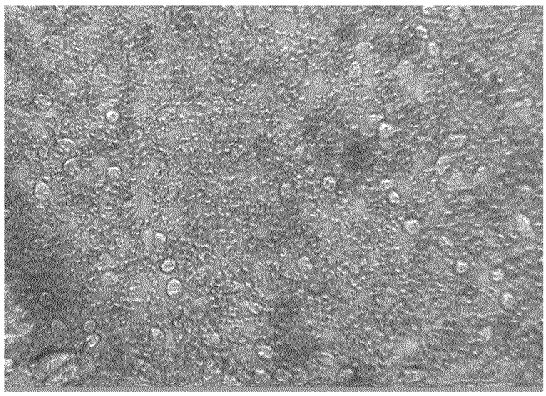
FIGURE 1

Control: Fibroblasts + 2%KSOS



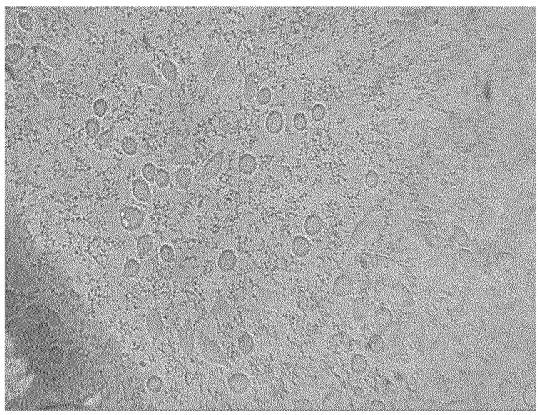
(a)

Fibroblasts + Staphylococcus aureus



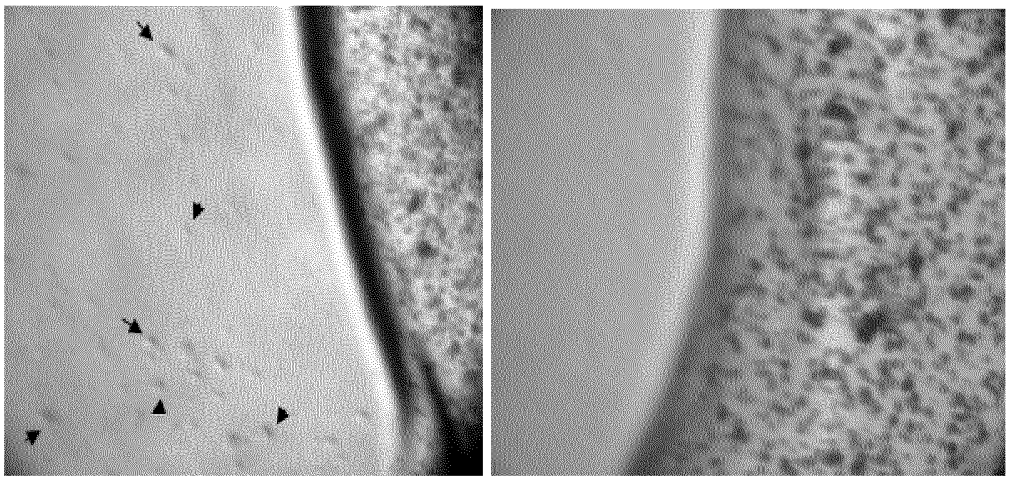
(b)

Fibroblasts + Staph. aureus + 2%KSOS



(c)

FIGURE 2



(a) (b)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/067482

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P31/00 A61P31/04 A61P31/10 A61P11/02 A61K31/7024
A61K47/36 A61K9/00 A61L29/10 A61L29/16 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 A61L

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)
EPO-Internal, BIOSIS, CHEM ABS Data, 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	WO 03/035656 A1 (INTERALIA S R L [IT]; CALDERINI GABRIELLA [IT]; PROSDOCIMI MARCO [IT];) 1 May 2003 (2003-05-01)	1-6
Y	page 7, lines 9-15; example 3 page 8, line 8 - page 13, line 18 claims 6,7	1-21

Y	CA 2 020 199 C (BUKH MEDITEC [DK]) 20 August 2002 (2002-08-20) page 8, line 17 - page 9, line 14 page 13, lines 13-29 page 15, lines 12-25 page 16, lines 10-13 page 18, lines 1-6 pages 33-34; example 15 claims 1,6,8,19,21,23,28,37,41	1-21

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2013	27/09/2013

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	Gradassi, Giulia

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/067482

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/43333 A1 (ALTHEXIS COMPANY [US]; US HEALTH [US]; NAVIA MANUEL A [US]; QUINN THOM) 2 September 1999 (1999-09-02) page 5, lines 18-29 page 15, lines 13-17 page 23, line 20 - page 24, line 4 page 28, lines 7-12 page 33, lines 1-11 pages 35-37; examples claims -----	1-21

4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/067482

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