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Title: ANTIMICROBIAL MIXTURE AND A COVERING SUPPORTING WOUND HEALING, HAVING AN ANTIMICROBIAL ACTIVITY

Fig. 1

Abstract: The invention relates to an antimicrobial mixture for healing of superficial wounds based on a physiologically acceptable hyaluronic acid salt, optionally with one or more other polysaccharides, and a compound having an antimicrobial activity, which can be used for healing of superficial wounds, especially chronic wounds, for example varicose ulcers, and to a covering which contains said mixture.
Antimicrobial mixture and a covering supporting wound healing, having an antimicrobial activity

Field of the Invention

The invention relates to a mixture for healing of superficial wounds based on a physiologically acceptable hyaluronic acid salt, optionally with one or more other polysaccharides, and a compound having an antimicrobial activity, which can be used for healing of superficial wounds, especially chronic wounds, for example varicose ulcers, and to a covering which contains said mixture.

Background of the Invention

Wound healing, especially anomalous wound healing, is a complicated process, which can be supported by a covering providing adequate healing conditions and/or containing active compounds. Anomalous wound healing includes conditions of excessive wound healing (such as fibrosis, adhesions and contractures), or, what is more frequent, insufficient wound healing (such as chronic varicose ulcers or diabetic wounds). Despite many recent significant advances in the field, anomalous wound healing still represents the cause of high costs, sickness rate and mortality; This all stresses out the importance of providing an optimal environment for wound healing which can positively influence the healing process (Stephanie R. Goldberg, Robert F. Diegelmann: Wound Healing Primer, Surgical Clinics of North America, Volume 90, Issue 6, December 2010, p. 1133-1 146).

A brief review of the literature dealing with the topic of wound healing is presented for example in the publication of James R. Hanna, Joseph A. Giacopelli: A review of wound healing and wound dressing products, The Journal of Foot and Ankle Surgery, Volume 36, Issue 1, January-February 1997, p: 2-14. The authors discuss especially the importance of an appropriate wound covering which ensures the optimal environment for wound healing. It is described that modern coverings visually consist of more than one layer and that the contact layer has the greatest influence on healing.

There are lots of commercial products on the market nowadays which, however, do not sufficiently fulfill the important requirements imposed on wound coverings or the production costs are high, and therefore, the mass employment is limited. Usually, various gel-forming natural or synthetic substances which are able to regulate moisture are used. But these substances mostly only regulate the moisture within the wound and do not support self-regeneration ability of the body. Moreover, the application of such substances reduces gas
permeability, and therefore, the respiration of the wound is blocked. Infections are eliminated by applying disinfecting agents before the bandage is applied, which means that the disinfecting effect is only for a short-term. These wound coverings cannot be applied to chronic wounds because there is the risk that the moist environment can promote infection multiplication and thereby significantly deteriorate the condition of the patient.

Many patents and publications describe the application of hyaluronan as the main component of wound coverings but due to the expensiveness these formulations are not commercially used very much. Therefore, the practical use of hyaluronan remains aimed only at intra-somatic (intra-articular injections), optionally at special applications (ophthalmology, eye drops for eye surface humectation for patients with contact lenses).

Commercial application of hyaluronan is illustrated by the preparation based on hyaluronan combined with another active substance described by U.S. Patent 4736024. The product is designated especially for ophthalmology and the pharmaceutically active substances used include for example kanamycin, neomycin, tetracycline, chloramphenicol and combinations thereof.

U.S. Patent 5128136 describes a preparation useful for prevention of wound dehydration and infection, i.e. for a similar purpose as the presented preparation. However, in the contrary to the preparation according to the invention it contains soluble collagen as the main component which does not affect the wound healing, and it does not contain hyaluronic acid or salts thereof. The disadvantage of the U.S. Patent 5128136 is that collagen can cause undesirable reactions, such as inflammation. Another disadvantage is that after the application the liquid transforms its phase into a solid gel which blocks the gas permeability.

Subject-matter of the Invention

The above mentioned disadvantages of the existing compositions supporting wound healing are eliminated by the mixture according to the invention, comprising a physiologically acceptable hyaluronic acid salt and an antimicrobial substance selected from the group comprising benzalkonium chloride, benzalkonium bromide, chlorhexidine, hibitane, polyhexamethylene biguanide, cetyltrimethylammonium bromide, cetyltrimethylammonium bromide and a mixture thereof in any ratios. The mixture can further comprise one or more polysaccharides selected from the group comprising native or modified polysaccharides such as hyaluronan, xanthan, schizophyllan, chitosan, glut, alginate, cellulose, glucan, 1-3-glucane and a mixture thereof; and optionally also an adjuvant increasing antimicrobial effect, for example a chelating agent such as EDTA,
betaine and 2-phenoxyethanol can optionally comprise also a saccharide selected from the group comprising glucose, fructose and saccharose, and/or an electrolyte selected from the group comprising sodium chloride, potassium iodide, magnesium chloride, sodium hydrogen phosphate, sodium dihydrogen phosphate, zinc sulphate, and/or a plant extract or another natural product selected from the group comprising bee propolis, olive oil, tea tree extract from oak, calendula, mint or citruses, or a mixture of any combination of said substances.

The mixture according to the invention preferably comprises hyaluronan and octenidine dihydrochloride as the antimicrobial substance, preferably in the weight ratio of 500:1.

The term modified polysaccharide means a polysaccharide with a covalently bound molecule or a functional group, wherein this molecule may be the same polysaccharide or a different polysaccharide, synthetic polymer or polymerized or cross-linked polysaccharide consisting of identical or different molecules.

The mixture can have the form of a chemical or physical mixture, wherein the chemical mixture is preferably an aqueous solution containing alcohol and the physical mixture is preferably a layer of polysaccharide fibres coiffing an antimicrobial substance in the structure thereof.

Furthermore, the invention relates to the covering for supporting the wound healing which is in the form of a single-layer or multi-layer formation. The multi-layer formation comprises layers arranged in the following manner in the direction from the wound: the contact layer (K), which is provided on one side which is to be in contact with the wound, with the coating (P) made of polysaccharides with an antimicrobial substance, i.e. made of the above-mentioned mixture according to the invention (i.e. chemical or the physical mixture), then one or more absorption layers (A) and the surface layer (R). Therefore, the lower, contact layer (K) contains at one (lower) side destined to be contacted with the wound, the coating (P) made of the mixture of polysaccharides with an antimicrobial substance, and the other (upper) side is abutted by one or more absorption layers (A, or 1:1; A-2 etc.). The upper side of the last upmost absorption layer which is the most distant from the wound abuts to the surface layer (R). All layers can be welded together at the edges, forming the so-called tea bag having the dimensions of for example 10 x 10 cm. In case of the one-layer covering, the formation consists only of the contact layer (K) having the coating (P) made of the mixture of polysaccharides with an antimicrobial substance, wherein the contact layer (K) can be composed of fabric with a high absorption capacity (it can be a superabsorbent too).
The contact layer is preferably made from woven or knitted fabric made of polyamide (PAD) monofilaments, optionally of staple fibers; from non-woven fabric or a porous membrane, polyurethane, polyester, viscose, mixtures of said fibers or other materials, for example synthetic fibres like polypropylene. The coating (P): can be preferably in the form of a lyophilizate or a dried coating, more specifically in the form of a lyophilized or dried layer of the chemical mixture or a layer of the physical mixture. The absorption layer or absorption layers (A) are preferably made from materials selected from the group comprising polyester, viscose, polyamide, polyethylene, polypropylene, polysaccharide, for example xanthan or cellulose derivative, superabsorption material, a combination of woven or non-woven textile fibres and superabsorbents, or a mixture of said materials. In case of more absorption layers (A), it is preferred to arrange them in such a way that the absorption capacity gradient increases in the direction from the wound, e.g. the absorption layer (A-1) close to the wound is made from 100% polyester and the absorption layer (A-2) further from the wound is made from polyester and viscose mixture 1:1. The surface layer (R), preferably prepared from non-woven polyester fabric, can optionally have an antimicrobial modification by means of impregnation by a suitable antimicrobial substance or by means of antimicrobial fibres content, for example obtained from bamboo, or by means of silver micro-particles content.

The coating (P) can be composed of a layer of precipitated fibres of polysaccharide, coated with a layer of an antimicrobial substance solution, wherein the amount of the polysaccharide coating is at least 0.1 mg/m² and the amount of antimicrobial substance coating is at least 0.0001 mg/m². If hyaluronan is used as the polysaccharide and octenidine dichloride is used as the antimicrobial substance, then the amount of the hyaluronan coating is within the range from 1 to 50 g/m² and the amount of the Octenidine dihydrochloride coating is within the range from 0.001 to 0.5 g/m². The most preferred amount of the hyaluronan coating is within the range from 5 to 20 g/m² and the amount of the octenidine dihydrochloride coating is within the range from 10 to 40 mg/m².

The contact layer (K) and the surface layer (R) can be attached together by the borders thereof, in order to close the absorption layer or absorption layers (A) between the contact layer (K) and the surface layer (R), e.g. the borders of the contact layer (K) and the surface layer (R) can be welded together.

**Brief Description of the Drawings**

Figure 1 represents a cross Elevation of the covering, the lower side being the contact layer K composed of 100% polyamide with the coating P of the mixture of polysaccharides...
and the antimicrobial substance, then the first absorption layer A-1 composed of 100% polyester, the second absorption layer A-2 composed of a mixture of 50% polyester and 50% viscose and over this, the surface layer R composed of 100% polyester. The layers K and R are welded together like a "tea bag", inside of which there are absorption layers instead of tea.

Figure 2 represents a view of the covering area from the direction from the polysaccharide coating. The border S is formed by a weld (the layers marked as K a R in Figure 1 are welded), part N is the coating composed of the mixture of the polysaccharides and the antimicrobial substance.

Figure 3 represents a graph showing the effectivity of wound healing on a healthy rat model, where the healed area of the wound was measured versus the healing time, using the covering of Example 1 and using a bandage consisting of a gauze without any preparation (i.e. without polysaccharides and without antimicrobial substance).

Figure 4 represents a graph showing the effectivity of wound healing on a healthy miniature pig model, where the area healed of the wound was measured versus the healing time, using me-covering of Example 1 and using a control bandage having the same structure, made of the same fabric materials, but 'without any preparation' coating (i.e. without polysaccharides and without antimicrobial substance).

Figure 5 represents a graph showing the effectivity of the overall antimicrobial effect of the preparation of Example 1 and the control bandage (see the description relating to Figure 4) on a miniature pig model.

Figure 6 represents a graph showing the antimicrobial effect of the preparation of Example 1 and the control bandage (see the description relating to Figure 4) on a miniaturized pig model.

Examples

Example 1

Covering with hyaluroriah, octenidine dihydrochloride and two different absorbent layers, lyophilized.

Octenidine dihydrochloride (10 mg) is dissolved in 1 ml of absolute ethyl alcohol, then 30 µl of this solution is taken to 10 ml with sterile water for injections. Sodium hyaluronate (150 mg) with molecular weight 1 650 000 g/mol is gradually added to this solution while stirring and is stirred for 12 hours. The resulting viscous solution is uniformly applied using a template having the width of 1 mm and the hole of 10 x 10 cm on the support fabric (contact layer) having the dimension of 13 x 13 cm, the support fabric being a 40 g/m² knitted lattice.
made of monofilaments of 22 dtex polyamide "M". The contact layer with the applied coating attached to a suitable support, is immediately freeze-dried to -70°C and then transferred into lyophilizer (the coating must stay frozen) and lyophilized. The resulting spongy porous elastic lyophilizate of white colour firmly sticks to the contact layer, partly it is within the material of the fabric, but the major part of the deposited material is on one side of the fabric. The contact layer is placed in such a manner that the lyophilizate is in contact with the base where it lies, for example with the table or the frame on which the fabric is stretched (i.e. the major part of the coating is directed towards the base), and a square of 10 x 10 cm of a 140 g/m² non-woven fabric made of polyester (the first absorption layer) is placed thereon. On the first absorption layer, the second absorption layer is placed (a square of 10 x 10 cm of a 140 g/m² non-woven fabric made of polyester and viscose 1:1). On the second absorption layer, the surface layer made of 30 g/m² non-woven polyester, having the dimensions of 13 x 13 cm, is placed. The covering is completed by welding the contact and the surface layers in a suitable distance from the absorption layers to prevent their damage by heat. The final product, a square of approximately 11 x 11 cm is then produced by cutting the excess border of the surface and contact layers.

The resulting square cohering is applied on the wound with the contact layer towards the wound and then fixed by % secondary covering. On a highly exudative wound, a dry covering is applied. On slightly exudative wounds, the contact layer is moisturized by 5 to 10 ml of physiological solution, sterile water or drinking water prior to application. On dry wounds it is always necessary to moisturize the covering by 10 ml of a suitable liquid (examples mentioned above). This covering can be left on the wound for up to 3 or 4 days, then it is re-bandaged. In case of massive exudation only the secondary covering is changed as often as necessary, the primary covering is not changed. The absorption capacity of the secondary covering can be increased; by using any commercially available superabsorbent or another appropriate system.

Example 2
Covering with hyaluronan, octenidine dihydrochloride and two different absorbent layers, dried

Octenidine dihydrochloride (10 mg) is dissolved in 1 ml of absolute ethanol and 30 µ of this solution is taken to 10 ml with sterile water for injections and isopropanol 1:1 (i.e. 50% solution of isopropanol). Sodium hyaluronan (150 mg) with the molecular weight 1 650 000 g/mol is gradually added to this solution while stirring and is stirred for 30 minutes. The
resulting viscous solution is uniformly applied using a template having the width of 1 mm and the hole of 10 x 10 cm on the support fabric (contact layer) having the dimension of 13 x 13 cm, the support fabric being a 40 g/m² knitted lattice made of monofilaments of 22 dtex polyamide "M". The contact layer with the deposited solution attached to a suitable support is oven dried at 40°C for 2.5 hours, or a suitable drying profile is used (e.g. drying at 40°C for 60 minutes, heating to 70°C for 30 minutes, delay at 70°C for 15 minutes and then cooling to 40°C). The resulting glassy elastic clear (colourless) film firmly sticks to the contact layer, partly it is within the material of the fabric, but the major part of the deposited material is on one side of the fabric and has the same structure as the surface of the support to which the fabric was fixed; if the support is smooth, the film is smooth too; a more preferred is a relief with slight depressions. The contact layer is placed in such a manner that the coating is in contact with the base (i.e. the major part of the coating is directed towards the base), and a square of 10 x 10 cm of a 140 g/m² short-woven fabric made of polyester (the first absorption layer) is placed thereon. On the first absorption layer, the second absorption layer is placed which is a square of 10 x 10 cm of a 140 g/m² non-woven fabric made of polyester and viscose 1:1. On the second absorption layer, the surface layer made of 30 g/m² non-woven polyester, having the dimensions of 13 x 13 cm is placed. The covering is completed by welding of the contact and surface layers in a suitable distance from the absorption layers to prevent damage by heat. The final product (a square of approximately 11 x 11 cm) is then produced by cutting excess boarders of the surface and contact layers.

The resulting covering is applied on the wound. With the contact layer directed towards the body in the same way as in Example 1. The difference between the film and the lyophilizate is that the film is less porous for gases and dissolves more quickly. Therefore, it is preferred to use the film especially in the first phase of the healing process when the chronic wound must be converted to the standard healing process. When the second phase begins, i.e. when the massive granulation tissue starts to form, it is possible to use the lyophilized or other coverings instead, in case of intergrowing of the granulation tissue into the covering.

Example 3

Covering with hyaluronic acid in dihydrochloride and fibrous

Sodium hydroxide (6 g) is dissolved in 100 ml of sterile water and then 8 g of sodium hyalurónan with molecular weight 1650 000 g/mol is added gradually while stirring and the
solution is stirred for 12 hours. The resulting viscous solution is filled into a syringe and is extruded into the precipitating bath, which is composed of 400 ml of concentrated acetic acid (about 98%) and 100 ml of isopropanol (about 99%). The resulting fibre is left in the bath and the length thereof can be modified for example by quickly rotating knives (e.g. in a machine similar to the common mixer). The solution of shorter fibres is then filtered through the contact fabric and a suitable porous diaphragm (e.g. a solid panel with bored holes, on which the fabric is placed) to form a layer of fibres having the capacity of about 15 g/m². Optionally, the coating of fibres can be prepared on a porous diaphragm and then it is transferred (e.g. by "reprint" on the contact layer). The fibres layer on the contact fabric is washed with a suitable liquid, e.g. a mixture of isopropanol and water (the concentration of isopropanol must be higher then 60% to ensure that the fibres do not dissolve). The washed layer is then pressed in a defined manner in order to attach the fibres to the base and to one another, without damaging the porous structure. The size of the support or the contact fabric and the layer of fibres weared in such a way that a square of 13 x 13 cm of the contact layer with the coating of fibres having the size of 10 x 10 cm is obtained. Octenidine dihydrochloride (10 mg) is dissolved in 1 ml of absolute ethanol, then 30 μl of this solution is taken to 2 ml of a mixture of sterile water for injections and isopropanol 1:1 (i.e. 50% isopropanol). Octenidine dihydrochloride solution is then homogenously applied on the whole area of the fibre coating, e.g. by spray coating, and optionally the fibres are pressed (a slight dissolving of the fibres surface occurs, which improves the attachment of the fibres together and the attachment of the fibres to the contact fabric). In this way, octenidine dichloride, i.e. the antimicrobial substance, penetrates into the polysaccharide fibres structure and a physical mixture of polysaccharide(s) and antimicrobial substance is formed. The contact layer, with fibres-coating attached to a suitable support is oven dried at 40°C for 2.5 hours or a suitable drying profile is used (e.g. drying at 40°C for 60 minutes, heating to 70°C for 30 minutes, delay at 70°C for 15 minutes and then cooling to 40°C). Further processing to obtain the Wound covering and the use thereof is the same as in Example 1. The fibre coating is more porous than the film prepared in Example 2, but at the same time, the structure of the pores is different from that of the lybpnilikaïe described in Example 1. The properties of the covering with the fibres coating are more similar to those of the iybphtilizate than to those of the film.

Example 4

Substitution of part of hyalurorian by another substance or mixture of substances
An analogous process is used as in Examples 1, 2 or 3, but 0.1 to 99.9 % of hyaluronan is replaced by another polysaccharide, for example by cellulose derivatives, xanthane, alginate, schizofyllane, chitosane, glucane, or by a saccharide, for example glucose, fructose, saccharose, or by an electrolyte, for example by sodium chloride, potassium chloride, potassium iodide, magnesium chloride, sodium hydrogen phosphate, sodium dihydrogen phosphate, zinc sulphate, or a plant extract or a suitable natural product, for example propolis, olive oil, tea tree oil, extract from oak tree, calendula, mint or citruses, or a mixture of any combinations of said substances. The formulation can be slightly modified by using 0.1 to 99.9% of the initial hyaluronan quantity and a higher quantity of another substance or mixture of substances than the original quantity of hyaluronan. For example, in the formulation according to Example 1 only 10 mg of hyaluronan instead 150 mg of hyaluronan is used and 150 mg of xanthan is added there to, so the total quantity of polysaccharides is higher than the original quantity of hyaluronan (160 mg of the mixture compared to the original 150 mg). Other substances or mixture of substances can be used up to the concentrations corresponding to their saturated solutions. The aim of this modification is to achieve a hypertonic environment, which is beneficial for some phases of particular wound healing, mostly of chronic wounds (especially necrosis dissolution).

Example 5
Substitution of octenidine dihydrochloride by another substance or mixture of substances

An analogous process is used as in Examples 1, 2, 3 or 4, but octenidine dihydrochloride is replaced by another suitable antimicrobial substance in a suitable concentration (here calculated per area of 10 x 10 cm). For example, 1.5 mg of benzalconium chloride can be used; 3.5 mg of chlorohexidine; 0.5 mg of PHMB; or a mixture of any combination various amounts of said substances with octenidine dihydrochloride, optionally without octenidine dihydrochloride.

Example 6
Evaluation of the efficiency of the preparation (tests of antimicrobial efficiency, preclinical assessment)

Models used, brief description

The antimicrobial effectivity was tested in vitro. The samples were tested batch-wise in several days. The samples were transferred by sterile forceps into sterile glass tubes (in case of solid coverings), liquid mixtures were transferred into tubes by a pipette. 15 ml of
culture medium BHI (Brain Heart Infusion Broth; Hi-Media) were added to each tube and samples were inoculated by the following microorganisms: Staphylococcus aureus subsp. aureus CCM 4516 (gram-positive coccus), Escherichia coli CCM 4517 (gram-negative rod), Pseudomonas aeruginosa CCM 1961 (gram-negative non-fermenting rod), Bacillus subtilis subsp. spizizenii CCM 1999 (sporulating gram-positive rod), Aspergillus niger CCM 8222 (fungus). The growing properties of the culture medium were tested. The tubes were capped with a metallic cover and transferred for cultivation into a constant-temperature chamber. Liquid samples were cultivated for 24 and 48 hours, solid covering samples were cultivated for one week at the temperature within the range from 30 to 35°C and the following week at 20 to 25°C. After the said period, the presence or absence of cloudiness was examined. Clouded samples were plated by sterile inoculating loop to Petri dishes with blood agar KA (Merck) for cultivation verification of the present microorganisms. The healing effect was assessed by using excision wound model in healthy rats (male ZDF rats). Wounds 2 × 2 cm were prepared and the healing was measured twice a week based on the wound contraction using computer evaluation of photographic recording of the wound. Also, the healing effect was assessed by minnesVtk type rhinitis pig model [1] with deep full profile excisions of 25 × 25 mm on each side; prepared in anesthesia by the method described by Van Dorp Verhoeven MC, Koerten HK, Van Der Nat-Van Der Meij TH, Van Blitterswijk CA, Vonec M.  "Derrnai regeneration in full-thickness wounds in calves miniature pigs using a biodegradable copolymer," Wound Repair Regen. 1998; 6(6):356-68: The wounds were then covered with a control and experimental covering until the healing was complete. Regular re-bandaging and documentation scraping for microbiological examination were performed twice a week. Based on the image analysis, the rate of wound closing (contraction, epithelization) was measured and based on the cultivation tests, also the effect of the covering on the wound microbial colonization was measured.

Microbial efficiency of the active mixture (liquid according to Example 1)

A sample of the liquid mixture (viscous solution) used for coating according to Example 1 showed the total (i.e. 100%) inhibition of E. coli strains growth (initial suspension 20 000 CFU/ml), S. aureus (20 000 CFU/ml), P. aeruginosa (40 000 CFU/ml) and B. subtilis (1000 CFU/ml) when the content of octenidine dihydrochloride was 4 mg. When 1 mg of octenidine dihydrochloride was used, the inhibition was 100% only for B. subtilis and
S. aureus strains, the inhibition for E. coli strain was 99.95% and for P. aeruginosa strain 90%.

A sample of the finished covering showed an inhibition zone around the contact layer, however, in a greater distance from the covering the microorganisms growth was not inhibited. On the other hand, this fact shows evidence that the antimicrobial substance is not released from the covering to the surrounding environment and therefore, the patient will not be adversely affected.

Microbial efficiency of the active mixture (liquid according to Example 5)

A sample of the liquid mixture (viscous solution) used for the coating according to Example 5 showed total (i.e. 100%) inhibition of E. coli strains growth (initial suspension 1 800 CFU/ml), S. aureus (600 CFU/ml) and A. niger (68 CFU/ml) with the content of 16 mg of benzalconium chloride (BAC); or 40 mg of chlorhexidine or 40 mg of PHMB. When 1.6 mg of BAC was used, the inhibition was 100% for S. aureus strains, the inhibition of E. coli strain was 16% and inhibition of A. niger strain was 89%. When 4 mg of chlorhexidine was used, the inhibition of E. coli growth was 34% S. aureus 95% and A. niger 32%. When 4 mg of polyhexamethylene biguanide (PHMB) was used, E. coli growth inhibition was 44% and A. niger 57%.

Samples of the finished coverings showed inhibition zones in a similar way as was described for the preparation of Example 1. Therefore, when a suitable quantity of a suitable antimicrobial substance is used, the coverings have similar microbial inhibition properties which are based on the overall construction and preparation process of the covering, not only on the particular antimicrobial substance.

Covering of Example 1, healing effect, healthy rat model

When the covering of Example 1 was used, a significant acceleration of the wound contraction was observed, which in the used model represents the scale of healing, or of healing efficiency. Compared to the not treated control wound (see Figure 3) it is clear that the application of the covering according to Example 1 shortens time of wound healing especially thanks to the fact that the wound healing process starts immediately after the injury. In case of the not treated wound, the healing process starts approximately three days after the injury. The intensity of the subsequent wound healing is then similar in both cases, as can be seen in the similar slope of both functions in Figure 3. Since healthy rats were used, the efficiency on chronic wound cannot be assessed.
Covering of Example 1, healing effect, healthy miniature pig model

It was found out that the covering of Example 1 positively influenced the rate of wound closing (Figure 4) and also the total bacteria content in the wounds decreased (Figure 5), of which especially the gram-negative bacteria were monitored (Figure 6).
CLAIMS

1. A mixture for supporting wound healing comprising a physiologically acceptable hyaluronic acid salt, characterized by that it further comprises an antimicrobial substance selected from the group comprising octenidine dihydrochloride, cetrimide, benzalconium chloride, benzalconium bromide, chlorhexidine, chlorhexidine bigluconate, polyhexamethylene biguanide, carbethopendecinium bromide, cetyltrimethylammonium bromide and a mixture thereof in any ratio.

2. The mixture according to claim 1, characterized by that it further comprises one or more polysaccharides selected from the group comprising native or modified polysaccharides hyaluronan, xanthan, schizofyllan, chitosane, glucane, alginate, cellulose, β-1-3-glucane and a mixture thereof.

3. The mixture according to claim 1 or 2, characterized by that said antimicrobial substance further comprises one or more polysaccharides selected from the group comprising native or modified polysaccharides hyaluronan, xanthan, schizofyllan, chitosane, glucane, alginate, cellulose, β-1-3-glucane and a mixture thereof.

4. The mixture according to any one of claims 1 to 3 characterized by that it comprises a physiologically acceptable hyaluronic acid salt and octenidine dihydrochloride at the weight ratio of 500:1.

5. The mixture according to any one of claims 1 to 4, characterized by that said modified polysaccharide is a polysaccharide with a synthetic polymer or polymerized of Cross-linked polyacchatide consisting of the same or different molecules,

6. The mixture according to any of the preceding claims, characterized by that it further comprises an adjuvant increasing the antimicrobial effect, for example a chelating agent such as EDTA, betaine and 2-phenoxyethanol.

7. The mixture according to any of the preceding claims, characterized by that said mixture further comprises a saccharide selected from the group comprising glucose, fructose and saccharose; or electrolyte selected from the group comprising sodium chloride, potassium chloride, potassium iodide, magnesium chloride, sodium hydrogen phosphate, sodium dihydrogen phosphate, zinc sulphate, and/or plant extract or other natural product selected from the group comprising bee propolis, olive oil, tea tree oil, extract from oak tree, calendula, mint or citrus, or any combination of said substances.

8. The mixture according to any of the preceding claims, characterized by that it is in the form of a chemical mixture or a physical mixture.
9. The mixture according to claim 8, characterized by that the chemical mixture is an aqueous solution with an alcohol content.

10. The mixture according to claim 8, characterized by that the physical mixture is a layer of polysaccharide fibres, containing an antimicrobial substance in its structure.

11. A covering supporting the wound healing, having an antimicrobial effect, characterized by that it comprises a contact layer (K), which contains on the side to be contacted with the wound a coating (P) formed of the mixture of any one of claims 1 to 10.

12. The covering according to claim 11, characterized by that it is in the form of a multilayer formation and further comprises one or more absorption layers (A) and a surface layer (R), wherein all layers are arranged in the following order in the direction from the wound: contact layer (K), which contains on the side to be contacted with the wound or on both sides, a coating (P) made of the mixture of any one of claims 1 to 10; one or more absorption layers (A) and a surface layer (R).

13. The covering according to any one of claims 11 or 12, characterized by that the contact layer is preferably made from a woven or knitted fabric made of polyamide (PAD) monofilaments, optionally of staple fibres; from non-woven fabrics or porous membrane, polyurethane, polyester, viscose; mixtures of said fibres or from other materials, for example from synthetic fibres such as polypropylene.

14. The covering according to any one of claims 11 to 13, characterized by that the coating (P) made of the mixture of any one of claims 1 to 10 can be in the form of a lyophilizate or a dried coating.

15. The covering according to any one of claims 11 to 14, characterized by that the coating (P) is a lyophilized or dried layer of the chemical mixture according to claim 9 or a layer of the physical mixture according to claim 10.

16. The covering according to any one of claims 12 to 15, characterized by that it comprises one or more absorption layers (A) prepared from materials selected from the group comprising polyester, viscose; polyamide, polyethylene, polypropylene, polysaccharide of the xanthan type or cellulose; deriyate, superabsorbioiit material, a combination of woven or non-woven fabric fibres and superabsorbents, or a mixture of said materials.

17. The covering according to any one of claims 12 to 16, characterized by that it comprises more absorption layers (A) arranged in such a way that the absorption capacity gradient increases in the direction from the wound.

18. The covering according to claim 17, characterized by that it comprises absorption layers (A-1) and (A-2), wherein the absorption layer (A-1) is closer to the wound and is made of
100% polyester and the absorption layer (A-2) is further from the wound and is made of a 1:1 mixture of polyester and viscose.

19. The covering according to any one of claims 12 to 18, characterized by that the surface layer (R) is preferably made from a non-woven polyester fabric which can optionally be antimicrobial treated by means of impregnation by a suitable antimicrobial substance or by means of antimicrobial fibres content, obtained for example from bamboo, or by means of silver microparticles content.

20. The covering according to any one of claims 11 to 19, characterized by that the coating (P) is composed of precipitated polysaccharide fibres layer, on which layer a layer of antimicrobial substance solution is applied, wherein the amount of the polysaccharide coating is at least 0.1 mg/m² and the amount of antimicrobial substance coating is at least 0.0001 mg/m².

21. The covering according to claim 20, characterized by that the polysaccharide is hyaluronan and the polysaccharide coating quantity is within the range from 1 to 50 g/m², and the antimicrobial substance as octenidine dichloride and the quantity of octenidine dihydrochloride coating is within the range from 0.001 to 0.5 g/m².

22. The covering according to claim 21, characterized by that the quantity of hyaluronan coating is within the range from 5 to 20 g/m² and the quantity of octenidine dihydrochloride coating is within the range from 10 to 40 mg/m².

23. The covering according to any one of claims 12 to 22, characterized by that the contact layer (K) and the surface layer (R) are attached together by the edges thereof in order to close the absorption layer or absorption layers (A) between the contact layer (K) and the surface layer (R).
Fig. 3

Fig. 4
The total number of bacteria in the wound

Fig. 5

The total number of Gram-negative rods in the wound

Fig. 6
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

| INV. | A61L 15/22 | A61L 15/28 | A61L 15/44 |

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)

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, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C.

See patent family annex.

**Date of the actual completion of the international search**

10 May 2012

**Date of mailing of the international search report**

18/05/2012

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk.
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Fax (+31-70) 340-3016

Authorized officer

Lamer, W. Fram
**INTERNATIONAL SEARCH REPORT**

**International application No**

PCT/CZ2012/00021

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