A composition for reducing scarring, comprises resveratrol, and an MCP-1 inhibitor. An exemplary composition includes resveratrol in an amount of 0.1 to 1.0 micromoles/liter, an siRNA MCP-1 inhibitor in an amount of 0.1 to 1.0 micromoles/liter, hyaluronic acid tetramer in an amount of 1.0 to 400 micromoles/liter, calcium in an amount of 0.1 to 1.0 millimoles/liter, and magnesium in an amount of 0.1 to 1.0 millimoles/liter.
METHOD OF REDUCING SCARRING

BACKGROUND

Wound healing is a complex process, and involves the regulation of numerous cellular functions including the interactions of fibroblasts/fibrocytes, osteoblasts, chondrocytes, endothelial cells, inflammatory cells, epithelial cells and smooth muscle cells, with the extracellular matrix. Normal healing results in scar formation in humans. However, it is well known that certain animals, and even the human fetus, are capable of regenerative healing of wounds which is indistinguishable from surrounding skin.

Although the intricate details of wound healing are still being discovered, the process follows along a typical time line having four phases:

Hemostasis Phase - This phase includes vasoconstriction lasting for the first 5-10 minutes after the injury.

Inflammation Phase - This phase includes vasodilation and a cellular response by inflammatory macrophages, neutrophils and fibroblasts. Neutrophils undergo cannibalization to produce transforming growth factor beta-1 (TGF-β1), which stimulates production of type I collagen (the mature collagen present in normal skin) and stimulates fibroblast to myofibroblasts mediated by hyaluronic acid and epidermal growth factor receptor (EGFR). Bacteria, foreign particles and damaged cells are removed from the wound. Vasodilation starts at about 10 minutes after the initial injury, and the cellular response typically starts 30 minutes after the initial injury. Keratinocytes detach from the basement membrane and migrate to cover the exposed wound and connective tissue, and the wound clot is replaced with epithelial cells and granulation tissue (type III collagen). Differentiating keratinocytes also produce TGF-β1. The cellular response may last 7 to 8 days.

Proliferation Phase - This phase includes re-epithelialization of the wound, fibroplasia, including collagen synthesis and wound contraction. During this phase skin cells multiply and spread, covering the wound. Re-epithelialization typically starts 24
hours after the injury. Fibroplasia typically starts in 3 to 4 days after the injury. Myofibroblasts (present in granulation tissue) express alpha-smooth muscle actin and are responsible for wound contraction, which typically starts 7 days after the injury.

Remodeling Phase - This phase includes scar/collagen remodeling. The newly formed collagen matrix becomes cross linked and organized starting about 3 weeks from wound initiation and lasting as long as 1 year.

Scar formation is a typical response for normal healing in humans. As compared with normal skin, a scar contains an overproduction of type III and type I collagens, and the mixture is disorganized. The scar itself is not very elastic and is of a different color than normal skin. The scar is also missing the layer of keratinocytes found on normal skin. Furthermore, depending on how deep was the original wound, the scar may be missing the normal underlying layers of muscle, fat, blood vessels, and many layers of the skin; these missing layers may result in the scar forming a depression compared to the level of the surrounding skin.

Some animals are capable of scar free healing. In axolotls, there is a substantial reduction in neutrophil infiltration and a relatively long delay in production of new extracellular matrix during scar free healing. Studies with athymic nude mice indicate that up-regulation in metalloproteinase-9 (MMP-9) throughout the remodeling phase may contribute to scar free healing. Matrix metalloproteinases (MMP's) are a family of zinc dependent enzymes capable of degradation of extracellular matrix and are vital to the remodeling of the matrix and migration of cells. During normal human wound healing, MMP-9 degrades the type IV collagen of the basement membrane allowing keratinocytes to detach from the basement membrane and migrate to cover the exposed wound and connective tissue.

Human oral healing of wounds results in little to no scar formation. Oral mucosal wounds show a robust early up-regulation of MMP-1, MMP-2 and MMP-9 at 3 days after the initial injury, as compared to skin wounds at 14 days after the initial injury. The human fetus, which also shows scar free healing, is surrounded by amniotic fluid which
contains high molecular weight hyaluronic acid, and furthermore in the early trimesters the fetus lacks a mature immune system which may contribute to the lack of scarring. High molecular weight hyaluronic acid is known to increase expression of MMP-2 and MMP-9. Although high molecular weight hyaluronic acid application at a wound site can reduce scarring, a scar is nevertheless still formed.

Resveratrol (trans-3,4',5-trihydroxystilbene), a stilbenoid, is a grape polyphenol present in various plants, some food products, red wine and grapes. Resveratrol has anti-inflammatory, anti-carcinogenic and anti-oxidant properties, and has been extensively studied. Huge interest in resveratrol was created when it was discovered that it was able to active the SIRT1 gene, a gene implicated in the life span extension associate with calorie-restricted diets. However, resveratrol is poorly absorbed when consumed as a dietary supplement, and is subject to metabolic degradation, and beneficial effect have been difficult to observe in human clinical studies.

SUMMARY

In a first aspect, the present invention is a composition for reducing scarring, comprising resveratrol, and an MCP-1 inhibitor. For example, the composition may contain resveratrol in an amount of 10 to 400 micromoles/liter, an siRNA MCP-1 inhibitor in an amount of 0.5 to 7.5 micromoles/liter, hyaluronic acid tetramer in an amount of 10 to 400 micromoles/liter, calcium in an amount of 0.1 to 1.0 millimoles/liter, and magnesium in an amount of 1.0 to 10 millimoles/liter.

In a second aspect, the present invention is a composition for reducing scarring, prepared by mixing resveratrol in an amount of 10 to 400 micromoles/liter, an siRNA MCP-1 inhibitor in an amount of 0.5 to 7.5 micromoles/liter, hyaluronic acid tetramer in an amount of 10 to 400 micromoles/liter, calcium in an amount of 0.1 to 1.0 millimoles/liter, and magnesium in an amount of 1.0 to 10 millimoles/liter.
In a third aspect, the present invention is a method for reducing scarring, comprising applying into a wound, any of the preceding compositions. The wound was formed at most one day before the applying, and no part of the skin surface of the wound is more than 3 cm from uninjured skin.

In a fourth aspect, the present invention is a composition for reducing scarring, comprising resveratrol, chemokine-binding protein (CBP), calcium, and magnesium.

In a fifth aspect, the present invention is a composition for reducing scarring, prepared by mixing resveratrol in an amount of 10 to 400 micromoles/liter, chemokine-binding protein (CBP) in an amount of 0.5 to 7.5 micromoles/liter, calcium in an amount of 0.1 to 1.0 millimoles/liter, and magnesium in an amount of 1.0 to 10 millimoles/liter.

DEFINITIONS

"Resveratrol" means trans-3,4',5-trihydroxystilbene, salts of trans-3,4',5-trihydroxystilbene (such as trans-resveratrol-3-sulfate), esters of trans-3,4',5-trihydroxystilbene, and mixtures thereof.

"MCP-1 inhibitor" means a chemical or drug which reduces the activity of monocyte chemoattractant protein-1 (MCP-1). MCP-1 inhibitors include anti-MCP-1 antibodies, fragments thereof, and conjugates thereof; anti-sense oligonucleotides; ribozymes and deoxyribozymes; interfering RNA, including siRNA such as double-stranded interfering RNA; aptamers; chemokine-binding protein (CBP); and mixtures thereof. Although resveratrol is believed to also down regulate MCP-1, "MCP-1 inhibitor" does not include resveratrol, as all composition of the present application include resveratrol.

"Innate immunity suppressor" means a chemical or drug which reduces the Toll-like receptor-3 (TLR3)-dependent expression of cytokines. Innate immunity suppressors include hyaluronic acid oligosaccharides such as hyaluronic acid tetramer; proteases from a number of viruses, such as Enterovirus 68 3C Protease, and the 3CD
protease-polymerase precursor of the hepatitis A virus; aptamers to TLR3 or TRIF; anti-TLR3 antibodies; and anti-TRIF antibodies.

"Hyaluronic acid", "hyaluronic acid oligosaccharides" and "hyaluronic acid tetramer" means the free acids, as well as the salts and esters of these compounds, such as the sodium salt.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photograph of the right forearm of the patient before scar excision. Proximal pre-operative site (left side of picture); distal arm pre-operative site (right side of picture).

FIG. 2 is a photograph of the left forearm of the patient before scar excision.

FIG. 3 is a photograph of the right forearm at time of treatment. Left side was treated with resveratrol/Ca/Mg/siRNA/TetraHA/HPMC, and right side was treated with resveratrol/Ca/Mg/HPMC.

FIG. 4A, 4B and 4C are photographs of the treatment and control sites 24 hours post incision. A. Control incision. B. Resveratrol/Ca/Mg/siRNA/TetraHA/HPMC. C. Resveratrol/Ca/Mg/HPMC.

FIG. 5A, 5B and 5C are photographs of the treatment and control sites 5 days (120 hours) post incision. A. Control incision. B. Resveratrol/Ca/Mg/siRNA/TetraHA/HPMC. C. Resveratrol/Ca/Mg/HPMC.

FIG. 6A, 6B and 6C are photographs of the treatment and control sites 3 weeks post incision. A. Control incision. B. Resveratrol/Ca/Mg/siRNA/TetraHA/HPMC. C. Resveratrol/Ca/Mg/HPMC.
FIG. 7A, 7B and 7C are photographs of the treatment and control sites 5 months post incision. A. Control incision. B. Resveratrol/Ca/Mg/siRNA/TetraHA/HPMC. C. Resveratrol/Ca/Mg/HPMC.

FIG. 8A, 8B and 8C are photographs of the treatment and control sites 6 months post incision. A. Control incision. B. Resveratrol/Ca/Mg/siRNA/TetraHA/HPMC. C. Resveratrol/Ca/Mg/HPMC.

DETAILED DESCRIPTION

Wound healing with application of resveratrol results in rapid epithelialization within 24 hours, resulting in an attenuated scar, and in some area an almost invisible scar. However, a small attenuated scar persists and is visible with microscopic examination.

The present invention makes use of the discovery that a reduction in the activity of monocyte chemoattractant protein-1 (MCP-1), an inflammatory chemokine that plays a pivotal role in mediating monocyte recruitment and macrophage activation, together with resveratrol, results in even less scarring that the use of resveratrol alone. Therefore, application of resveratrol and an MCP-1 inhibitor will further reduce scarring than resveratrol alone. The present invention includes methods for reducing scarring including application to a wound a composition containing resveratrol and an MCP-1 inhibitor, as well as compositions containing resveratrol and an MCP-1 inhibitor.

It has been discovered that if a wound or incision is completely healed in less than 3 days, before fibroplasia begins, then almost no scar will be formed at the location of the wound or incision. Therefore compositions containing resveratrol and an MCP-1 inhibitor will allow for scar free healing when applied to wounds or incisions that do not have any injured or missing tissue which is more than 3 cm from uninjured tissue. Examples include almost all incisions purposefully created by a surgeon, because the surgeon is able to bring the edges of the skin at the location of the incision to well within
3 cm of each other. Preferably, no part of the skin surface of the wound is more than 3 cm from uninjured skin, more preferably no part of the skin surface of the wound is more than 2 cm from uninjured skin, even more preferably no part of the skin surface of the wound is more than 1 cm from uninjured skin, and most preferably no part of the skin surface of the wound is more than 0.5 cm from uninjured skin.

Compositions containing resveratrol and an MCP-1 inhibitor, either as the sole active agents or in combination with other active agents, is preferably applied to a wound or incision at any time from prior to formation of a wound or incision up until at most one day after the formation of a wound or incision; more preferably prior to formation of a wound or incision, up until at most 1 hour after the formation of a wound or incision; and most preferably prior to formation of a wound or incision, up until at most 10 minutes after the formation of a wound or incision. Preferably, only a single application of a composition containing resveratrol and an MCP-1 inhibitor is used. For example, a composition containing resveratrol and an MCP-1 inhibitor may be applied topically to an incision site, or injected below an incision site, then the skin may be cut, optionally followed by closing the incision; for example the deep structures which have been cut under the skin may be tied down using VICRYL™ (polyglactin 910) sutures, and then skin sutured or sealed using DERMABOND ADVANCED™ topical skin adhesive or NEW-SKIN® liquid bandage. Alternatively, a composition containing resveratrol and an MCP-1 inhibitor may be applied to the incision or wound after it is formed, followed by closing the wound or incision as described above.

In some forms, such as gels and pastes, the delivery medium limits contact with the surrounding tissue, the surrounding tissue rapidly degrades the resveratrol, and the tissue itself will absorb the resveratrol, resulting in a much lower effective concentration of resveratrol. Accordingly, preferably resveratrol is present in a composition at a concentration of at least 1 micromole/liter, more preferably at a concentration of at least 10 micromoles/liter, and most preferably at a concentration of at least 50 micromoles/liter. Preferably, resveratrol is present in those compositions at a
concentration of at most 1000 micromoles/liter. Examples include 7.5, 8.0, 9.0, 10, 12.5, 15, 16, 17, 18, 19, 20, 21, 21.9, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 and 500 micromoles/liter.

MCP-1 inhibitors include anti-MCP-1 antibodies, fragments thereof, and conjugates thereof; anti-sense oligonucleotides; ribozymes and deoxyribozymes; interfering RNA, including siRNA such as double-stranded interfering RNA; CBP and mixtures thereof. Preferably, the MCP-1 inhibitor is an siRNA or CBP. Although resveratrol is believed to also down regulate MCP-1, "MCP-1 inhibitor" does not include resveratrol, as all composition of the present application include resveratrol.

A direct way to reduce the activity of MCP-1 is through protein binding. Chemokine-binding protein (CBP) is a protein secreted by parapoxviruses such as orf virus, bovine papular stomatitis virus (BPSV) and pseudocowpox virus. CBP shows high-affinity binding for human and mouse CC, CXC and C chemokines, particularly MCP-1 (CCL2), and prevents inflammatory monocyte recruitment. Binding MCP-1 blocks monocytes, which prevents the monocytes from being converted to fibrocytes and contributing to scar formation.

CBP may be produced recombinantly from parapoxvirus DNA. A general method for isolating and producing parapoxvirus proteins is described in Inder, M.K. et al., "Bovine papular stomatitis virus encodes a functionally distinct VEGF that binds both VEGFR-1 and VEGFR-2", Journal of General Virology, vol. 88, pp. 781-791 (2007). The protein may be tagged, for example using FLAG octapeptide. Recombinant FLAG-tagged proteins may be expressed in suitable cells, such as 293-EBNA cells. The expressed protein may then be purified and/or quantified. A preferred source of CBP is BPSV strain V660. CBP activity may be assessed using an ELISA. CBP will bind to CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES), CCL19 (MIP-3β) and XCL1 (lymphotactin); will interact with CXCL2 (MIP-2) and CXCL4 (PF4); but will not bind to CXCL8 (IL-8), CXCL10 (IP10), or CXCL12 (SDF-1). The DNA sequence of the BPSV
V660 gene encoding CBP has been deposited in GenBank under accession no. KM400588 and is identified as SEQ ID NO: 1 in the attached Sequence Listing. SEQ ID NO: 2 is the translated protein sequence of the CBP encoded by the gene of SEQ ID NO: 1. For a general discussion of CBP isolated from BPSV V660, see Lee, S. et al., "Effect of a broad-specificity chemokine-binding protein on brain leukocyte infiltration and infarct development", Stroke, vol. 46, pp. 537-544 (2015).

CBP may be present in the composition in a concentration of at least 0.1 micromoles/liter, preferably at least 0.5 micromoles/liter, and more preferably at least 1.0 micromoles/liter, including 0.25 to 25 micromoles/liter, 1.0 to 10 micromoles/liter, such as 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 micromoles/liter. Similar concentrations of other MCP-1 inhibitors may also be used.

Alternatively, the activity of MCP-1 may be reduced by reducing its production at the site of the wound, by using a small interfering RNA (siRNA), which inhibits MCP-1 translation. When used as an inhibitor of protein expression, the siRNA includes a strand of RNA which is complementary to a portion of the mRNA transcribed from the gene for the protein. The strand of RNA must be long enough to reliably bind to the mRNA and to be specific for the mRNA of the protein. Preferably, the siRNA is 20 to 100 bases long, including 25, 27, 30 and 35 bases long. Preferably, a mixture of different siRNAs, all specific for the protein of interest, for example 2, 3 or 4 different siRNAs, is used together. Preferably, the siRNA is double-stranded, complemented with a RNA strand which non-translatable, for example a universal scrambled negative non-translatable RNA strand having the same length as the siRNA.

An especially preferred MCP-1 inhibitor is a mixture of three double strand interfering RNAs available from AMS Biotechnology (Europe) Limited (Milton Park, Abingdon UK), called "CCL2 (ID 6347) Trilencer-27 Human siRNA". Other MCP-1 inhibitors include a mixture of three double strand interfering RNA available from Santa Cruz Biotechnology, Inc. (Dallas, Texas), called "MCP-1 siRNA (h): sc-43913"; and an
aptamer specific for MCP-1 from NOXXON Pharma AG (Berlin, Germany), called Emapticap pegol (NOX-E36).

[36] siRNA may be present in the composition in a concentration of at least 0.1 micromoles/liter, preferably at least 0.5 micromoles/liter, and more preferably at least 1.0 micromoles/liter, including 0.25 to 25 micromoles/liter, 1.0 to 10 micromoles/liter, such as 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 micromoles/liter.

[37] siRNA may be coupled to a nanoparticle or administered with an innate immunity suppressor to avoid unwanted inflammation. Suitable innate immunity suppressors include hyaluronic acid oligosaccharides such as hyaluronic acid tetramer; proteases from a number of viruses, such as enterovirus 68 3C protease, and the 3CD protease-polymerase precursor of the hepatitis A virus; aptamers to TLR3 or TRIF; anti-TLR3 antibodies; and anti-TRIF antibodies. Preferably, the innate immunity suppressor is hyaluronic acid tetramer.

[38] Hyaluronic acid tetramer may be present in the composition at a concentration of at least 1 micromole/liter, preferably at a concentration of at least 10 micromoles/liter, and more preferably at a concentration of at least 50 micromoles/liter. Preferably, hyaluronic acid tetramer is present in those compositions at a concentration of at most 1000 micromoles/liter, more preferably at most 400 micromoles/liter. Examples include 7.5, 8.0, 9.0, 10, 12.5, 15, 16, 17, 18, 19, 20, 21, 21.9, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300 and 350 micromoles/liter. Other innate immunity suppressors may be used at similar concentrations.

[39] Preferably, the composition contains calcium. Calcium may be added to the composition as calcium chloride. The concentration of calcium is preferably at least 0.05 millimoles/liter, more preferably at least 0.1 millimoles/liter, including 0.15 to 3.0 millimoles/liter or 0.2 to 1.0 millimoles/liter, including 0.25, 0.30, 0.35, 0.40, 0.45 and 0.5 millimoles/liter.
Preferably, the composition contains magnesium. Magnesium may be added to the composition as magnesium chloride. The concentration of magnesium is preferably at least 0.50 millimoles/liter, more preferably at least 1.0 millimoles/liter, including 1.5 to 30 millimoles/liter or 2.0 to 10 millimoles/liter, including 2.5, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 4.0, 4.5 and 5.0 millimoles/liter.

Resveratrol has a very low solubility in water, however only that portion which is dissolved in water will exert its effects. Furthermore, if the resveratrol is applied dissolved in a hydrophobic medium, it may slowly diffuse into the surrounding aqueous medium, and undesirably extend the effective application time. Therefore, it is preferable that the compositions be applied as a solution in an aqueous medium. For ease of application in a clinical setting, preferably the aqueous medium is a gel, paste, foam, suspension or thickened solution. Examples include aqueous compositions containing hydroxypropyl methylcellulose, high molecular weight hyaluronic acid, polyethylene glycol, agar, dextrin, pectin, trehalose, xanthan gum, polyoxyethylene alkyl ethers, chitosan, guar gum and sodium alginate. Other vehicles, adjuvants and excipients, which are hydrophilic or have hydrophilic moieties, and are compatible which application into wounds, may also be used. Other pharmaceutically acceptable adjuvant, excipients and vehicles may also be included.

Premeasured amounts of the compositions may also be used. These are referred to as unit dosage forms, since each premeasured amount is intended to be used on a single patient for one or more application, all used at the same time. Examples include prefilled syringes, pouches, packets and tubes. Another example would be a tube or dispenser which may be used to form foam of its contents just prior to application, for example by shaking or using a foaming agent. A self-foaming tablet, which forms foam when placed into water, could also be used. The volume of material present in these unit dosage forms may be 0.1 to 100 ml, or 1 to 50 ml, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40 and 45 ml.
Other active agents may be included, such as other activators of SIRT1; HDAC2 (a class I histone deacetylase) inhibitors, such as trichostatin A; agents which stimulate the production of certain growth factors such as EGF, FGF-10 and IGF-1; luteolin; tretinoin (all-trans retinoic acid); and high molecular weight hyaluronic acid. Although it is not known exactly how resveratrol reduces scarring, resveratrol does up-regulate and increase the expression of a variety of agents which are involved in wound healing. One possible explanation is that resveratrol causes the over-expression of MMP-9, interleukin-8 (IL-8) and SIRT1, and increases expression of EGFR on the keratinocyte membrane and nucleus. SIRT1 may then promote differentiation, motility and proliferation of keratinocytes, and deacetylation and inactivation of p53 protein thus inhibiting p53-dependent cell death from apoptosis in response to stress in human tenocytes (fibroblast-like tendon cells). SIRT1 may induce nitric oxide (NO) production, which inhibits class I HDAC 2 from blocking growth factors, including epithelial growth factor, keratinocyte growth factor 2, fibroblast growth factor 10 (FGF-10), and insulin-like growth factor 1 (IGF-1). SIRT1 may also decrease inflammation and apoptosis through a variety of mechanisms. IL-8 has a direct and profound stimulatory effect on the migration of keratinocytes, which is likely due via the PLC-gamma pathway, and furthermore IL-8 may recruit neutrophils. As noted above, MMP-9 degrades the type IV collagen of the basement membrane. EGFR may cause keratinocyte and fibroblast migration and may protect and repair tissue through nuclear DNA repair. Resveratrol may also inhibit NF-kB dependent proinflammatory and matrix degrading gene products induced by IL-1β and nicotinamide.

Although resveratrol is believed to also down regulate MCP-1, the degree of down regulation may be insufficient to result in scarless healing. The MCP-1 inhibitor further down regulates MCP-1, further reducing scaring as compared to resveratrol alone. However, an MCP-1 inhibitor without resveratrol delays healing, appears to be detrimental to healing and may even increase scaring.

EXAMPLES
Example 1: *In vivo* application resveratrol and anti-sense RNA against MCP-1 mRNA in a human pilot study.

Resveratrol was able to partially reduce MCP-1 activation but additional reduction was believed necessary. Additional MCP-1 reduction was tested with the introduction of anti-sense RNA against MCP-1 mRNA, together with resveratrol. The healing was accelerated, but the double strand RNA elicited a Toll Receptor-3 (TLR-3) inflammatory response.

Example 2: *In vivo* application of resveratrol and anti-sense RNA against MCP-1 mRNA, together with an innate immunity suppressor, in a human pilot study.

Male subject with previous 10 cm scar on right forearm and 3 cm scar left forearm. The right treatment site forearm scar was demarcated into two equal portions and the full experimental composition was used on the distal scar revision wound and composition without hyaluronic acid tetramer and without siRNA against MCP-1 mRNA used on the proximal scar revision wound. The left forearm scar served as control and was excised in traditional fashion without any gel composition placed at the time of excision.

The full composition used on the *distal* right forearm:
- Resveratrol - 100 microMolar
- Calcium chloride - 0.3 milliMolar
- Magnesium chloride - 3.3 milliMolar
- siRNA against MCP-1 - 2 microMolar
- Hyaluronate tetramer ("TetraHA") - 100 microMolar
- Hydroxypropyl Methylcellulose Gel 8% ("HPMC")

Composition used on the *proximal* right forearm:
- Resveratrol - 100 microMolar
- Calcium chloride - 0.3 milliMolar
Magnesium chloride - 3.3 milliMolar
Hydroxypropyl Methylcellulose Gel 8%

[53] No composition was used on the left forearm.

[54] Both the right arm and left arm treatment site scars were prepped and draped in a sterile fashion and demarcated with a surgical skin marker encompassing the entire existing scars. The sites were infiltrated with lidocaine 1% and epinephrine 1:100,000. After adequate anesthesia, the scars were excised with #15c blade, removing the old scar. After hemostasis was assured, the sites were inspected and the appropriate gel, or no gel, instilled into each section of the wounds with a 27G micro-cannula. After topical instillation of the gels (or no gel), the sites were closed in sections with 5-0 nylon in a running subcuticular fashion. An occlusive TEGADERM™ clear dressing was placed over the sites for 24 hours. The TEGADERM™ was removed after 24 hours and the incisions were allowed to heal without intervention for 7 days, at which point the subcuticular sutures were removed.

[55] Results:

[56] FIGS. 1 through 8 are photographs of the treatment sites and control site from before removal of the scars, until 6 months post treatment.

[57] The two right forearm treatment sites were compared and each site compared to the control site on the left arm. A significant acceleration in skin re-epithelialization was noted for both treatment sites (right forearm) with re-epithelialization occurring during the first 24 hours compared to the control site (left forearm) crusting persisting past the 10th day. A significant reduction in erythema was noted in both treatment sites (right forearm) with persistent control site (left forearm) erythema until the second week.

[58] Evaluation of the treatment incision sites (right forearm) revealed smooth, nearly scar free healing until the third week. There was no significant difference in erythema between treatment sites (right forearm) until the third week, when the side without
siRNA/TetraHA (proximal right forearm) gradually increased in erythema and scar size. Also at the third week, a new raised scar appeared on the side without siRNA/TetraHA (proximal right forearm) and wispy erythema was noted over the full treatment site (distal right forearm). Final scar size was significantly reduced in the distal right forearm (resveratrol/Ca/Mg/siRNA/TetraHA) compared to the proximal right forearm (resveratrol/Ca/Mg). Scar height was negligible in the distal right forearm (resveratrol/Ca/Mg/siRNA/TetraHA) whereas the control site (left forearm) and proximal right forearm (resveratrol/Ca/Mg) had significant scar height.

[59] Example 3: In vivo application of resveratrol and CBP in a human pilot study (prophetic)

[60] A composition will be prepared from the following ingredients:
- Resveratrol - 100 microMolar
- Calcium chloride - 0.3 milliMolar
- Magnesium chloride - 3.3 milliMolar
- CBP - 2 microMolar
- Hydroxypropyl Methycellulose Gel 8% ("HPMC")

[61] A subject with an existing scar will be identified as a candidate for therapy. The scar will be revised. The composition will be instilled on the scar revision wound at the time of excision.


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WHAT IS CLAIMED IS:

1. A composition for reducing scarring, comprising:
   resveratrol, and
   an MCP-1 inhibitor.

2. The composition of claim 1, wherein the MCP-1 inhibitor is an siRNA, and
   the composition further comprises an innate immunity suppressor.

3. The composition of claim 2, wherein the innate immunity suppressor is
   hyaluronic acid tetramer.

4. The composition of any of the preceding claims, further comprising
   calcium.

5. The composition of any of the preceding claims, further comprising
   magnesium.

6. The composition of any of the preceding claims, wherein the resveratrol is
   present in an amount of 1 to 1000 micromoles/liter.

7. The composition of any of the preceding claims, wherein the MCP-1
   inhibitor is present in an amount of 0.1 to 10 micromoles/liter.

8. The composition of any of claims 2-7, wherein the innate immunity
   suppressor is present in an amount of 1 to 1000 micromoles/liter.

9. The composition of any of claims 4-8, wherein the calcium is present in an
   amount of 0.05 to 3.0 millimoles/liter.
10. The composition of any of claims 5-9, wherein the magnesium is present in an amount of 0.50 to 30 millimoles/liter.

11. The composition of claim 5, wherein:
   the resveratrol is present in an amount of 10 to 400 micromoles/liter,
   the MCP-1 inhibitor is an siRNA, and is present in an amount of 0.5 to 7.5 micromoles/liter,
   the innate immunity suppressor is hyaluronic acid tetramer, and is present in an amount of 10 to 400 micromoles/liter,
   the calcium is present in an amount of 0.1 to 1.0 millimoles/liter, and
   the magnesium is present in an amount of 1.0 to 10 millimoles/liter.

12. The composition of any of the preceding claims, wherein the composition is a gel, paste, foam, suspension or thickened solution.

13. The composition of any of the preceding claims, wherein the composition is a gel or thickened solution.

14. The composition of any of the preceding claims, wherein the composition further comprises at least one member selected from the group consisting of hydroxypropyl methylcellulose, high molecular weight hyaluronic acid, polyethylene glycol, agar, dextrin, pectin, trehalose, xanthan gum, polyoxyethylene alkyl ethers, chitosan, guar gum and sodium alginate.

15. The composition of any of the preceding claims, wherein the composition further comprises at least one member selected from the group consisting of hydroxypropyl methylcellulose and high molecular weight hyaluronic acid.

16. The composition of any of the preceding claims, wherein the composition further comprises hydroxypropyl methylcellulose.
17. The composition of any of the preceding claims, wherein the composition is provided as a unit dosage form.

18. The composition of claim 35, wherein the unit dosage form has a volume of 0.1 to 100 ml.

19. The composition of claim 35, wherein the unit dosage form has a volume of 5 to 10 ml.

20. The composition of any of the preceding claims, wherein the unit dosage form is selected from the group consisting of a prefilled syringe, a pouch, a packet and a tube.

21. A method for reducing scarring, comprising: applying into a wound, the composition of any of the preceding claims, wherein the wound was formed at most one day before the applying, and no part of the skin surface of the wound is more than 3 cm from uninjured skin.

22. The method of claim 21, wherein the wound was formed at most one hour before the applying.

23. The method of claim 21, wherein the wound was formed at most 10 minutes before the applying.

24. The method of any of claims 21-23, wherein no part of the skin surface of the wound is more than 2 cm from uninjured skin.

25. The method of any of claims 21-23, wherein no part of the skin surface of the wound is more than 1 cm from uninjured skin.
26. The method of any of claims 21-23, wherein no part of the skin surface of the wound is more than 0.5 cm from uninjured skin.

27. A composition for reducing scarring, prepared by mixing:
resveratrol, in an amount of 10 to 400 micromoles/liter,
an siRNA MCP-1 inhibitor, in an amount of 0.5 to 7.5 micromoles/liter,
hyaluronic acid tetramer, in an amount of 10 to 400 micromoles/liter,
calcium, in an amount of 0.1 to 1.0 millimoles/liter, and
magnesium, in an amount of 1.0 to 10 millimoles/liter.

28. The composition of claim 27, wherein the composition is a gel, paste, foam, suspension or thickened solution.

29. The composition of claim 27, wherein the composition is a gel or thickened solution.

30. The composition of claim 27, prepared by further mixing in at least one member selected from the group consisting of hydroxypropyl methylcellulose, high molecular weight hyaluronic acid, polyethylene glycol, agar, dextrin, pectin, trehalose, xanthan gum, polyoxyethylene alkyl ethers, chitosan, guar gum and sodium alginate.

31. The composition of claim 27, prepared by further mixing in at least one member selected from the group consisting of hydroxypropyl methylcellulose and high molecular weight hyaluronic acid.

32. The composition of claim 27, prepared by further mixing in hydroxypropyl methylcellulose.

33. The composition of claim 1, wherein the MCP-1 inhibitor is chemokine-binding protein (CBP).
34. The composition of claim 33, wherein the chemokine binding protein (CBP) is encoded by the gene comprising the nucleotide sequence of SEQ ID NO: 1.

35. The composition of claim 33, wherein the chemokine binding protein (CBP) has the amino acid sequence of SEQ ID NO: 2.

36. A composition for reducing scarring, comprising:
   resveratrol,
   chemokine-binding protein (CBP),
   calcium, and
   magnesium.

37. The composition of claim 35, wherein:
   the resveratrol is present in an amount of 10 to 400 micromoles/liter,
   the chemokine-binding protein (CBP) is present in an amount of 0.5 to 7.5 micromoles/liter,
   the calcium is present in an amount of 0.1 to 1.0 millimoles/liter, and
   the magnesium is present in an amount of 1.0 to 10 millimoles/liter.

38. A composition for reducing scarring, prepared by mixing:
   resveratrol, in an amount of 10 to 400 micromoles/liter,
   chemokine-binding protein (CBP), in an amount of 0.5 to 7.5 micromoles/liter,
   calcium, in an amount of 0.1 to 1.0 millimoles/liter, and
   magnesium, in an amount of 1.0 to 10 millimoles/liter.
**INTERNATIONAL SEARCH REPORT**

**International application No**

PCT/US2015/054758

**A. CLASSIFICATION OF SUBJECT MATTER**


A61K31/05 A61K31/7088 A61K31/7088 A61K33/06 A61P17/02

**ADD.**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>ISMAI YAMAN ET AL: &quot;Effects of resveratrol on incisional wound healing in rats&quot;, SURGERY TODAY, vol. 43, no. 12, 15 December 2012 (2012-12-15), pages 1433-1438, XP055144076, ISSN: 0941-1291, DOI: 10.1007/S00595-012-0455-7</td>
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<td>EP 2 522 330 AI (DSM IP ASSETS BV [NL])</td>
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<td>paragraph [0049]; claims 1-6</td>
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* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"A" document member of the same patent family

Date of the actual completion of the international search: 23 February 2016

Date of mailing of the international search report: 03/03/2016

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

Bbhmerova, Eva
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<td>US 2009/220450 AI (GREEN COLIN R [NZ] ET AL) 3 September 2009 (2009-09-03) paragraph s [0032], [0137], [0286], [0326], [0327]; figure s 5A,5B</td>
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<td>HYERAIYUNG CHOI ET AL: &quot;Oligosaccharides of hyaluronic acid increase epidermal cell stemness by modulation of integrin expression&quot;, JOURNAL OF COSMETIC DERMATOLOGY, vol. 11, no. 4, 23 December 2012 (2012-12-23), page s 290-296, XP055249937, GB ISSN: 1473-2130, DOI: 10.1111/j.co.1.2009 abstract page 291, left-hand column, paragraph 2</td>
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<td>WO 2014/126370 A1 (DONG A PHARM CO LTD (KR)) 21 August 2014 (2014-08-21) paragraphs [0036] - [0038], [0042], [0043]; claims 1, 5, 6</td>
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This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

2. Claims Nos.: 34, 35 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

Remark on Protest
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.
Continuation of Box II.2

Claims Nos.: 34, 35

The specific sequences of claims 34, 35 have, according to PCT Rule 13ter. l.d., not been searched since the Sequence Listing as present in the description does not comply with WIPO Standard ST 25 prescribed in the administrative instructions under Rule 5.2. The Sequence Listing has not been furnished in machine readable form as provided for in the same instructions and the applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation pursuant to PCT Rule 13ter. l.a.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on a matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the applicant proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.
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