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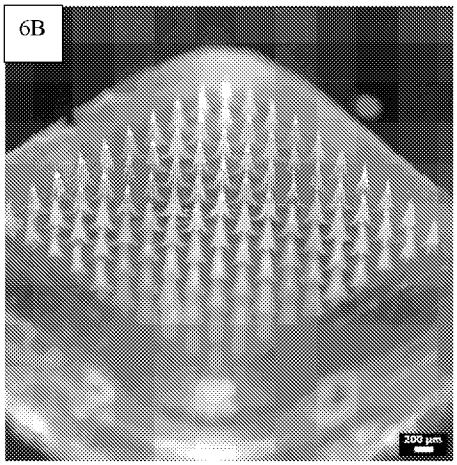

(57) Abstract: Provided herein are rapidly dissolving microneedle arrays comprising nanoparticles disposed distally in said microneedles, methods of manufacturing same, and uses of same in treating a disease or disorder in a subject in need thereof. The microneedles dissolve rapidly upon administration depositing the nanoparticles comprising pharmaceutically active agents into the viable epidermis layer of the skin.

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RAPIDLY DISSOLVING MICRONEEDLE ARRAYS COMPRISING NANOPARTICLES

FIELD OF THE INVENTION

[001] The present disclosure relates to soluble microneedle arrays suitable for transdermal delivery of active pharmaceutical agents. More specifically, the present disclosure relates to arrays of rapidly dissolving biodegradable microneedles, comprising nanoparticles of a biodegradable polymer and a pharmaceutically active agent deposited in the tip of said needles. The present disclosure also refers to methods of manufacturing of such arrays, as well as their use in treatment of diseases or disorders responsive to said active pharmaceutical agent.

BACKGROUND

[002] Transdermal drug delivery systems are a useful minimally invasive alternative to other drug administration routes. Microneedles approach has gained a significant attention during the last two decades; they are micron-sized structures, usually in the height range of 50 to 1500 μm, allowing for painless penetration into the skin via the SC, viable epidermis, and upper dermis without contacting nerves or blood vessels. Biodegradable polymeric microneedles may be used in controlled-release drug delivery due to their tunable properties and ease of patient self-administration. Different types of MNs were developed such as solid, hollow, coated, dissolving, and hydrogel-forming MNs, which differ in their delivery strategy, manufacturing methods, geometry, and the materials used in the manufacturing. Several constituent materials such as metals, ceramics, sugars, silicon, and biodegradable polymers, are widely used to prepare MNs due to their advantages including low cost, simple manufacturing, biocompatibility, stability, folding ability, and favorable drug loading capacity (Salwa et al, Journal of Drug Delivery Science and Technology 2021, 65, 102711, doi:10.1016/j.jddst.2021.102711; Guillot, A.J et al, Pharmaceutics 2020, 12, 569, doi:10.3390/pharmaceutics12060569; Aldawood, F.K. et al, Polymers 2021, 13, 2815, doi:10.3390/polym13162815).

[003] Microneedles have been manufactured from a variety of materials, including biodegradable polymers, e.g., polyesters. Polyesters are biodegradable polymers that are often used for sustained

drug release because of their intrinsic properties including biocompatibility, biodegradability, and favorable mechanical performance. Microneedle arrays for intradermal delivery of PLGA-based microneedle tips has been described in He, M. et al. **2020,** *J Pharm Sci*, *109* (6), 1958–1966. https://doi.org/10.1016/j.xphs.2020.02.009. The publication discloses the manufacturing and biopharmaceutical properties of controlled-release etonogestrel-loaded PLGA microtips, which remain implanted in the skin after application. The optimized drug loading was between 140-166 micrograms, which was apparently sufficient for the high-potency low-dose drug, such as sex hormones. Additionally, US patent application US 20180078498 discloses similar matrix-type PLGA tips disposed on the distal ends of microneedles.

[004] Since MNs can by design penetrate the skin easily and provide a site-specific delivery a minimally invasive manner, they can serve as a vehicle for the treatment of several skin diseases such as psoriasis, dermatitis, eczema, acne, and skin cancer. Topical corticosteroids are one of the most widely used treatment modalities for inflammatory skin diseases. Topical corticosteroids can be formulated in different dosage forms such as creams, ointment, and lotions. However, these dosage forms frequently lead to poor patient compliance due to their greasy texture, odor, stickiness, dosage frequency, potential systemic, and local side effects.

[005] Therefore, there is a particular need in the art to provide an alternative means for administration of drugs for topical administration, e.g., topical steroids, in an accurate and repeatable manner, the drugs that suffer among others from same drawbacks. As demonstrated in the appended examples, microneedle arrays comprising dexamethasone have been successfully prepared in PLGA nanoparticles and embedded in rapidly dissolving microneedles made of sodium alginate (NP-MNs) for improved treatment of skin diseases. It has now been unexpectedly found that soluble microneedle arrays possess enhanced skin puncturing properties, rapidly and accurately release the active agent, e.g., dexamethasone, in the desired skin layer.

SUMMARY

[006] Disclosed herein rapidly dissolving microneedle arrays comprising nanoparticles, methods of manufacturing thereof, and uses thereof in treatment of a disease or a condition in a patient in need thereof. Degradable microneedle arrays are useful drug delivery systems for transdermal

delivery of pharmaceutically active agents. Some soluble microneedles have been disclosed before. However, the skin penetration of these soluble microneedles is usually rather weak due to various reasons, inter alia, due to plasticizing effect of water on soluble polymers, and high amounts of the polymer may be required and not always useful. Therefore, crosslinking is considered as generally required, leading to decreased dissolution rate of the microneedles.

[007] It has now been unexpectedly found that the penetration of the microneedles through the skin and skin surrogates can be significantly improved by providing nanoparticles, e.g., nanospheres, nanocapsules, or other nano-sized particulate matter, deposited selectively to the tips of the microneedles. Without being bound by a particular theory it is believed that the mechanical strength of the nanoparticles, particularly when composed of a polyester, such as polylactides, permits the primary puncturing of the upper layers of the skin and delivery of the tip to deeper layers. Preferably, the nanoparticles disposed at the tips of the microneedles, are advantageously associated with the nanoparticle suspension stabilizers, such as surfactants, which are usually present during the manufacturing of the nanoparticles but are generally discarded during the purification process of the nanoparticles. The association of nanoparticles with these stabilizing modalities may in fact accelerate the disintegration of the microneedle tips and the microneedles themselves. As demonstrated herein, the tips of the microneedles degraded within as little as only about 15 seconds post administration to a human skin simulating model, and the microneedles dissolved completely after merely several minutes. Moreover, without the incorporation of nanoparticles, the microneedles demonstrated significantly poorer skin penetration performance and significantly higher deformation and height reduction.

[008] It has further been unexpectedly found that when the microneedle tips are loaded with the nanoparticles, the microneedle array may be manufactured only when the microneedle precursor solution is supplied to the microneedle array in a reduced-pressure atmosphere, but without the need to heat the array or the solution, and the process may be carried out at room temperature according to European Pharmacopeia, e.g., between 15 °C and 25 °C. When manufacturing of the microneedle arrays was attempted with conventional techniques, arrays of poor quality were obtained, if at all.

[009] Without being bound by a particular theory it is believed that utilizing nanoparticles-loaded tips it may be possible to employ a variety of water-soluble polymers that are generally considered less suitable for the use in microneedle arrays for transdermal delivery, and to provide rapidly dissolving microneedle array, having, e.g., an advantageously low required retention time before removal, e.g., by washing.

[0010] Thus, in a first aspect provided herein is a microneedle array comprising a plurality of microneedles and a backing layer. Each said microneedle comprises a plurality of nanoparticles associated with a nanoparticle suspension stabilizer which is preferably a surface-active agent, and said nanoparticles and said nanoparticle suspension stabilizer are disposed at the portion of said microneedle that is distal to said backing layer. Said nanoparticles comprise a pharmaceutically active agent and a polyester selected from the group of polylactic acid, polyglycolic acid, poly(lactic-co-glycolic) acid, polycaprolactone, and combinations of any of these. Said microneedles and said backing layer, individually, comprise a soluble polymer. Optionally, said soluble polymer is selected from the group consisting of a povidone, a hypromellose (hydroxypropyl methylcellulose), a hydroxypropyl cellulose, a carboxymethyl cellulose and/or salts thereof, and an alginate. Optionally, said soluble polymer is an alginate, preferably sodium alginate. Optionally, the weight ratio between said nanoparticles associated with said nanoparticle suspension stabilizer and said water-soluble polymer, preferably an alginate, in said microneedles is between 1:4 to 1:1. Optionally, the weight ratio between said nanoparticles and said water-soluble polymer, preferably an alginate, in said microneedles is between 1:6 to 5:6. Optionally, said nanoparticle suspension stabilizer is selected from the group consisting of polyoxyethylated fatty esters, preferably PEG(15)-hydroxystearate, a polysorbate, a sorbitan ester, a soluble polymer preferably selected from the group consisting of sodium alginate, sodium carboxymethyl cellulose, a povidone, a hypromellose, and a hydroxypropyl cellulose, and combinations thereof. Optionally, said nanoparticle suspension stabilizer is PEG(15)-hydroxystearate. Optionally, the weight ratio between said nanoparticles and said nanoparticle suspension stabilizer is between 7:1 and 1:2, preferably between 7:2 and 3:5. Optionally, the pharmaceutically active agent in the microneedle array has an aqueous solubility at 25°C of less than 1 mg/mL. Optionally, said pharmaceutically active agent has a therapeutic dose of below 5-10 mg per day. Optionally, said pharmaceutically active agent is an anti-inflammatory, preferably a steroid or an NSAID, an analgesic, an antibiotic,

a fungicide, a retinoid, an anti-cancer agent, preferably an anti-melanoma agent, an anesthetic, or a hormone. Optionally, said microneedles and said backing layer consist essentially of an alginate. Optionally, a weight ratio between said nanoparticles and said soluble polymer, preferably an alginate, in said microneedles is between 1:6 to 5:6. Further optionally, said nanoparticle suspension stabilizer is PEG(15)-hydroxystearate, wherein a weight ratio between said nanoparticles and said nanoparticle suspension stabilizer is between 7:1 and 1:2, and said pharmaceutically active agent is a steroid.

[0011] In a further aspect provided herein is a method of manufacturing a nanoparticle-bearing microneedle array, said method comprising applying an aqueous solution of a water-soluble polymer, preferably an alginate, onto a microneedle array mold under reduced pressure and preferably at room temperature. Optionally, the concentration of said soluble polymer in said aqueous solution is between 1 %wt and 10 %wt. Optionally, said concentration of said soluble polymer in said aqueous solution is between 2 %wt and 6 %wt. Optionally, said method of manufacturing a nanoparticle-bearing microneedle array further comprises applying a microneedle tip precursor solution comprising a suspension of said nanoparticles comprising said nanoparticle suspension stabilizer onto a microneedle array mold. Optionally, said applying of said microneedle tip precursor solution precedes said applying an aqueous solution of a water-soluble polymer. Optionally, said method comprises repeatedly applying said microneedle tip precursor solution until the desired drug loading is achieved. Optionally, the method further comprises drying said mold with said microneedle array. Optionally, the method s further comprises packaging said microneedle array. Optionally, the method further comprises sterilizing said microneedle array.

[0012] Thus, in particular embodiments, provided herein is a method of manufacturing a microneedle array. The method comprises providing a mold for microneedle array and providing a microneedle tip precursor solution. The microneedle tip precursor solution comprises a suspension of nanoparticles, and a nanoparticle suspension stabilizer, preferably a surface-active agent. The method further comprises applying the microneedle tip precursor solution to the mold, optionally under a reduced pressure. The method further comprises providing an aqueous microneedle precursor solution and applying the aqueous microneedle precursor solution to the mold under a reduced pressure and optionally at room temperature, e.g., between 15°C and 25 °C.

The method further comprises applying an aqueous backing layer solution, and one or more drying steps according to the need, of the mold for microneedle array containing applied said microneedle tip precursor solution, and/or the aqueous microneedle precursor solution, and/or the aqueous backing layer solution. The aqueous backing layer solution may comprise a water-soluble polymer, preferably an alginate, and optionally a polyol, and the aqueous microneedle precursor solution comprises also a water-soluble polymer, preferably an alginate.

[0013] In a further aspect, provided herein is a use of the microneedle array as described herein, or obtainable by a process as described herein, for the treatment a disease or disorder in a subject in need thereof, by applying said microneedle array to the skin of said subject, pressing with sufficient force to effect penetration of said microneedles into said skin, and retaining said microneedle array pressed to said skin for a time interval of between about 30 seconds to about 10 minutes. Optionally, said time interval is between about 30 seconds to about 3 minutes. Optionally, further comprising washing off the remainder of said microneedle array after said time interval. Optionally, said microneedle array is applied at a frequency of three times a day, twice a day, once daily, once every two days, twice a week, and once a week. Optionally, said disease or disorder is selected from the group consisting of inflammation-related disorders, for example psoriasis, dermatitis, or eczema, infections, such as bacterial, fungal, or protozoal infections, viral infections, such as Herpes Zoster, acne, cancer, for example in-situ cutaneous carcinomas, local irritation, and localized pain.

[0014] In yet a further aspect provided herein is a method of treatment of a disease or disorder in a subject in need thereof, said treatment comprising applying a microneedle array according to the use as generally described herein.

BRIEF DESCRIPTION OF FIGURES

[0015] **Fig. 1** demonstrates FTIR spectra of the nanoparticle components as exemplified in Example 1.

[0016] **Fig. 2** demonstrates DSC thermograms of Dexamethasone, PLGA, their physical mixture (1:3 ratio), and DEX-NPs as exemplified in Example 1.

[0017] **Fig. 3a** demonstrates XRD patterns of neat dexamethasone, and **Fig. 3b** demonstrates the diffractogram of DEX NPs and blank PLGA NPs, as exemplified in Example 1.

[0018] **Fig. 4** schematically demonstrated an elaborate preparation scheme of nanoparticle-loaded microneedles, by a vacuum-deposition micromolding method using a polydimethylsiloxane (PDMS) microneedle mold, as exemplified in Example 2.

[0019] **Fig. 5** Demonstrates representative SEM micrographs of the obtained microneedles according to Example 2. **Fig. 5A**: SA-MNs, **Fig. 5B**: close-up view of SA-MNs, **Fig. 5C**: NP-MNs, and **Fig. 5D**: close-up view of NP-MNs.

[0020] **Fig. 6** demonstrates representative images of the obtained microneedles, as exemplified in Example 2: **Fig. 6A:** blank SA-MNs, and **Fig. 6B**: nanoparticles-loaded NP-MNs.

[0021] **Fig. 7** demonstrates representative images of insertion test in the Parafilm™ model, as exemplified in Example 2. **Fig. 7A**: NP-MNs, **Fig. 7B**: blank NP-MNs, and **Fig. 7C**: blank MNs.

[0022] **Fig. 8** demonstrates representative images of the tested mechanical strength applied onto SA-MNs and NP-MNs, as exemplified in Example 2. **Fig. 8A**: Optical images of the deformation of blank microneedles (SA-MNs), and **Fig. 8B**: nanoparticle-loaded microneedles (NP-MNs), under weights of 50, 500, and 1000 g, scale bar 200 μm.

[0023] **Fig. 9** demonstrates a graph of MN height reduction for MNs after compression, results expressed as means ± s.d., n=10.

[0024] **Fig. 10** demonstrates *ex-vivo* and *in vitro* dissolution studies of NP-MNs: images of NP-MNs before the test (**Fig. 10A** and **Fig. 10E**), and after insertion in *ex vivo* chicken skin for 15 seconds (**Fig. 10B**), 45 seconds (**Fig. 10C**), and 120 seconds (**Fig. 10D**), and in agarose gel after 15 seconds (**Fig. 10F**), 45 seconds (**Fig. 10G**), and 120 seconds (**Fig. 10H**). Scale bar 100 μm.

[0025] **Fig. 11** demonstrates an i*n vitro* DEX release from PLGA NPs (pH 7.4, 37 °C). Inset shows the drug release profile over the initial 6 h. Values are mean ± s.d. of four experiments.

DETAILED DESCRIPTION

[0026] Thus, in a first aspect, provided herein a microneedle array for the delivery of a pharmaceutically active agent into and/or under the skin of a patient in need thereof. The "microneedle array" used alone or with a qualifier, such as "rapidly dissolving", "nanoparticles-bearing" and the like, is interchangeably referred to herein as just "array", "microarray", "microneedles", "patch", and in like terms, unless the context clearly dictates otherwise. The microneedle array, as described in greater detail below, comprises a plurality of micro-sized needles, disposed on a backing layer. The backing layer supports the microneedles. As demonstrated in the examples' section below, the microneedles are rapidly dissolving upon contact with an aqueous medium, releasing the drug-containing nanoparticles. The rate of the drug release from the nanoparticles is what in turn controls the overall release pattern of the drug upon the dissolution of the microneedles in the skin. The nanoparticles are disposed distally from the microneedle base, specifically, in the tips and/or the edges of the microneedles. The nanoparticles comprise a polyester, preferably selected from the group of polylactic acid, polyglycolic acid, poly(lactic-co-glycolic) acid, polycaprolactone, and combinations of any of these. Without being bound by a particular theory or explanation, it is currently believed that the selective disposition of the nanoparticles, made of polymers having a significantly different mechanical properties than the microneedles themselves, in the tips and/or along the edges of the microneedles, creates a reinforced structure facilitating the puncturing of the corneal skin layer (*stratum corneum*), but being discrete nanoparticles, they retain the properties thereof, such as dispersibility, surface charge, etc. This is particularly true since the microneedles comprise not only the nanoparticles but also the nanoparticle suspension stabilizer, which is usually discarded during the preparation of the nanoparticles. It has been unexpectedly found that the original nanoparticle dispersion without purification may be beneficially retained and applied in the manufacturing of the microneedles, facilitating the rapid dispersion of the nanoparticles from the microneedles upon wetting. Therefore, the soluble polymer, which is not infrequently sensitive to water or humidity to the extent as to impair the mechanical strength of the microneedles, e.g., as seen in the examples below for the blank microneedles, may be made nonetheless into useful microneedle array,

preserving the advantages of soluble polymers, e.g., the washability upon application, and demonstrating an unexpectedly rapidly dissolution rate.

[0027] Thus, in some embodiments, it is provided a rapidly dissolving microneedle array comprising a plurality of microneedles and a backing layer. Each microneedle comprises a plurality of nanoparticles and a nanoparticle suspension stabilizer, which is preferably a surface-active agent. The nanoparticles and the nanoparticle suspension stabilizer are being disposed at the portion of the microneedle that is distal to the backing layer. The nanoparticles comprise a pharmaceutically active agent and a polyester selected from the group of polylactic acid, polyglycolic acid, poly(lactic-co-glycolic) acid, polycaprolactone, and combinations of any of these. The microneedles and the backing layer, individually, comprise a water-soluble polymer.

[0028] The rapidly dissolving microneedle array comprises nanoparticles. As used herein, the term "nanoparticles" as used in reference to the drug-bearing composition disposed in the soluble microneedle matrix, refers to nanoparticles per se, and alto to nanospheres, nanocapsules, and other nano-sized particulate matter regardless of its exact shape and/or constitution. The nanoparticles, as used herein, preferably have a particle size of between 10 and 1500 nm, preferably between 50 and 500 nm. Currently preferably, the nanoparticles have an essentially spherical shape, thus having their size dictated by the sphere diameter. Preferably, nanoparticles comprise a polyester. The polyester in the microneedles layer may have any suitable molecular weight and polydispersity, provided, that it forms a readily pourable solution in the solvent, as defined herein, e.g., at a concentration between 0.5% and 10% by weight, e.g., between 0.5%, or 1%, or 1.5%, or 2%, or 2.5%, or 3%, or 3.5%, or 4%, or 4.5%, or 5%, and 20%, or 15%, or 12%, or 10%, or 9%, or 8%, or 7%, or 6.5%, or 6%, or 5.5%, by weight. The polyester is thus selected such that a viscosity of an acetone solution (or, alternatively, a solution in other solvent as enumerated below) at a concentration by weight as defined herein, is between 5 and 5,000 mPa*s, such that the viscosity of the solution allows undisturbed flow into and filling of the microneedles' mold cavities, as described herein. Preferably, the polydispersity of the polymer is less than 4, further preferably less than 3. The molecular weight of the polyester may usually be between 4,000 and 200,000 Dalton. The polyester is preferably polyester selected from the group of polylactic acid,

polyglycolic acid, poly(lactic-co-glycolic) acid, polycaprolactone, and combinations of any of these. Currently preferably, the polyester is poly(lactic-co-glycolic) acid.

[0029] The nanoparticles contain a drug, as elaborated below. The drug may preferably be a pharmaceutically active agent suitable for the local treatment of the skin, or for the systemic delivery. As described in further detail below, the drug may be an anti-inflammatory, e.g., a steroid or an NSAID, an analgesic, an antibiotic, a fungicide, a retinoid, an anti-cancer agent, e.g., an anti-melanoma agent, an anesthetic, or a hormone. In some embodiments, the drug may comprise a combination of at least two drugs. Suitable steroids and NSAIDs may include without being limited dexamethasone, aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, desoximetasone, difluorocortolone, fluclorolone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluocortin butyl, fluorocortisone, fluorocortolone, fluorometholone, flurandrenolone, fluticasone, halcinonide, hydrocortisone, icomethasone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, tixocortol, triamcinolone, piroxicam, aspirin, flurbiprofen, diflunisal, ibuprofen, fenoprofen, fenamate, ketoprofen, nabumetone, naproxen, diclofenac, indomethacin, sulindac, tolmetin, etodolac, ketorolac, oxaprozin, celecoxib, meclofenamate, mefenamic acid, oxyphenbutazone, phenylbutazone, salicylates, and phytosphingosine, or respective pharmaceutically acceptable salts or derivatives thereof. . Suitable antibiotics may be cutaneously active antibiotics, including without being limited to ampicillin, dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V, ticarcillin, rifampin, tetracycline, fusidic acid, lincomicyn, novobiocine, and spectinomycin, or respective pharmaceutically acceptable salts or derivatives thereof . Suitable fungicides may be cutaneously active antifungal agents, including without being limited to clotrimazole, itraconazole, ketoconazole, fluconazole, voriconazole, posaconazole and ravuconazole, or respective pharmaceutically acceptable salts or derivatives thereof . Suitable local anesthetics include, but not limited to, lidocaine benzocaine, procaine, chloroprocaine, prilocaine, tetracaine, bupivacaine, cinchocaine, and ropivacaine.

[0030] The particular drug may be usually selected based on several important properties, including, but not limited to, the elimination half-life, the therapeutic dose, the desired concentrations' range of the drug in the body, etc. As to the pharmacokinetic parameters, the drug should preferably have a short elimination half-life, e.g., between 10 minutes and 5 hours. The therapeutic dose should be accommodated in the microneedle array, and readily controlled by the array's dimensions and density, as described above. The fact of intradermal delivery should also be taken into account, such that the kinetics of drug elimination from the skin should be considered as well. Currently preferably, the pharmaceutically active agent has a therapeutic dose of below 5-10 mg per day, e.g., a cutaneous therapeutic dose, although administration of higher doses may be possible by using larger arrays and/or application to multiple sites during the same day. The term "drug" as used herein interchangeably with the terms "pharmaceutically active agent" or "active pharmaceutical ingredients", and the like, refer to the substance exerting pharmacological effect on the body or a system of a subject to whom said drug is administered, or on a biological model thereof.

[0031] The drug should also be selected such that it is soluble in at least one solvent that dissolves appreciable amounts of the polyester used in the nanoparticles. Exemplary solvents that dissolve both the drug and the polyester include chloroform, dichloromethane, acetone, and any organic solvent comprising a mixture of one or more of the above with each other or with other solvents. Without being bound by a particular theory it is believed that this property of solubility in a mutual solvent would allow successful incorporation of the drug inside the nanoparticle, regardless of the final solid state wherein the drug is present in the nanoparticle, i.e., a dispersion of amorphous powder, a dispersion of a crystalline powder, a solid solution in the polyester, and any combination of these states, i.e., some of the drug being present in a crystalline form, some as amorphous powder, and some amount of the drug being dissolved in the polymer. The drug should preferably also have a comparable solubility in other solvents to the polymer used, such that the drug and the polymer remain in a solution together, and if the solvent conditions are changed and the polymer is precipitated, the drug would precipitate as well at very similar conditions. Therefore, the drug may have a limited solubility in distilled water, e.g., at room temperature (between 15 °C and 25°C). The drug may have in improved solubility in aqueous buffer solutions, particularly in buffers of physiological pH values, e.g., between 5.0 and 7.5, than its solubility in distilled water

at same temperature. The solubility property as generally referred herein, unless the context dictates otherwise refers to the equilibrium concentration of a saturated solution comprising undissolved components under standard pressure and a temperature of 25°C.

[0032] Given the desirability of the drug solubility being close to the solubility of polyester used in the nanoparticles, the solubility of the drug in distilled water should therefore be at or below 1 mg per milliliter, preferably, below 10 mg per liter, e.g., less than 1 mg per liter, less than 0.5 mg per liter, and less than 0.1 mg per liter. This property of similar solubility in at least a pair of solvents is advantageously utilized in the manufacturing of the nanoparticles, as well as to limit the diffusion of the drug to the base of the microneedle array, e.g., to the backing layer and proximal parts of the microneedles in the array. Other characteristics of the drug may also include the log P of between about 1.5 and about 7. Currently preferably, the pharmaceutically active agent has an aqueous solubility at 25°C of less than 1 mg/mL.

[0033] The drug loading in the nanoparticles may be between 5 and 50% by weight, preferably between 6 and 15 % by weight. Without being bound by a particular theory it is believed that the nanoparticles should contain a significant amount of the polyester, which is a polymer having stronger mechanical properties than the soluble polymer of the microneedles, to enable the reinforcement and "cutting edge" function useful in facilitating the puncturing of the skin and thus the administration of the drug payload.

[0034] The drug release from the nanoparticles may be tailored to the needs of a particular formulation of the microneedle array, e.g., by choosing a suitable blend of the polymers with different degradation and/or water penetration properties, as known in the art. The drug is releasable from the microneedles upon exposure to an aqueous environment, e.g., an artificial fluid for dissolution testing, or body fluid, if the array is applied to the skin. The drug is preferably controllably releasable from the nanoparticles, e.g., at a specific rate, and not immediately. The rate of drug release may be controlled by the formulation of the nanoparticles, and may release a fraction of drug as function of time, the fraction being proportional to various parameters, e.g., to the square root of time, or to the time in any other power between 0.3 and 1. An initial relatively rapid release may also be observed, particular for a drug wherein a rapid onset may be desired.

This initial rapid release may account for between 5% to 70% of the deliverable dose or of the label claim, released between 15 minutes to 8 hours. The average duration of the release may be adjusted according to the needs, but generally the microneedles release at least 80% of the drug in controlled manner, as described herein and demonstrated in the appended examples, e.g., during 48 hours-interval, or during 12 hours-interval, or during 36 hours-interval, or during 24-hours interval, or during 72-hours interval. Depending on the pharmaceutically active agent and its potency, the microneedles may be formulated to release the at least 80% of the drug in controlled manner within one week, two weeks, or even one month. The release duration may be conveniently determined in dissolution studies, when the microneedle array is subjected to a liquid medium at sink conditions (i.e., when the amount of the medium is sufficient to completely dissolve at least 300% of the drug present at the test, preferably at least 1000% of the drug). Whereas these dissolution studies may be indicative of the inherent controlled release potential of the nanoparticles, the actual drug release rate in vivo may be significantly slower, and the release duration consequently significantly longer. Without being bound by a particular theory, it is believed that the in vivo release may be slower that the in-vitro under sink conditions, due to sub-sink momentary conditions at the application site.

[0035] The nanoparticles contained in the microneedles are associated with the nanoparticles' suspension stabilizer. As described in greater detail below, the nanoparticles' suspension may be used as obtained without further purification, thereby depositing upon evaporation of the solvent also the excipients of the nanoparticles' suspension. Generally, the nanoparticles associated with nanoparticles' suspension stabilizer are usually essentially colocalized topologically in the specific parts of the microneedles. Such, for example, the nanoparticles may be disposed distally from the backing layer, and most of the amount of the nanoparticles' suspension stabilizer may be found distally from the backing layer. The nanoparticles' suspension stabilizer is preferably a surface-active agent. Without being bound by a particular theory it is believed that the suspension stabilizer acts upon wetting as a dispersing agent, facilitating the disintegration of the part wherein the nanoparticles are concentrated in the microneedle, i.e., in the tips and/or along the edges thereof. The surface-active agents may include but not be limited to polysorbates, sorbitan esters, e.g., fatty acid esters, poloxamers, polyoxyethylated fatty acid ethers, preferably PEG(15)-hydroxystearate, a soluble polymer, preferably selected from the group consisting of sodium alginate, sodium

carboxymethyl cellulose, a povidone, hypromellose, and hydroxypropyl cellulose, and combinations thereof. Currently preferably, the surface-active agent is a polyoxyethylated fatty acid ether, e.g., a compound with the fatty acid residue having a number of carbons between 12 and 22 and the polyoxyethylene group having the number of repeating units between 12 and 18, such as PEG-15-hydroxystearate.

[0036] The nanoparticles' suspension stabilizer may usually be present in the microneedles in an amount generally as required and dictated by the process of manufacturing of nanoparticles to stabilize same during their formation. Thus, based on the nature and identity of the nanoparticles' suspension stabilizer, a weight ratio between the nanoparticles and the nanoparticle suspension stabilizer may be, e.g., between 1:10 and 10:1, but preferably the ratio may be between about 7:1 and 1:2, further preferably about 7:2 and about 3:5, e.g., between about 2:1 and about 4:5.

[0037] As disclosed above, the microneedle array comprises a water-soluble polymer, preferably, the whole array being readily water-soluble. The water-soluble polymers suitable for the use in the microneedle arrays should generally conform with the requirements for the manufacturing of the arrays, i.e., should form a pourable solution readily penetrating and filling the cavities of the microneedle array mold, and should have a sufficient mechanical strength such that upon the reinforcement by the nanoparticles as described herein, the microneedles possess sufficient stiffness to puncture the corneal layer of the skin. Suitable water-soluble polymers include but not limited to a povidone, hypromellose, hydroxypropyl cellulose, carboxymethyl cellulose and/or salts thereof, an alginate, and the like. The water-soluble polymers on the microneedles may be the same or different from the water-soluble polymers used in the backing layer. Additionally, the backing layer may further comprise additional components, as generally described below, but notably plasticizers. The plasticizers, if used in the backing layer, are preferably polyols with low diffusivity in the water-soluble polymer. The polyols in the backing layer may be mono- or oligosaccharides, or smaller molecules, such as glycerin, propylene glycol, and like, provided they do not compromise the long-term stability of mechanical properties of the microneedles. Some currently preferred polyols include sorbitol, and xylitol. However, currently preferably, the water-soluble polymer both in the microneedles and the backing layer is the same water-soluble polymer. Nevertheless, polymers of different grade, e.g., viscosity, molecular weight, and like, may be used

for the microneedles and the backing layer, e.g., to impart certain flexibility to the backing layer without impairing the mechanical stiffness of the microneedles. Currently preferably, the water-soluble polymer is an alginate. In some particular embodiments, the microneedle array, e.g., both the microneedles and the backing layer, consists essentially of an alginate, e.g., sodium alginate, as the matrix former, and further contain drug-loaded nanoparticles associated with nanoparticles suspension stabilizer, in the tips and/or along the edges of the microneedles.

[0038] Thus, the microneedle array preferably comprises an alginate. As used herein, the term "alginate" refers to a polymer having alginic acid backbone, and at least a part of the carboxylic acids thereof being ionized and forming a salt. As known in the art, alginic acid is a polysaccharide linear polymer formed by beta-D-mannuronate and alpha-L-guluronate, via 1-4 glycosidic linkage. Alginic acid may have alternating mannuronate and guluronate blocks, and sometimes may have homopolymeric blocks of polymannuronate and polyguluronate. Alginic acid is available from a variety of sources, e.g., from various species of multicellular algae, or from bacterial sources. It is currently believed that the particular source of alginate is immaterial. Preferably, the salt of the alginic acid is with an alkali metal or an alkali earth metal, provided that the salt is soluble in water. Most preferably, the alginate is sodium alginate. Also preferably, alginate is a fully neutralized alginate, e.g., comprising equal amount of equivalent of the metal cation and the carboxylic acid residues. The alginate may have any suitable molecular weight and polydispersity, provided that it forms a readily pourable solution in water, at a concentration between 0.5% and 10% by weight. The alginate is thus selected such that a viscosity of an aqueous solution at a concentration between 0.5% and 10% by weight is between 5 and 5000 mPa*s. Preferably, the polydispersity of alginate is less than 4, further preferably less than 3. The molecular weight of alginate may usually be between 5000 and 200000 Dalton.

[0039] The nanoparticles and the nanoparticles' suspension stabilizers associated therewith are preferably disposed distally from the microneedle array backing layer. Preferably, the nanoparticles are disposed in the tips of the microneedles, and/or along the edges thereof. The distal disposition of the nanoparticles in relation to the backing layer is preferably such that most of the nanoparticles and/or nanoparticle suspension stabilizer is found in the distal 1/2 to 1/5 of the microneedle length (i.e., height), preferably at the distal 25% to 40% of the microneedles' length.

Thus, the concentration of nanoparticles in the parts of microneedles that are adjacent to the backing layer is preferably negligible or nil. Generally, over 70% of the nanoparticles are present distally to the backing layer, preferably, over 75%, or over 80%, or over 85%, or over 90%, or over 95% of the nanoparticles and/or nanoparticles' suspension stabilizer are present in the distal 25% to 40% portion of the microneedle length.

[0040] Generally, the rapidly dissolving microneedles comprise the soluble polymer as the major matrix-forming component. This ensures that the drug-loaded tips are inserted into the layers of viable epidermis upon application, and, since the microneedles generally dissolve rapidly in water and in the skin, decreases the risk of dose wasting if the microneedles are prematurely removed. Although even highly loaded matrix with nanoparticles loading exceeding 50%-70% by weight may be envisaged, currently preferably the soluble polymer constitutes at least 40% by weight of the microneedles. Thus, a weight ratio between the nanoparticles associated with the nanoparticle suspension stabilizer, and the soluble polymer, preferably alginate, in the microneedles of the array, may be between about 1:10 and about 1:2, e.g., between about 1:4 to about 1:1. It has been unexpectedly found that the microneedles with these amounts of nanoparticles may be readily manufactured, with the nanoparticles depositing in the cutting edges and the tip of the microneedles, thereby providing significant tolerability to the nature of the microneedle matrix polymer, which can now be water-soluble without significantly impairing the performance.

[0041] In currently preferred embodiments, the microneedle array as described herein, has a weight ratio between the nanoparticles and the soluble polymer, preferably an alginate, in the microneedles, of between 1:6 to 5:6, has PEG(15)-hydroxystearate as nanoparticle suspension stabilizer, has a weight ratio between the nanoparticles and the nanoparticle suspension stabilizer between about 7:1 and about 1:2, preferably about between 7:2 and about 3:5, and has a steroid as pharmaceutically active agent.

[0042] The microneedles in the array are usually evenly distributed throughout the array, e.g., they are essentially evenly spaced one from another, although arrays with irregularly distributed microneedles may also be envisaged. The even distribution may be expressed in a value called array pitch, or just "pitch", which, as used herein, should be construed as an average distance

between microneedle tips. The pitch may usually be a measure of microneedles density in the array. It is evident that depending on the base dimensions, the pitch would vary accordingly, with the densest possible configuration being when the pitch is equal to the base dimension of the microneedle. The pitch may also be adjusted to afford the flexibility of the microneedles array, such that to avoid friction between the array elements upon bending or other handling. For example, when microneedles' base is about 200 micrometers, the pitch may preferably vary between 300 and 700 micrometers, further preferably between 400 and 600 micrometers. Without being bound by a particular theory it is believed that the denser microarrays would allow higher absolute drug loading per a unit of area, due to a larger number of microneedles and thus the microneedles' tips. As used herein the term "absolute drug loading", used herein interchangeably with the terms "loading capacity" and the like, should be construed as amount of drug in weight units, e.g., in micrograms or in milligrams, per unit or area, or per one array, unless the context clearly dictates otherwise, e.g., when discussing the relative drug loading in a formulation, which is usually expressed in percentage.

[0043] The microneedles of the array, in their turn, may be of any suitable shape and size to perform their basic functions, which include puncturing the corneal layer of the skin and substantially penetrating beneath it. Thus, the height of the microneedles is selected such that it would be greater than, e.g., 100 micrometers, to ensure penetration of the corneal layer of the skin. On the other hand, it may be advantageous to provide microneedles of greater dimensions, of greater volume in particular, as this would allow increasing absolute drug loading. However, large microneedles may cause significant distress to the skin, *inter alia* by reaching the enervated tissues and triggering nociception. Such upper limit dimensions are readily known in the art. Depending on the base size, the microneedles may have the height of between 300 and 1000 microns, preferably between 400 and 600 microns. The base of the microneedles may be of any suitable shape, e.g., a circle, an oval, an ellipse, a polygon, or an irregular figure. However, to maximize the force transduction when it is applied onto the microneedles' array, it is preferable that the base be a regular geometric figure, e.g., a circle, or an equilateral polygon, such as an equilateral triangle, a square, a hexagon, etc. The microneedles may thus be in a form of pyramids or cones. Preferably, the microneedles have the tip projection positioned at the geometrical center of the base, e.g., at diagonals crossing point for the square base, or the center of the circle. The

microneedles may also be of a more complex shape, e.g., prism or cylinder at a base, and a pyramid or a cone at the tip, provided that the tip is sufficiently thin to enable puncturing the skin upon application of pressure onto the microarray. Embodiments wherein at least part of microneedles have different shape from the others are also envisaged. In some currently preferred embodiments, the microneedles are in form of pyramids.

[0044] The microneedles' array may be of any suitable size and shape per se. Generally, the number of microneedles per array and their distribution density may be such that the microarray has comfortable dimensions for application and wearing, and contains sufficient number of microneedles to carry and administer the desired dose of the drug. For example, the microneedles array that is demonstrated in the examples below, has 100 microneedles evenly spaced on a 5 mm per 5 mm square-shaped area. The number of microneedles per array, may therefore be, depending on the drug loading, from 100 microneedles as exemplified herein, and up to 200,000 microneedles, for a microneedle array of exemplary dimensions of about 20 cm x 20 cm and slightly increased microneedles' density. Larger arrays are also envisaged for applications requiring larger doses, or longer duration of release.

[0045] Further external layers may be applied to the microneedle arrays, over the backing layer, e.g., a woven or non-woven tissue layer to prevent accidental adherence of the array from the rear side thereof, an aluminum foil, e.g., to prevent residual moisture loss, and others, as known in the art. However, provided short application times as generally described below and demonstrated in the examples, the microneedle arrays may be devoid of further layers insoluble in water.

[0046] Other components may be present in the microneedle arrays as described herein, in addition to ones described above, provided that they do not impair the mechanical properties of the microneedles. The nanoparticles, microneedles and the backing layer, may comprise, together or individually, any one of the following excipients below. The microneedle arrays may include surface active agents, e.g., to modify the release of the drug from the nanoparticles, or to facilitate the wash-off of the backing layer, such as polysorbates, sorbitan fatty acid esters, poloxamers, polyoxyethylated fatty acid ethers, and others. The microneedle array may comprise fillers, e.g., to increase the mechanical strength of the microneedles, or to render the backing layer less tacky

in dry form. Antioxidants and preservatives may be used, e.g., to slow down the degradation of the active ingredient or the polymers, and to inhibit microbial growth in the final dosage form. Buffers may be used, in particularly in the backing layer, to maintain the pH of the alginate. Lubricating agents, usually poorly miscible with the other components of the microneedle array, may be used to facilitate detachment of the microarrays from the molds. Suitable excipients are enumerated in various compendia and are well-known to the skilled artisan, e.g., appear in the Handbook of Pharmaceutical Excipients (*Rowe, R. C., et al. "Handbook of Pharmaceutical Excipients, 7th edn, 784–790." (2012).*), or in the Internet site of the US Food and Drug Administration, as currently listed in Inactive Ingredients in Approved Drug Products database.

[0047] Thus, in a further aspect, provided herein a method of manufacturing of nanoparticle-bearing microneedle arrays, comprising a step applying an aqueous solution of a soluble polymer, preferably an alginate, onto a microneedle array mold under reduced pressure, and preferably at room temperature. It has been unexpectedly found that applying the soluble polymer solution differently, e.g., at ambient pressure followed by degassing under reduced pressure, results in unsatisfactory filling of the mold, poor adherence to the deposited nanoparticles, and consequently, inoperable array. The process may be advantageously performed without the need of increased temperature, e.g., at ambient conditions, i.e., at a temperature between 15 °C and 25 °C. These advantages may be particularly relevant for sensitive drugs, such as proteins. Preferably, the concentration of the water-soluble polymer in the aqueous solution is between about 1 %wt to about 10 %wt, preferably about 2 %wt and about 6 %wt. The term “reduced pressure” as used herein refers to conditions wherein the ambient air pressure is reduced, e.g., by means of a vacuum pump, to below 50% of the ambient pressure, preferably to below 30%, or below 20%, or below 10%, or below 5%, or below 4%, or below 3%, or below 2%, or below 1% of the ambient air pressure. The term may also encompass high vacuum with pressure significantly below 0.1% of the normal ambient air pressure.

[0048] To obtain the microneedles with nanoparticles disposed at the tips and/or along the edges thereof, method further comprises applying a suspension of the nanoparticles onto the microneedle array mold, e.g., prior to applying the aqueous solution of water-soluble polymer. The suspension

of nanoparticles usually comprises the nanoparticle suspension stabilizer, preferably, as used during the manufacturing process of the nanoparticles. The applied nanoparticles' suspension may then be dried or degassed and dried, e.g., at reduced pressure and/or elevated temperature. Degassing may be performed at reduced pressure, for a time interval sufficient to remove the entrapped air bubbles, e.g., between about 5 and about 30 minutes. Drying may be performed at ambient or reduced pressure, and preferably at an elevated temperature, e.g., between about 35°C and 50 °C, for a time interval of between 30 and 150 minutes, until the solvent of the applied nanoparticles solution is substantially completely evaporated. The step of applying nanoparticles' suspension may be repeated several times, until the desired drug loading is achieved in the microarray mold. Without being bound by a theory it is believed that sequential application of nanoparticles' suspension not only allows adjusting the drug loading, but also facilitating the deposition of the nanoparticles along the edges of the mold, since the edges have increased surface area and may be capable of interacting with the polyester nanoparticles as described herein above.

[0049] Therefore, the method of manufacturing comprises providing a microneedle array mold. The mold may be produced as known in the art, e.g., as described in WO2015122838, in any suitable material, preferably in a durable flexible material, e.g., in polydimethylsiloxane, polyvinyl siloxane (PVS), or the mold can be 3-D printed. The mold may have receptacle element, with a plurality of cavities at the bottom thereof. These cavities are usually essentially perpendicular to the base of the receptacle and are reciprocal in shape to the microneedles to be formed. The cavities in the receptacle may therefore have the shape and the density as described above for the microneedles. The mold may be of a size of an actual microneedle array, or may be at any suitable larger size, e.g., to enable manufacturing of multiple microneedle arrays concomitantly. In this case the cavities in the mold may be grouped into cavity groups corresponding in size to the contemplated microneedle array. These groups may be separated from one another, e.g., by an increased distance between the cavities, or by a protrusion or series of protrusions encircling the groups of cavities on the bottom of the receptacles.

[0050] At any time of the manufacturing process, the mold may be coated with a thin layer of a suitable lubricant, to facilitate the demolding of the arrays.

[0051] The mold is usually first filled with a microneedle tip precursor solution. This solution comprises a suspension of nanoparticles, and further comprising a nanoparticle suspension stabilizer, preferably a surface-active agent. The suspension of nanoparticles may be readily obtained as known in the art, e.g., by nanoprecipitation method, as elaborated in Far et al, ACS Omega 2020, 5, 7432–7439, doi:10.1021/acsomega.0c00111, or in Abu Ammar et al, Drug Deliv. and Transl. Res. 2019, 9, 76–84, doi:10.1007/s13346-018-00603-0. Briefly, an organic solution of the polymer and the solution or dispersion of a drug in a solvent that is at least partially soluble in water, e.g., in acetone, chloroform, or dichloromethane. The solvent may also be a solvent mixture that comprises these solvents, and may further comprise additional solvents that do not significantly alter the solubility of the polymer and the drug in the solvent mixture or in water. The polymer is completely dissolved and forms a true solution in the solvent / solvent system, but the drug may be either completely dissolved, or may be partially dispersed in form of colloidal particles. Preferably, the amount of the drug dissolved in the nanoparticle organic precursor solution is above 50% of the total drug amount by weight, introduced into the solution, further preferably above 60%, or above 70%, or above 80%, or above 90%, or above 95%, or above 99%, or above 99.9%. To prepare the nanoparticle organic precursor solution, the polymer and the drug are added consecutively or concomitantly to the solvent or to the solvent mixture, and mixed using mixing means as known in the art, e.g., using a suitable mixer, such as mechanical overhead mixer equipped with an impeller. The mixture is mixed until complete dissolution. The mixture may be heated to facilitate the dissolution of the polymer and/or the drug. Preferably, the heating is below the temperature whereat the pharmaceutically active agent begins decomposition, and in some cases should be avoided altogether. When the mixture is heated, it may be heated to a temperature between 25 °C and a temperature several degrees lower than the boiling temperature of the solvent or of any of its component. Therefore, when chloroform or acetone are present in the solvent, the mixture may be heated to a temperature between 25 °C and 52-55 °C, and when dichloromethane is present in the solvent, the mixture may be heated to a temperature between 25 °C and 36-37 °C.

[0052] The aqueous phase receiving the organic nanoparticle precursor solution is prepared likewise, by adding nanoparticles suspension stabilizer(s) and mixing till dissolution. The organic solution is then introduced into the aqueous solution. The weight ratio between the organic and

aqueous phase is preferably between 1:1 and 1:5. Generally, the higher quantity of the aqueous phase in reference to the organic solution generally leads to higher kinetics of nanoprecipitation, but also results in more diluted nanoparticles' suspension. The final suspension may be concentrated to a desired volume, e.g., by evaporating the excessive water, or by centrifugation and reconstitution of the pellet in a fresh aqueous phase comprising a suspension stabilizer.

[0053] The obtained microneedle tip precursor solution is applied to the mold. Applying of the solution to the mold may be carried out by transferring the solution onto the mold. The mold is preferably levelled prior to transferring of the microneedle precursor solution thereon, to avoid overflow of the receptacle and to ensure an equal distribution of the solution on the mold. The solution may be fed into the mold as known in the art, e.g., by pouring it onto the mold via one or more outlets, by transferring the solution, e.g., with a suitable pump, onto the mold. The solution may also be fed into the mold via a manifold feeder positioned over or inside the cavities of the mold. The particular means to apply the solution will be dictated by the needs of the process. The amount of the solution may be such that it is sufficient to fill the cavities of the mold; that is, the volume of the microneedle tip precursor solution applied is usually less than the combined volume of the microneedles, or the combined remaining volume of microneedles if a previous tip precursor solution has been applied to the mold. The filling of the mold with microneedles tip precursor solution may be performed under reduced pressure, but may preferably be performed at a temperature between 15 °C and 25 °C.

[0054] The mold with the applied solution thereon may then be degassed, e.g., by placing the mold under reduced pressure, e.g., into a vacuum chamber. Degassing of the solution in the mold usually accomplishes several tasks: one is to remove the trapped air bubbles at the tips of the cavities in the mold, and thus ensure uniform formation of the microneedles. Another goal is to remove the air bubbles to ensure uniform coverage of the mold by the microneedle precursor solution. A further goal is to effect a partial evaporation of the solvent, as need may be. Degassing may be performed at reduced pressure, for a time interval sufficient to remove the entrapped air bubbles, e.g., between about 5 and about 30 minutes.

[0055] The mold with optionally degassed microneedle tip precursor solution is then dried to substantially complete evaporation of the aqueous medium of the precursor solution. Preferably, that the process be performed on a levelled surface, as mentioned above, and therefore drying may be carried out in a vacuum oven. Additionally, the drying may be performed in a desiccator. Drying may be performed at ambient or reduced pressure, and preferably at an elevated temperature, e.g., between about 35°C and 50 °C, for a time interval of between 30 and 150 minutes, until the solvent of the applied nanoparticles solution is substantially completely evaporated. The step of applying nanoparticles' suspension may be repeated several times, until the desired drug loading is achieved in the microarray mold.

[0056] An aqueous microneedle precursor solution may then be provided in a manner similar to furnishing of other solutions. Briefly, the water-soluble polymer is combined with water and mixed until dissolution. Further components, if present, may be added consecutively or concomitantly with the water-soluble polymer. Preferably, the concentration of the water-soluble polymer in the aqueous solution is between about 1 %wt to about 10 %wt, e.g., between about 2 %wt and about 6 %wt. In currently preferred embodiments, the water-soluble polymer is sodium alginate. The aqueous microneedle precursor solution is then applied to the mold containing the substantially dried microneedle tips deposited at the bottom of the mold. The applying of the aqueous microneedle precursor solution is performed under reduced pressure. When the same polymer and same composition is used for the microneedles and the backing layer, the amount of the aqueous microneedle precursor solution applied to the mold is sufficient to fill the microneedles' cavities and the backing later. Preferably, the aqueous microneedle precursor solution is applied in several steps as well, with at least partial drying performed between the applications. The drying may be performed as generally descried above.

[0057] When a backing layer composition is different from the microneedle composition, the backing layer solution may be applied after the drying of the aqueous microneedle precursor solution. The backing layer solution is also prepared for the application to the mold as described generally herein. To prepare the backing layer solution, the water-soluble polymer, e.g., alginate, and optionally additional components, such as plasticizers, e.g., polyols, are added consecutively or concomitantly to the solvent or to the solvent mixture, and mixed using mixing means as known

in the art, e.g., using a suitable mixer, such as mechanical overhead mixer equipped with an impeller. The mixture is mixed until complete dissolution. The mixture may be heated to facilitate the dissolution of the components of the backing layer solution. Preferably, the heating is to a temperature between 25 °C and 60 °C.

[0058] The finally filled mold may be dried by means known in the art, e.g., in a suitable oven. Preferably, that the process be performed on a levelled surface, as mentioned above, and therefore drying may be carried out in a vacuum oven. Additionally, the drying may be performed in a desiccator. Drying may be performed until the composition in the mold is completely solidified and non-tacky. Suitable in-process quality controls may be employed to determine the endpoint of drying of the microneedles array, e.g., gravimetry, or water determination, e.g., via Karl-Fischer titration.

[0059] The dried microneedles array may be detached from the mold, and may further be processed as needed, e.g., further dried, sterilized, packaged, etc.

[0060] Thus, provided herein a method of manufacturing a nanoparticle-bearing microneedle array, comprising providing a mold for microneedle array, providing a microneedle tip precursor solution, the microneedle tip precursor solution comprising a suspension of nanoparticles and further comprising a nanoparticle suspension stabilizer which is preferably a surface-active agent, applying the microneedle tip precursor solution to the mold, optionally under a reduced pressure, and drying the applied microneedle tip precursor solution to obtain a mold partially filled with microneedle tips, optionally further applying the needed number of times the microneedle tip precursor solution to the mold and drying same, providing an aqueous microneedle precursor solution, applying the aqueous microneedle precursor solution to the mold under a reduced pressure and optionally at room temperature, drying the applied microneedle precursor solution, and optionally applying an aqueous backing layer solution, wherein the aqueous backing layer solution comprises a water-soluble polymer, preferably an alginate and optionally a polyol, and wherein the aqueous microneedle precursor solution comprises a soluble polymer, preferably an alginate.

[0061] The obtained microneedle array may be sterilized as known in the art, preferably by gamma irradiation, and packaged in a suitable package. When the sterilizing is performed by gamma irradiation, the microneedle array may be first packaged in the final package and then sterilized.

[0062] Thus, provided herein is a nanoparticle-bearing microneedle array, manufactured according to the process as generally described herein above. However, certain equivalent but not explicitly disclosed or similar procedures may be envisaged for the manufacturing of the nanoparticle-bearing rapidly dissolving microneedle array as generally disclosed herein. Therefore, the provided herein nanoparticle-bearing microneedle array may be essentially identical to the one that is manufactured according to the process as generally described herein above, i.e., it may be obtainable thereby.

[0063] The microneedles arrays as generally described herein may be used in treatment of patients in need thereof. For example, the microneedles arrays may be used to treat or ameliorate the symptoms of a disease or disorder responsive to the pharmaceutically active agent in the microneedles array. The symptom or disease may be selected from inflammation-related disorders, e.g., psoriasis, dermatitis, and eczema, infections, e.g., bacterial, fungal, or protozoal infections, Herpes Zoster, acne, cancer, e.g., in-situ cutaneous carcinomas, local irritation, and localized pain.

[0064] The microneedle arrays are usually administered to a patient in need thereof by applying the microneedle array to a skin of the patient, and pressing to effect the penetration of the microneedles into the skin. As demonstrated in the examples' section below, as little as 15 seconds may be sufficient to effect the dissolution of the microneedles and the deposition of the nanoparticle payload in the viable epidermis. Therefore, the array may be retained in place for a period of between 1 minute and 30 minutes, preferably above 3 minutes, or above 4 minutes, or above 5 minutes. The upper limit of the patch retention in place is immaterial, and the remaining array may be washed off by gentle rubbing with soap and warm water at any suitable time point beyond the retention interval disclosed herein.

[0065] Usually, the array may be applied as needed, e.g., up to several times a day. However, it may be advantageous to utilize microneedle arrays that contain a significant absolute drug loading and release the drug over an extended time interval. Therefore, the array may be applied at any suitable frequency as known in the art, e.g., three times a day, twice a day, once daily, and also once every two days, twice a week, or even once a week.

EXAMPLES

Materials

[0066] Dexamethasone (Alfa Aesar) and sodium alginate (Fisher Chemical) were purchased from Holland-Moran Inc. Israel. PLGA-Purasorb PDLG 5010 (50:50) was donated by Corbion Purac (Gorinchem, The Netherlands). Solutol HS 15 was supplied from BASF (Ludwigshafen, Germany). Silicone MPatch microneedle templates were purchased from Micropoint Technologies Pte Ltd. (Pioneer Junction, Singapore) and were pyramidal in shape with a dimension of 10 × 10 needle array, 200 µm base, 500 µm height and 500 µm pitch. Phosphate buffer saline (PBS) was purchased from Hyclone Laboratories. Organic solvents were obtained from Sigma-Aldrich (Rehovot, Israel).

Example 1 - Preparation of dexamethasone nanoparticles for the microneedle arrays

Preparation of DEX-loaded PLGA NPs

[0067] DEX-loaded NPs were prepared using the nanoprecipitation method. Briefly, 2 mg of DEX and 6 mg of PLGA were dissolved in 1 mL acetone. The organic phase was added rapidly into an aqueous phase (2 mL) containing 0.5% (w/v) solutol HS 15, which was continuously stirred at room temperature (25 °C, 900 rpm) for 24 h to evaporate the organic solvent, followed by adjustment of the formulation volume to 2 mL by distilled water. The vials were wrapped with aluminum foil to avoid drug degradation due to light exposure.

Physicochemical characterization of DEX NPs

[0068] Particle size (hydrodynamic diameter), polydispersity index (PDI), and zeta potential were determined by the dynamic light scattering (DLS) method using Malvern ZetaSizer nano-ZS laser particle size distribution analyzer (Zetasizer Pro, Malvern Instruments, UK). Samples were diluted

at 1:100 (v/v) with water and added to folded capillary zeta cells (DTS1070) to analyze at a 90° angle at room temperature (25 °C). For each sample, the mean value ± s.d. of three determinations was established.

Determination of drug encapsulation efficiency and loading content

[0069] Encapsulation efficiency (EE) and drug loading content (DLC) were determined by first separation of the unloaded drug (free DEX) by filtration/centrifugation using of Amicon® Ultra-15 (molecular weight cut-off 100 kDa) centrifugal filter unit (Merck Millipore Ltd.). The formulation samples were added to the upper chamber of the Amicon® tube and then washed with equal volume of distilled water three times by centrifugation at 3000 rpm for 1 min each. Finally, the amount of free DEX was determined from the filtrate using spectrophotometrically (GeneQuant 1300; Biochrom, UK) at a wavelength of 242 nm, versus a calibration curve (linear range between 0-25 µg/mL). The EE and DLC were calculated using the following equations:

$$EE\ (\%) = \frac{amount\ of\ DEX\ fed\ initially - amount\ of\ free\ DEX}{amount\ of\ DEX\ fed\ initially} \times 100$$

$$\text{DLC}\ (\%) = \frac{amount\ of\ DEX\ fed\ initially - amount\ of\ free\ DEX}{amount\ of\ formulation\ components} \times 100$$

[0070] Afterwards, the nanoparticles from the upper chamber of the Amicon® tube were centrifuged at 3000 rpm for 1 min to remove the polymeric debris and polymeric debris was analyzed to ensure that no drug residues are present.

[0071] The DEX-loaded PLGA nanoparticles were prepared with a mean particle size of 93.7 nm, narrow size distribution (PDI < 0.3), and negative zeta potential values, as seen in Table 1 below. DEX was successfully incorporated into the PLGA NPs exhibiting an encapsulation efficiency of 86.6% and adequate drug loading content of 9.1 %.

Table 1. Physicochemical properties, encapsulation efficiency and drug loading content of blank and DEX-loaded PLGA NPs (n = 3, mean ± s.d.).

Formulation	Mean Diameter (nm)	Polydispersity index (PDI)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Drug loading content (%)
DEX NPs	93.7 ± 5.10	0.27 ± 0.04	-27.5 ± 3.31	80 ± 0.6	9.1 ± 0.1
Blank PLGA NPs	116 ± 1.92	0.13 ± 0.03	-35.6 ± 1.63	-	-

Infrared spectroscopy

[0072] Attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) spectra were measured by a PerkinElmer Spectrum 100S spectrometer equipped with a universal ATR sampling accessory. For each spectrum 4 scans were collected with a resolution of 2 reciprocal cm, and the scan range was between 4000 and 650 reciprocal cm.

[0073] ATR-FTIR spectra of DEX, PLGA, physical mixture, DEX-NPs, and blank-NPs are presented in Figure 1. In the Figure, the legends read as follows: the label "Dexamethasone" refers to the spectrum obtained by dexamethasone, the label "PLGA" refers to the spectrum obtained by PLGA, the label "DEX-PLGA 1:3" refers to the spectrum obtained by physical mixture of PLGA and dexamethasone in ratio 1:3, the label "DEX NPs" refers to the spectrum obtained by dexamethasone nanoparticles, and the label "PLGA-NPs" refers to the spectrum obtained by PLGA blank nanoparticles.

[0074] In the spectrum of DEX, the characteristic peaks were observed at 1704.01, 1662.17 and 1617.57 reciprocal centimeters, as known in the art. These peaks were attributed to the stretching vibrations of C = O and C = C (in ring) double bond framework conjugated to C = O bonds in DEX. The characteristic peak of PLGA was identified at 1747.51 reciprocal centimeters due to the ester group. The spectrum of the physical mixture exhibited relatively similar peaks of DEX and PLGA. The spectra of blank-NPs and DEX-NPs showed a peak at 1735 cm^{-1}, which was ascribed to the used surfactant, solutol HS 15, and overlapped with the C = O ester stretching vibration of PLGA. The peak corresponding to C = O stretch in DEX was observed at 1662.67 cm1 in the case of drug-containing NPs, where it was absent in the spectrum of blank NPs, suggesting the successful entrapment of DEX within PLGA NPs with no significant shift that would indicate chemical interaction between the drug and the polymer.

Differential scanning calorimetry (DSC)

[0075] The thermal behavior of the DEX-loaded nanoparticles, PLGA, dexamethasone, and a physical mixture thereof, was analyzed by a DSC 1 Star System apparatus equipped with Star Software (Mettler Toledo, Greifensee, Switzerland) and a DSC131 Evo (SETARAM Instrumentation, Caluire-etCuire, France). Weighed samples of 6–11 mg were placed into 100 μL

aluminum crucibles and the samples were scanned from 25 °C to 300 °C at a constant heating rate of 20 °C/min.

[0076] The DSC thermograms are presented in Figure 2. In the Figure, the legends read as follows: the label "Heat flow (mV)" refers to the heat flow expressed in mV, the label "Exo" refers to the direction of an exotherm, the label "Endo" refers to the direction of an endotherm, the label "Temperature (°C)" refers to the temperature expressed in degrees Celsius, the label "Dexamethasone (DEX)" refers to the spectrum obtained by dexamethasone, the label "PLGA" refers to the spectrum obtained by PLGA, the label "DEX-PLGA 1:3" refers to the spectrum obtained by physical mixture of PLGA and dexamethasone in ratio 1:3, the label "DEX NPs" refers to the spectrum obtained by dexamethasone nanoparticles, and the label "PLGA-NPs" refers to the spectrum obtained by PLGA blank nanoparticles.

[0077] PLGA exhibited a characteristic endothermic peak at 50 °C depicting its glass transition temperature. The thermal behavior of DEX was characterized by an endothermic peak at 255°C, which is attributed to the melting point of the crystalline drug. No endothermic melting peak was determined for DEX NPs, which may be because DEX was dispersed at molecular state within the nanoparticles in an amorphous or disordered crystalline state.

X-ray diffraction (XRD)

[0078] XRD analysis was used to corroborate the physical state of DEX and the degree of its incorporation in the polymeric NPs. X-ray powder diffraction measurements were performed on the D8 Advance diffractometer with LYNXEYE-XE-T detector (Bruker AXS, Karlsruhe, Germany) operating in 1D mode. The diffractograms are presented in thermograms are presented in Figure 3. In the Figure, the legends read as follows: the label "Intensity (counts)" refers to the intensity expressed in the number of counts, the label "2 Theta (°)" refers to the 2θ angle expressed in degrees, the label "DEX NPs" refers to the spectrum obtained by dexamethasone nanoparticles, and the label "PLGA-NPs" refers to the spectrum obtained by PLGA blank nanoparticles.

[0079] Low-background quartz sample holders were carefully filled with the powder samples. XRD patterns within the range 2° to 75° 2θ were recorded at room temperature using CuKα

radiation (λ=1.5418 Å) with the following measurement conditions: tube voltage of 40 kV, tube current of 40 mA, step-scan mode with a step size of 0.02° 2θ and counting time of 0.5 sec/step.

[0080] Fig. 3 shows XRD patterns of DEX, DEX NPs and blank PLGA NPs. The presence of numerous distinct peaks in the DEX diffractogram implies its crystalline nature as known in the art. The diffraction pattern of DEX NPs shows a broad hump in the region of 10°–25°. This is indicative of the amorphous polymeric matrix, and the presence of some characteristic peaks of DEX indicates the presence of it in the NPs. The intensity of these peaks was reduced suggesting that significant loss of crystallinity of DEX occurred while being incorporated in the polymeric matrix, thus supporting that it is dispersed at molecular state within the nanoparticles, in agreement with the DSC thermogram of DEX NPs, in which the DEX crystalline peak at 255 °C was not observed. This indicates a degree of amorphization of the drug within the PLGA matrix.

Example 2 – Preparation of microneedle arrays

[0081] Sodium alginate at a concentration of 4% (w/v) was chosen from preliminary screening tests for the preparation of dissolving MNs. In the case of NP-MNs, the obtained nanoparticles were encapsulated into the tips of the microneedles, being concentrated at the tips rather than being dispersed in the base layer, to provide more efficient delivery, by decreasing drug overload and waste. The MNs were prepared as a 10 × 10 array mold of 200x200x500 [base length x width x height] through a vacuum-deposition micromolding method as schematically shown in Figure 4. In the Figure, the legends read as follows: the label "1. Casting DEX NPs into PDMS mold" refers to the step of casting DEX NPs into PDMS mold, the label "2. Vacuum (10 min)" refers to a step of applying vacuum for 10 minutes, the label "3. Drying at 37°C (1 h)" refers to a step of drying at 37 °C for 1 hour, the label "4. Repetition of steps 1-3" refers to a step repetition of steps 1-3, the label "5. Casting 4% w/v SA solution under vacuum (10 min)" refers to a step of casting 4% w/v sodium alginate solution under vacuum over 10 minutes, the label "6. Drying at 37°C (1 h)" refers to a step of drying at 37 °C for 1 hour, the label "7. Casting 4% w/v SA solution" refers to a step of casting 4% w/v sodium alginate solution, the label "8. Drying at 37°C (1.5 h)" refers to a step of drying at 37 °C for 1.5 hours, the label "9. Demolding" refers to the step of demolding, the label "DEX NPs" refers to dexamethasone nanoparticles, and the label "SA layer" refers to sodium alginate layer.

[0082] NP-MNs were prepared using a vacuum-deposition micromolding method, adopted and optimized as follows. In brief, a polydimethyl siloxane (PDMS) micromold was placed into a vacuum flask, and 150 µL of DEX-loaded nanoparticle dispersion was injected onto the micromold surface, followed by a vacuum for 10 minutes and drying at 37 °C for 1 h. This process was repeated once more, and then 150 µL of 4% w/v sodium alginate solution was injected under vacuum through the septum onto the micromold surface, held for 10 min, and allowed to dry at 37 °C for 1 h. Afterwards, 100 µL of 4% w/v SA solution was added to form the base of MNs and dried at 37 °C for 1.5 h before being demolded.

[0083] Blank microneedle arrays were prepared by the same method as nanoparticle-loaded, without adding NPs at any stage of the preparation.

[0084] Morphological characterization of the loaded and the blank microneedle arrays was performed by scanning electron microscope (SEM, Apreo 2 Thermo Scientific). The micrographs are shown in Figure 5. In the Figure, the legends read as follows: the scale bars at the right lower corners of the Figs. 5A-5D labelled with "400 µm" and "100 µm" refer to the corresponding size of 400 µm and 100 µm, respectively.

[0085] As seen in Fig. 5, when characterized by SEM, the MN formulations had a quadrangular pyramidal shape and were uniformly distributed on the substrate. SA-MNs and NP-MNs were successfully formed with dimensions of approximately 500 µm height and 200 µm width of the base as represented in Table 2 below. Moreover, it can be seen that sharper tips and smoother surfaces were obtained with the NP-MNs compared to blank microarrays (SA-MNs).

Table 2. Summary of microneedle dimensions (n = 10, mean ± s.d.).

Formulation	Base (µm)	Height (µm)
SA-MN	199.9 ± 1.3	500.2 ± 0.4
NP-MNs	200.3 ± 1.9	500.2 ± 0.1

[0086] Light microscopy of the obtained microneedle arrays was performed using a stereomicroscope (Olympus-SZ61, Japan). The observations demonstrated clear semi-transparent

structures, with opaque drug payloads clearly observable on the tips of NP-MNs Fig. 6B, as opposed to clear uniform blank microneedles of Fig. 6A. In the Figures, the scale bars at the right lower corners of the Figs. 6A-6B labelled with "200 μm" refer to the corresponding size of 200 μm.

Insertion capabilities of MN Array in Parafilm™

[0087] To evaluate the insertion properties of MNs and to analyze MN insertion depth, commercial Parafilm™ sealant film was used as a skin simulant for preliminary assessment instead of biological tissues. SA-MNs and NP-MNs were manually pushed into 8 layers of parafilm (thickness of 140 ± 10 μm each) stacked together for MN insertion by manual pressure (using a thumb), to imitate the practical use in clinical settings. The pressure was applied for 30 seconds, and then the individual layers were observed under the light stereomicroscope to study the insertion efficiency and MN insertion depth. The insertion was expressed in the number of pores created in each parafilm layer, and the change of the height (μm) of 10 randomly selected MNs was calculated and plotted as the percentage of reduction in MN height.

[0088] Both SA-MNs and NP-MNs created 100 pores only in the first Parafilm™ layer, indicating that the MN insertion depth is approximately 140 μm. This suggests that MNs readily pierce at least the outermost layer of the skin, the *stratum corneum* (~50 μm thickness), and allow for MN insertion into the epidermis. Fig. 7 shows images of the first layer of Parafilm™ from the tests of NP-MNs (Fig. 7A), blank NP-MNs (Fig. 7B), and blank MNs (Fig. 7C). In the Figures, the scale bars labelled with "200 μm" refer to the corresponding size of 200 μm. It can be readily seen that NP-MNs and blank-NP-MNs created square-shaped pores, while blank SA-MNs created pores with less defined shape. Without being bound by a theory it is presumed that this effect was due to the different structure toughness and the structural change occurred during the insertion process. This could be supported by the extensive bending observed in the blank microneedle arrays, whereas the nanoparticle-loaded microneedles essentially retained their original shape. The mean percentage reduction in height of SA-MNs and NP-MNs was 25.5%, and 7.4%, respectively. This demonstrates that nanoparticle-loaded microneedles became slightly compressed and were mechanically stronger than SA-MNs.

Evaluation of mechanical properties

[0089] The mechanical strength of MNs against to static forces was measured by placing different weights against the MNs. Briefly, the microneedle patches were placed facing upward, onto which weights of 50 g, 500 g, and 1000 g were placed gently on the top of each patch, respectively. After 5 minutes, the weights were removed, and morphological changes were evaluated by a HAYEAR 4K UHD microscope camera. The rate of change of the height (μm) of the MNs was calculated and plotted as the percentage of reduction in MN height. As shown in Fig. 8, the sharp tips of MNs exhibited reduction and deformation in the vertical direction as a function of the static force ranged from 50 g (~ 4.9 mN/needle) to 1000 g (~ 98 mN/needle). The MNs showed adequate mechanical strength, and no fractures or broken MNs were noted, even though they were pressed by 1000 g weight. In the Figure 8, the legends read as follows: the scale bars labelled with "200 μm" refer to the corresponding size of 200 μm, the label "0 g" refers to the load of 0 grams (i.e., no load), the label "50 g" refers to the load of 50 grams, the label "500 g" refers to the load of 500 grams, and the label "1000 g" refers to the load of 1000 grams.

[0090] Significant morphological changes and differences in the average percent height reduction of SA-MNs and NP-MNs were observed after applying different weights, as shown in Figure 9, ascertaining that the incorporation of DEX NPs into the MNs improved their mechanical strength, in agreement with the results of the Parafilm™ insertion test. In the Figure, the legends read as follows: the label "SA MNs" refers to the blank sodium alginate microneedles, the label "NP MNs" refers to the nanoparticle-bearing microneedles, the label "50 g" refers to the load of 50 grams, the label "500 g" refers to the load of 500 grams, and the label "1000 g" refers to the load of 1000 grams, and the label "MN height reduction (%)" refers to the reduction of height of the microneedles as expressed in percentage of initial height.

Dissolution of MNs in ex vivo chicken skin and agarose gel

[0091] The dissolving capability of the polymeric tips affects the release rate of NPs from MNs. Therefore, the MNs dissolution study after application to *ex vivo* chicken skin and skin-mimicking agarose gel was performed to determine the time required for MNs dissolution. The MN arrays were applied on *ex vivo* chicken skin obtained from a local slaughterhouse, and to agarose gel 3% (w/v). A weight of 500 g was placed above the arrays, and MNs were removed after pre-determined

times of 15, 45 and 120 seconds. The MNs were imaged before and after dissolution using a microscope camera, as seen in Fig. 10. In the Figure, the legends read as follows: the label "t=0" refers to the time point of 0 seconds (i.e., the initial time point, the label "t=15 s" refers to the time point of 15 seconds, the label "t=45 s" refers to the time point of 45 seconds, and the label "t=2 min" refers to the time point of 2 minutes.

[0092] It can be readily seen that the NP MNs were gradually dissolved within 2 min after insertion into *ex vivo* chicken skin, with a significant softening and bending already visible after 15 seconds. The needle height of NP-MNs decreased to over ca. 50% already after 15 seconds, releasing the nanoparticles instantaneously. In agarose gel a similar picture was observed, with the needle height decreasing to ca. 25%. Without being bound by a particular theory or explanation, it is currently believed that the effect observed was due to high water solubility of sodium alginate, that the MNs could dissolve very quickly and thoroughly. It is likewise presumed that Solutol HS 15 affected the wetting of the polymeric matrix, thus increasing the water penetration through the MNs and enhancing dispersion of the nanoparticles, thereby eventually decreasing the overall dissolution time.

[0093] These results suggest that the recommended application time of the MN patch could be set as over 15 seconds, but not necessarily much longer. Once the MNs dissolve in the skin and the drug-loaded NPs are released, the base may be readily washed off with water, promoting skin recovery and restoring its barrier function, thus minimizing potential skin infections via the microchannels created by the MNs.

In-vitro drug release studies

[0094] DEX NPs samples (200 μL) were placed into dialysis bags, with a molecular cutoff of 8000 Da, soaked in 10 mL of PBS pH 7.4, and maintained at 37 °C (50 rpm) in a rotary incubator. At predetermined time intervals, 0.5 mL of the release medium was sampled and was replenished immediately with the same volume of fresh prewarmed PBS (37 °C) maintaining sink condition throughout the experiment for 120 h. The experiment was performed in four replicates.

[0095] The release data presented in Figure 11. In the Figure, the legends read as follows: the label "Cumulative DEX release (%)" refers to the cumulative dexamethasone release expressed in percentage, and the label "Time (h)" refers to the time elapsed in hours.

[0096] The release data indicates that the release of DEX was apparently biphasic, and an initial burst drug release observed in the first 6 h followed by sustained release over the rest of the experiment. This release profile would effectively suppress both acute and chronic inflammation responses.

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[0097] Various other features according to the invention as described herein for the aspect of the microneedle arrays are applicable *mutatis mutandis* to the aspects of methods of preparation of said arrays, methods of treatment of a disease or disorder in subject in need thereof by the means of microneedle arrays, the microneedle use in these methods according to the teachings herein, and the use of the microneedle arrays in the methods according to the teachings herein.

[0098] The herein described preferred embodiments demonstrating some of the embodiments of the present disclosure are provided to better understand the present disclosure, which however does not limit the invention in any respect. Variants and equivalents may be readily envisaged by the skilled artisan; the invention therefore encompassing all these variations and equivalents.

[0099] It must also be noted that, as used in this specification and the appended claims: all scientific and technical terms have meanings commonly used in the art unless otherwise specified; the definitions as provided herein are given with the purpose to facilitate understanding of certain terms used frequently herein and are not necessarily meant to limit the scope of the present disclosure; as used herein the term "about", "c.a.", "~", and like, as used interchangeably herein, refers to the value and the range of ± 10 %; the terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to", with this terms also encompassing the terms "consisting of" and "consisting essentially of", which have their narrower meaning as known in the art, thus an embodiment described as comprising something

also discloses embodiments consisting essentially of same and consisting exclusively of same; the singular forms “a”, “an”, and “the”, include plural referents unless the content clearly dictates otherwise; as used herein, a phrase in the form “A and/or B” means a selection from the group consisting of (A), (B) or (A and B); as used herein, a phrase in the form “at least one of A, B, and C” means a selection from the group consisting of (A), (B), (C), (A and B), (A and C), (B and C) or (A, and B, and C), and further combinations are envisaged for the lists comprising larger number of terms.

[00100] It is appreciated that, certain features of the invention, which are, for brevity, described in the context of separate embodiments, may also be provided in combination with other features in a single embodiment, unless technically infeasible. Conversely, features described in specific combinations of various features, which are, for clarity and demonstration, are described in the context of a single embodiment, may also be provided as separate embodiments individually or in any suitable sub-combination with other features and/or embodiments, as reasonable to the skilled artisan, suitable and operative. Certain features described in the context of various embodiments, including preferred features, are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Claims

1. A microneedle array comprising a plurality of microneedles and a backing layer,

wherein each said microneedle comprises a plurality of nanoparticles associated with a nanoparticle suspension stabilizer, said nanoparticles and said nanoparticle suspension stabilizer being disposed at the portion of said microneedle that is distal to said backing layer,

wherein said nanoparticles comprise a pharmaceutically active agent and a polyester selected from the group of polylactic acid, polyglycolic acid, poly(lactic-co-glycolic) acid, polycaprolactone, and combinations of any of these, and

wherein said microneedles and said backing layer, individually, comprise a soluble polymer.

2. The microneedle array according to claim 1, wherein nanoparticle suspension stabilizer is a surface-active agent.

3. The microneedle array according to any one of the preceding claims, wherein said soluble polymer is selected from the group consisting of a povidone, a hypromellose, a hydroxypropyl cellulose, a carboxymethyl cellulose and/or salts thereof, and an alginate.

4. The microneedle array according to any one of the preceding claims, wherein said soluble polymer is an alginate.

5. The microneedle array according to claim 4, wherein said alginate is sodium alginate.

6. The microneedle array according to any one of the preceding claims, wherein a weight ratio between said nanoparticles associated with said nanoparticle suspension stabilizer and said water-soluble polymer in said microneedles is between 1:4 to 1:1.

7. The microneedle array according to claim 6, wherein said water-soluble polymer is an alginate.

8. The microneedle array according to any one of the preceding claims, wherein said weight ratio between said nanoparticles and said water-soluble polymer, in said microneedles is between 1:6 to 5:6.

9. The microneedle array according to claim 8, wherein said water-soluble polymer is an alginate.

10. The microneedle array according to any one of the preceding claims, wherein said nanoparticle suspension stabilizer is selected from the group consisting of polyoxyethylated fatty esters, a polysorbate, a sorbitan ester, a soluble polymer, and combinations thereof.

11. The microneedle array according to claim 10, wherein said soluble polymer is selected from the group consisting of sodium alginate, sodium carboxymethyl cellulose, a povidone, a hypromellose, and a hydroxypropyl cellulose.

12. The microneedle array according to any one of the preceding claims, wherein said nanoparticle suspension stabilizer is PEG(15)-hydroxystearate.

13. The microneedle array according to any one of the preceding claims, wherein a weight ratio between said nanoparticles and said nanoparticle suspension stabilizer is between 7:1 and 1:2.

14. The microneedle array according to claim 13, wherein said ratio is between 7:2 and 3:5

15. The microneedle array according to any one of the preceding claims, wherein pharmaceutically active agent has an aqueous solubility at 25°C of less than 1 mg/mL.

16. The microneedle array according to claim 15, wherein said pharmaceutically active agent has a therapeutic dose of below 5-10 mg per day.

17. The microneedle array according to any one of claims 15 or 16, wherein said pharmaceutically active agent is an anti-inflammatory, optionally wherein said anti-inflammatory agent being a steroid or an NSAID, an analgesic, an antibiotic, a fungicide, a retinoid, an anti-cancer agent, an anesthetic, or a hormone.

18. The microneedle array according to any one of claims 15 to 16, wherein said anti-cancer agent is an anti-melanoma agent.

19. The microneedle array according to any one of the preceding claims, wherein said microneedles and said backing layer consist essentially of an alginate.

20. The microneedle array according to any one of the preceding claims, wherein a weight ratio between said nanoparticles and said soluble polymer, in said microneedles is between 1:6 to 5:6, wherein said nanoparticle suspension stabilizer is PEG(15)-hydroxystearate, wherein a weight ratio between said nanoparticles and said nanoparticle suspension stabilizer is between 7:1 and 1:2, and wherein said pharmaceutically active agent is a steroid.

21. The microneedle array according to claim 20, wherein said soluble polymer is an alginate.

22. A method of manufacturing a nanoparticle-bearing microneedle array as described in any one of the preceding claims, said method comprises applying an aqueous solution of a water-soluble polymer onto a microneedle array mold under reduced pressure.

23. The method according to claim 22, wherein said water-soluble polymer is an alginate.

24. The method according to any one of claims 22 or 23, wherein said applying is performed at a temperature between 15°C and 25 °C.

25. The method according to any one of claims 22 to 24, wherein the concentration of said soluble polymer in said aqueous solution is between 1 %wt and 10 %wt.

26. The method according to any one of claims 22 to 25, wherein the concentration of said soluble polymer in said aqueous solution is between 2 %wt and 6 %wt.

27. The method according to any one of claims 22 to 26, wherein said method further comprises applying a microneedle tip precursor solution comprising a suspension of said nanoparticles comprising said nanoparticle suspension stabilizer onto a microneedle array mold.

28. The method according to claim 27, wherein said applying of said microneedle tip precursor solution precedes said applying an aqueous solution of a water-soluble polymer.

29. The method according to any one of claims 27 or 28, wherein said method comprises repeatedly applying said microneedle tip precursor solution until the desired drug loading is achieved.

30. The method according to any one of claims 22 to 29, further comprising drying said mold with said microneedle array.

31. The method according to any one of claims 22 to 30, further comprising packaging said microneedle array.

32. The method according to any one of claims 22 to 31, further comprising sterilizing said microneedle array.

33. The method of manufacturing a microneedle array, said method comprising

providing a mold for microneedle array,

providing a microneedle tip precursor solution, said microneedle tip precursor solution comprising a suspension of nanoparticles, and further comprising a nanoparticle suspension stabilizer,

applying said microneedle tip precursor solution to said mold, optionally under a reduced pressure,

providing an aqueous microneedle precursor solution,

applying said aqueous microneedle precursor solution to said mold under a reduced pressure and optionally at room temperature, and

applying an aqueous backing layer solution,

drying according to the need said mold for microneedle array containing applied said microneedle tip precursor solution, and/or said aqueous microneedle precursor solution, and/or said aqueous backing layer solution,

wherein said aqueous backing layer solution comprises a water-soluble polymer, and optionally a polyol, and

wherein said aqueous microneedle precursor solution comprises a water-soluble polymer.

34. The method according to claim 33, wherein said nanoparticle suspension stabilizer is a surface-active agent.

35. The method according to any one of claims 33 or 34, wherein said water-soluble polymer is an alginate.

36. Use of the microneedle array according to any one of claims 1-21, or obtainable by a process according to claims 22-35, in treating of a disease or disorder in a subject in need thereof, by applying said microneedle array to the skin of said subject, pressing with sufficient force to effect penetration of said microneedles into said skin, and retaining said microneedle array pressed to said skin for a time interval of between about 30 seconds to about 10 minutes.

37. The use according to claim 36, wherein said time interval is between about 30 seconds to about 3 minutes.

38. The use according to any one of claim 36 or 37, further comprising washing off the remainder of said microneedle array after said time interval.

39. The use according to any one of claim 36 to 38, wherein said microneedle array is applied at a frequency selected from the group consisting of three times a day, twice a day, once daily, once every two days, twice a week, and once a week.

40. The use according to any one of claim 36 to 39, wherein said disease or disorder is selected from the group consisting of inflammation-related disorders, infections, acne, cancer, local irritation, and localized pain.

41. The use according to claim 40, wherein said inflammation-related disorders are psoriasis, dermatitis, or eczema.

42. The use according to claim 40, wherein said infections are bacterial, fungal, protozoal, or Herpes Zoster infections.

43. The use according to claim 40, wherein said cancer is an in-situ cutaneous carcinoma.

44. A method of treatment of a disease or disorder in subject in need thereof, by applying a microneedle array according to the use as defined in any one of claims 36 to 43.

Fig. 1

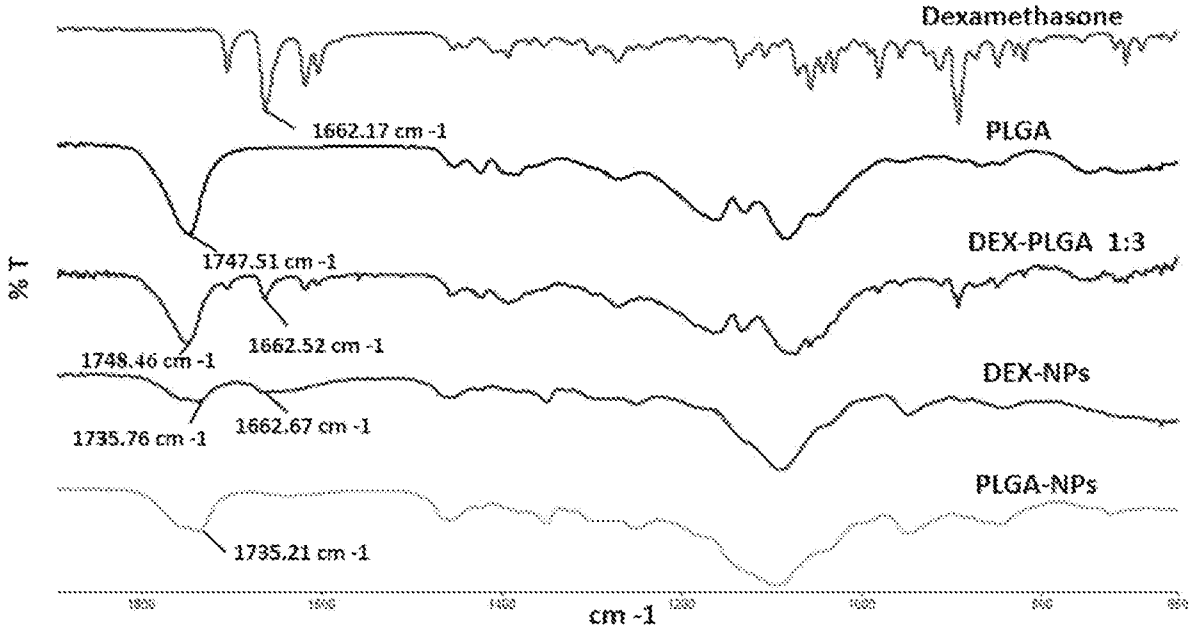

Dexamethasone
1662.17 cm -1
PLGA
1747.51 cm -1
DEX-PLGA 1:3
% T
1748.46 cm -1
1662.52 cm -1
DEX-NPs
1735.76 cm -1
1662.67 cm -1
PLGA-NPs
1735.21 cm -1
cm -1

Fig. 2

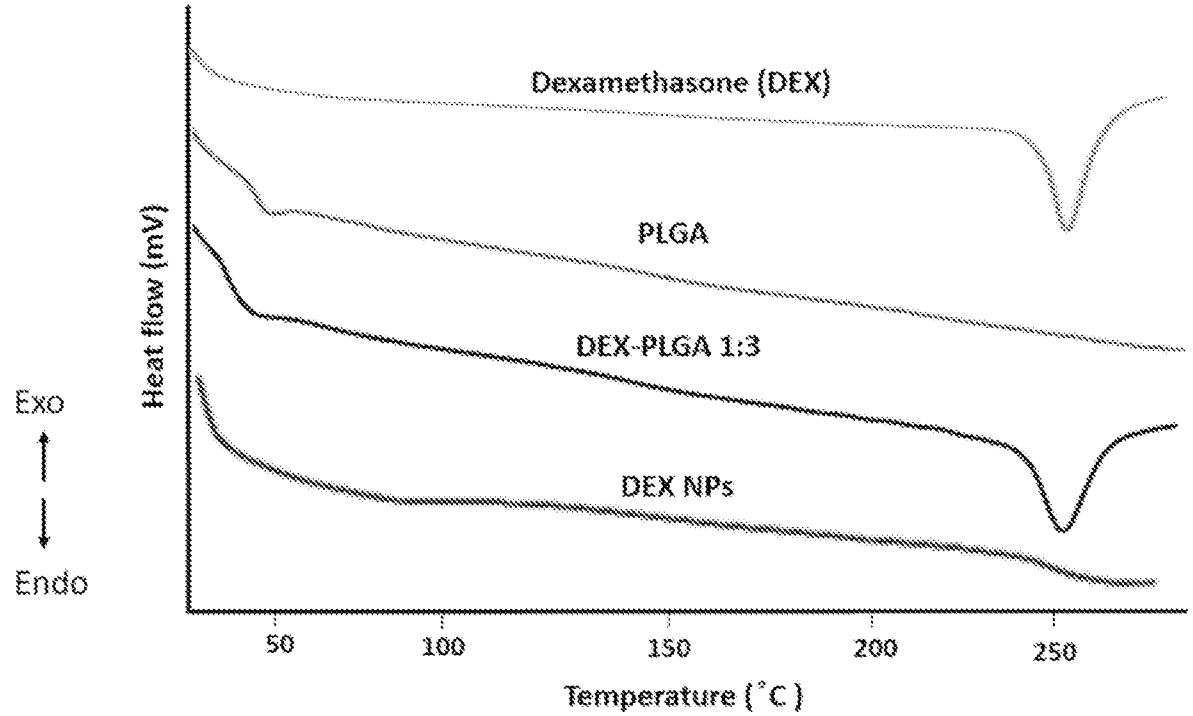

Dexamethasone (DEX)
PLGA
DEX-PLGA 1:3
DEX NPs
Heat flow (mV)
Exo
Endo
50
100
150
200
250
Temperature (˚C)

Fig. 3A

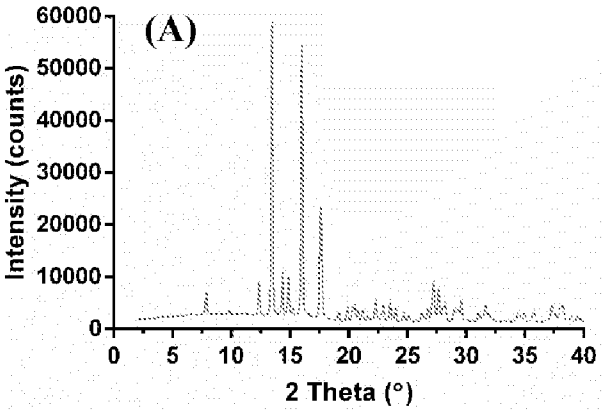


Fig. 3B

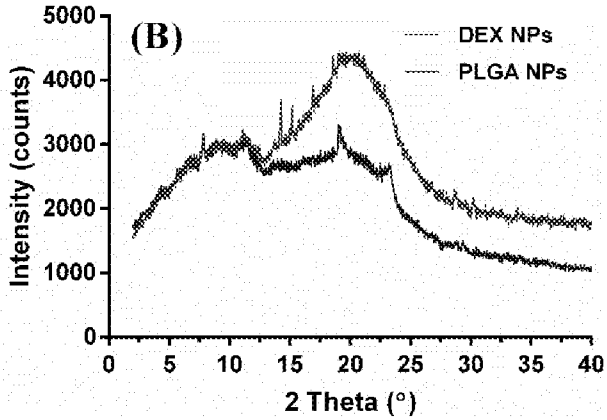


Fig. 4

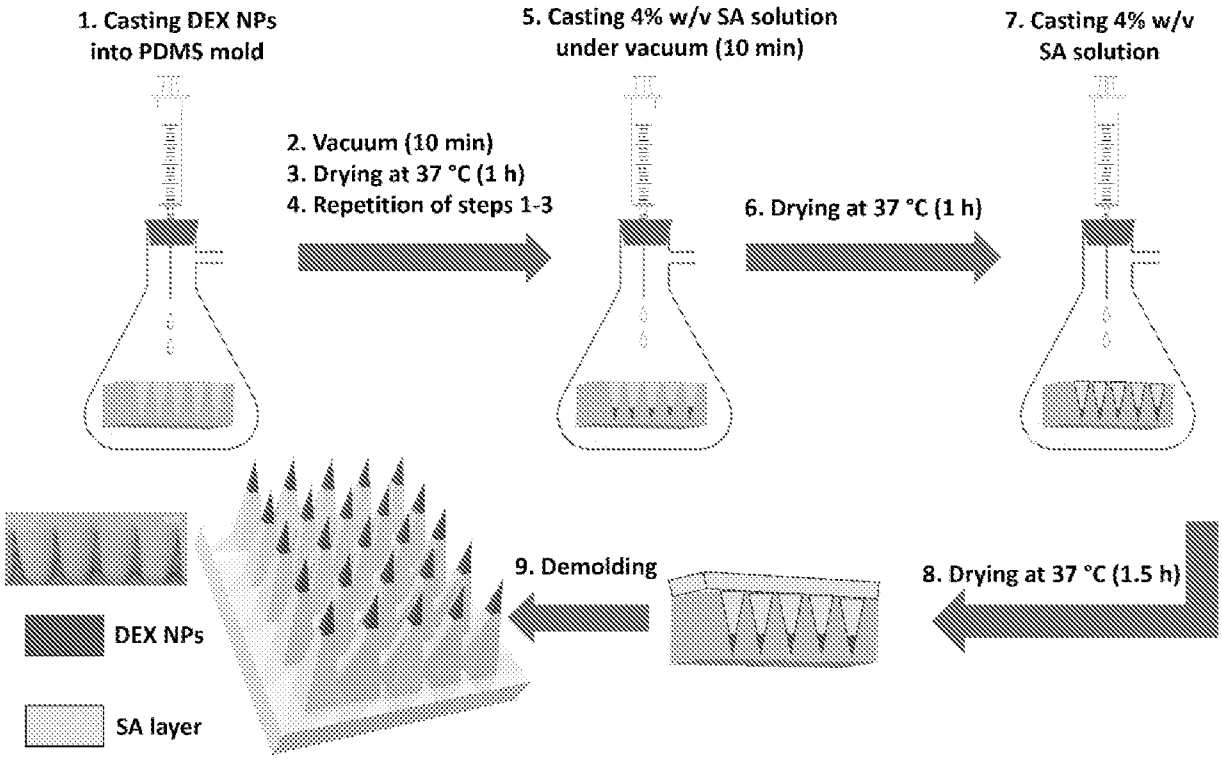


Fig. 5

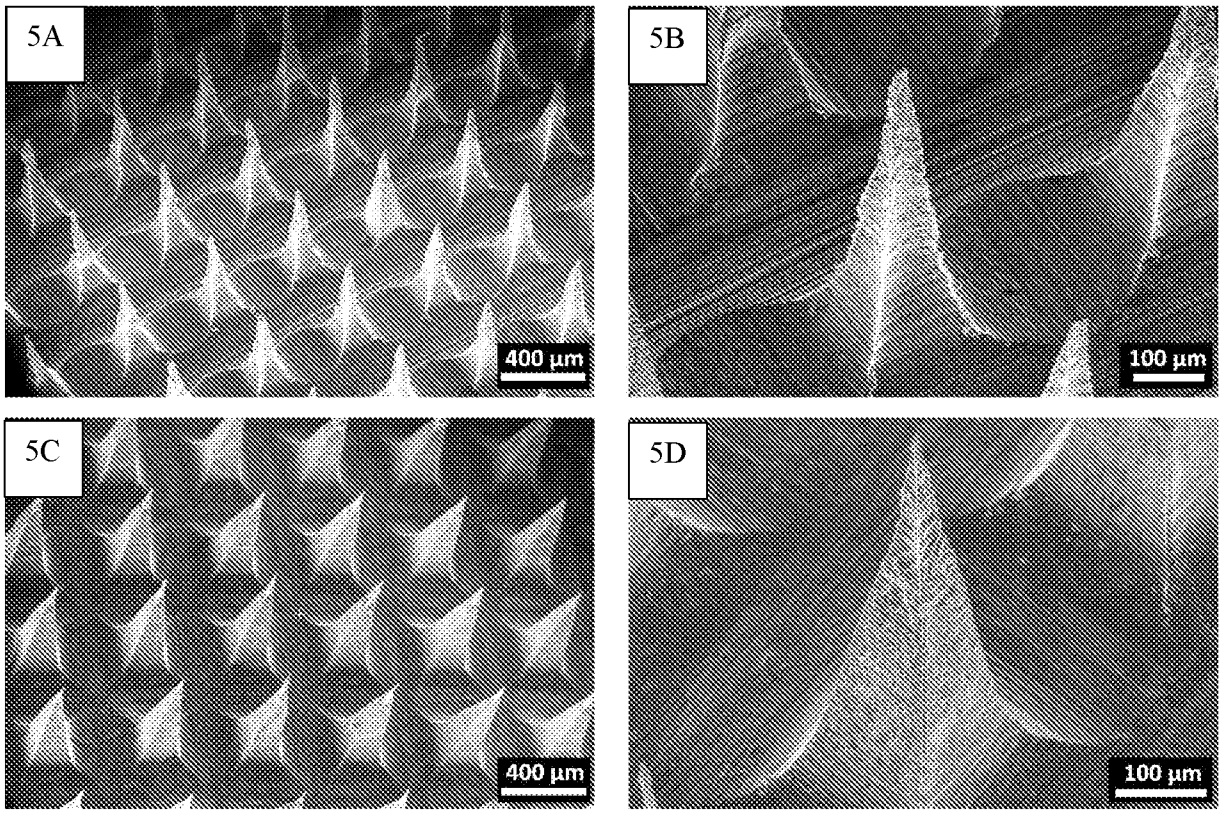


Fig. 6

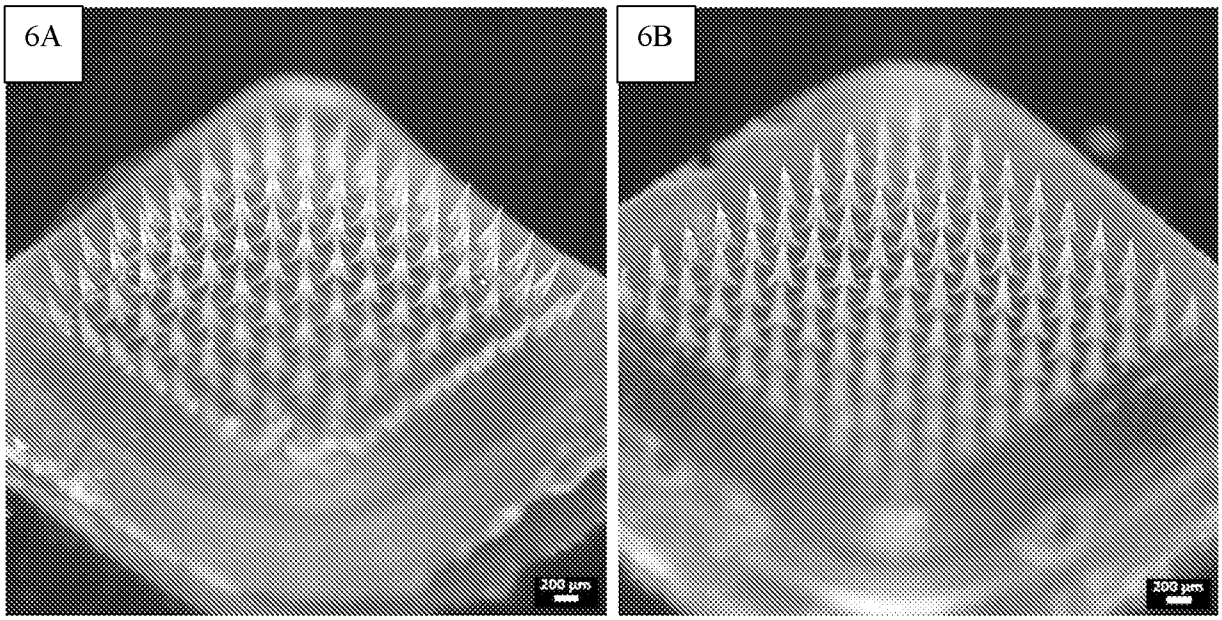


Fig. 7

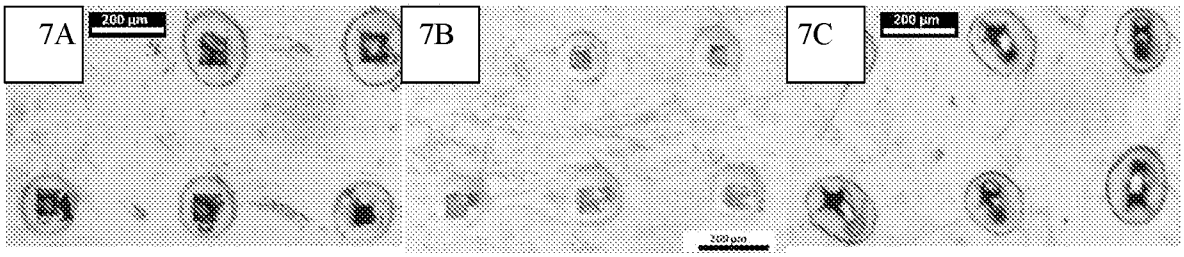


Fig. 8

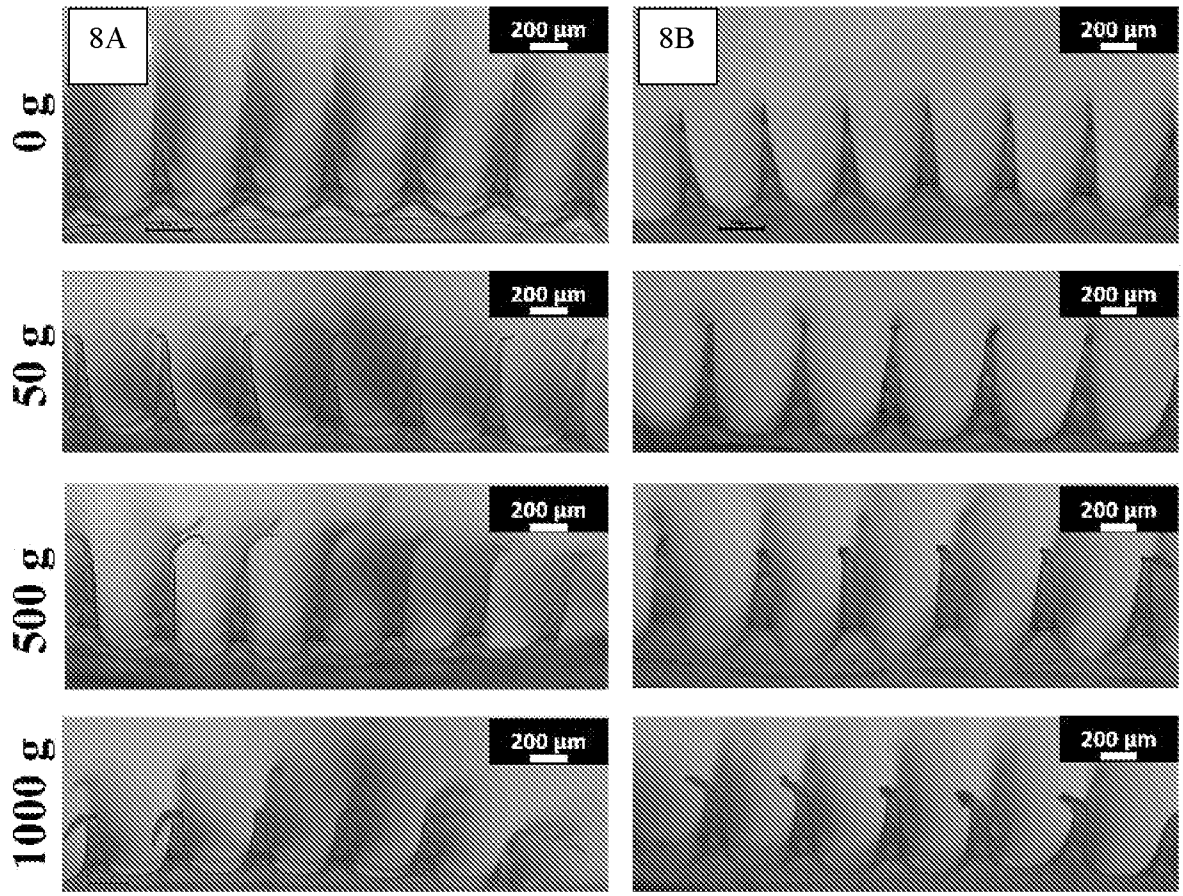


Fig. 9

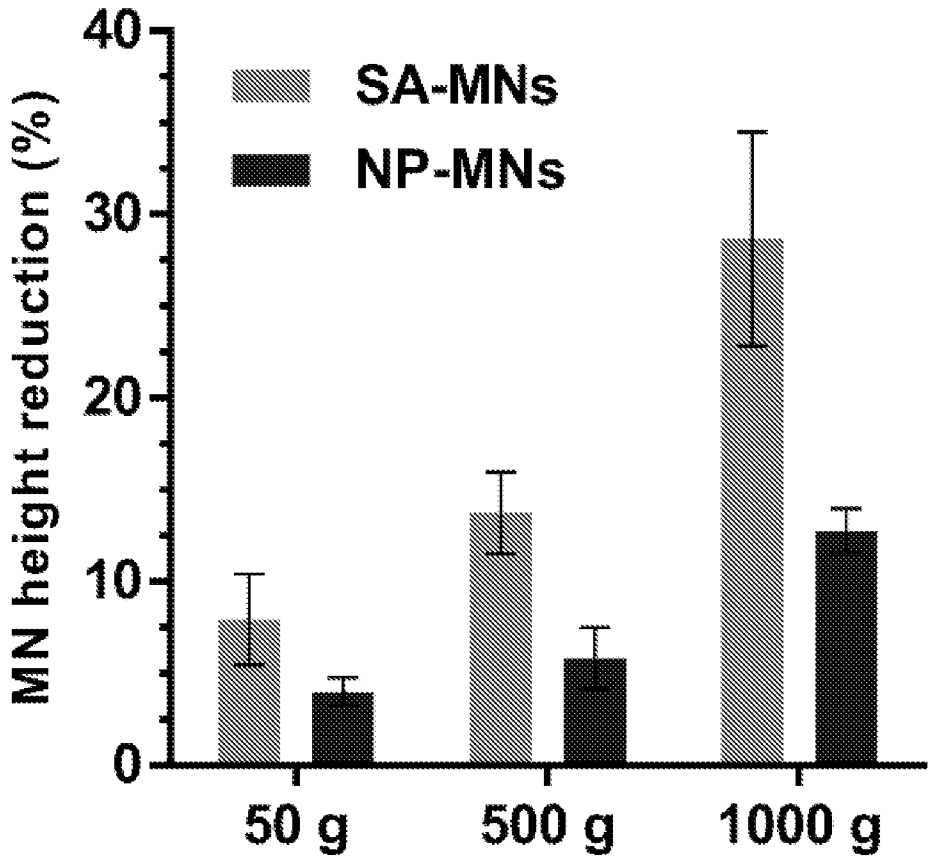

40
30
20
10
0
MN height reduction (%)
SA-MNs
NP-MNs
50 g
500 g
1000 g

Fig. 10

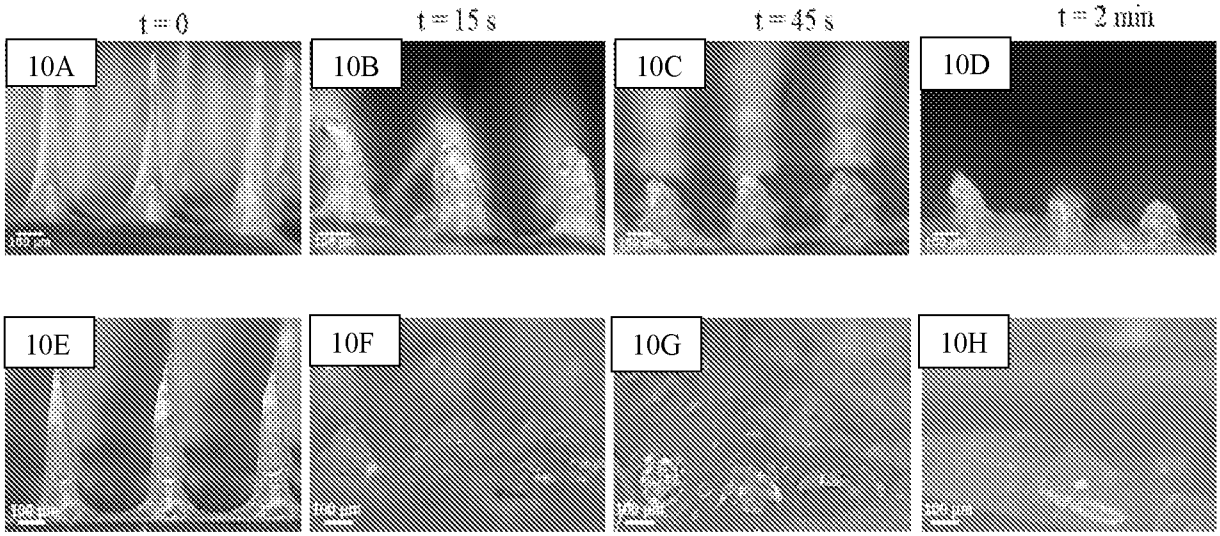
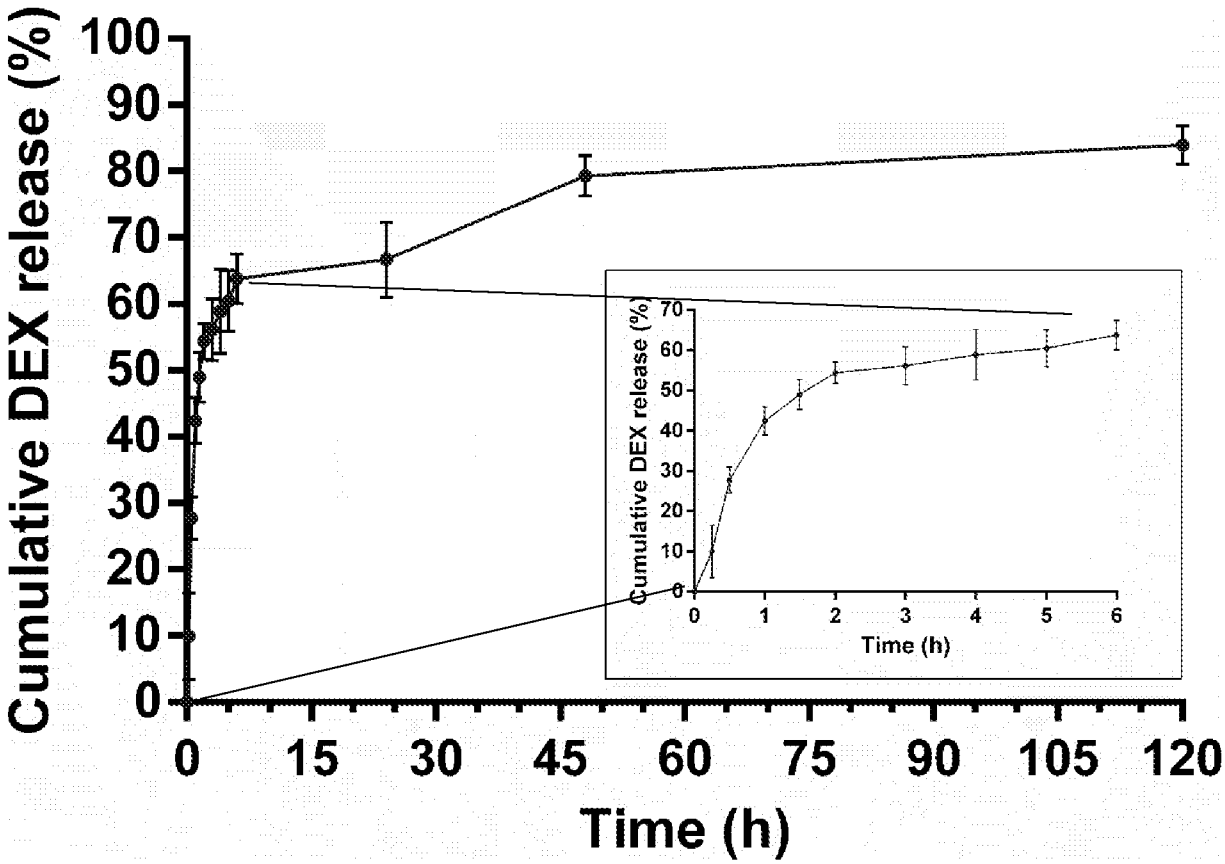

t = 0
t = 15 s
t = 45 s
t = 2 min
10A
10B
10C
10D
10E
10F
10G
10H

Fig. 11


Cumulative DEX release (%)
100
90
80
70
60
50
40
30
20
10
0
0
15
30
45
60
75
90
105
120
Time (h)
Cumulative DEX release (%)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time (h)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2024/050080

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/57(2024.01)i; ***A61K 9/00***(2024.01)i; ***A61K 9/48***(2024.01)i; ***A61M 37/00***(2024.01)i
CPC:A61K 31/57; A61K 9/00; A61K 9/4808; A61M 37/00

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 31/57; A61M 37/00
CPC:A61K 31/57; A61M 37/00

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)

Databases consulted: Esp@cenet, Google Patents, Orbit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020238065 A1 (GEORGIA TECH RES INCT [US]) 30 July 2020 (2020-07-30) whole document	1-44

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

* Special categories of cited documents:
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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
13 May 2024	**19 May 2024**

Name and mailing address of the ISA/IL	Authorized officer
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2024/050080

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		CA 3115572 A1	18 April 2019
		CN 111465387 A	28 July 2020
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		US 2023381477 A1	30 November 2023
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