(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
06 December 2018 (06.12.2018)

(10) **International Publication Number**
WO 2018/222874 A1

(51) **International Patent Classification:**
A61K 39/35 (2006.01) *A61P 37/00* (2006.01)
A61K 39/39 (2006.01) *A61P 37/02* (2006.01)
A61M 5/158 (2006.01)

(21) **International Application Number:**
PCT/US2018/035399

(22) **International Filing Date:**
31 May 2018 (31.05.2018)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/512,884 31 May 2017 (31.05.2017) US
62/576,176 24 October 2017 (24.10.2017) US

(71) **Applicant: TEXAS TECH UNIVERSITY SYSTEM** [US/US]; Office of Research Commercialization, P.O. Box 42007, Lubbock, TX 79409-2007 (US).

(72) **Inventors: GILL, Harvinder, Singh**; 3110 81st Street, Lubbock, TX 79423 (US). **SHAKYA, Akhilesh, K.**; 1602 68th Street, Lubbock, TX 79412 (US).

(74) **Agent: CHALKER, Daniel, J.** et al.; Chalker Flores, LLP, 14951 North Dallas Parkway, Suite 400, Dallas, TX 75254 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** METHODS AND DEVICES FOR THE TREATMENT OF FOOD ALLERGIES

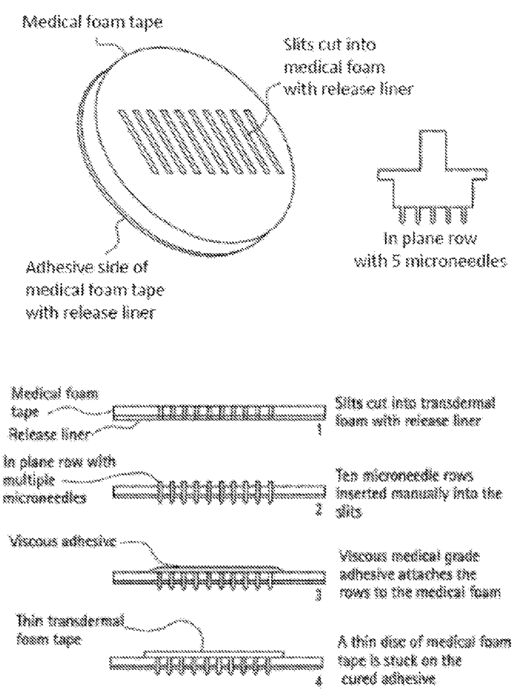

FIG. 1

(57) **Abstract:** Methods and devices are provided for treating a food allergy in a subject in need thereof. The method entails delivering an effective amount of an allergen associated with the food allergy into the subject's cutis skin layer. Delivering the allergen is carried out by inserting one or more allergen-coated solid microneedles into the subject's skin. The one or more solid microneedles each has a base, shaft and tip, and when inserted in the subject, do not extend beyond the cutis. The allergen is allowed to dissociate from the one or more microneedles while inserted in the subject's cutis. Once the allergen disassociates, the one or more microneedles is removed from the subject's skin.

WO 2018/222874 A1

[Continued on next page]

Published:

— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

METHODS AND DEVICES FOR THE TREATMENT OF FOOD ALLERGIES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods, devices and compositions for the treatment of food allergies.

INCORPORATION-BY-REFERENCE OF MATERIALS FILED ON COMPACT DISC

The present application includes a Sequence Listing, which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 30, 2018, is named TECH2103WO_SeqList and is 6 kilobytes in size.

BACKGROUND OF THE INVENTION

Peanut allergy is a life-threatening condition. About 1% of the U.S. population (~3 million people) has peanut allergies, and there is no FDA-approved treatment. Strict avoidance, and a peanut-free diet is the only option available to manage peanut allergies, which imposes severe limitations on the lifestyle of the patient and their families. Patients are also advised to carry an epinephrine injection to mitigate anaphylaxis, which can occur due to accidental peanut exposure. Adherence to a peanut-free diet imposes severe limitations on the lifestyle of the patient and their families, and reduces their quality of living. Importantly, restricted food diets in children can lead to nutritional deficiencies, and as such, methods for the treatment of peanut allergies are of great interest.

Peanut allergy is associated with an abnormal immune response to peanut proteins and it is mediated by peanut specific IgE antibodies. Allergic reaction to peanut in food can produce diverse symptoms including skin rashes, gastrointestinal reactions such as pain and vomiting, and even a severe life-threatening anaphylactic reaction (Sampson et al. (2014 J Allergy Clin Immunol. 134(5), pp. 1016-1025 e43). Oral immune-therapy (OIT) for peanut allergies is relatively new and experimental, and it aims to modulate this aberrant IgE response. The first open-label trial for peanut OIT was published in 2009 (Hofmann et al. (2009). J Allergy Clin Immunol. 124(2), pp. 286-291, 291.e1-6; Jones et al. (2009). J Allergy Clin Immunol. 124(2), pp. 292-300.e97). The published protocols for peanut OIT (and food OIT in general) typically involve oral delivery of peanut flour/protein or extract in: (i) a rapid/rush dose escalation phase lasting one day (peanut protein dose increased from about 100 μg to 50 mg), (ii) a gradual dose buildup phase lasting many months (peanut protein dose increased to hundreds and thousands of milligram), and (iii) maintenance phase lasting months to years (peanut protein dose maintained at several thousand milligram). (Wood (2016). J Allergy Clin Immunol. 137(4), pp. 973-982;

Deol and Bird (2014). Hum Vaccin. Immunother. 10(10), pp. 3017-21; Sampson (2013). J Allergy Clin Immunol.: In Practice 1(1), pp. 15-21.

The end-point of most peanut OITs has been to reorient the abnormal immune response and to desensitize the patient to peanut (Deol and Bird (2014). Hum Vaccin. Immunother. 10(10), pp. 3017-21; Sampson (2013). J Allergy Clin Immunol.: In Practice 1(1), pp. 15-21; Commins et al. (2016). Current Allergy and Asthma Reports 16(5), p. 35; Wood (2017). J Investig Allergol Clin Immunol, p. 0). Desensitization means increasing the patient's threshold to peanut reactivity (i.e., the amount of peanut that can be safely tolerated by the patient). To maintain desensitization, the patient is required to continue ingesting peanuts at a maintenance dose at regular intervals. However, sustained unresponsiveness, i.e., the ability of the patient to be non-responsive to peanut ingestion after completion of OIT without the need to be on a maintenance dose, is the desirable treatment endpoint. In 2011 the first data of sustained unresponsiveness was published, and it was shown that 50% of the subjects were unresponsive to peanut 4 weeks after OIT (Vickery et al. (2014). J Allergy Clin Immunol. 133(2), pp. 468-475.e6). In another study, it was found that 7/20 subjects were unresponsive 3 months after stopping OIT, and only 3 out of these 7 were unresponsive another 3 months later (i.e., 3/20 were unresponsive 6 months after stopping OIT) (Syed et al. (2014). J Allergy Clin Immunol. 133(2): pp. 500-510.e11).

Current peanut OIT protocols require daily ingestion of peanut, and the dose is continuously increased to thousands of milligrams of peanut protein. Adverse events such as abdominal pain, vomiting, upper respiratory reactions (sneezing and congestion), and skin rashes/hives are very common, especially during the initial rush dose escalation from micrograms to tens of milligram in a single day (Hofmann et al. (2009). J Allergy Clin Immunol. 124(2), pp. 286-91, 291.e1-6), and the dose buildup phase when the peanut dose is raised from tens to thousands of milligrams (Vickery et al. (2017). J Allergy Clin Immunol. 139(1), pp. 173-181 e8). In one study, a direct correlation was observed between asthma and peanut OIT, wherein it was found that asthmatic patients experienced respiratory adverse events (Hofmann et al. (2009). J Allergy Clin Immunol 124(2), pp. 286-91, 291.e1-6.).

Allergy shots, which are subcutaneously delivered, have a proven track record to provide long term treatment for environmental allergens that cause respiratory symptoms such as allergic rhinitis, allergic conjunctivitis, allergic asthma, or insect allergy (including bee venom) (Cox et al. (2011). The Journal of allergy and clinical immunology 127(1 Suppl), pp. S1-55). Building on this success, peanut subcutaneous immunotherapy was attempted in 1990s (Oppenheimer et al. (1992). J Allergy Clin Immunol 90(2), pp. 256-62; Nelson et al. (1997). J Allergy Clin Immunol. 99(6 Pt 1), pp. 744-51). Patients underwent rush immunotherapy, whereby they received four injections per day of increasing doses of peanut extract for five consecutive days, and then received eight injections (1/week) of maintenance dose. This was a small clinical study, and in three of the six patients, 67-100% reduction in symptoms was seen when they were orally challenged with peanut (Nelson et al. (1997). J Allergy Clin Immunol. 99(6 Pt 1), pp. 744-51). However, the systemic reactions were high. During rush immunotherapy, 23% of the injections given to the patients led to a systemic reaction needing epinephrine injection, while during maintenance phase this reaction rate was 33%. Based in part on these high reaction rates, the studies were halted. Subcutaneous injections have not been attempted again.

To circumvent the high reaction rate seen when peanut allergen is injected subcutaneously, a skin patch containing peanut allergen (100, 250 or 500 μg) was developed (34-38). This patch has now also completed a clinical trial (Jones et al. (2017). J Allergy Clin Immunol. 139(4), pp. 1242-1252), for which the patch was applied to the skin continuously for 24 hr. After 24 hr., the patch was removed and a new patch was immediately placed on a different skin site. Thus, the patient received a skin patch continuously, every day for one year. Low reaction rates were seen, and mostly these were observed topically on the skin. None of the patients could successfully complete the oral food challenge of 1044 mg (Jones et al. (2016). Consortium of Food Allergy, Epicutaneous immunotherapy for the treatment of peanut allergy in children and young adults. J Allergy Clin Immunol). In contrast, peanut oral immunotherapy, although associated with a higher number of adverse events, has allowed patients to successfully complete oral challenges with about 4000 mg peanut protein (Hofmann et al. (2009). J. Allergy Clin. Immunol. 124(2), pp. 286-91, 291.e1-6; Jones et al. (2009). J. Allergy Clin. Immunol. 124(2), pp. 292-300.e97; Blumchen et al. (2010). J. Allergy Clin. Immunol. 126(1), pp. 83-91).

Based on the results of these clinical studies, new treatment options for peanut and other food allergies would be beneficial and an advance in the field. The present invention addresses this and other needs.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a method for treating a food allergy in a subject in need thereof. In one embodiment, the method for treating the food allergy comprises delivering an effective amount of an allergen associated with the food allergy into the subject's cutis. The delivering comprises inserting one or more solid microneedles each having a base, shaft and tip into the subject's cutis for an administration period. At least one of the one or more solid microneedles is coated with allergen associated with the food allergy and the one or more microneedles do not extend beyond the cutis once inserted. The allergen is allowed to dissociate from the microneedles while inserted in the subject's cutis. Once the allergen dissociates, the one or more microneedles are removed from the subject's skin. In one embodiment, at least one of the one or more solid microneedles is further coated with an adjuvant. The adjuvant coating can be on the same microneedle(s), or a different microneedle(s), than the allergen coating.

The subject in one embodiment is a human. In a further embodiment, the human subject is from about 2 to about 12 years old, e.g., from about 4 to about 12 years old or from about 4 to about 10 years old. In one embodiment, the one or more solid microneedles comprise a microneedle array of two or more solid microneedles, for example, from about 10 to about 100 solid microneedles.

In one embodiment of the method, the one or more solid microneedles extends from an adhesive substrate. In one embodiment, the one or more solid microneedles are stainless steel. In a further embodiment, the average length of the one or more solid microneedles is from about 100 μm to about 1000 μm, as measured from the base of the tip. In even a further embodiment, the average length of the one or more solid microneedles is from about 200 μm to about 900 μm.

The one or more solid microneedles used in the methods and devices provided herein, in one embodiment, comprises from about 10 to about 150 microneedles, for example, from about 10 to about 100 microneedles, from about 10 to about 80 microneedles, or from about 20 to about 70 microneedles.

The methods provided herein are not limited to the type of food allergy that is treatable. For example, in one embodiment, the food allergy is a groundnut, peanut, milk, egg, tree nut, seed, fish, shellfish, crustacean, cereal, legume allergy, or a combination thereof. In one embodiment, the food allergy is a peanut allergy. In embodiments where a peanut allergy is treated, the peanut allergen can comprise Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara h6, Ara h7, Ara h8, Ara h9, Ara h10, Ara h11, Ara h12, Ara h13, a peptide fragment thereof, or a combination thereof. In a further embodiment, the peanut allergen comprises Ara h1, Ara h2, Ara h6, a peptide fragment thereof, or a combination thereof. Additionally, where a peanut allergen is coated on the microneedle array, it can be provided as peanut protein extract, protein flour, or a combination thereof. The peanut allergen can be delivered alone, or in combination with an adjuvant. The peanut allergen coating can be on the same microneedle(s), or a different microneedle(s), then the adjuvant coating.

In one embodiment of the method, once inserted into the cutis, the tip(s) of the one or more microneedles do not extend beyond the epidermis skin layer. In another embodiment, once inserted into the cutis, the tip(s) of the one or more microneedles do not extend beyond the dermis skin layer.

As provided above, in one embodiment, a method for treating a food allergy is provided comprising in part, inserting one or more solid microneedles into the subject's cutis, wherein at least one microneedle of the one or more solid microneedles is coated with allergen associated with the food allergy, and the at least one coated microneedle of the array does not extend beyond the cutis once inserted. In a further embodiment, each microneedle of the one or more microneedles does not extend beyond the cutis once inserted. The allergen is allowed to dissociate from the at least one microneedle while inserted in the subject's cutis. In a further embodiment, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis. Disassociation of the allergen in one embodiment, is carried out for about 1 minute to about 10 minutes, for example from about 1 minute to about 6 minutes or from about 1 minute to about 5 minutes.

In one embodiment, a subject is treated for a food allergy via delivering an effective amount of an allergen associated with the food allergy into the subject's cutis for an administration period. The delivering is carried out via inserting one or more solid microneedles into the subject's cutis, wherein the one or more solid microneedles each comprises a base, shaft and tip. At least one microneedle of the one or more solid microneedles is coated with allergen associated with the food allergy and the at least one coated microneedle of the array does not extend beyond the cutis once inserted. Optionally, the one or more solid microneedles are coated with an adjuvant. In a further embodiment, each microneedle of the one or more solid microneedles does not extend beyond the cutis once inserted. The allergen is allowed to dissociate from the at least one microneedle while inserted in the subject's cutis. Once the allergen dissociates, the one or more solid microneedles is removed from the subject's skin. In one embodiment, the method is carried out once daily, twice daily, three times daily, every other day, twice a week, or once weekly during the administration period. In a further embodiment, the delivering an effective amount of an allergen results in delivering substantially the same amount of allergen each time the method is carried out during the administration period. In another embodiment, the delivering an effective amount of an allergen results in delivering an escalating dosage of the allergen at least once, at least twice, or at least three times during the administration period.

In one embodiment of the method of treating food allergy, the treating results in desensitization to the allergen, e.g., peanut allergen. For example, in one embodiment, desensitization is by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, about 70%, about

75%, at least about 80%, at least about 85%, or at least about 90% as compared to the subject prior to commencing the treatment, a subject receiving a placebo or a subject not receiving the treatment.

In one embodiment of the method of treating food allergy, the treating results in a decrease in the number of allergen specific IgE antibodies in the subject, as compared to the number secreted prior to the treating. In another embodiment, the treating results in an increase in the number of allergen specific IgG antibodies in the subject, as compared to the number secreted prior to the treating. In a further embodiment, the subject is human and the allergen specific IgG antibodies are allergen specific IgG4 antibodies. In another embodiment, the treating results in a decreased number of mast cells in the subject, as compared to the number secreted prior to the treating. In a further embodiment, the decreased number of mast cells is at the site of allergen exposure (e.g., the gastrointestinal (GI) tract for food allergens) in the subject, as compared to the number secreted at the site prior to the method being carried out. In yet another embodiment, the treating results in a decreased number of basophils in the subject, as compared to the number secreted prior to the treating. In a further embodiment, the decreased number of basophils is at the site of allergen exposure (e.g., the GI tract for food allergens) in the subject, as compared to the number secreted at the site prior to the method being carried out. In even another embodiment, the treating results in an increased cytokine production in the subject, as compared to the number secreted prior to the treating. In a further embodiment, the cytokine is IL-10 or TGF-β. In yet another embodiment, the treating results in an increased number of T-regulatory cells in the subject, as compared to the number of T-regulatory cells prior to the treating.

In one embodiment of the method of treating food allergy provided herein, the treating results in an increase in the eliciting dose of the allergen, as compared to the eliciting dose prior to initiation of treatment. For example, in one embodiment, the increase in the eliciting dose of the allergen is an increase by 10%, by 20%, by 30%, by 40%, by 50%, by 60%, by 70%, by 80%, by 90%, by 100%, by 500%, by 1000%, by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 80%, by at least about 90%, by at least about 100%, by at least about 500% or by at least about 1000%.

In one embodiment of the method of treating food allergy provided herein, the treating results in a sustained unresponsiveness to the allergen. In a further embodiment, the sustained unresponsiveness lasts for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months or at least about 12 months after therapy has ended.

In yet another aspect of the invention, a device is provided for the treatment of a food allergy. The device comprises a microneedle array comprising a plurality of solid microneedles extending from a common substrate. Each microneedle of the plurality has a base, shaft and tip, and at least one microneedle of the array is coated with a food allergen. In some embodiments, at least one microneedle of the array is coated with an adjuvant. The adjuvant coating can be on the same microneedle as the allergen coating (e.g., a combination coating of allergen and adjuvant) or on a different microneedle(s). In a further embodiment, the substrate is adhesive on at least one side. The substrate in one embodiment, is rigid. However, in another embodiment, the substrate is flexible.

In one embodiment of the device, the average length of the microneedles in the array is from about 100 µm to about 1000 µm, as measured from the base of the tip of the microneedles. In a further embodiment, the average length of the microneedles in the array is from about 100 µm to about 900 µm, or from about 100 µm to about 800 µm, or from about 100 µm to about 700 µm, or from about 100 µm to about 600 µm, or from about 100 µm to about 500 µm.

The device in one embodiment, comprises a microneedle array comprising from about 20 to about 150 microneedles, for example, from about 20 to about 150 microneedles, from about 30 to about 100 microneedles, or from about 40 to about 100 microneedles.

The device in one embodiment, comprises solid microneedles coated with a groundnut, peanut, milk, egg, tree nut, seed, fish, shellfish, crustacean, cereal, legume allergen, or a combination thereof.

The device in one embodiment, comprises solid microneedles coated with a peanut allergen. In a further embodiment, the peanut allergen can comprise Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara h6, Ara h7, Ara h8, Ara h9, Ara h10, Ara h11, Ara h12, Ara h13, a peptide fragment thereof, or a combination thereof. In a further embodiment, the peanut allergen comprises Ara h1, Ara h2, Ara h6, a peptide fragment thereof, or a combination thereof. Additionally, where a peanut allergen is coated on the microneedle array, it can be provided as peanut protein extract or peanut flour or a combination thereof.

DESCRIPTION OF THE FIGURES

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention long with the accompanying figures and in which:

Figure 1 illustrates one embodiment of a process for assembling a microneedle patch including coated in-plane microneedle rows as described herein.

Figure 2 is a cross sectional view of microneedles in a microneedle array which are dipped into an allergen coating liquid using a physical mask to control deposition of coating, with the mask having multiple dip holes built into the mask.

Figure 3 is a cross sectional view of microneedles in a microneedle array which are dipped into an allergen coating liquid using a physical mask to control deposition of coating, with a single reservoir in fluid communication with open dip holes.

Figure 4 is a scanning electron micrograph of one embodiment of a microneedle array.

Figure 5 is a fluorescent micrograph of a microneedle array prior to insertion into the skin. The insets are of a single microneedle prior to insertion (left) and after insertion (right).

Figure 6 is a bar graph showing the amount of ovalbumin (Ova) delivered into mouse skin via microneedles, as well as the amount of Ova remaining on microneedles and on the skin surface. Ova was quantified via fluorescein–conjugated Ova (λex437 nm / λem515 nm) through spectrofluorometer.

Figure 7 shows the schedule and doses for sensitization and oral peanut challenge in Balb/c mice with peanut extract (PE).

Figure 8 (left) is a graph showing the body temperature (°C) as a function of time for naïve and sensitized mouse groups, after oral challenge with PE, as measured with a rectal probe. Figure 8 (right) is a graph of the anaphylactic score as a function of time in naïve and sensitized mouse groups after oral challenge with PE. An anaphylactic scoring system based on mouse activity was used to evaluate anaphylactic severity as described previously by Li and McCaskill (Li et al. (1999). J Allergy Clin Immunol 103(2 Pt 1), pp. 206-14; McCaskill et al. (1984). Immunology 51(4), pp. 669-77, the disclosure of each of which is hereby incorporated by reference in its entirety for all purposes). 0: No symptoms; 1: Hypersensitivity to touch, irritation/aggression; 2: Puffiness around the eyes, pilar erection, reduced activity with increase respiratory rate; 3: Cyanosis around the mouth and tail, labored breathing, lying flat; 4: Loss of consciousness, no activity upon prodding, tremor or convulsions; 5: Death. All data illustrated as mean±SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

Figure 9 (left) is a graph of PE-specific IgE antibodies in naïve and sensitized mouse groups after oral challenge with PE. Figure 9 (right) is a graph showing histamine release (concentration in plasma pg/mL) in naïve and sensitized mouse groups after oral challenge with PE. All data illustrated as mean ± SEM. $*p<0.05$, $**p<0.01$.

Figure 10 is an immunotherapy schedule employed with peanut coated microneedles. Schedule shows the effect of PE dose on immune response after vaccination with PE coated MNs. Microneedles coated with 1, 5 or 25 μg PE were used at one dose per week. Six weeks later (at day 56), mice were bled to check for anti-PE responses.

Figure 11 (top left) is a graph of anti-PE IgG antibodies, as measured by ELISA at different serum dilutions. Figure 11 (top right) is a graph of anti-PE IgG1 antibodies, as measured by ELISA at different serum dilutions. Figure 11 (bottom left) is a graph of anti-PE IgG2a antibodies, as measured by ELISA at different serum dilutions. Figure 11 (bottom right) is a graph of anti-PE IgE antibodies, as measured by ELISA at different serum dilutions.

Figure 12 is a bar graph showing PE specific antibody response for the three microneedle groups. At day 56, mice were euthanized and bone marrow was aseptically collected. Bone marrow cells were cultured in RPMI medium containing penicillin-streptomycin and 10% FBS. After 72 hr., supernatant was collected to analyze PE specific antibodies using ELISA. Data illustrated as mean ± SEM; ns: not significant.

Figure 13 are graphs showing cytokine response of mice after splenocyte restimulation (IL-2, top left; IFN-γ, top right; IL-4, bottom left; IL-5, bottom right). At day 56, mice were euthanized and spleen was aseptically collected. Splenocytes were restimulated with PE (200 μg/ml) in an in vitro culture. After 12 hr. of restimulation, supernatant was collected to analyze IL-2, while other cytokines were analyzed after 72 hr. of restimulation. All data are illustrated as mean ± SEM. $*p<0.05$, and ns: not significant.

Figure 14 is a microneedle immunotherapy schedule. Mice were sensitized orally to peanut and treated with 5 μg PE coated on microneedles (MNs)-CIT (cutaneous immunotherapy) every week. Three weeks post-immunotherapy, mice were challenged orally with high dose of PE (20 mg: 10 mg+10mg at 30 min interval).

Figure 15 are graphs of anti PE antibodies in plasma (at different dilutions) after oral challenge. IgG – left graph. IgG1 – right graph. Plasma was collected 5 minutes post challenge.

Figure 16 are graphs of anti PE antibodies in plasma (at different dilutions) after oral challenge. IgG2a – left graph. IgE – right graph. Plasma was collected 5 minutes post challenge.

Figure 17 are graphs showing various anaphylactic indicators for mouse treatment groups (after oral challenge with peanut extract (PE)). Figure 17, top left: Post PE oral challenge, mice were monitored and their symptoms were scored and are shown as 'anaphylactic score'; 0: no symptoms; 1: hypersensitivity to touch, irritation/aggression; 2: puffiness around the eyes, pilar erection, reduced activity with increase respiratory rate; 3: cyanosis around the mouth and tail, labored breathing, lying flat; 4: loss of consciousness, no activity upon prodding, tremor or convulsions; 5: death. Figure 17, top right: Anaphylaxis mediator histamine in plasma collected 5 minutes after challenge for the four mouse groups. Figure 17, bottom left: Anaphylaxis mediator mast cell protease 1 (MCPT-1) in plasma collected 5 minutes after challenge for the four mouse groups. All data are illustrated as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Figure 18 are graphs for anti-ovalbumin (Ova) response in mice treated with Ova coated MNs with or without stimulator of interferon genes (STING) ligand adjuvants. Mice were treated at day (d) 0 and d28 with Ova (25 µg) with or without STING adjuvants cGMP (25 µg) or cAMP (25 µg) using coated MNs. Serum was collected on d28 and d60 to determine anti-Ova antibody response using the ELISA method. (Figure 18, Left) anti-Ova IgG and (Figure 18, Right) anti-Ova gG2a response in mice serum. Individual mouse serum was diluted to 1:100 and used in analysis. All data illustrated as mean ± SEM. ***p<0.0005, **** p<0.0001 and ns; not significant. ELISA: Enzyme-linked immunosorbent assay.

Figure 19 are graphs for anti-Ova response in mice treated with Ova coated MNs with or without CpG adjuvant. Mice were treated on day (d) 0, d7, d14 with Ova (25 µg) ± CpG (25 µg) and bled at d21 and d35 to determine anti-Ova antibody response using the ELISA method. (Figure 19, Left) anti-Ova IgG, and (Figure 19, Right) anti-Ova IgG2a response in mice serum. Individual mouse serum was diluted at 1:20 dilution and used in analysis. All data illustrated as mean ± SEM. ***p<0.0005, **** p<0.0001 and ns; not significant. ELISA: Enzyme-linked immunosorbent assay.

Figure 20 are graphs for anti-peanut extract (PE) response in mice treated with PE coated MNs with or without CpG adjuvant. Mice were treated on day (d) 0, d7, and d14 with PE (25 µg) ± CpG adjuvant (25 µg) coated on MNs. Serum was collected on d56 to determine anti-Ova antibody response with ELISA method. (Figure 20, Left) anti-PE IgG, (Figure 20, Middle) anti-PE IgG2a and (Figure 20, Right) anti-PE IgE response in mouse serum. Individual mouse serum was used in analysis. All data illustrated as mean ± SEM. *p<0.05, **p<0.005 and ns: not significant.

Figure 21 shows a peanut allergy immunotherapeutic schedule for examining effect of adjuvant. Immunotherapy schedule; mice were sensitized orally every week up to six weeks with 1 mg peanut extract (PE) +10 µg cholera toxin (CT). Three weeks later, sensitized mice were treated with MNs coated with PE (5 µg) ± CpG (5 µg). Four weeks post-immunotherapy, mice were challenged orally with a high dose of PE (20 mg).

Figure 22 shows therapeutic efficacy of CpG adjuvant in peanut allergy treatment. Five minutes after oral peanut challenge, mice were assessed for allergic reaction. Figure 22, Left: 'Anaphylactic score'; 0: no symptoms; 1: hypersensitivity to touch, irritation/aggression; 2: puffiness around the eyes, pilar erection, reduced activity with increase respiratory rate; 3: cyanosis around the mouth and tail, labored breathing, lying flat; 4: loss of consciousness, no activity upon prodding, tremor or convulsions; 5: death. Figure 22, Middle: Histamine level in serum. Figure 22, Right: MCPT-1 level in serum. All data illustrated as mean ± SEM. ****$p<0.0001$ and ns: not significant.

Figure 23 (left) is a stereomicroscope brightfield image of a microneedle array whose alternate diagonal rows are coated with two different dyes (green fluorescent fluorescein isothiocyanate and red fluorescent sulforhodamine) to simulate an allergen and an adjuvant coating on separate rows of microneedles: Figure 23 (right) is a stereomicroscope fluorescent and brightfield mixed-light image of the same array. Scale bar for both images is 500 µm.

DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not limit the invention, except as outlined in the claims.

Peanut allergy is a life-threatening condition. About 1% of the US population (~3 million people) has peanut allergies, and there is no FDA-approved treatment. As such, strict avoidance, i.e., a peanut-free diet, is the only option available to manage peanut allergies. Moreover, although oral immunotherapy, classical allergy shots and transdermal patches have all been attempted for the treatment of peanut allergy, each has drawbacks such as the lack of sustained unresponsiveness, and the presence of adverse events.

Specifically, a major limitation of oral immunotherapy is that the peanut oral dose is escalated to thousands of milligrams, which can cause various side effects. Additionally, peanut oral immunotherapy has only been shown in some instances to offer short term desensitization.

Moreover, allergy shots, when administered for peanut allergy, resulted in systemic reactions after rush immunotherapy. Transdermal patches, currently being developed, are also met with challenges. Appreciating that skin is impermeable to large molecules such as proteins (see, e.g., Karande and Mitragotri (2010). Annu. Rev. Chem. Biomol. Eng. 1, pp. 175-201; Prausnitz et al. (2004). Nat. Rev. Drug Discov. 3(2), pp. 115-124, the disclosure of each of which is incorporated by reference in its entirety for all purposes), the delivery of peanut proteins from such a skin patch is in all likelihood very low, and as such, it is not surprising that the immune modulating effect is not that strong. Indeed, none of the patients is the transdermal patch study could successfully complete the oral food challenge of 1044 mg (Jones et al. (2017). J Allergy Clin Immunol. 139(4), pp. 1242-1252, the disclosure of which is incorporated by reference in its entirety for all purposes).

The present invention addresses the need in the art for a new treatment method for food allergy, and specifically, peanut allergy by providing microneedle arrays for the application to a patient's skin. Microneedles (MNs) are sharp microstructures, and due to their small size, MNs can be non-invasive and painless. Due to their micrometer dimensions, coated MNs also have the potential to allow targeting of the allergens to dendritic cells, e.g., Langerhans cells (LCs) that reside in the topmost hundred micrometers of the skin epidermis.

Skin dendritic cells (DCs) play a central role in the initiation of allergic skin responses. Following encounter with an allergen, DCs become activated and undergo maturation and differentiate into immunostimulatory DCs and are able to present antigens effectively to T-cells. (Toebak et al. (2009). Contact Dermatitis 60(1), pp. 2-20, incorporated by reference herein in its entirety for all purposes). Without wishing to be bound by theory, it is thought that because MNs can target dendritic cells in the cutis, e.g., LCs in the epidermis, they can help in dose reduction of the respective food antigen, e.g., peanut antigen.

In one aspect, the present invention is directed to a method for treating a food allergy in a subject in need thereof. In one embodiment, the method for treating the food allergy comprises delivering an effective amount of an allergen associated with the food allergy into the subject's cutis (i.e., the outer layer of skin comprising the epidermis and dermis layers) for an administration period. The delivering step comprises inserting one or more microneedles (e.g., present as a microneedle array) into the subject's cutis, wherein the one or more microneedles each has a base, shaft and tip. At least one microneedle of the one or more microneedles is coated with allergen associated with the food allergy and the at least one coated microneedle of the array does not extend beyond the cutis once inserted into the subject's skin. Optionally, the one or more microneedles are coated with an adjuvant. In some embodiments described herein, each microneedle of the array does not extend beyond the cutis once inserted. In a further embodiment, substantially all the microneedles are coated with the allergen. The allergen is allowed to dissociate from the one or more microneedles while inserted in the subject's cutis. Once the allergen dissociates, the one or more microneedles is removed from the subject's skin.

In one embodiment, the microneedle tips of the one or more microneedles extend into the epidermis layer of the subject's skin once inserted. In a further embodiment, the microneedle tips extend into the epidermis layer of the skin and do not extend into the dermis layer once inserted into the subject. However, in some embodiments, the microneedle tips do extend into the dermis layer. It should be noted that unlike a subcutaneous injection, the microneedles provided herein do not extend beyond the dermis layer of the skin, i.e., the microneedles do not extend into the subcutis. Additionally, microneedles of the one or more microneedles can be fabricated having different lengths. As a result, different microneedles can extend to different depths in the cutis. In one embodiment, the microneedles of different length are present on a single array. In another embodiment, the microneedles of different length are present on separate microneedle arrays.

The one or more microneedles can be present as an array of two or more microneedles, i.e., as a microneedle array. The one or more microneedles (e.g., microneedle array) comprises at least one solid microneedle coated with or associated with one or more food allergens that are specific to the food allergy being treated. In one embodiment, substantially all of the microneedles in the array are coated with the one or more food allergens, an adjuvant, or a combination thereof. In another embodiment, a majority of the microneedles in the array are coated with the one or more food allergens, an adjuvant, or a combination thereof.

Because microneedles are very small structures, they are painless and therefore, should promote patient compliance when used as a vehicle for allergen administration. In embodiments of the treatment methods provided herein, a microneedle array comprising one or more microneedles coated with an allergen is inserted into the subject's skin one or more times during an administration period. The administration period, in one embodiment, is a time sufficient to cause a protective immune response, e.g., desensitization or sustained unresponsiveness to the allergen. The allergen dose can be the same or different for each insertion/application during the administration period. For example, microneedle arrays can be applied serially, and deliver an escalating dose of the allergen, or combinations of allergens, during the administration period.

The one or more microneedles (e.g., microneedle array) in one embodiment is inserted into a subject's skin one or more times during the administration period. In one embodiment, the one or more microneedles remain inserted for about 1 min. to 1 hr., for example, from about 1 min.

to about 10 min., or from about 1 min. to about 5 min for each insertion (also referred to as an application) during the administration period. Where the one or more microneedles is inserted into a subject's skin multiple times (i.e., multiple applications) during the administration period, in one embodiment, there is an "off period" in between the multiple applications/insertions. The "off period" in one embodiment, is 12 hrs., one day, two days, three days, four days, five days, six days, seven days or 14 days. As such, the one or more microneedles (e.g., the microneedle array) can be applied at various frequencies during the administration period until desensitization and/or sustained unresponsiveness to the allergen is achieved. In one embodiment, the one or more microneedles (e.g., microneedle array) is inserted into a subject's skin once daily, twice daily, every other day, every third day, or once a week during the administration period until a protective immune response is achieved. In one embodiment, the administration period is about 1 month, about 3 months, about 6 months, about 9 months, about12 months, about 15 months, about 18 months, about 24 months, about 27 months, about 30 months, about 33 months or about 36 months. In one embodiment, the administration period is at least about 1 month, at least about 3 months, at least about 6 months, at least about 9 months, at least about12 months, at least about 15 months, at least about 18 months, at least about 24 months, at least about 27 months, at least about 30 months, at least about 33 months or at least 36 months. The administration period, in one embodiment, is the amount of time sufficient to achieve desensitization and/or long term unresponsiveness to the allergen being administered.

As used herein, the term "subject" is used to mean an animal, for example a mammal, including a human or non-human. The terms subject and patient can be used interchangeably. The subject can be a child or an adult. In one embodiment, a subject is from about 2 to about 30 years old. In a further embodiment, the subject is human. In another embodiment, the subject is human and is from about 2 years old to about 12 years old. In a further embodiment, the subject is a human subject and is from about 4 years old to about 11 years old or about 4 years old to about 10 years old.

As used herein, the term "treating" or "treatment" refers to the ability to achieve desensitization to the respective allergen, and/or long term unresponsiveness (also referred to as sustained unresponsiveness). In one embodiment, the desensitization is characterized relative to the same subject, prior to commencing therapy, or compared to a subject receiving placebo or not receiving treatment. In one embodiment, the subject is desensitized by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, about 70%, about 75%, at least about 80%, at least about 85%, or at least about 90% as compared to the subject prior to commencing therapy, a subject receiving a placebo or a subject not receiving treatment.

An “effective amount” of an allergen is an amount of allergen that can provide desensitization to the allergen, and/or the increase in eliciting dose of the allergen. The effective amount can refer to a single dose as part of multiple doses during an administration period, or as the total dosage of allergen given during an administration period. The “effective amount” of allergen can be present with or without an adjuvant. The treatment regimen can include substantially the same dose for each allergen administration, or can comprise at least one, at least two or at least three escalating dosages. An “effective amount” of an allergen can be present on a single microneedle. In another embodiment, an “effective amount” of an allergen is the amount present on a plurality of microneedles of a microneedle array.

Successful desensitization can be characterized in one embodiment, by a decrease in the number of allergen specific IgE antibodies, and/or increased production of T regulatory cells. The T-regulatory cells in one embodiment, are Tr1 cells (produce IL-10, IL-10+), (ii) Th3 cells (produce TGF-β, latency associated peptide:LAP+), (iii) CD4+CD25+forkhead box P3:Foxp3+ Tregs, or a combination thereof.

In another embodiment, successful desensitization is characterized by an increase in cytokine production (e.g., IL-10, TGF-β), increased production of IgG allergen specific antibodies (e.g., IgG4 in humans, IgG2a in mice), decreased number of mast cells (e.g., at the site of allergen exposure (e.g., the gastrointestinal tract (GI) in the case of food allergens) as compared to prior to treatment), decreased number of basophils (e.g., at the site of allergen exposure (e.g., the gastrointestinal tract (GI) in the case of food allergens), or a combination of the foregoing.

Successful treatment can also be measured by an increase in the eliciting dose of the allergen, as compared to the eliciting dose prior to initiation of treatment. The “eliciting dose” of an allergen or allergenic food, as used herein, is the lowest dose of allergen or allergenic food containing the allergen, that causes a response in a subject that is sensitized to the allergen, e.g., symptoms of an allergic reaction. “Eliciting dose” can also be used interchangeably with “threshold dose”. The symptoms can be skin inflammation/redness, upper airway (eyes, nose, and throat), lower airway (lungs), gastrointestinal, cardiovascular and/or neurological symptoms, as assessed by one of ordinary skill in the art. In one embodiment, the symptom is a mild, objective symptom in a sensitized subject, e.g., a highly sensitized subject. See, e.g., Taylor et al. (2004). Clin Exp. Allergy 34, pp. 689-695, the disclosure of which is incorporated herein in its entirety for all purposes.

Low dose challenges can begin, e.g., at 10 μg of the allergen and can continue to increase based on the judgement of one of ordinary skill in the art. In one embodiment, a 30 minute or 1 hr. interval is used between doses. In one embodiment, the dose increase is an increase in an order of magnitude.

In one embodiment, a peanut allergen challenge comprises the administration of a peanut flour to a subject. The peanut flour can be defatted, and can comprise Florunner, Virginia, or Spanish peanut flour, or a combination thereof. In one embodiment, the peanut flour comprises equal parts Florunner, Virginia and Spanish peanut flour. In another embodiment, roasted peanuts are used as the challenge material. The foregoing compositions can also be used to coat the microneedles provided herein.

"Long term unresponsiveness" and "sustained unresponsiveness" are used interchangeably herein, and refers to the lack of clinical reactivity to the ingested food allergen for 1 month to 1 year after therapy has ended. In one embodiment, the sustained unresponsiveness lasts for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months or about 12 months after therapy has ended, i.e., after the last dose of allergen given during the administration period. In one embodiment, the sustained unresponsiveness lasts for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months or at least about 12 months after therapy has ended, i.e., after the last dose of allergen given during the administration period.

In one embodiment, the food allergy is a milk, fish, shellfish or nut allergy. In one embodiment, the food allergy is a nut allergy. In a further embodiment, the nut allergy is a soy or a peanut allergy. In even a further embodiment, the nut allergy is a peanut allergy.

The one or more microneedles (e.g., present as a microneedle array) provided herein can be used to delivery one or more food allergens to a subject in need thereof in order to desensitize the subject to the allergen, and/or to obtain a sustained unresponsiveness to the allergen. The one or more microneedles, in one embodiment, comprise at least one microneedle coated with an adjuvant.

The term "allergen" refers to an immunogenic molecule (or a combination of immunogenic molecules) involved in an allergic reaction contained in food. The allergen in one embodiment, is a lipid, carbohydrate, protein, peptide, polypeptide, or a combination thereof. In one embodiment, the allergen is a native food preparation, a food extract, or a purified protein, polypeptide and/or peptide composition. The allergen may be in a natural state, or produced artificially (e.g., by recombinant and/or enzymatic techniques, and or de novo synthesis for instance). The allergen in one embodiment, is structurally altered or modified to improve its stability or immunogenicity. The allergen in on embodiment is delivered with one or more other constituents, such as an adjuvant (e.g., via an admixture on individual microneedles or as separate coatings on microneedles of the same microneedle array). The allergen may be a mixture of several molecules (e.g., an extract such as a peanut protein extract). The allergen may be present in combination with other allergens, or in combination with other molecules from the food that are not immunogenic. In one embodiment, one or more adjuvants are included in a composition comprising the allergen, coated on one or more microneedles of a microneedle array.

The invention may be used with any food or food allergens such as, without limitation, groundnut, peanut, milk, egg, tree nuts and seeds (such as but not limited to: hazelnut, cashew, walnut, pecan, brazil nut, macadamia, chestnut, pistachio, coconut, almond, sesame, mustard), fish, shellfish, crustaceans, cereals (e.g., wheat, corn, oat, barley, rye, rice, sorghum, spelt), legumes (e.g., soy, kidney bean, black bean, common bean, chickpea, pea, cow pea, lentils, lupine), or mixtures thereof.

In one embodiment, the allergen is a peanut allergen or a combination of peanut allergens. The peanut allergen in one embodiment is in the form of a peanut protein extract. Thirteen peanut allergens (Ara h1 through Ara h13) have been recognized by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies (Zhou et al. (2013). International Journal of Food Science, V. 2013, Article ID 909140, incorporated by reference herein in its entirety). In one embodiment, the peanut allergen comprises one or more of Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara h6, Ara h7, Ara h8, Ara h9, Ara h10, Ara h11, Ara h12 or Ara h13, or a peptide fragment of one of the foregoing, or a combination thereof. In a further embodiment, the peanut allergen comprises Ara h1, Ara h2, Ara h3, a peptide fragment thereof, or a combination thereof. In yet another embodiment, the peanut allergen comprises Ara h1, a peptide fragment thereof, or multiple peptide fragments thereof.

Peanut Flour (PF) for use as an allergen composition can be obtained commercially, for example, from the Golden Peanut Company (Alpharetta, GA). The PF can be defatted, and can comprise in one embodiment, Florunner, Virginia, or Spanish PF, or a combination thereof. In one embodiment, the peanut flour comprises equal parts Florunner, Virginia and Spanish PF. In another embodiment, roasted peanuts are used as a source of allergen for the allergen composition. Peanut extract for use as an allergen composition in another embodiment, can be obtained commercially, for example, from Greer Labs (Lenoir, NC).

In one embodiment, the peanut allergen comprises Ara h1 (or a peptide fragment thereof), Ara h2 (or a peptide fragment thereof), and Ara h6 (or a peptide fragment thereof).

Representative linear epitopes for peanut allergens are provided in Zhou et al. (Zhou et al. (2013). International Journal of Food Science, V. 2013, Article ID 909140, incorporated by reference herein in its entirety). For example, for Ara h1, epitope sequences that can be incorporated into the peanut allergen include PGQFEDFF (Ara h1 epitope # 7, SEQ ID NO:1), YLQGFSRN (Ara h1 epitope # 8, SEQ ID NO:2), FNAEFNEIRR (Ara h1 epitope # 9, SEQ ID NO:3), QEERGQRR (Ara h1 epitope # 10, SEQ ID NO:4), DITNPINLRE (Ara h1 epitope # 11, SEQ ID NO:5), NNFGKLFEVK (Ara h1 epitope # 12, SEQ ID NO:6), GNLELV (Ara h1 epitope # 13, SEQ ID NO:7), RRYTARLKEG (Ara h1 epitope # 14, SEQ ID NO:8), ELHLLGFGIN (Ara h1 epitope # 15, SEQ ID NO:9), HRIFLAGDKD (Ara h1 epitope # 16, SEQ ID NO:10), IDQIEKQAKD (Ara h1 epitope # 17, SEQ ID NO:11), KDLAFPGSGE (Ara h1 epitope # 18, SEQ ID NO:12), KESHFVSARP (Ara h1 epitope # 19, SEQ ID NO:13), NEGVIVKVSKEHVEELTKHAKSVSK (Ara h1 epitope # 21, SEQ ID NO:14), or a combination thereof.

Peptides that may be incorporated into an Ara h2 peanut allergen include HASARQQWEL (Ara h2 epitope # 1, SEQ ID NO:15), QWELQGDRRC (Ara h2 epitope # 2, SEQ ID NO:16), DRRCQSQLER (Ara h2 epitope # 3, SEQ ID NO:17), LRPCEQHLMQ (Ara h2 epitope # 4, SEQ ID NO:18), KIQR.DEDSYE (Ara h2 epitope # 5, SEQ ID NO:19), YERDPYSPSQ (Ara h2 epitope # 6, SEQ ID NO:20), SQDPYSPSPY (Ara h2 epitope # 7, SEQ ID NO:21), DRLQ..GRQQEQ (epitope # 8, SEQ ID NO:22), KRELRNLPQQ (Ara h2 epitope # 9, SEQ ID NO:23), QRCDLDVESG (epitope # 10, SEQ ID NO:24), or a combination thereof.

Peptides that may be incorporated into an Ara h3 allergen include IETWNPNNQEFECAG (Ara h3 epitope # 1, SEQ ID NO:25), GNIFSGFTPEFLAQA (Ara h3 epitope # 2, SEQ ID NO:26), VTVRGGLRILSPDRK (Ara h3 epitope # 3, SEQ ID NO:27), DEDEYEYDE--EDRRRG (Ara h3 epitope # 4, SEQ ID NO:28), or a combination thereof.

In one embodiment, the allergen is a legume allergen or a tree nut allergen. For example, the allergen in one embodiment is soy. In another embodiment, the allergen is almond, pecan, hazelnut, walnut or a combination thereof. It should be noted that certain patients are sensitized against more than one type of food allergen (Sicherer et al. (1998). Pediatrics 102(1), p. e6; Sicherer et al. (2001). J Allergy Clin Immunol. 108(1), pp. 128-132, each of which is incorporated by reference herein in its entirety for all purposes). As such, some embodiments of the invention are directed to the delivery of multiple allergens to a patient in the treatment methods provided herein. Alternatively, an allergen is cross reactive to two different food substances, and therefore, in one embodiment, a cross reactive allergen can be used to desensitize a patient to multiple food allergens. In cross-reactivity, IgE antibodies against one allergen can bind to a different homologous allergen and trigger the adverse reaction similar to that elicited by its binding to the first allergen. Homologous allergens share structural similarity or common epitopes, which increases the chances of cross-reactivity. For example, peanut proteins share structural homology within the legume family (e.g. soy protein), and with certain tree nuts (e.g. almond, pecan, hazelnut, and walnut) (Sicherer et al. (2000). Allergy 55(6), pp. 515-521; de Leon et al. (2003). Clin. Exp. Allergy 33(9), pp. 1273-1280; Rosenfeld et al. (2012). Int. Arch. Allergy Immunol. 157(3), pp. 238-245, each of which is incorporated by reference herein in its entirety for all purposes).

The one or more allergens provided herein are delivered to a subject in need thereof in an allergen composition coated on one or more microneedles, e.g., microneedles of a microneedle array. The allergen composition includes at least one allergen in a pharmaceutically acceptable vehicle. In one embodiment, the allergen composition further comprises one or more adjuvants. In another embodiment, the one or more adjuvants are present in a separate composition from the allergen, and are present on separate microneedles of the same microneedle array that delivers the allergen.

An "adjuvant" is substance delivered with one of the allergens provided herein to increase the allergen's immunogenicity, as compared with its immunogenicity in absence of the adjuvant. An adjuvant may be included in an allergen composition provided herein, for example, to increase the efficacy of the allergen and/or to induce or enhance an immune response that is not sufficiently induced in the absence of the adjuvant. In some embodiments, the adjuvant enables a lower dose of the allergen. The adjuvant, in one embodiment, alters the abnormal allergic Th2 skewed IgE response of an allergen to a Th1 response. In another embodiment, the adjuvant enables a more rapid immune response. The practical result of the more rapid immune response is a reduction in a multi-dosing regimen to a fewer number of doses, and in some cases, a single dose.

The adjuvant can be mixed with the allergen and present in the same microneedle coating. Alternatively, the adjuvant and allergen can be coated on separate microneedles. In one embodiment where an adjuvant is delivered with an allergen with a microneedle array, the allergen and adjuvant are coated on different microneedles of a microneedle array. In a further embodiment, individual rows of a microneedle array are coated with either the allergen or the adjuvant. See, e.g., Figure 23 left and right.

Figure 23 (left) is a stereomicroscope brightfield image of a microneedle array whose alternate diagonal rows are coated with two different dyes (green fluorescent fluorescein isothiocyanate and red fluorescent sulforhodamine) to simulate an allergen and an adjuvant coating on separate rows of microneedles: Figure 23 (right) is a stereomicroscope fluorescent and brightfield mixed-light image of the same array. Scale bar for both images is 500 μm.

In one embodiment, the adjuvant is an aluminum salt, inulin, l-Tyrosine, algammulin, combination of inulin and aluminum hydroxide, monophosphoryl lipid A (MPL), l-Tyrosine in combination with MPL, resiquimod, muramyl dipeptide (MDP), N-glycolyl dipeptide (GMDP), poly IC, CpG oligonucleotide, an interferon (e.g., interferon gamma (IFN-γ)), aluminum hydroxide with MPL, any water in oil emulsion, any oil in water emulsion that contains one or more of the following constituents: squalene or its analogues or any pharmaceutically acceptable oil, tween-80, sorbitan trioleate, alpha-tocopherol, cholecalciferol, calcium phosphate or a combination of two or more of the foregoing. In one embodiment, the adjuvant is IFN-γ. IFN-γ is a type-II interferon and is produced by T-cells and NK cells upon stimulation by microbes. Since IFN-γ promotes the Th1 pathway, without wishing to be bound by theory, it is thought that IFN-γ can alter the abnormal allergic Th2 skewed IgE response to a Th1 response, and promote long term desensitization.

In one embodiment of the allergen composition provided herein, the composition comprises a stimulator of interferon genes (STING) ligand adjuvant. The STING ligand in one embodiment, is a cyclic dinucleotide or a xanthenone derivative. In a further embodiment, the STING ligand is cyclic guanosine monophosphate (cGMP), cyclic di-GMP (c-diGMP), cyclic adenosine monophosphate (cAMP), cyclic-di-AMP (c-di-AMP), cyclic-GMP-AMP (cGAMP, e.g., 2'2'-cGAMP, 2'3'-cGAMP or 3'3'-cGAMP). STING ligands are available commercially, e.g., from Invivogen (San Diego, CA, USA).

In another embodiment, the adjuvant is an oil and water emulsion (for example, complete Freund's adjuvant and incomplete Freund's adjuvant, Corynebacterium parvum, Bacillus Calmette Guerin, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, certain synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, Avridine (N, N-dioctadecyl-N′,N′-bis(2-hydroxyethyl)-propanediamine), paraffin oil, muramyl dipeptide or a combination thereof.

In one embodiment, the allergen composition comprises alum as an adjuvant.

In one embodiment, the adjuvant in the allergen composition is l-Tyrosine. Various animal studies have shown l-Tyrosine to be a safe and effective adjuvant, with high adsorptive power for proteins, and enhancement of antibody indication as well as a short-term depot. See, e.g., Baldrick et al. (2002). J. Appl. Toxicol. 22, pp. 333-344, the disclosure of which is incorporated by reference herein in its entirety for all purposes.

In another embodiment, the adjuvant in the allergen composition is monophoshoryl lipid A (MPL).

In another embodiment, the adjuvant in the allergen composition is l-Tyrosine in combination with monophoshoryl lipid A (MPL).

In one embodiment, the adjuvant in the allergen composition is a CpG oligonucleotide (ODN). For example, the CpG adjuvant is one or more adjuvants disclosed in U.S. Patent Application Publication No. 2017/0136119, the disclosure of which is incorporated by reference in its entirety for all purposes.

In yet another embodiment, the allergen is delivered with an adjuvant selected from alum; a CpG oligonucleotides (ODN); polyA-polyU; dimethyldioctadecylammonium bromide (DDA), N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine, carbomer, chitosan (see, e.g., U.S. Pat. No. 5,980,912 for example, the disclosure of which is incorporated herein by reference in its entirety for all purposes.

The adjuvant, in another embodiment, comprises a lipophile, a polymer of acrylic or methacrylic acid, saline, cholesterol, a saponin, sodium hydroxide, or a combination thereof. For example, one or more of the adjuvants disclosed in U.S. Patent Application Publication No. 2017/0202959 and U.S. Patent No. 9,730,987, the disclosure of each of which is incorporated herein by reference in its entirety for all purposes.

The devices and methods provided herein employ one or more microneedles to deliver an allergen into a subject’s cutis. In one embodiment, a single microneedle is employed. However, in another embodiment, two or more microneedles are employed. The two or more microneedles can be in the form of a microneedle array. A microneedle patch can be employed in embodiments described herein, and includes one or more microneedles extending from a common substrate. Where two or more microneedles are employed, e.g., as an array, each microneedle need not be coated with allergen. However, in one embodiment, substantially every microneedle is coated with allergen.

“Microneedle array” as used herein, refers to two or more microneedles extending from a common substrate. Each microneedle includes a base, a tip portion and a shaft between the base and tip portion. The two or more microneedles in the array need not be homogenous with respect to size, shape and/or material. In other words, a microneedle array may include a mixture of different microneedles. For example, an array may include microneedles having various lengths, base portion diameters, tip portion shapes, spacings between microneedles, drug coatings, etc. However, in one embodiment, the two or more microneedles in the array are substantially the same size and shape, and are fabricated from the same material. In a further embodiment, the two or more microneedles in an array are each fabricated from stainless steel, and are solid microneedles. In one embodiment, the microneedle comprises between 2 and 1000 (e.g., between 2 and 500) microneedles. In one embodiment, the microneedle array comprises between 2 and 250 microneedles, for example, between 2 and 100 microneedles, or from 10 to 100 microneedles.

The microneedles provided herein can be fabricated of different biocompatible materials, including metals, glasses, semi-conductor materials, ceramics, or polymers. Examples of suitable metals include pharmaceutical grade stainless steel, gold, titanium, nickel, iron, tin, chromium, copper, alloys thereof, and combinations thereof. In one embodiment, microneedles are fabricated from stainless steel.

In another embodiment, the microneedle is fabricated from a polymer substrate. The polymer can be biodegradable or non-biodegradable. Examples of suitable biocompatible, biodegradable polymers include polylactides, polyglycolides, polylactide-co-glycolides (PLGA), polyanhydrides, polyorthoesters, polyetheresters, polycaprolactones, polyesteramides, poly(butyric acid), poly(valeric acid), polyurethanes and copolymers and blends thereof. Representative non-biodegradable polymers include polyacrylates, polymers of ethylene-vinyl acetates and other acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonate polyolefins, polyethylene oxide, blends and copolymers thereof. Biodegradable microneedles can provide an increased level of safety compared to non-biodegradable ones, such that they are essentially harmless even if inadvertently broken off into the biological tissue. In embodiments where biocompatible polymers are employed, the allergen can either be coated on the surface of the microneedle, or encapsulated with the polymer for example, as described in PCT Publication WO 2014/182932, the disclosure of which is incorporated by reference herein in its entirety. For example, a solution of biocompatible polymer can be mixed with allergen and cast in a mold to form microneedles.

The microneedles employed herein can be solid or hollow. In addition, the microneedles can be porous or non-porous. The microneedles may be planar, cylindrical, or conical. The microneedles can have a straight or tapered shaft. In one embodiment, the microneedle array comprises two or more solid microneedles. In a further embodiment, each microneedle in the array is a solid microneedle.

In one embodiment, the diameter of one or more of the microneedles in the array is greatest at the base end of the microneedle (i.e., the portion attached to the substrate) and tapers to a point at the end distal the base. In a further embodiment, each of the microneedles in the array has a diameter that is greatest at the base, which tapers to a point at the end distal to the base. The microneedles can also be fabricated to have a shaft that includes both a straight (i.e., untapered) portion and a tapered portion. In another embodiment, one or more microneedles in the array are straight, while one or more microneedles are tapered. In yet another embodiment, the microneedles in the array comprise shafts that have a circular cross-section in the perpendicular. However, in another embodiment, the microneedles in the array comprise shafts that have a non-circular cross-section.

Each microneedle employed herein includes a tip portion. The tip portion can have a variety of configurations. The tip portion can be symmetrical or asymmetrical about the longitudinal axis of the shaft. Moreover, the tip portion in one embodiment, is beveled, tapered, squared-off, or rounded. The tip portion, in one embodiment, has a length that is less than 50% of the total length of the microneedle.

Microneedle length selection, as an initial matter, is selected considering whether the entire length of the microneedles is inserted, or whether a portion of the microneedles is inserted with a portion that remains uninserted. The length of a microneedle is measured from the base, i.e., the portion of the microneedle attached to the substrate, to the tip of the microneedle. In the case of a microneedle array, where two or more microneedles are employed, the microneedles can have substantially the same length, or different lengths. Different lengths can be employed, for example, to deliver allergen to different depths in the subject's cutis. In one embodiment, the average length of the microneedles in a microneedle array is from about 50 μm to about 5000 μm, from about 100 μm to about 1500 μm, from about 200 μm to about 1000 μm, from about 200 μm to about 800 μm, from about 200 μm to about 700 μm. In one embodiment, the average length of the microneedles in the array is from about 500 μm to about 1000 μm. In yet another embodiment, the average length of the microneedles in the array is about 150 μm, about 250 μm, about 300 μm, about 500 μm, about 600 μm, about 700 μm, about 750 μm, about 800 μm or about 850 μm.

The cross-section of the microneedle, or width, is tailored to provide, among other things, the mechanical strength to remain intact for the delivery of the drug or for serving as a conduit (i.e., in the case of a hollow microneedle), while being inserted into the skin, while remaining in place during its functional period, and while being removed (unless designed to break off, dissolve, or otherwise not be removed). In various embodiments, the base portions of the microneedles in the array have an average width or cross-sectional dimension from about 20 μm to about 500 μm, for example from about 50 μm to about 350 μm, or from about 100 μm to about 250 μm. In one embodiment, the width of the microneedles in the array is substantially the same in the base and the shaft of the microneedles.

The one or more microneedles, in one embodiment, have an average aspect ratio (width:length) of from about 1:1 and 1:10. The tip of the microneedle can sharpen gradually as in the case of microneedles with a conical, pyramidal, or triangular cross-section. In another embodiment the tip can be suddenly formed into a sharp point as in the case of microneedles with cylindrical cross-section. In one embodiment, the microneedles have an aspect ratio of about 1:3.5 with a cross-section that is rectangular for about 70% of its length followed by a tapering triangular shape constituting the remaining about 30% of the top, and culminating in to a sharp tip.

The one or more microneedles, in one embodiment, includes a microneedle comprising a pocket. As used herein, "pocket" refers to an aperture extending crosswise into the microneedle shaft (e.g., perpendicular to the direction of microneedle movement during the process of insertion into skin). The pocket can extend through the shaft. However, the pocket in another embodiment, is closed at one end, distal the opening in the shaft. This is distinct from a hollow bore wherein a concentric space extends substantially through the axial length of the shaft. The pockets are considered to be part of the surface of the microneedle. In one embodiment, the pocket is included in a solid microneedle, and includes coating material which may be particularly advantageous in certain embodiments where the coating material needs to be protected from mechanical forces during the insertion process, e.g., when the coating comprises a liquid or particles. Without wishing to be bound by theory, it is thought that such coating materials are more likely than others to be prematurely dislodged from the microneedle during insertion into skin, diminishing the complete delivery of the complete dosage of the coating. However, the pockets of the microneedles advantageously function to shield the coating material therein from the mechanical forces of insertion. The pockets may be made in various shapes (e.g., circular, square, rectangular) and of various numbers and dimensions and different spacings within the microneedle.

The microneedles in the arrays provided herein can be fabricated by a variety of methods known in the art. In one embodiment, a wet etch process is employed. For example, the wet etch processes described in Ma et al. (2014). Pharmaceutical Research 31(9), pp. 2393-2403; Jain et al. (2016). Journal of Controlled Release 239, pp. 72-81, each of which is incorporated by reference herein in its entirety for all purposes, can be employed.

Details of other manufacturing techniques amenable for use with the microneedles described herein are described, for example, in U.S. Patent Application Publication No. 2006/0086689, U.S. Patent Application Publication No. 2006/0084942, U.S. Patent Application Publication No. 2005/0209565, U.S. Patent Application Publication No. 2002/0082543, U.S. Patent No. 6,334,856, U.S. Patent No. 6,611,707, U.S. Patent No. 6,743,211, each of which is incorporated herein by reference in its entirety for all purposes.

In one embodiment, the microneedles are cut from stainless steel or other metal sheets using a laser (e.g., an infrared laser) or other techniques known in the art.

In one embodiment, an electropolishing technique is used to produce clean, smooth, and sharp solid microneedles. Electropolishing can remove slag deposits from the microneedles, as laser-cutting of metals such as stainless steel may produce microneedles with rough edges covered with slag deposits. In one embodiment, laser cut stainless steel microneedles are electropolished in a solution that includes glycerin, ortho-phosphoric acid (85%), and water. In one example, a copper plate is used as the cathode and the metal microneedles serve as the anode. The anode may be vibrated using means known in the art to help remove gas bubbles generated at the anodic surface during electropolishing. Electropolishing is believed to be especially effective, because current density (i.e., etching rate) is largest at sites of high curvature, which inherently targets sites of surface roughness for removal. In some embodiments, the electropolishing process has an output rate of finished microneedle arrays of one 50-needle array every 30 minutes using a single laser. This rate can be increased by process optimization and use of multiple lasers.

In one embodiment, the microneedle array used in the methods provided herein (or the single microneedle) includes a substantially planar foundation from which two or more microneedles extend (or the single microneedle extends), typically in a direction normal (i.e., perpendicular or out-of-plane) to the foundation. Alternatively, microneedles may be fabricated on the edge of a substrate ‘in-plane’ with the substrate. In one embodiment, the microneedle array extends from a flexible base substrate. In another embodiment, the microneedle array extends from a curved base substrate. The curvature of the base substrate typically would be designed to conform to the shape of the tissue surface. The curved base substrate can be flexible or rigid.

In one embodiment, the one or more microneedles extends from an adhesive patch substrate. The patch comprises one or more microneedles, for example, an array of tens or hundreds of microneedles (e.g., from about 10 to about 500 microneedles or from about 10 to about 100 microneedles). The patch, in one embodiment, comprises an adhesive component to secure the patch to the skin. The patch includes a plurality of linear rows of in-plane microneedles, a plurality of individual arrays of out-of-plane microneedles, or a combination thereof.

The patch, e.g., adhesive patch, can be a flexible or rigid substrate which includes a pressure sensitive adhesive as known in the art.

In one embodiment, the microneedles and adhesive component are configured such that the microneedles extend through apertures in the adhesive layer. Individual microneedles or subgroups of microneedles (e.g., rows) can extend through a single aperture. Without wishing to be bound by theory, it is thought that when the adhesive surface is adjacent the microneedles, the adhesive is able to better hold the microneedles down and to compensate for the recoiling-tendency of skin and/or a rigid substrate for out-of-plane microneedles.

In one embodiment, in-plane microneedles are fabricated with a uniform adhesive layer in between the microneedles. For example, rows of microneedles can be assembled into a patch by forming slits (equal to the length of an in-plane row) in a material, e.g., polyethylene medical foam tape. Such cutting can be performed by any suitable technique known in the art, such as laser cutting. The microneedle rows can be manually or robotically inserted into each slit from the non-adhesive side of the foam tape and glued to the foam tape using a medical grade adhesive. The adhesive is then allowed to cure. Optionally, a medical foam tape of sufficient thickness can then be cut into a disc and affixed onto the dried glue area to provide a cushioned backing to facilitate pressing the patch during insertion. See Figure 1. In one embodiment, the thickness of the medical foam tape is from about 0.4 mm to about 1.0 mm, or from about 0.6 mm to about 1.0 mm, or from about 0.7 mm to about 0.9 mm, e.g., 0.8 mm.A "row" of microneedles, as used herein, refers to two or more microneedles arranged linearly. In embodiments described herein, individual microneedle rows can be coated with the same coating or a different coating. For example, in one embodiment, alternating rows of allergen coated microneedles and adjuvant coated microneedles are present on a microneedle array. See, e.g., Figure 23.

In another embodiment, a microneedle patch is assembled using out-of plane microneedles, a circular disc of a single-sided medical foam tape and a thick double-sided medical tape. In the middle of the disc, a rectangular piece of adhesive release liner equal in dimensions to the periphery of the array can be cut out and peeled off. The microneedle array can then be attached to this exposed adhesive. To provide a layer of pressure-sensitive adhesive on the stainless steel substrate of the affixed array itself, a double-sided, carrier tape first perforated with holes corresponding to the microneedles can be attached by slipping it over the microneedles using an alignment device. In one embodiment, the carrier tape is a polyethylene terephthalate (PET) carrier tape.

In one embodiment, microneedle array patches are assembled into transdermal patches containing pressure-sensitive adhesive to adhere to the skin. To secure microneedles in the skin at all times until ready to be removed, microneedles in one embodiment, are integrated into a Band-Aid-like patch. The patch had pressure-sensitive adhesive on one complete side, with microneedles protruding therefrom. The adhesive secured the microneedles and compensated for the recoiling tendency of the skin and the rigid stainless steel material of the out-of-plane microneedles (i.e., microneedles normal to the patch substrate). Patches can be fabricated using either multiple linear rows of in-plane microneedles or individual arrays of out-of-plane microneedles.

In-plane microneedles, in one embodiment, are fabricated with a uniform adhesive layer in between the microneedles. In this embodiment, a set of rows of microneedles (e.g., 10 rows), each containing, for example, 5-10 microneedles each, can be assembled into a patch of, for example, 50-100 microneedles. In one embodiment, slits are laser cut into a single sided medical foam tape. Each slit is cut to the length of a row of microneedles, and the number of slits corresponds to the number of microneedle rows in the patch. Microneedle rows can be manually or robotically inserted into each slit from the non-adhesive side of the foam tape, and glued to the foam tape using a medical grade adhesive. The adhesive can then be allowed to cure for a sufficient amount of time, for example from about 12 hours to about 48 hours, for example about 24 hours. A medical foam tape can then be cut to size of the assembled array, and affixed onto the dried glue area to provide a cushioned backing to facilitate pressing the patch during insertion.

In one embodiment, a microneedle patch is assembled with out-of plane microneedles. In this embodiment, a circular disc of appropriate diameter is cut from a single-sided medical foam tape, for example, using a CO2 laser. One of ordinary skill in the art will appreciate that the diameter of the disk will be dictated by the size and shape (e.g., number of rows) of the microneedle array. In the middle of this disc, a rectangular piece of the adhesive release liner equal in dimensions to the periphery of the array can be cut out, e.g., using a CO2 laser, and subsequently peeled off. The stainless steel microneedle array can then be attached to this exposed adhesive. To provide a layer of pressure-sensitive adhesive on the stainless steel substrate of the affixed array itself, a double-sided carrier tape (e.g., polyethylene terephthalate (PET) tape) can be attached. The carrier film is first perforated with holes at the same spacing as the microneedles using a CO2 laser. The tape is then slipped over the microneedles using a custom-built alignment device and pressed to stick against the stainless steel microneedles.

The coated solid microneedles provided herein can be fabricated via methods known to those of ordinary skilled in the art. For example, in one embodiment, the coated microneedles are fabricated via the methods disclosed in U.S. Patent No. 9,364,426, the disclosure of which is incorporated by reference in its entirety for all purposes. Coated microneedle arrays can include microneedles with the same coating or different coatings. In one embodiment, individual rows of a microneedle array are coated with a different coating. For example, in one embodiment, alternating rows of allergen coated microneedles and adjuvant coated microneedles are present on a microneedle array.In one embodiment where an adjuvant is delivered with an allergen with a microneedle array, the allergen and adjuvant are coated on different microneedles of the array. In a further embodiment, individual rows of a microneedle array are coated with either the allergen or the adjuvant. See, e.g., Figure 23.

In one embodiment, prior to coating the microneedle or microneedles with allergen (with or without adjuvant), the microneedle or microneedles are treated with oxygen or air plasma. Such treatment has been reported to increase the surface energy and wettability of certain substrates such as stainless steel. See, e.g., Tang et al. (2004). Korean J. Chem. Eng. 21(6), pp. 1218-1223, the disclosure of which is incorporated by reference in its entirety for all purposes. Moreover, an oxygen or air plasma treatment may result in additives not being needed in the subsequent allergen coating (e.g., additive to facilitate coating adhesion), and can serve to sterilize the microneedle surface.

In some embodiments, prior to coating the one or more microneedles (e.g., microneedle array) with the allergen (e.g., with or without adjuvant) or combination of allergens (e.g., with or without adjuvant), a precoating to at least one surface of the microneedles is performed, in order to increase the surface energy of the surface, or to otherwise modify the surface energy properties of the microneedles. In another embodiment, the coating liquid is modified to decrease the surface tension of the coating liquid. A combination of the aforementioned can also be carried out. It should also be noted that a precoating need not be applied to all microneedles of the one or more microneedles. Nor does the coating liquid need to be modified for all microneedles, when a modification of the coating liquid is performed.

The coating liquid in one embodiment, is disposed in one or more reservoirs. Microneedles can be dipped directly into the reservoir containing the allergen or combination of allergens. In another embodiment, a physical mask having a plurality of apertures therethrough, each aperture having cross-sectional dimensions larger than the at least one microneedle to be coated is provided over the reservoir. In this embodiment, the microneedle array is aligned with the plurality of apertures, and the array is inserted through the aligned aperture and into the coating liquid. The coated microneedle array is then removed from the coating liquid and from the apertures. The one or more reservoirs may be defined in a secondary structure or the physical may have a plurality of the reservoirs defined therein.

By utilization of a physical mask, access of the coating liquid is restricted only to the microneedle shaft and tip, thereby preventing contamination of the substrate from which the microneedles extend. Thus, any meniscus rise or capillary action that may cause contact of the coating liquid to an adjacent microneedle or with the substrate is avoided such that the coating is on the surface of the microneedle shafts and tips, and the base substrate is free of the coating.

In one embodiment, the physical mask is in the form of a plate having a one or more discrete apertures therethrough. The apertures, in one embodiment, are in the form of one or more holes or slits which closely circumscribe each microneedle, a single row of microneedles, multiple rows of microneedles, or another subset of microneedles of the array. As used herein, the term "closely circumscribe" means that the physical mask is effective to restrain, for example, by surface tension forces, the coating liquid to the reservoir and apertures, preventing it from "climbing up" the microneedle shaft substantially beyond the dipped portion of the microneedle which it is desired to coat. Surface energy properties of the coating system (physical mask, microneedle, and coating fluid) and operating conditions (e.g., temperature, dipping/withdrawal speed) can impact the selection of appropriate dimensions for the holes and slits.

In one embodiment, the physical mask is in the form of a substantially rigid plate secured to the reservoir (see, e.g., Figure 2). The plate includes an array of micron-sized holes which are used for inserting the microneedles to be coated. When aligned, for example using micropositioners or pre-aligned parts moving on a rail, each of the microneedles can be simultaneously inserted through the micron sized holes and into the coating liquid, resulting in a controlled micro-dip-coating process. The use of one or more micropositioners can be used to provide control over the microneedle length being coated, that is how much of the microneedle length is actually coated. In one embodiment, physical stops in the form of thick sheets or protruding cylinders in between the physical mask and microneedles, or a combination thereof, are used to control the microneedle length being coated. The coating device can be configured to coat single microneedles, in-plane rows of microneedles, and out-of-plane arrays of microneedles.

In another embodiment, the physical mask acts as a coating liquid reservoir or reservoirs. For example, in one embodiment, the physical mask includes reservoirs, closed at one end, that can be filled with the coating liquid (see, e.g., Figure 3). Single microneedles or multiple microneedles of an array can be dipped into each reservoir. In one embodiment, the apertures of the mask have a closed bottom, and the coating liquid is filled in these apertures from the open top. However, an inlet port can be present on the bottom of the apertures to fill coating liquid. Apertures can be periodically or continually refilled to maintain a constant amount of coating liquid in the reservoir(s).

To reduce propensity of air bubbles in the reservoir and/or apertures in the plate, vent holes designed to release entrapped air can be provided in the coating apparatus. To prevent evaporation of coating liquid (or solvent thereof) from the coating liquid, a pumping device (e.g., an automated or manually pulsated syringe plunger) can be included with the coating apparatus to fill the coating liquid reservoir and to oscillate/mix the coating liquid in dip-coating holes. The coating liquid in the reservoir may be flowed or agitated to facilitate maintenance of a uniform coating liquid composition during the dipping process. Alternatively, or additionally, the coating process may be performed at a reduced temperature (relative to ambient) to reduce the rate of evaporation of the coating liquid or solvent portion thereof.

In one embodiment, the coating process includes the step of volatilizing at least a portion of the solvent to form a solid coating. This may be referred to as “drying” the coating or coating liquid. A similar step may be included when using molten coating liquids, wherein the coated liquid is permitted to (or actively caused to) cool the molten material sufficiently to cause it to solidify, forming a solid coating on at least a portion of the microneedles of the array.

Microneedles can be coated with a single coating or multiple coatings. For example, the coating method in one embodiment includes inserting at least one coated microneedle of a coated microneedle array into the same or a different coating liquid and then removing the microneedle from said same or different coating liquid. The composition of the coating liquid may include a solvent to dissolve part of the previous coating, if desired. In another embodiment, the coating method includes the step of applying a second coating liquid onto the solid coating or onto a second surface of the microneedle in need of coating. The composition of the second coating liquid may include a second antigenic epitope. Multiple such dippings into the same or a different coating liquid may be repeated.

The coating process can also include an optional intervening dip into a cleaning solvent, e.g., to thin or remove part of a coating layer. This may be useful to build complete coating structures, e.g., where one coating composition is located on one part of the microneedle (e.g., a first pocket) and a second coating composition is located on another part of the microneedle (e.g., a second pocket).

To obtain uniform coatings on microneedle surfaces, it is generally desired that the surface tension of the coating liquid is lower than the surface energy of the microneedle surface material (material of construction or overcoat deposition). A slow (taking more than a second) or rapid (taking less than a second, e.g., less than a tenth of a second or less than a hundredth of a second) withdrawal of the microneedle from the immersed state to outside the coating liquid will provide a uniform coating on the microneedle. Addition of a viscosity enhancer to the coating solution increases the coating thickness by increasing the film thickness of the entrained liquid during withdrawal. However, the requirement of coating liquid surface tension being lower than the microneedle material can be overcome by conducting the coating process at a rate faster than is needed to achieve thermodynamic equilibrium. For instance, by increasing the viscosity and withdrawing at a rapid speed, the microneedle will entrain a significant volume of the liquid on the surface. If the solvent then evaporates before the liquid film can contract to form an island in the middle of the microneedle surface, the solid coating will become uniformly deposited onto the microneedles. Another way to overcome the surface tension barrier to obtain uniform coatings is to use a non-aqueous solvent that has lower surface tension, possibly lower than the microneedle material. Similarly, while coating only the pockets, advantage can be made of the kinetic effect by utilizing a high surface energy liquid/solution that will not wet the microneedle surface but will fill the pockets. Again, the speed must be sufficiently slow so that liquid does not entrain on the surface, but only gets into the pockets.

EXAMPLES

The present invention is further illustrated by reference to the following Examples. However, it should be noted that these Examples, like the embodiments described above, are illustrative and are not to be construed as restricting the scope of the invention in any way.

Example 1 – Intradermal Delivery of Model Antigen Via Coated Microneedles

Microneedle arrays were fabricated from 50 μm-thick stainless steel (304) sheets using a wet etch process. Each microneedle measured about 700 μm in length and about 200 μm in width, and each microneedle array contained 57 microneedles. Microneedle arrays were fabricated as described previously (see, e.g., Ma et al. (2014). Pharmaceutical Research 31(9), pp. 2393-2403; Jain et al. (2016). Journal of Controlled Release 239, pp. 72-81, each of which is incorporated by reference herein in its entirety for all purposes. The individual microneedles were then manually bent to make them perpendicular to the metal sheet (Figure 4). Microneedles were coated using a micro-precision dip coating station developed in-house. It comprised of an automated x-y linear computer-controlled stage on to which microneedles were mounted. The coating solution was housed in an orifice in to which the microneedles were dipped through motion control of the x-y stage, as described by Ma et al. (Ma et al. (2014). Pharmaceutical Research 31(9), pp. 2393-2403, incorporated by reference in its entirety for all purposes).

The coating solution was composed of 1% (w/v) carboxymethylcellulose (CMC) sodium salt (low viscosity, USP grade, CarboMer, San Diego, CA, USA), 0.5% (w/v) Lutrol F-68 NF (BASF, Mt. Olive, NJ, USA), and fluorescent OVA labeled with fluorescein as a model allergen. CMC and Lutrol F-68 are FDA approved for injection, and are thus safe excipients to use. Coated microneedle arrays were inserted in mouse skin for 5 min (Figure 5). Mouse skin was first prepared by carefully trimming the hair and then by applying hair-removing lotion. The mass of OVA on fresh microneedle array (M1), on microneedle array after insertion (M2), and on skin surface (obtained by using a cotton tip and extracting in water) (M3) was quantified using fluorescent spectroscopy and a standard curve of fluorescein-OVA. The amount of OVA delivered into skin was then obtained (M1-M2-M3), and converted into percent delivered by dividing with M1. Greater than 70% of OVA coated on MNs was delivered into the mouse skin (Figure 6).

Example 2 – Mouse Model for Peanut Allergy

To assess the therapeutic efficacy of peanut extract (PE) coated microneedles, a mouse peanut allergy model was established. Using a previously published protocol (Dioszeghy et al. (2014). Clin. Exp. Allergy 44(6): pp. 867-881, incorporated by reference herein in its entirety for all purposes) mice were sensitized to peanut by oral gavage with 1 mg PE + 10 μg cholera toxin (CT) weekly for six weeks (Figure 7). To check if mice were successfully made allergic to PE, the mice were challenged orally with 20 mg PE (10 mg + 10 mg at 30 min interval), and body temperature and clinical scores were recorded. Significant drop ($p<0.0001$) in body temperature (Figure 8, left) and significantly higher anaphylactic score (Figure 8, right) in sensitized mice in comparison to control naïve mice verified the progression of allergic reaction in sensitized mice. Five minutes post challenge, blood was also collected to analyze anti-PE IgE antibodies and histamine, which is released by mast cells and basophils during an allergic reaction. An elevated level of anti-PE IgE (Figure 9, left) and histamine (Figure 9, right) further verified successful development of the mouse peanut allergy model.

Example 3 – Generation of Peanut Extract Specific Antibodies

To determine the ability of peanut extract (PE) coated microneedles to generate an immune response, microneedles coated with 1, 5 or 25 μg PE were used to immunize naïve mice three times (one dose per week) (Figure 10). Six weeks later (at day 56), mice were bled to check for anti-PE responses. The mice were then euthanized and their bone marrows and spleens were aseptically collected. All three PE doses were able to induce PE-specific IgG, IgG1 and IgG2a antibodies (Figure 11). The 5 μg and 25 μg PE doses had similar antibody levels, while the 1 μg PE dose induced slightly lower anti-PE antibodies, although the difference was not statistically significant ($p > 0.05$).

The antibody response from peripheral B cells of the bone marrow cells was also evaluated. All IgG subtypes and IgE were detectable in supernatant of bone marrow cultures irrespective of the dose (Figure 12). Low anti-PE IgE responses are indicative that microneedle-based allergen immunotherapy does not cause sensitization to peanut. Without wishing to be bound by theory, it is thought that the ability to detect antibody secretion in the bone marrow offers the possibility that long-term plasma cells that secrete anti-PE antibodies might be stimulated, which might imply the ability to generate long term sustained unresponsiveness to peanut allergen through microneedle based peanut immunotherapy.

Example 4 – Assessment of Immune Response

To assess the nature of immune response (Th1 vs Th2) induced by PE-coated microneedles, splenocytes (from spleens as collected in Example 3, above) were cultured in vitro, and re-stimulated with PE (200 μg/ml). After 72 hr. of re-stimulation, supernatants were collected to analyze the secreted cytokines. Both Th1 (IL-2 & IFN-γ) and Th2 cytokines (IL-4 & IL-5) were secreted irrespective of PE dose. Expression of IL-2 was higher in 5 μg PE group than the 25 μg PE, while IFN-γ was observed higher in 1 μg PE group (Figure 13). There was no considerable difference observed in IL-4 and IL-5 expression between the 1, 5, and 25 μg PE doses. Microneedles thus appear to induce a mixed Th1/Th2 response.

Example 5 – Assessment of Anaphylactic Shock in Peanut Sensitized Mice

In this experiment, it was determined whether peanut extract (PE) coated microneedles provide a therapeutic effect in peanut sensitized (allergic) mice. The experimental protocol is shown in Figure 14. First, mice were sensitized to peanut as described above in Example 2. Then, after a rest of three weeks, the microneedle cutaneous immunotherapy (CIT) group received 5 μg PE coated on microneedles every week for a total of three weeks. After a rest of three more weeks, mice were challenged orally with a high dose of PE (20 mg/mouse: 10 mg + 10 mg delivered at 30 min interval via oral gavage) (Figure 14).

The following control groups were included: (i) peanut sensitized mice that did not receive microneedle-CIT treatment but received oral PE challenge, (ii) naive mice that received oral PE challenge, and (iii) naïve mice without treatment or oral challenge. For all groups, five minutes after oral PE challenge, mice were bled to collect plasma for analysis of inflammatory markers. Mice were monitored every 10 min. to assess the severity of anaphylaxis based on a scoring system described previously (see, McCaskill et al. (1984). Immunology 51(4), pp. 669-77, incorporated herein by reference in its entirety for all purposes), and for change in body temperature measured with a rectal probe. The microneedle-CIT group had higher anti-PE IgG, IgG1 and IgG2a in plasma as compared to the allergic/sensitized but untreated group (Figures 15 and 16). Anti-PE IgE levels were significantly lower in the microneedle-CIT group as compared to the untreated group. Moreover, lower score of anaphylaxis, and low expression of histamine and mast cell protease-1 (MCPT-1) in plasma of mice that were treated with microneedle-CIT as compared to untreated group further demonstrated the therapeutic efficacy of microneedle-CIT (Figure 17). No considerable differences were observed in change of body temperature between the different groups.

Example 6 – Peanut Allergen Dose Titration

Naïve mice will be given 0.1 μg, 0.3 μg, 0.6 μg, 1 μg, 2 μg, or 5 μg peanut allergen, in the form of peanut extract (PE) coated on microneedles, once a week for 12 weeks. Blood will be collected every two weeks to measure anti-PE antibodies set forth in Figure 11 and the corresponding Example. At the end of the 12-week period, mice will be euthanized, and bone marrow and spleen will be collected to analyze antibody secreting cells in bone marrow, and cytokines from splenocyte restimulation (e.g., the antibodies set forth in Figure 11 and the cytokines set forth in Figure 13, and the corresponding Examples). Sham (microneedles coated with excipients but no PE) and naïve groups will be included as controls.

Example 7 – Comparison of Microneedle Lengths

The immune response generated from microneedles (MNs) of various lengths (e.g., 200 μm, 300 μm, 400, μm, 500 μm, 600 μm and 700 μm will be assessed.

The delivery efficiency from MNs of different lengths will be evaluated as by coating MNs with fluorescent Ova and measuring the fluorescence delivered via the microneedles (see, e.g., Figure 6).

Immune response will be characterized by measuring the antibodies and/or cytokines described previously in Figures 11 and 13 and the corresponding Examples.

Example 8 – Comparison of allergen delivery with and without adjuvant

The effect of cGMP and cAMP, which are ligands of Stimulator of Interferon Genes (STING) (also known as transmembrane protein 173 (TMEM173)) was evaluated (Figure 18). cGMP and cAMP were added to coated microneedle formulation containing ovalbumin as a model allergen. Figure 18 shows an increased Th1 response (higher IgG2a) for the compositions containing the STING ligands, as compared to ovalbumin composition alone.

The effect of CpG as an adjuvant was evaluated on IgG response (Figure 19). CpG (#1826, a mouse specific CpG, 5'-tccatgacgttcctgacgtt-3': 20 nucleotides with bases having phosphorothioate bonds to make it nuclease resistant) was added to microneedle coating compositions containing ovalbumin as a model allergen. Addition of CpG adjuvant increased total IgG and IgG2a (Th1 type response) as compared to ovalbumin without CpG as adjuvant (Figure 19).

The effect of CpG was assessed with peanut extract as the allergen. Results are shown in Figure 20. When CpG (25 μg) was included in compositions containing 25 μg peanut extract and coated on microneedles, there was some increase in total IgG and IgG2a as compared to compositions containing PE without CpG. Further, CpG significantly reduced anti-peanut IgE levels (Figure 20).

Addition of adjuvants in MN coating compositions for the treatment of peanut allergy was tested. The schedule set forth in Figure 21 was followed. Mice were sensitized to peanut. Allergic mice were treated with either peanut extract (PE) or peanut extract + CpG (PE+CpG), and subsequently, mice were orally challenged with peanut to test the treatment efficacy. None of the mice in PE+CpG group (0/8) had an anaphylactic score of greater than 2 while in the PE group (no CpG usage) 3/8 mice had a score >3 (Figure 22). The anaphylactic score is a measure of severity of the anaphylactic reaction with lower score indicating a less severe reaction.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of" or "consisting of". As used herein, the phrase "consisting essentially of" requires the specified integer(s) or steps as well as those that do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited integer (e.g., a feature, an element, a characteristic, a property, a method/process step or a limitation) or group of integers (e.g., feature(s), element(s), characteristic(s), property(ies), method/process steps or limitation(s)) only.

The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

As used herein, words of approximation such as, without limitation, "about", "substantial" or "substantially" refers to a condition that when so modified is understood to not necessarily be absolute or perfect but would be considered close enough to those of ordinary skill in the art to warrant designating the condition as being present. The extent to which the description may vary will depend on how great a change can be instituted and still have one of ordinary skill in the art recognize the modified feature as still having the required characteristics and capabilities of the unmodified feature. In general, but subject to the preceding discussion, a numerical value herein that is modified by a word of approximation such as “about” may vary from the stated value by at least ±1, 2, 3, 4, 5, 6, 7, 10, 12 or 15%.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

To aid the Patent Office, and any readers of any patent issued on this application in interpreting the claims appended hereto, applicants wish to note that they do not intend any of the appended claims to invoke paragraph 6 of 35 U.S.C. § 112, U.S.C. § 112 paragraph (f), or equivalent, as it exists on the date of filing hereof unless the words “means for” or “step for” are explicitly used in the particular claim.

For each of the claims, each dependent claim can depend both from the independent claim and from each of the prior dependent claims for each and every claim so long as the prior claim provides a proper antecedent basis for a claim term or element.

CLAIMS

1. A method for treating a food allergy in a subject in need thereof, comprising,

delivering an effective amount of an allergen associated with the food allergy into the subject's cutis skin layer, wherein the delivering step is carried out one or more times during an administration period, and comprises,

(i) inserting one or more solid microneedles each comprising a base, shaft and tip into the subject's skin, wherein at least one microneedle of the one or more solid microneedles does not extend beyond the cutis once inserted, and is coated with an amount of the allergen; and

(ii) allowing the allergen to dissociate from the at least one microneedle while inserted in the subject's cutis; and

(iii) removing the one or more solid microneedles from the subject's skin.

2. The method of claim 1, wherein the one or more solid microneedles extends from an adhesive substrate.

3. The method of claim 1 or claim 2, wherein the one or more solid microneedles is stainless steel.

4. The method of any one of claims 1-3, wherein the one or more solid microneedles is present in a microneedle array comprising a plurality of microneedles extending from a common substrate.

5. The method of claim 4, wherein the microneedles of the plurality are stainless steel.

6. The method of claim 4 or claim 5, wherein the microneedles of the plurality are substantially the same length.

7. The method of claim 4 or claim 5, wherein two or more of the microneedles have different lengths.

8. The method of any one of claims 1-7, wherein the average length of the one or more microneedles is from about 100 μm to about 1000 μm, as measured from the base of the tip.

9. The method of claim 8, wherein the average length of the one or more microneedles is from about 100 μm to about 900 μm, or from about 100 μm to about 800 μm, or from about 100

μm to about 700 μm, or from about 100 μm to about 600 μm, or from about 100 μm to about 500 μm.

10. The method of any one of claims 1-9, wherein the average width of the one or more microneedles, as measured at the widest cross section of each respective microneedle of the one or more microneedles, is from about 20 μm to about 500 μm, or from about 50 μm to about 350 μm, or from about 100 μm to about 250 μm.

11. The method of any one of claims 1-10, wherein the one or more microneedles comprises a plurality of microneedles, and substantially all the microneedles of the plurality are coated with the allergen.

12. The method of any one of claims 1-10, wherein the one or more microneedles comprises a plurality of microneedles, and at least one microneedle of the plurality is not coated with the allergen.

13. The method of any one of claims 1-12, wherein the one or more microneedles comprises from about 10 to about 200 microneedles.

14. The method of claim 13, wherein the one or more microneedles comprises from about 20 to about 150 microneedles.

15. The method of claim 13, wherein the one or more microneedles comprises from about 20 to about 150 microneedles.

16. The method of claim 13, wherein the one or more microneedles comprises from about 30 to about 100 microneedles.

17. The method of claim 13, wherein the one or more microneedles comprises from about 40 to about 100 microneedles.

18. The method of any one of claims 1-17, wherein the food allergy is a groundnut, peanut, milk, egg, tree nut, seed, fish, shellfish, crustacean, cereal, legume allergy, or a combination thereof.

19. The method of any one of claim 1-17, wherein the food allergy is a peanut allergy.

20. The method of claim 18, wherein the food allergy is a seed allergy.

21. The method of claim 20, wherein the seed allergy is a hazelnut, cashew, walnut, pecan, brazil nut, macadamia, chestnut, pistachio, coconut, almond, sesame, or a mustard seed allergy, or a combination thereof.

22. The method of claim 20, wherein the food allergy is a legume allergy.

23. The method of claim 22, wherein the legume allergy is a soy, kidney bean, black bean, common bean, chickpea, pea, cow pea, lentil or lupine allergy, or a combination thereof.

24. The method of claim 19, wherein the peanut allergen comprises Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara h6, Ara h7, Ara h8, Ara h9, Ara h10, Ara h11, Ara h12, Ara h13, a peptide fragment thereof, or a combination thereof.

25. The method of claim 19 or claim 24, wherein the peanut allergen comprises Ara h1, Ara h2, Ara h6, a peptide fragment thereof, or a combination thereof.

26. The method of any one of claims 19 and 24-25, wherein the peanut allergen is provided as peanut protein extract.

27. The method of any one of claims 1-26, wherein the one or more solid microneedles is coated with an adjuvant.

28. The method of claim 27, wherein the adjuvant is alum.

29. The method of claim 27, wherein the adjuvant is L-Tyrosine.

30. The method of claim 27, wherein the adjuvant is monophoshoryl lipid A (MPL).

31. The method of claim 27, wherein the adjuvant is L-Tyrosine and monophoshoryl lipid A (MPL).

32. The method of claim 27, wherein the adjuvant is an aluminium salt, inulin, L-Tyrosine, algammulin, combination of inulin and aluminium hydroxide, monophosphoryl lipid A (MPL), L-Tyrosine in combination with MPL, resiquimoid, muramyl dipeptide (MDP), N-glycolyl dipeptide (GMDP), poly IC, CpG oligonucleotide, resiquimod, aluminium hydroxide with MPL or a water in oil emulsion.

33. The method of claim 32, wherein the adjuvant is a water in oil emulsion.

34. The method of claim 27, wherein the adjuvant is IFN-gamma (IFN-γ).

35. The method of claim 33, wherein the water in oil emulsion contains one or more of squalene or its analogues or any pharmaceutically acceptable oil, tween-80, sorbitan trioleate, alpha-tocopherol, cholecalciferol, calcium phosphate or a combination two or more of the foregoing.

36. The method of claim 33, wherein the adjuvant is complete Freund's adjuvant.

37. The method of claim 33, wherein the adjuvant is incomplete Freund's adjuvant.

38. The method of claim 27, wherein the adjuvant is *Corynebacterium parvum, Bacillus Calmette Guerin*, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, a synthetic polymer such as a poly amino acid or a co-polymer of amino acids, saponin, Avridine (N, N-dioctadecyl-N′,N′-bis(2-hydroxyethyl)-propanediamine), paraffin oil, muramyl dipeptide or a combination thereof.

39. The method of claim 27, wherein the adjuvant is a CpG oligonucleotide (ODN).

40. The method of claim 27, wherein the adjuvant is alum; a CpG oligonucleotide (ODN); polyA-polyU; dimethyldioctadecylammonium bromide (DDA), N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine, carbomer or chitosan.

41. The method of claim 40, wherein the adjuvant is N ,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine.

42. The method of claim 27, wherein the adjuvant is a stimulator of interferon genes (STING) ligand adjuvant.

43. The method of claim 42, wherein the STING adjuvant is a cyclic dinucleotide or a xanthenone derivative.

44. The method of claim 43, wherein the STING ligand is a xanthenone derivative.

45. The method of claim 43, wherein the STING ligand is a cyclic dinucleotide.

46. The method of claim 45, wherein the STING ligand is cyclic di-GMP (c-diGMP).

47. The method of claim 45, wherein the STING ligand is cyclic-di-AMP (c-di-AMP).

48. The method of claim 45, wherein the STING ligand is cyclic-GMP-AMP (cGAMP).

49. The method of claim 48, wherein the STING ligand 2'2'-cGAMP, 2'3'-cGAMP or 3'3'-cGAMP.

50. The method of claim 42, wherein the STING ligand is cyclic guanosine monophosphate (cGMP).

51. The method of claim 42, wherein the STING ligand is cyclic adenosine monophosphate (cAMP).

52. The method of any one of claims 27-51, wherein the adjuvant and allergen are coated on separate microneedles.

53. The method of claim 52, wherein the adjuvant and allergen are coated on separate rows of microneedles.

54. The method of any one of claims 1-53, wherein each microneedle of the one or more microneedles does not extend beyond the epidermis skin layer.

55. The method of any one of claims 1-53, wherein each microneedle of the one or more microneedles does not extend beyond the dermis skin layer.

56. The method of any one of claims 1-55, wherein at least about 40% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

57. The method of any one of claims 1-55, wherein at least about 50% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

58. The method of any one of claims 1-55, wherein at least about 60% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

59. The method of any one of claims 1-55, wherein at least about 70% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

60. The method of any one of claims 1-55, wherein at least about 80% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

61. The method of any one of claims 1-55, wherein at least about 90% of the allergen disassociates from the at least one microneedle while inserted in the subject's cutis.

62. The method of any one of claims 1-61, wherein the allowing step is carried out for about 1 minute to about 10 minutes.

63. The method of claim 62, wherein the allowing step is carried out for about 1 minute to about 8 minutes.

64. The method of claim 62, wherein the allowing step is carried out for about 1 minute to about 6 minutes.

65. The method of claim 62, wherein the allowing step is carried out for about 1 minute to about 4 minutes.

66. The method of claim 62, wherein the allowing step is carried out for about 5 minutes.

67. The method of any one of claims 1-66, wherein the delivering is carried out once daily during the administration period.

68. The method of any one of claims 1-66, wherein the delivering is carried out twice daily during the administration period.

69. The method of any one of claims 1-66, wherein the delivering is carried out three times daily during the administration period.

70. The method of any one of claims 1-66, wherein the delivering is carried out every other day during the administration period.

71. The method of any one of claims 1-66, wherein the delivering is carried out twice a week during the administration period.

72. The method of any one of claims 1-66, wherein the method is carried out once weekly during the administration period.

73. The method of any one of claims 1-72, wherein the delivering an effective amount of an allergen results in delivering substantially the same amount of allergen each time during the administration period.

74. The method of any one of claims 1-72, wherein the delivering an effective amount of an allergen results in delivering an escalating dosage of the allergen at least once during the administration period.

75. The method of any one of claims 1-72, wherein the delivering an effective amount of an allergen results in delivering an escalating dosage of the allergen at least twice during the administration period.

76. The method of any one of claims 1-72, wherein the delivering an effective amount of an allergen results in delivering an escalating dosage of the allergen at least three times during the administration period.

77. The method of any one of claims 1-76, wherein the treating results in desensitization to the allergen.

78. The method of claim 77, wherein the desensitization to the allergen is by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, about 70%, about 75%, at least about 80%, at least about 85%, or at least about 90% as compared to the subject prior to commencing the treatment, a subject receiving a placebo or a subject not receiving the treatment.

79. The method of any one of claims 1-78, wherein the treating results in a decrease in the number of allergen specific IgE antibodies in the subject, as compared to the number secreted prior to the treating.

80. The method of any one of claims 1-79, wherein the treating results in an increase in the number of allergen specific IgG antibodies in the subject, as compared to the number secreted prior to the treating.

81. The method of claim 80, wherein the subject is human and the allergen specific IgG antibodies are allergen specific IgG4 antibodies.

82. The method of any one of claims 1-81, wherein the treating results in a decreased number of mast cells in the subject, as compared to the number secreted prior to the treating.

83. The method of any one of claims 1-82, wherein the treating results in a decreased number of basophils in the subject, as compared to the number secreted prior to the treating.

84. The method of any one of claims 1-83, wherein the treating results in an increased cytokine production in the subject, as compared to the number secreted prior to the treating.

85. The method of claim 84, wherein the cytokine is IL-10.

86. The method of claim 84, wherein the cytokine is TGF-β.

87. The method of any one of claims 1-86, wherein the treating results in an increased number of T-regulatory cells in the subject, as compared to the number of T-regulatory cells prior to the treating.

88. The method of claim 87, wherein the T-regulatory cells are Tr1 cells, Th3, CD4+CD25+forkhead box P3:Foxp3+ T-regulatory cells, or a combination thereof.

89. The method of any one of claims 1-88, wherein the treating results in an increase in the eliciting dose of the allergen, as compared to the eliciting dose prior to initiation of treatment.

90. The method of claim 89, wherein the increase in the eliciting dose of the allergen is an increase by 10%, by 20%, by 30%, by 40%, by 50%, by 60%, by 70%, by 80%, by 90%, by 100%, by 500% or by 1000%.

91. The method of claim 89, wherein the increase in the eliciting dose of the allergen is an increase by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 80%, by at least about 90%, by at least about 100%, by at least about 500% or by at least about 1000%.

92. The method of any one of claims 1-91, wherein the treating results in a sustained unresponsiveness to the allergen.

93. The method of claim 92, wherein the sustained unresponsiveness lasts for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months or about 12 months after the final delivering step of the administration period.

94. The method of claim 89, wherein the sustained unresponsiveness lasts for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9

months, at least about 10 months, at least about 11 months or at least about 12 months after the final delivering step of the administration period.

95. The method of any one of claims 1-94, wherein the subject is a human subject.

96. The method of any one of claims 1-95, wherein the subject is from about 2 years old to about 12 years old.

97. The method of any one of claims 1-95, wherein the subject is from about 3 years old to about 12 years old.

98. The method of any one of claims 1-95, wherein the subject is from about 4 years old to about 12 years old.

99. The method of any one of claims 1-95, wherein the subject is from about 4 years old to about 11 years old.

100. The method of any one of claims 1-95, wherein the subject is from about 4 years old to about 10 years old.

101. The method of any one of claims 1-95, wherein the subject is from about 2 years old to about 9 years old.

102. The method of any one of claims 1-101, wherein the administration period is about 1 month, about 3 months, about 6 months, about 9 months, about12 months, about 15 months, about 18 months, about 24 months, about 27 months, about 30 months, about 33 months or about 36 months.

103. The method of any one of claims 1-101, wherein the administration period is at least about 1 month, at least about 3 months, at least about 6 months, at least about 9 months, at least about12 months, at least about 15 months, at least about 18 months, at least about 24 months, at least about 27 months, at least about 30 months, at least about 33 months or at least 36 months.

104. The method of any one of claims 1-101, wherein the administration period is from about 1 month to about 24 months.

105. The method of any one of claims 1-101, wherein the administration period is from about 1 month to about 18 months.

106. The method of any one of claims 1-101, wherein the administration period is from about 1 month to about 12 months.

107. The method of any one of claims 1-101, wherein the administration period is from about 3 months to about 12 months.

108. The method of any one of claims 1-101, wherein the administration period is from about 3 months to about 18 months.

109. The method of any one of claims 1-101, wherein the administration period is from about 6 months to about 18 months.

110. The method of any one of claims 1-109, wherein the administration period is the amount of time sufficient to achieve desensitization to the allergen being administered.

111. The method of any one of claims 1-110, wherein the administration period is the amount of time sufficient to achieve long term unresponsiveness to the allergen being administered.

112. A microneedle array comprising a plurality of solid microneedles extending from a common substrate, wherein each microneedle of the plurality has a base, shaft and tip, wherein at least one microneedle of the plurality is coated with an effective amount of a food allergen.

113. The microneedle array of claim 112, wherein the substrate is adhesive.

114. The microneedle array of claim 112 or 113, wherein the substrate is rigid.

115. The microneedle array of claim 112 or 113, wherein the substrate is flexible.

116. The microneedle array of any one of claims 112-115, wherein the average length of the plurality of microneedles in the array is from about 100 μm to about 1000 μm, as measured from the base of the tip.

117. The microneedle array of claim 116, wherein the average length of the plurality of microneedles in the array is from about 100 μm to about 900 μm.

118. The microneedle array of claim 116, wherein the average length of the plurality of microneedles in the array is from about 100 μm to about 800 μm.

119. The microneedle array of claim 116, wherein the average length of the plurality of microneedles in the array is from about 100 µm to about 700 µm.

120. The microneedle array of claim 116, wherein the average length of the plurality of microneedles in the array is from about 100 µm to about 600 µm.

121. The microneedle array of claim 116, wherein the average length of the plurality of microneedles in the array is from about 100 µm to about 500 µm.

122. The microneedle array of any one of claims 112-121, wherein the average width of the plurality of microneedles in the array, as measured at the widest cross section of each respective microneedle in the array, is from about 20 µm to about 500 µm.

123. The microneedle array of claim 122, wherein the average width of the plurality of microneedles, as measured at the widest cross section of each respective microneedle in the array, is from about 50 µm to about 350 µm, or from about 100 µm to about 250 µm.

124. The microneedle array of any one of claims 112-123, wherein the average aspect ratio (width:length) of the plurality of microneedles in the array is from about 1:1 and 1:10.

125. The microneedle array of any one of claims 112-124, wherein the microneedle array comprises from about 10 to about 200 microneedles.

126. The microneedle array of claim 125, wherein the microneedle array comprises from about 20 to about 150 microneedles.

127. The microneedle array of claim 125, wherein the microneedle array comprises from about 20 to about 150 microneedles.

128. The microneedle array of claim 125, wherein the microneedle array comprises from about 30 to about 100 microneedles.

129. The microneedle array of claim 125, wherein the microneedle array comprises from about 40 to about 100 microneedles.

130. The microneedle array of any one of claims 112-129, wherein the food allergen is a groundnut, peanut, milk, egg, tree nut, seed, fish, shellfish, crustacean, cereal, legume allergen, or a combination thereof.

131. The microneedle array of any one of claims 112-129, wherein the food allergen is a peanut allergen.

132. The microneedle array of claim 130, wherein the food allergen is a seed allergen.

133. The microneedle array of claim 132, wherein the seed allergen is a hazelnut, cashew, walnut, pecan, brazil nut, macadamia, chestnut, pistachio, coconut, almond, sesame, or a mustard seed allergen, or a combination thereof.

134. The microneedle array of claim 130, wherein the food allergen is a legume allergen.

135. The microneedle array of claim 134, wherein the legume allergen is a soy, kidney bean, black bean, common bean, chickpea, pea, cow pea, lentil or lupine allergen, or a combination thereof.

136. The microneedle array of claim 131, wherein the peanut allergen comprises Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara h6, Ara h7, Ara h8, Ara h9, Ara h10, Ara h11, Ara h12, Ara h13, a peptide fragment thereof, or a combination thereof.

137. The microneedle array of claim 131, wherein the peanut allergen comprises Ara h1, Ara h2, Ara h6, a peptide fragment thereof, or a combination thereof.

138. The microneedle array of any one of claims 131 and 136-137, wherein the peanut allergen is provided as peanut protein extract.

139. The microneedle array of any one of claims 112-138, wherein at least one microneedle of the plurality comprises an adjuvant.

140. The microneedle array of claim 139, wherein the adjuvant is alum.

141. The microneedle array of claim 139, wherein the adjuvant is L-Tyrosine.

142. The microneedle array of claim 139, wherein the adjuvant is monophoshoryl lipid A (MPL).

143. The microneedle array of claim 139, wherein the adjuvant is L-Tyrosine and monophoshoryl lipid A (MPL).

144. The microneedle array of claim 139, wherein the adjuvant is an aluminium salt, inulin, L-Tyrosine, algammulin, combination of inulin and aluminium hydroxide, monophosphoryl lipid A (MPL), L-Tyrosine in combination with MPL, resiquimoid, muramyl dipeptide (MDP), N-glycolyl dipeptide (GMDP), poly IC, CpG oligonucleotide, resiquimod, aluminium hydroxide with MPL or a water in oil emulsion

145. The microneedle array of claim 144, wherein the adjuvant is a water in oil emulsion.

146. The microneedle array of claim 145, wherein the water in oil emulsion contains one or more of squalene or its analogues or any pharmaceutically acceptable oil, tween-80, sorbitan trioleate, alpha-tocopherol, cholecalciferol, calcium phosphate or a combination two or more of the foregoing.

147. The microneedle array of claim 145, wherein the adjuvant is complete Freund’s adjuvant.

148. The microneedle array of claim 145, wherein the adjuvant is incomplete Freund’s adjuvant.

149. The microneedle array of claim 139, wherein the adjuvant is *Corynebacterium parvum, Bacillus Calmette Guerin*, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, a synthetic polymer such as a poly amino acid or a co-polymer of amino acids, saponin, Avridine (N, N-dioctadecyl-N′,N′-bis(2-hydroxyethyl)-propanediamine), paraffin oil, muramyl dipeptide or a combination thereof.

150. The microneedle array of claim 139, wherein the adjuvant is a CpG oligonucleotide (ODN).

151. The microneedle array of claim 139, wherein the adjuvant is alum; a CpG oligonucleotide (ODN); polyA-polyU; dimethyldioctadecylammonium bromide (DDA), N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine, carbomer or chitosan.

152. The microneedle array of claim 151, wherein the adjuvant is N ,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine.

153. The microneedle array of claim 139, wherein the adjuvant is a stimulator of interferon genes (STING) ligand adjuvant.

154. The microneedle array of claim 153, wherein the STING adjuvant is a cyclic dinucleotide or a xanthenone derivative.

155. The microneedle array of claim 154, wherein the STING ligand is a xanthenone derivative.

156. The microneedle array of claim 154, wherein the STING ligand is a cyclic dinucleotide.

157. The microneedle array of claim 156, wherein the STING ligand is cyclic di-GMP (c-diGMP).

158. The microneedle array of claim 156, wherein the STING ligand is cyclic-di-AMP (c-di-AMP).

159. The microneedle array of claim 156, wherein the STING ligand is cyclic-GMP-AMP (cGAMP).

160. The microneedle array of claim 159, wherein the STING ligand 2'2'-cGAMP, 2'3'-cGAMP or 3'3'-cGAMP.

161. The microneedle array of claim 153, wherein the STING ligand is cyclic guanosine monophosphate (cGMP).

162. The microneedle array of claim 153, wherein the STING ligand is cyclic adenosine monophosphate (cAMP).

163. The microneedle array of claim 139, wherein the adjuvant is IFN-γ.

164. The microneedle array of any one of claims 139-163, wherein the allergen and adjuvant are coated on different microneedles.

165. The microneedle array of claim 164, wherein the allergen coated microneedles and adjuvant coated microneedles are present in separate microneedle rows.

166. The microneedle array of any one of claims 139-163, wherein at least a portion of the microneedles of the plurality comprise both an allergen coating and an adjuvant coating.

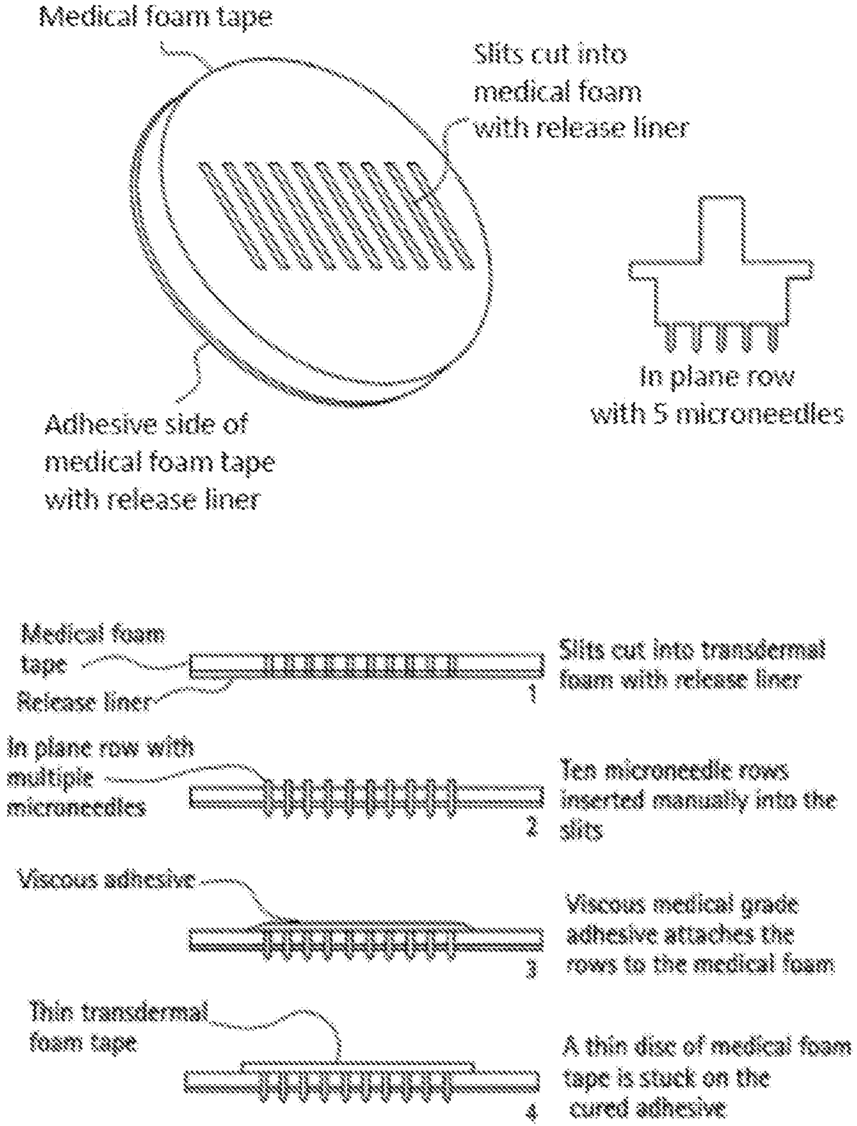
Medical foam tape
Slits cut into medical foam with release liner
In plane row with 5 microneedles
Adhesive side of medical foam tape with release liner
Medical foam tape
Release liner
1
Slits cut into transdermal foam with release liner
In plane row with multiple microneedles
2
Ten microneedle rows inserted manually into the slits
Viscous adhesive
3
Viscous medical grade adhesive attaches the rows to the medical foam
Thin transdermal foam tape
4
A thin disc of medical foam tape is stuck on the cured adhesive

FIG. 1

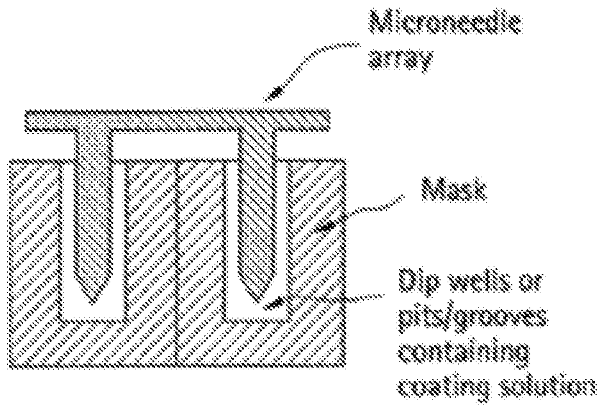
Microneedle
array
Mask
Dip wells or
pits/grooves
containing
coating solution

FIG. 2

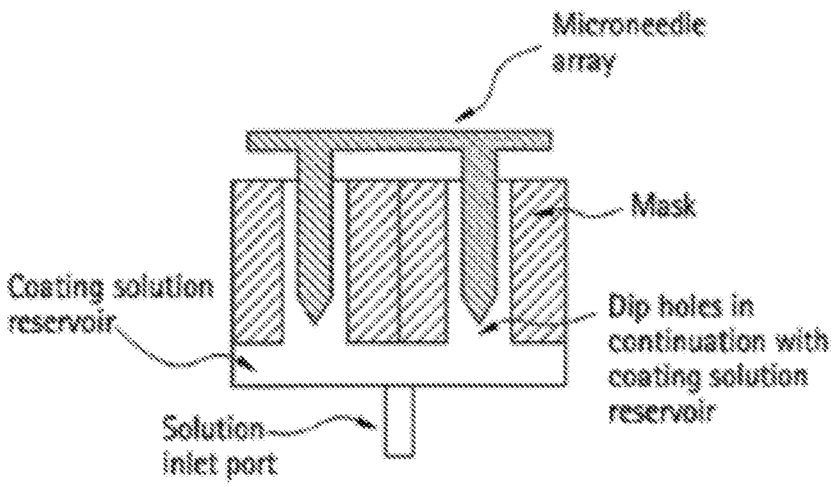
Microneedle
array
Mask
Coating solution
reservoir
Dip holes in
continuation with
coating solution
reservoir
Solution
inlet port

FIG. 3

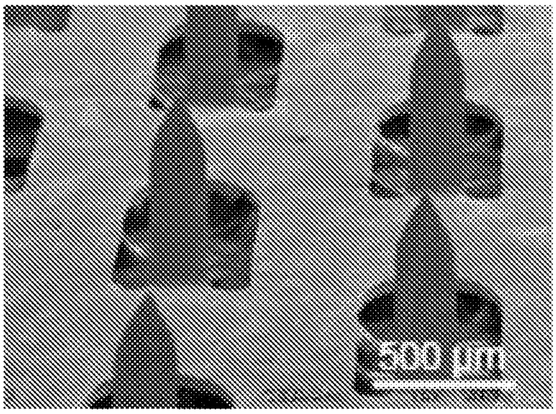

FIG. 4

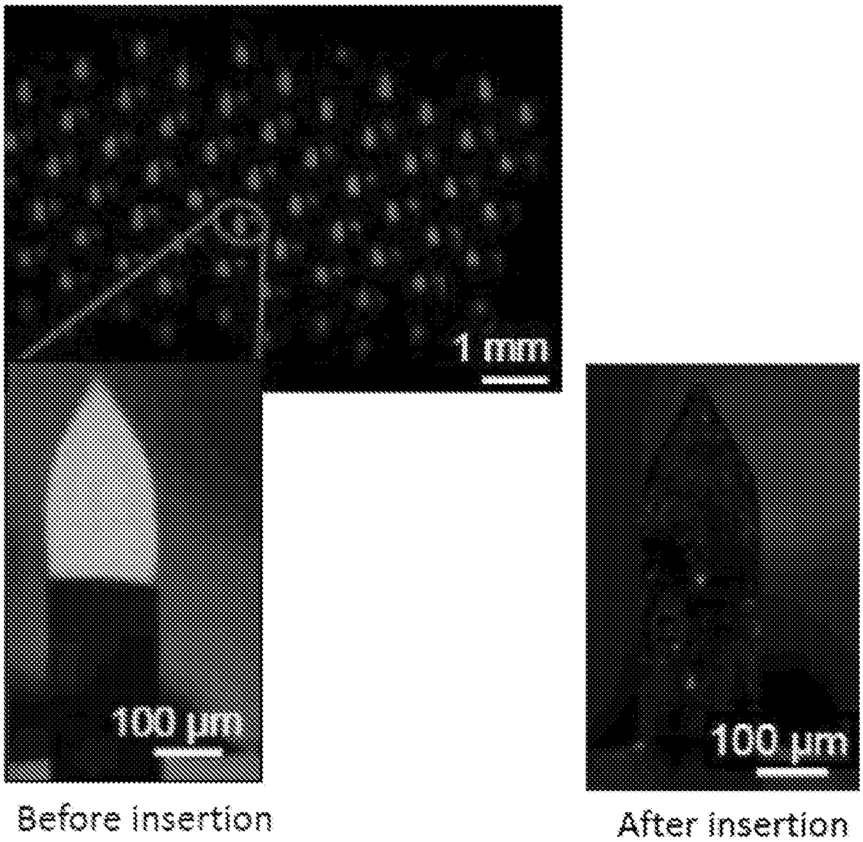

FIG. 5

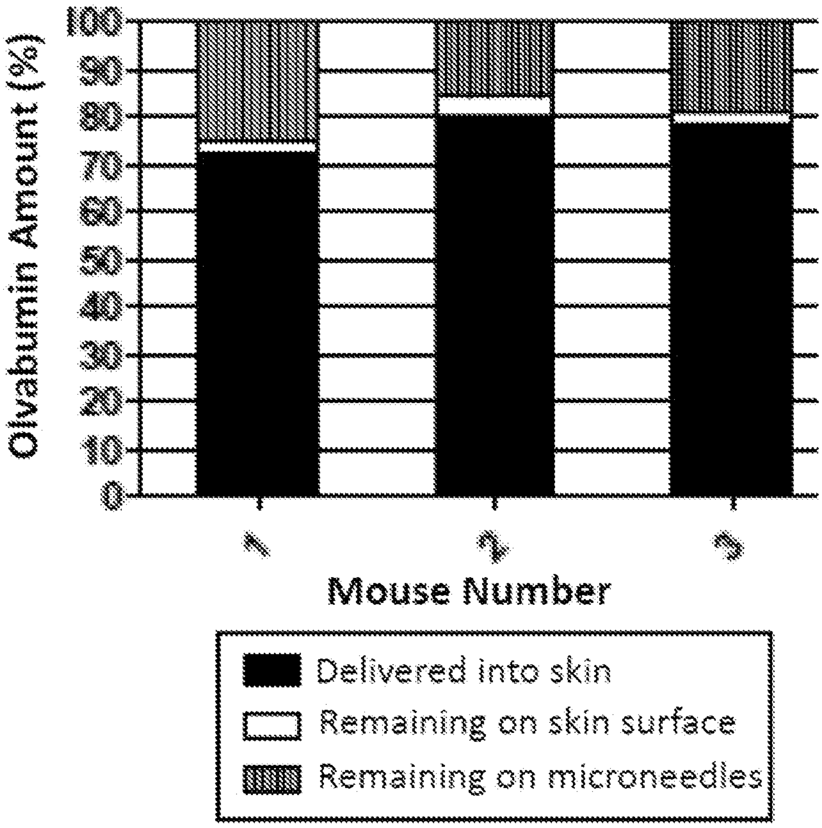
Olvabumin Amount (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
Mouse Number
Delivered into skin
Remaining on skin surface
Remaining on microneedles

FIG. 6

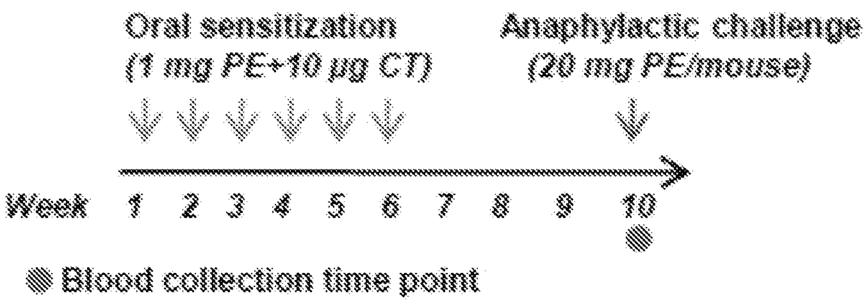
Oral sensitization
(1 mg PE+10 µg CT)
Anaphylactic challenge
(20 mg PE/mouse)
Week 1 2 3 4 5 6 7 8 9 10
Blood collection time point

FIG. 7

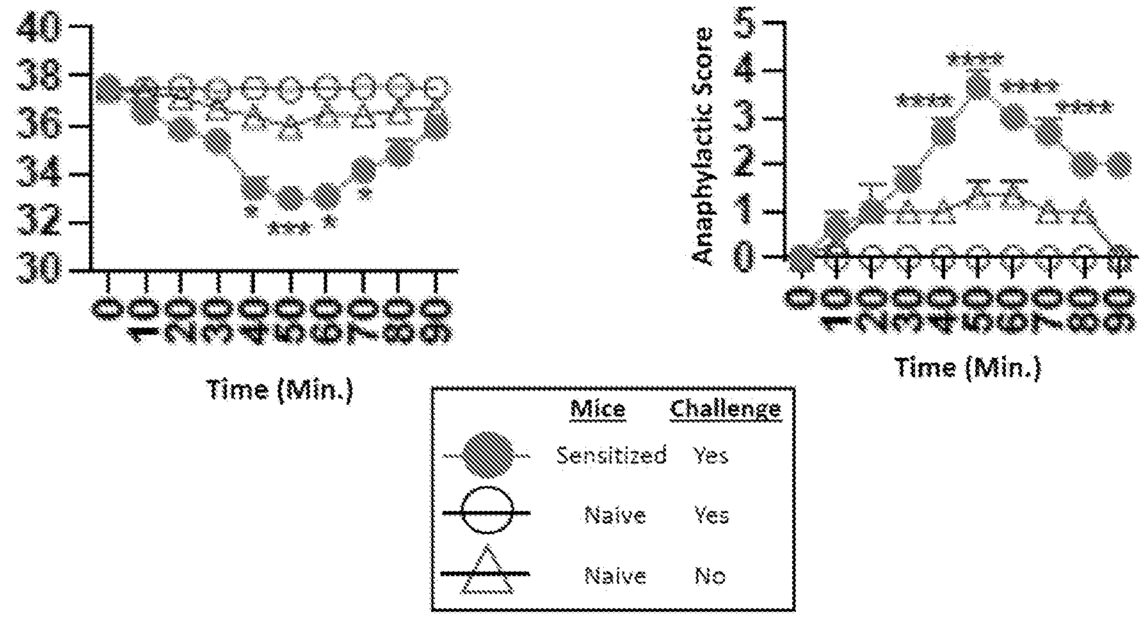
40
38
36
34
32
30
0
10
20
30
40
50
60
70
80
90
Time (Min.)
Anaphylactic Score
5
4
3
2
1
0
Time (Min.)
Mice
Challenge
Sensitized
Yes
Naive
Yes
Naive
No

FIG. 8

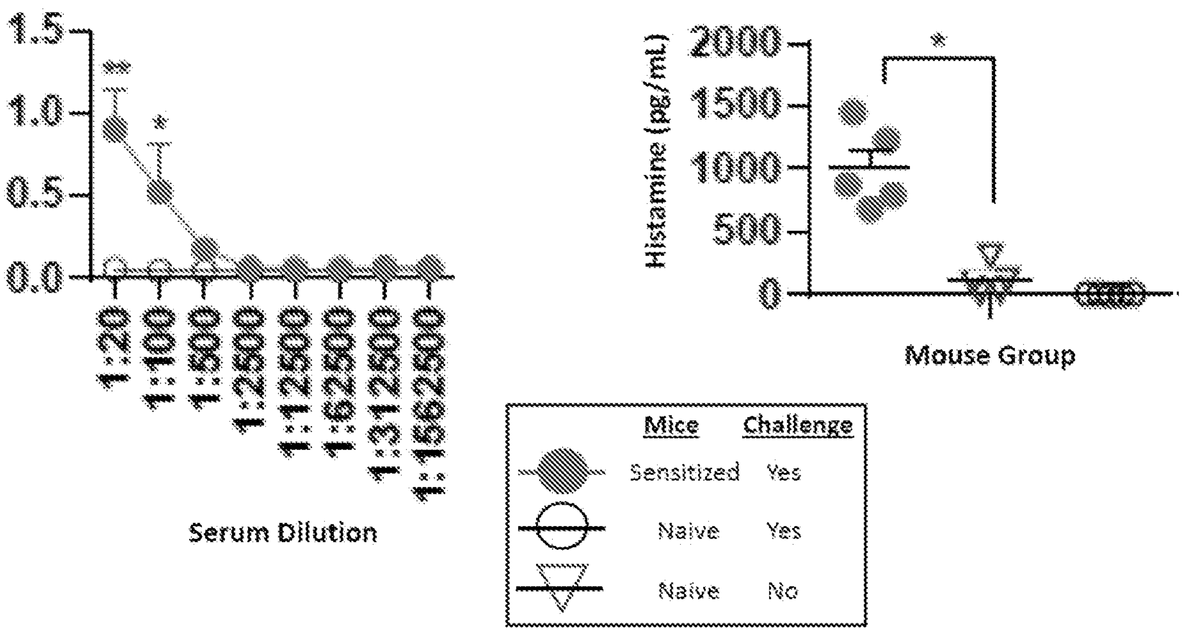
1.5
1.0
0.5
0.0
1:20
1:100
1:500
1:2500
1:12500
1:62500
1:312500
1:1562500
Serum Dilution
Histamine (pg/mL)
2000
1500
1000
500
0
Mouse Group
Mice
Challenge
Sensitized
Yes
Naive
Yes
Naive
No

FIG. 9

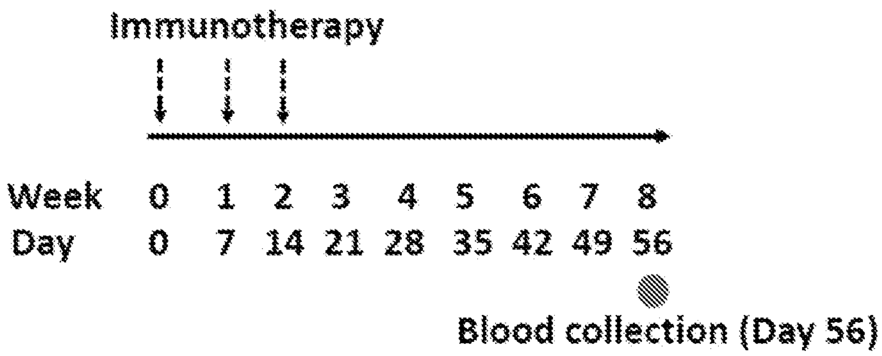
Immunotherapy
Week 0 1 2 3 4 5 6 7 8
Day 0 7 14 21 28 35 42 49 56
Blood collection (Day 56)

FIG. 10

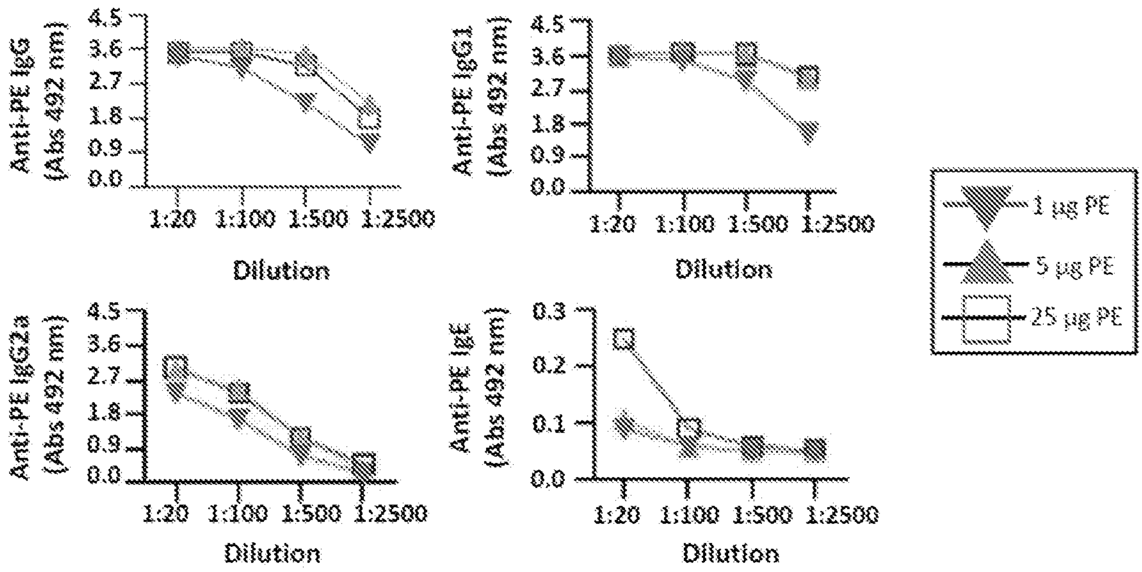
Anti-PE IgG (Abs 492 nm)
Anti-PE IgG1 (Abs 492 nm)
Anti-PE IgG2a (Abs 492 nm)
Anti-PE IgE (Abs 492 nm)
4.5 3.6 2.7 1.8 0.9 0.0
0.3 0.2 0.1 0.0
1:20 1:100 1:500 1:2500
Dilution
1 μg PE
5 μg PE
25 μg PE

FIG. 11

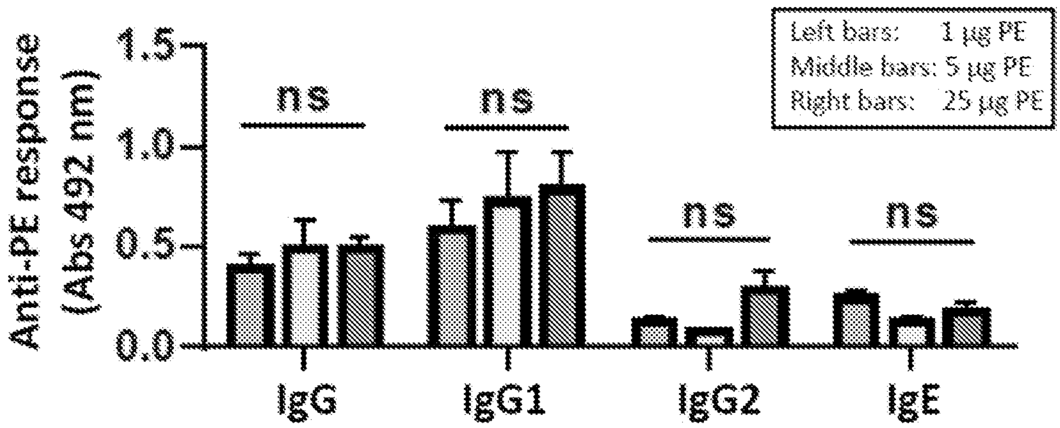

FIG. 12

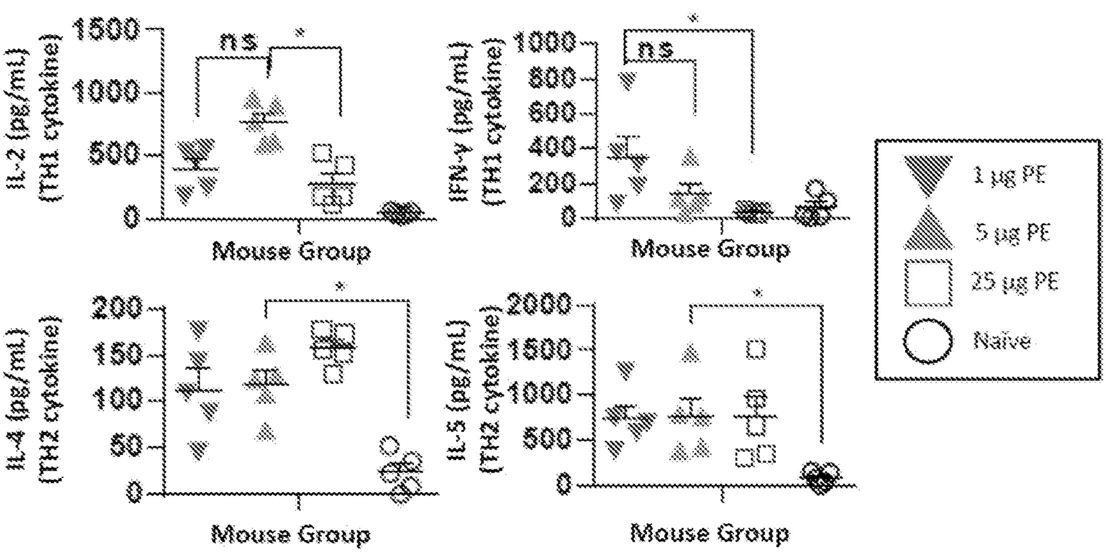

FIG. 13

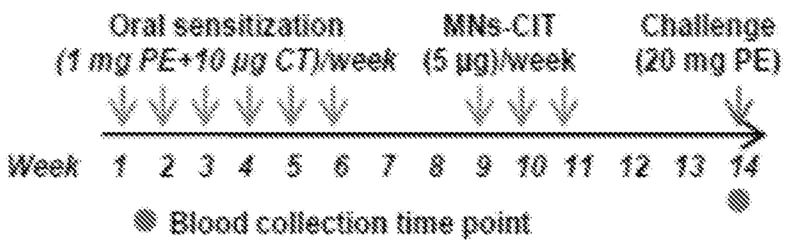

FIG. 14

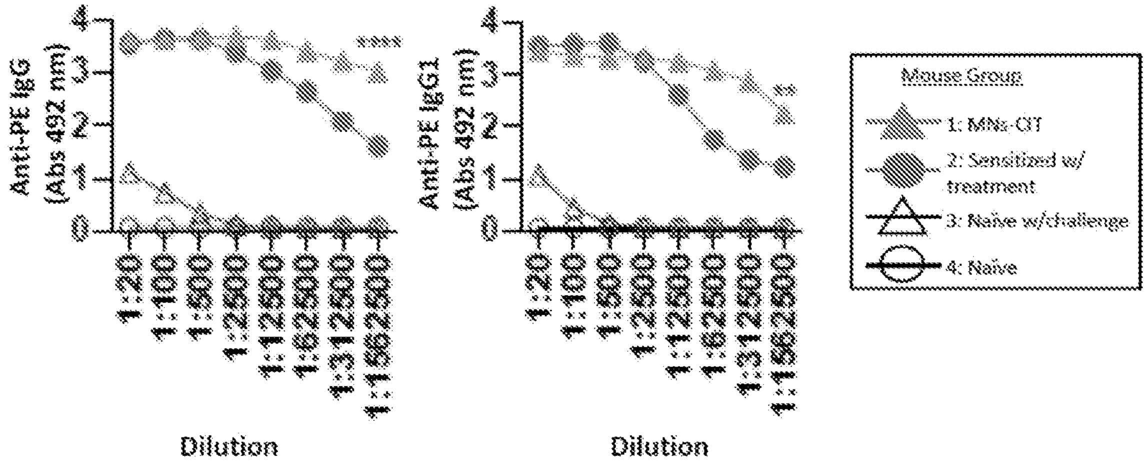
Anti-PE IgG (Abs 492 nm)
****
1:20
1:100
1:500
1:2500
1:12500
1:62500
1:312500
1:1562500
Dilution
Anti-PE IgG1 (Abs 492 nm)
**
Dilution
Mouse Group
1: MNs-CIT
2: Sensitized w/ treatment
3: Naive w/challenge
4: Naive

FIG. 15

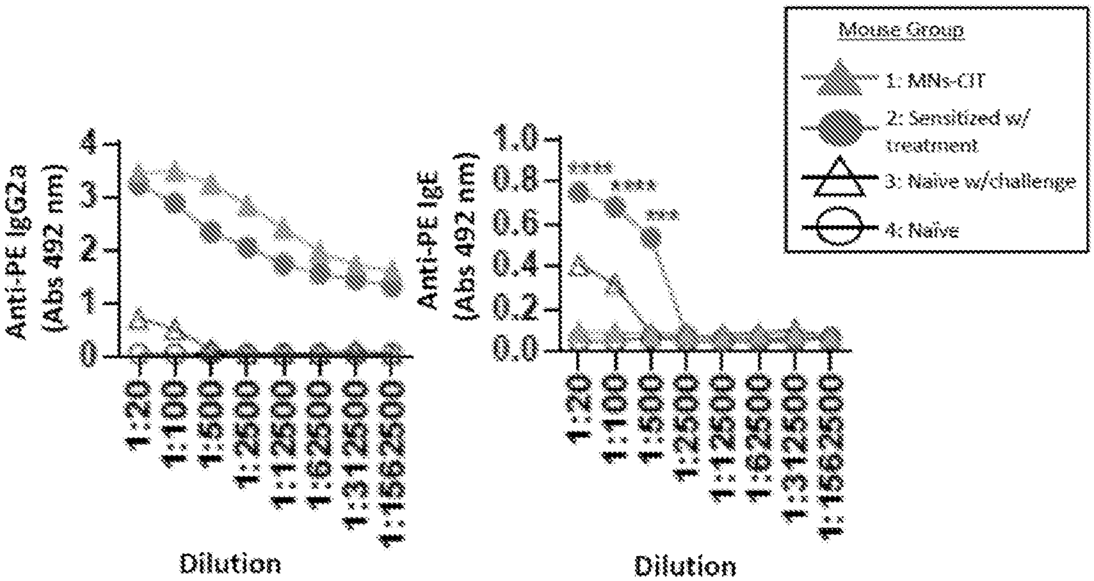
Mouse Group
1: MNs-CIT
2: Sensitized w/ treatment
3: Naive w/challenge
4: Naive
Anti-PE IgG2a (Abs 492 nm)
1:20
1:100
1:500
1:2500
1:12500
1:62500
1:312500
1:1562500
Dilution
Anti-PE IgE (Abs 492 nm)
****
****
***
Dilution

FIG. 16

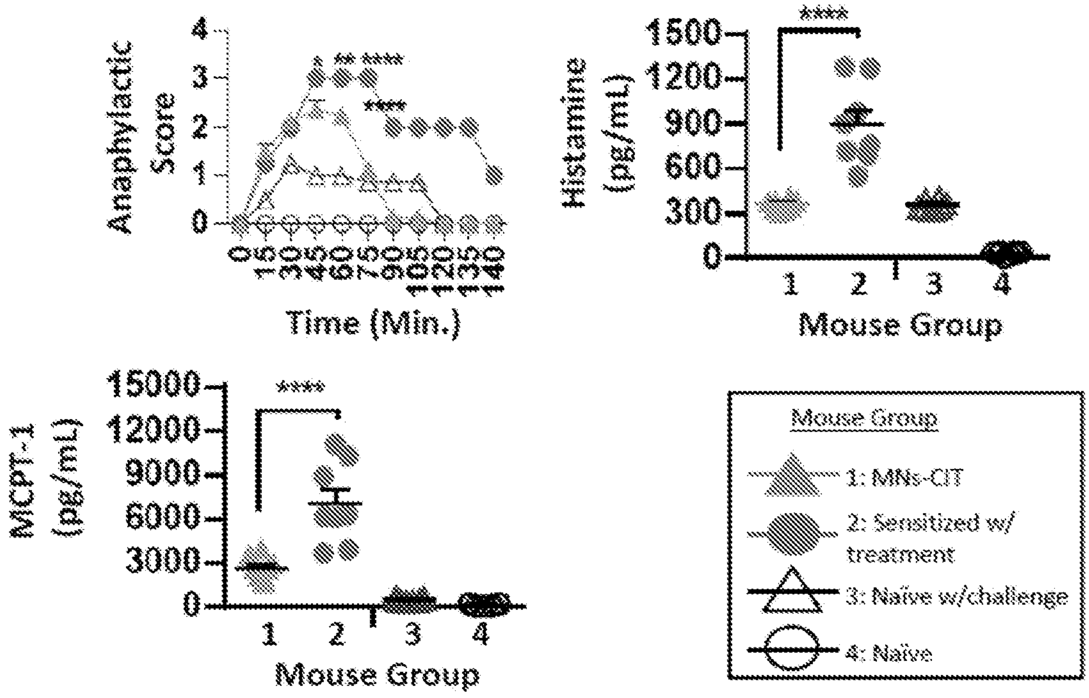
Anaphylactic Score
Time (Min.)
Histamine (pg/mL)
Mouse Group
MCPT-1 (pg/mL)
Mouse Group
Mouse Group
1: MNs-CIT
2: Sensitized w/ treatment
3: Naïve w/challenge
4: Naïve

FIG. 17

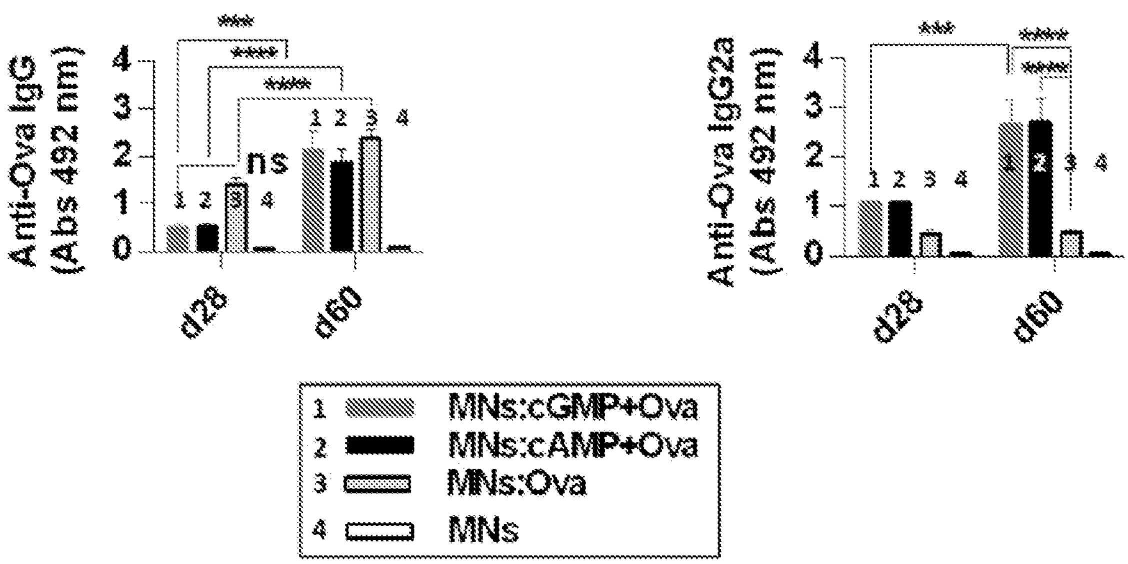
Anti-Ova IgG (Abs 492 nm)
ns
d28
d60
Anti-Ova IgG2a (Abs 492 nm)
d28
d60
1 MNs:cGMP+Ova
2 MNs:cAMP+Ova
3 MNs:Ova
4 MNs

FIG. 18

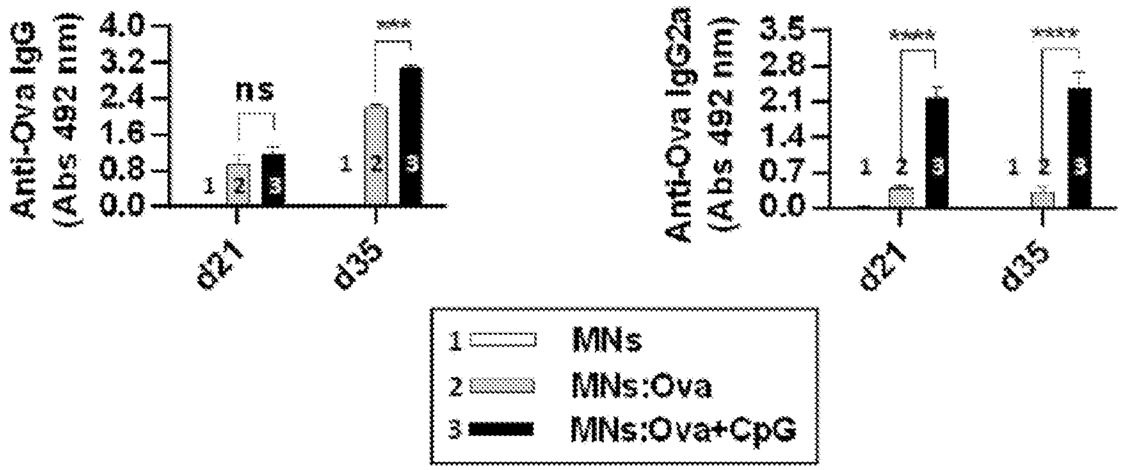

FIG. 19

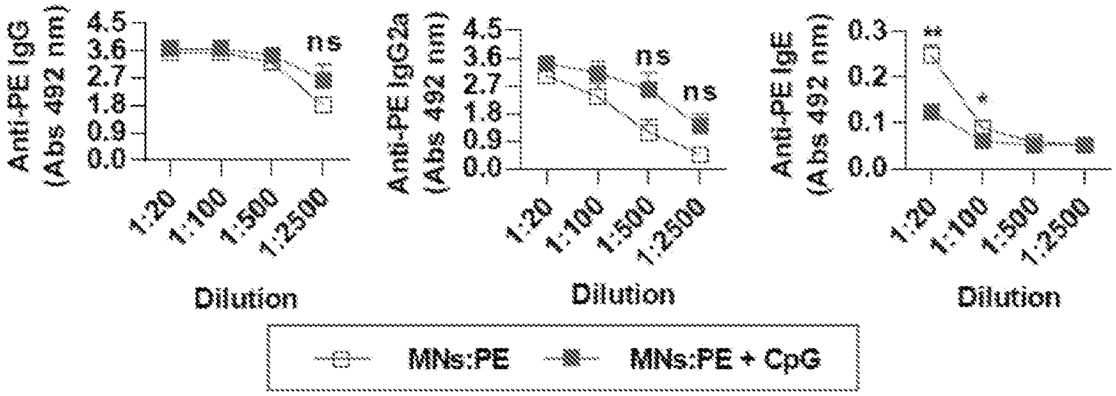

FIG. 20

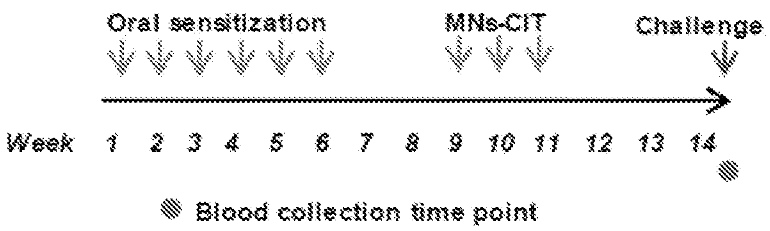

FIG. 21

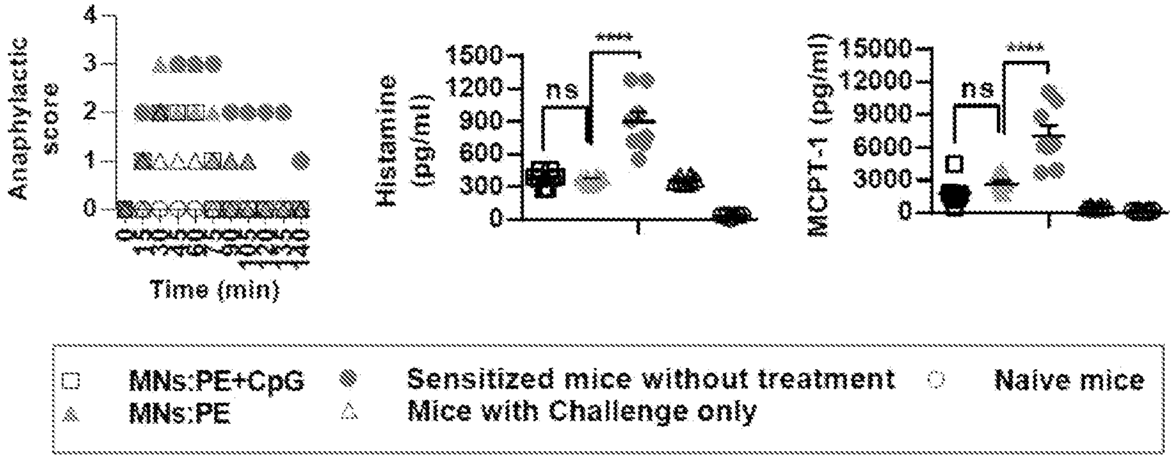
Anaphylactic score
Time (min)
Histamine (pg/ml)
ns
****
MCPT-1 (pg/ml)
ns
****
MNs:PE+CpG
MNs:PE
Sensitized mice without treatment
Mice with Challenge only
Naive mice

FIG. 22

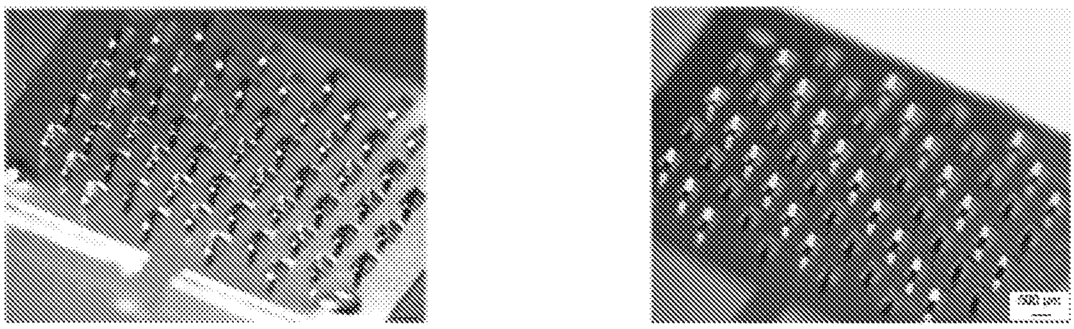

FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/035399

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/35 (2006.01) A61K 39/39 (2006.01) A61M 5/158 (2006.01) A61P 37/00 (2006.01) A61P 37/02 (2006.01)

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)

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)

PATENW, MEDLINE, CAPLUS, BIOSIS, EMBASE, FSTA: Food, peanut, nut, milk, dairy, egg, seed, fish, shellfish, crustacean, cereal, grain, wheat, legume, cashew, pecan, macadamia, pistachio, almond, sesame, mustard, soy, bean, pea, lentil, lupine, allergen, immunogen, microneedle, micropillar, microlancer, dermaroller, dermapen, viaskin, adminpatch and similar terms, A61K 39/35, A61M 5/158, A61K 39/39. **Internal databases provided by IP Australia, PubMed, Espacenet:** Applicant/Inventors and keywords search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C   [X] See patent family annex

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2018	01 August 2018

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
Email address: pct@ipaustralia.gov.au

Authorised officer

Lauren Howitt
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. +61262256130

INTERNATIONAL SEARCH REPORT	International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/US2018/035399

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SHAKYA, A.K. et al., 'Cutaneous vaccination with coated microneedles prevents development of airway allergy', Journal of Controlled Release. Published online 15 August 2017, Vol. 265, pages 75-82 See Abstract, Materials and methods, Figure 1, Table 1, Results and discussion, Conclusion	1-166
X	SHAKYA, A.K. et al., 'A comparative study of microneedle-based cutaneous immunization with other conventional routes to assess feasibility of microneedles for allergy immunotherapy', Vaccine. 2015, Vol. 33, No. 33, pages 4060-4064 See Abstract, Materials and methods, Figure 1, Discussion	1-166
X	WO 2014/182932 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 13 November 2014 See page 2 [0009] – page 3 [0010], [0017], [0020], [0040]-[0043], [0055]-[0060], [00101], [00110], Figures 1-2	112-138
X	WO 2013/033400 A2 (PEROSPHERE INC.) 07 March 2013 See Abstract, page 4, lines 3-9, page 6, lines 1-17, page 10, lines 3-25, page 11, line 17 – page 13, line 10, Example 1, claims 11-12	1-166
A	US 2004/0047902 A1 (DUPONT et al.) 11 March 2004 See Abstract, [0005], [0008], Examples 1-2	1-166
A	US 2010/0260821 A1 (DUPONT et al.) 14 October 2010 See Abstract, [0048], [0077]-[0078], Examples 1-3	1-166
A	US 2007/0161964 A1 (YUZHAKOV) 12 July 2007 See Abstract, [0061]	112-166

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   There was a sequence listing originally filed but it was not used for the purposes of this search and opinion.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/035399**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2014/182932 A1	13 November 2014	WO 2014182932 A1	13 Nov 2014
		US 2016058377 A1	03 Mar 2016
WO 2013/033400 A2	07 March 2013	WO 2013033400 A2	07 Mar 2013
		AU 2012301814 A1	27 Feb 2014
		AU 2018200963 A1	01 Mar 2018
		CA 2846858 A1	07 Mar 2013
		EP 2750705 A2	09 Jul 2014
		JP 2014525458 A	29 Sep 2014
		US 2014207105 A1	24 Jul 2014
		US 2017209567 A1	27 Jul 2017
US 2004/0047902 A1	11 March 2004	US 2004047902 A1	11 Mar 2004
		US 7722897 B2	25 May 2010
		AU 2002242807 B2	16 Sep 2004
		AU 2007242731 A1	01 Nov 2007
		AU 2007242731 B2	23 Aug 2012
		BR PI0710928 A2	31 May 2011
		CA 2438325 A1	19 Sep 2002
		CA 2650266 A1	01 Nov 2007
		CN 1496239 A	12 May 2004
		CN 101484075 A	15 Jul 2009
		EA 200301003 A1	26 Feb 2004
		EA 005143 B1	30 Dec 2004
		EA 200870478 A1	28 Apr 2009
		EA 014504 B1	30 Dec 2010
		EP 1367944 A1	10 Dec 2003
		EP 1367944 B1	13 Oct 2004
		EP 2010063 A2	07 Jan 2009
		FR 2822049 A1	20 Sep 2002
		FR 2822049 B1	01 Aug 2003
		HK 1065239 A1	15 Sep 2006
		IL 194882 A	30 Jul 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/035399**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		JP 2004527290 A	09 Sep 2004
		JP 4177110 B2	05 Nov 2008
		JP 2009534145 A	24 Sep 2009
		JP 5243408 B2	24 Jul 2013
		KR 20030082966 A	23 Oct 2003
		KR 100849757 B1	31 Jul 2008
		KR 20090049038 A	15 May 2009
		KR 101405332 B1	10 Jun 2014
		MX 2008013688 A	11 May 2009
		NZ 572804 A	30 Mar 2012
		US 2007048361 A1	01 Mar 2007
		US 7635488 B2	22 Dec 2009
		WO 02071950 A1	19 Sep 2002
		WO 2007122226 A2	01 Nov 2007
		ZA 200809988 B	30 Dec 2009

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/035399**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2010/0260821 A1	14 October 2010	US 2010260821 A1	14 Oct 2010
		US 9539318 B2	10 Jan 2017
		AU 2008333192 A1	11 Jun 2009
		AU 2008333192 B2	20 Mar 2014
		AU 2008339833 A1	02 Jul 2009
		AU 2008339833 B2	03 Apr 2014
		CA 2706020 A1	02 Jul 2009
		CA 2707013 A1	11 Jun 2009
		CN 101883584 A	10 Nov 2010
		CN 101883584 B	31 Jul 2013
		CN 101951952 A	19 Jan 2011
		CN 101951952 B	13 Apr 2016
		EP 2214704 A2	11 Aug 2010
		EP 2214704 B1	27 Nov 2013
		EP 2217272 A1	18 Aug 2010
		EP 2217272 B1	07 Sep 2016
		FR 2924349 A1	05 Jun 2009
		FR 2924349 B1	01 Jan 2010
		IL 205822 A	31 Mar 2016
		IL 206028 A	31 Jul 2017
		JP 2011505410 A	24 Feb 2011
		JP 5732681 B2	10 Jun 2015
		JP 2011505413 A	24 Feb 2011
		JP 5941246 B2	29 Jun 2016
		KR 20100103512 A	27 Sep 2010
		KR 101561986 B1	20 Oct 2015
		KR 20110015408 A	15 Feb 2011
		KR 101788226 B1	26 Oct 2017
		US 2010136093 A1	03 Jun 2010
		US 8202533 B2	19 Jun 2012
		US 2017128566 A1	11 May 2017
		US 10022439 B2	17 Jul 2018
		WO 2009071599 A1	11 Jun 2009
		WO 2009080934 A2	02 Jul 2009
US 2007/0161964 A1	12 July 2007	US 2007161964 A1	12 Jul 2007

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/035399**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 7658728 B2	09 Feb 2010
		EP 1973479 A2	01 Oct 2008
		EP 1973479 B1	28 Sep 2016
		US 2010130940 A1	27 May 2010
		US 8414548 B2	09 Apr 2013
		US 2013218083 A1	22 Aug 2013
		WO 2007081430 A2	19 Jul 2007

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.