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(54) Title: REMOVABLE TATTOO INK AND METHOD OF PRODUCING SAME

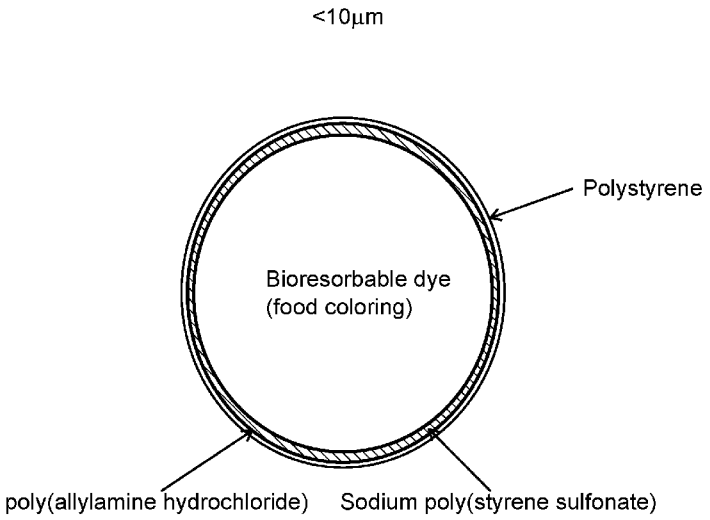


FIGURE. 1

(57) Abstract: Embodiments disclosed herein provide a method of making removable tattoo ink that is composed of colored microparticles that create permanent tissue markings, such as tattoos. The microparticles include an inner core housing a bioabsorbable chromophore and an outer shell, which includes polystyrene sulfonate and polyallylamine hydrochloride and is designed for rupture with external energy sources, such as ultrasonic energy. The microparticles can be implanted in the tissue of a subject, for example to create a tattoo and be ruptured *in situ* by the application of an external energy source, such as ultrasonic energy, to remove the tattoo.

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# REMOVABLE TATTOO INK AND METHOD OF PRODUCING SAME

## TECHNICAL FIELD

**[0001]** This disclosure relates generally to a new method of producing hollow microparticles capable of being ruptured upon the exposure to an energy source, including but not limited to ultrasonic energy, laser light operating in the near infrared, radio frequency ablation, radio frequency cryoablation, or ultraviolet light. The present invention more particularly relates to removable tattoo ink. More specifically, this disclosure relates to a method of producing removable tattoo ink comprising bio-absorbable colored microparticles having a polymer shell that is rupturable upon application of external energy sources.

## BACKGROUND

**[0002]** Tattooing involves the placement of pigment into the skin's dermis, the layer of dermal tissue underlying the epidermis. After initial injection, pigment is dispersed throughout a homogenized damaged layer down through the epidermis and upper dermis. As healing proceeds, the damaged epidermis flakes away (eliminating surface pigment) while deeper in the skin granulation tissue forms, which is later converted to connective tissue by collagen growth. This mends the upper dermis, where pigment remains trapped within fibroblasts, ultimately concentrating in a layer just below the dermis/epidermis boundary.

**[0003]** Several health issues related to embedding different pigments into the skin are already recognized, such as toxicity, especially for heavy metal pigments; potential carcinogenic risk; the slow dissolution of pigments; the photoreactivity of organic dyes; and the formation of dangerous radicals by titanium dioxide ($TiO_2$) pigments. These problems arise mainly as a result of direct contact between the dangerous compound or its surfaces with the tissue of the dermis, leading to an immune response in the body. Besides health risks, there are also several problems in the quality of tattooing, arising mainly from insufficient long-term stability of the tattoo inks. Insufficient stability of tattoo inks often leads to fading and shifts of color tones over time, which requires reapplication of the pigment. Several factors determine the instability of pigments in the skin, such as dissolution, chemical degradation, photobleaching and enzymatic degradation or bioclearance by the phagocytes of the immune system. Phagocytes take up foreign particles according to

recognition of the particles as being dangerous for the body. This recognition is based on the physicochemical surface properties and the size of particles. After uptake of those particles, the phagocytes can leave the skin in order to remove the pigment from the organism, which leads to fading.

**[0004]** The most common method of tattooing in modern times is the electric tattoo machine, which inserts ink into the skin via a single needle or a group of needles that are soldered onto a bar, which is attached to an oscillating unit. The unit rapidly and repeatedly drives the needles in and out of the skin, usually 80 to 150 times a second.

**[0005]** A significant number of individuals have tattoos making up approximately 15% of the world population. The demand for tattoos continues to increase primarily for body art adornment. Tattoos are also used for religious, cultural, and medical indications. Sixteen percent of tattoo consumers have been shown to eventually regret their tattoos, which is approximately 6.5 million people in the U.S. (Corso RA. Three in Ten Americans with Tattoo Say Having One Makes Them Feel Sexier. The Harris Poll. 2008). More than half of tattoo users are in the age group of 25-40 years of age with no significant difference between the number of male and female tattoo consumers (Corso RA. Three in Ten Americans with Tattoo Say Having One Makes Them Feel Sexier. The Harris Poll. 2008).

**[0006]** Tattoo removal methods have included overtattooing without ink, laser therapy, dermabrasion, and surgical excision, all of which usually leave unacceptable appearance and/or scarring.

**[0007]** Currently, the most common and most effective technique for tattoo removal is high energy lasers. However, laser treatments require a long period of time and multiple sessions for substantial removal and a cost up to $10,000 (Millennials' Judgments about Recent Trends Not So Different. Pew Research Center for the People and the Press. 2010). Laser treatments are also painful and have poor results often resulting in discoloration, raised or thickened burn scars, and residual smudged tattoos that are incompletely removed. According to the 2010 statistics of the U.S. Census Bureau, there were 1,430 establishments in the U.S. in the category of "tattoo services" with total revenue of about $200 million (2007 Economic Census. U.S. Census Bureau. Updated 2010. Accessed June 27, 2011). However, there are only 88 establishments in the category of "tattoo removal services" with revenue of $6 million

(2007 Economic Census. U.S. Census Bureau. Updated 2010. Accessed June 27, 2011).

**[0008]** Since current tattoo removal methods have not been overly successful, developing a non-permanent tattoo ink that is easily removable by a less expensive and painless removal method may be an optimal solution. Consumers should have the option of leaving no reminiscence of their tattoos while the manufacturers, tattoo artists, and doctors can profit financially from the popularity of this new ink. Therefore, there exists a need for tattoo inks that are amenable to removal. This disclosure meets this need.

## SUMMARY OF THE INVENTION

**[0009]** Some of the above-described problems can be eliminated by encapsulating the pigments in polymer shells, thus preserving their size and color but unifying and optimizing their surface properties. A thin, transparent, and homogenous coating with a thickness in the nanometer range is needed for the polymer shells. A viable solution is based on layer-by-layer (LbL) technology. The LbL process was developed on planar surfaces in 1966. Now, LbL technology is the subject of intensive research on many different applications, such as corrosion control, biomedical applications, and material science applications, including drug delivery, catalysis, and medicine.

**[0010]** LbL technology allows for charged surfaces to be coated with polymer films and for the thickness of these films to be controlled on the nanometer scale. This is demonstrated by coating a pigment to generate a negatively charged surface. If an aqueous solution of a positively charged polyelectrolyte, such as polyallylamine hydrochloride (PAH), is applied to the pigment, the PAH will be adsorbed onto the surface due to electrostatic attraction and an increase in entrophy. Not only are the negative charges on the pigment surface neutralized by the adsorption but also the pigment surface is reversed to a positive value. When a defined positive potential is achieved, the adsorption stops due to repulsion of further PAH molecules in the solution. After rinsing, excess PAH is removed and the process can be repeated by assembling a polyanion, such as polystyrene sulfonate (PSS), in a similar manner, which changes the surface potential back to a negative value. This can be repeated many times, yielding a constant thickness of approximately 4 nm for each double layer of PAH/PSS. A variety of more than 50 polyanions and also polycations with

different properties and functionalities are commercially available, giving this technology potential to easily modify the surface property of microparticles.

**[0011]** There exists a large demand for tattoo ink that can be easily removed, is inexpensive, and is a painless alternative to current laser removal. The present disclosure meets that need by providing UltraInk™ a new tattoo ink formed by encapsulation of bio-absorbable chromophores, such as food coloring, within a transparent, translucent or opaque outer shell that can be ruptured by the application of external energy sources, such as ultrasonic energy, laser light operating in the near infrared, radio frequency, or ultraviolet light. The resultant microparticles have the appearance, consistency, color and microscopic size similar to conventional tattoo ink.

**[0012]** Disclosed herein is removable tattoo ink, comprising colored microparticles. In some embodiments, the microparticles comprise an internal core, comprising one or more bio-absorbable chromophores and an outer shell, comprising at least one layer of polystyrene sulfonate (PSS) and at least one layer of polyallylamine hydrochloride (PAH). In certain example embodiments, the one or more bio-absorbable chromophores may comprise one or more of the outer shells or located between one or more layers of the outer shell. This outer shell is configured to be rupturable by the application of external energy sources, such as ultrasonic energy, laser light operating in the near infrared, radio frequency, or ultraviolet light. In some embodiments, the outer shell is further coated with polystyrene. The outer shell and the optional coating were developed to be substantially visibly transparent such that the chromophore is detectable through the outer shell and/or coating.

**[0013]** Further disclosed are methods of making the removable tattoo ink. In one embodiment, the method comprises forming an internal core of calcium carbonate ($CaCO_3$), coating the internal core with at least one layer of polystyrene sulfonate (PSS) and at least one layer of polyallylamine hydrochloride (PAH), dissolving the internal core of calcium carbonate to form hollow microparticles, loading one or more bio-absorbable chromophores into the hollow microparticles, and heating the microparticles to permanently entrap the bio-absorable chromophores.

**[0014]** In another embodiment, the method of making the removable tattoo ink comprises using a layer-by-layer core synthesis procedure, wherein the layers surrounding the core are alternating layers of oppositely charged polyelectrolytes. In

a preferred embodiment, the alternating layers of oppositely charged polyelectrolytes are either poly(allylamine hydrochloride) (PAH) or polystyrene sulfonate (PSS).

**[0015]** In other embodiments, the alternating layers of oppositely charged polyelectrolytes include, but are not limited to, poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid.

**[0016]** In other embodiments, the alternating layers of oppositely charged polyelectrolytes comprise polymers containing azobenzene moieties including, but not limited to, Brilliant Yellow (BY), or poly{1-4[4-(3-carboxy-4-hydroxyphenylazo) benzenesulfonamido]-1,2-ethanediyl sodium salt} (PAZO).

**[0017]** Further disclosed is a method of making the removable tattoo ink using a layer-by-layer core synthesis procedure, wherein the bio-absorbable chromophores (such as negatively charged white and/or black chromophores) are mixed with the PSS and then a layer of PAH, being positively charged, is absorbed on top of the PSS/chromophore layer. The result is a microparticle shell of alternating layers of PSS/chromophores and PAH.

**[0018]** It is to be understood that the present invention also includes using the organic, custom-multilayer microparticles or microspheres ranging from sub-micron to multi-micron size that is able to serve as a biodegradeable/bioresorbable carrier for the encapsulation of a range of molecules including, but not limited to, inks, small molecule drugs, vitamins, antibiotics and nutrients.

**[0019]** These and other aspects, objects, features and advantages of the example embodiments will become apparent to those having ordinary skill in the art upon consideration of the following detailed description of illustrated example embodiments.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0020]** Figure 1 is a schematic depiction of an exemplary embodiment of an example microparticle with a chromophore in the hollow core, eight alternating layers of PAH and PSS, and an outer shell layer of high molecular weight polystyrene.

**[0021]** Figure 2 provides a digital image of the formation of $CaCO_3$ microparticles.

**[0022]** Figure 3 provides a digital image of the formation of polyelectrolyte microparticles having alternating layers of polystyrene sulfonate (PSS) and poly(allylamine hydrochloride) (PAH).

**[0023]** Figure 4 provides a digital image of the formation of hollow microparticles prior to loading of the dye.

**[0024]** Figure 5 provides a digital image of the microparticles after loading with chromophores.

**[0025]** Figure 6 is a graph showing a two-week study to determine if there was leakage of dye from the microparticles depending upon the number of polyelectrolyte layers.

**[0026]** Figure 7 is a digital image of the formation of $CaCO_3$ microparticles using the Modified Core Synthesis of the present invention.

**[0027]** Figure 8 is a digital image of the formation of hollow microparticles prior to loading of the dye formed from the Modified Microparticle Preparation of the present invention.

**[0028]** Figure 9 provides a digital image of the microparticles, after loading with chromophores, using the Modified Dye Loading scheme of the present invention.

## DETAILED DESCRIPTION OF THE EXAMPLE EMBODIMENTS

### Terms

**[0029]** Unless otherwise noted, technical terms are used according to conventional usage. As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. As used herein, the term "comprises" means "includes." Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

**[0030]** To facilitate review of the various embodiments of the invention, the following explanations of terms are provided:

**[0031]** Chromophore: is a substance that has color or imparts a color to microparticles, for example by virtue of being inside a hollow microparticle.

**[0032]** Color: is broadly defined herein as a detectable (that is, visible or able to be made visible under certain lighting conditions, or able to be detected using a

device, for example, an infrared camera) property determined by a substance's electromagnetic absorption and/or emission spectrum (that is, in the ultraviolet, near-ultraviolet, visible, near-infrared, infrared, and other ranges). Black and white are colors under this definition.

**[0033]** Microparticle: A particle of a relatively small size, that can be implanted to form tissue markings and thus can be less than 50 nm to 100 microns or greater. Microparticles are also large enough on average and have a configuration on average such that when a plurality is implanted into tissue a sufficient number is retained to form a detectable marking, even though some number of the individual microparticles may be relocated from the tissue marking site. Microsphere, as used herein, is substantially equivalent to microparticle.

**[0034]** Subject: An animal, including both human and veterinary subjects.

**[0035]** Tattoo: A type of tissue marking where the tissue is typically the skin.

**[0036]** Tissue marking: A mark created by the introduction of microparticles disclosed herein into tissue, typically living tissue. Markings can be any color and must be detectable. The tissue markings, such as tattoos, of the present disclosure generally remain visible or otherwise detectable until it is exposed to an external energy source.

**[0037]** External energy source: A specific external energy, such as thermal, sonic (including ultrasonic, audible, and subsonic), light (including laser light, infrared light, or ultraviolet light), radio frequency (including diathermy, ablation and cryoablation), electric, magnetic, chemical, enzymatic, mechanical (such as shear force from rubbing or massaging), or any other type of energy or combination of energies.

**[0038]** Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

**Overview**

**[0039]** The present invention includes release of microsphere or microparticle contents by external stimuli, including, but not limited to, ultrasound, temperature, pH, and UV-light, thereby allowing for time and/or situation-specific delivery of the contents of the microsphere or microparticle. Examples include, but are not limited to, hyaluronic acid loaded LbL microcapsules for anti-aging and prolonged release of anti-aging products. This could be an injectable gel, since hyaluronic acid is popular

for lip injections and rejuvenation. Another example is for delivery of moisturizer, i.e., LbL capsules loaded with essential nutrients for skin.

**[0040]** Another embodiment of the present invention is for drug delivery wherein the LbL capsules loaded with a chemotherapeutic agent are targeted for cancer therapy. Targeting moieties can be attached to the outer shell of the microcapsule thereby allowing for targeted delivery of the microcapsule containing the chemotherapeutic agent. Because the LbL capsule can be made to respond to a change of pH conditions, the drug can be released upon internalization into the cell. Also contemplated as part of the present invention are glucose sensitive LbL capsules for the deliver of insulin for diabetic patients. A glucose sensitive polyelectrolyte can be incorporated into the shell of the microcapsule or microparticle, which will disassemble and release insulin in a high glucose environment. This is also applicable to sensors in general (i.e. oxygen sensors, etc.).

**[0041]** Another embodiment of the present invention includes pH sensitive LbL capsules, which are important in wound healing. The pH environment of a wound can help determine the treatment condition of the wound and aid in wound treatment. Chronic non-healing wounds generally have an alkaline environment. Being able to sense the pH of the environment can aid wound healing. A material is encapsulated that can be released in certain pH conditions. The release of material can be measured and the pH environment can be determined. Microcapsules can be applied onto the skin in alternative ways as well. For example, microneedling can be used to deposit an array of capsules under the skin. This is particularly useful in having a sensor embedded into the dermis in a less painful way. This is also useful for sensing UV exposure when a UV-sensitive component is incorporated into the layer-by-layer assembly of the capsules. Microspheres loaded with vitamin A/retinol or vitamin B in the moisturizer where the multilayered microsphere protects from UV/sunlight degradation.

**[0042]** Another embodiment of the present invention includes vaccine delivery of DNA based vaccines. By loading LbL capsules with DNA, the DNA then becomes targetable and the capsules can be modified to become deliverable.

**[0043]** The present invention can be used as a band aid or wound dressing wherein an antibiotic encapsulated microsphere is coated onto the dressing surface. Microspheres can contain an endotoxin- or LPS-sensitive rupturable element that is triggered as bacterial load increases during a local infection. This provides for

targeted (both location and time) antibiotic delivery, thereby limiting unnecessary antibiotic exposure.

**[0044]** The present invention also includes UV-responsive lotion to determine UV exposure levels. This lotion acts as a conventional moisturizer, but contains UV-sensitive microspheres. The higher the level of UV-exposure, the greater the degree of degradation of microspheres thereby releasing a visual indicator of the degree of exposure.

**[0045]** The present invention also includes an improvement for Desitin® in the field of diaper rash. The Desitin® is proportionally delivered according to the extent of rash which relates to the heat generated in the rash. In addition, diapers can be coated with a microsphere that responds to diaper content and thereby releases, pre-emptively, a moisturizer to minimize diaper rash. The diaper rash can be detected and treated before the rash becomes severe.

**[0046]** Another embodiment of the present invention is in the application of a topical analgesic using temperature sensitive microspheres. This would be appropriate for a sports application, whereby the degree of analgesia increases during a workout or game as the microspheres release the active agent.

**[0047]** The present invention can be used in a shampoo or conditioner wherein the microspheres are oil sensitive and are ruptured in the presence of hair sebum, thereby delivering more surfactant to cleanse the hair. Alternatively, damaged hair can be targeted by microspheres that release nutrients near the point of damage.

**[0048]** In the area of vision care, the present invention can be used to reduce the stinging and burning upon application of eye care products. Encapsulation of these active ingredients into a temperature-sensitive microsphere that releases more slowly once applied to the cornea minimizes the discomfort.

**[0049]** Another embodiment of the present invention is in military applications. The present invention can be used as military dog-tags that are safe for military personnel allowing for easier identification in multiple locations during battlefield trauma. These “military dog-tags” can be completely and safely removed when the soldiers exit service. The present invention can also be a chemical warfare alert system that can be injected into the skin, whereby the presence of the biological threat causes the release of specific antidotes to the threat in question.

**[0050]** The present invention can also be used as an antiaging eye cream, antiaging facial cream, antiaging body cream and an antibruising cream.

**[0051]** Embodiments disclosed herein also provide improved removable tattoo inks and methods of making the same. The tattoo ink compositions comprise transparent, translucent or opaque microparticles having a hollow inner core, which is filled with one or more chromophores. The outer shell of the tattoo ink compositions comprise alternating layers of oppositely charged polyelectrolytes. The relative size of the particle is large enough to avoid removal by macrophages or other elements of the lymphatic system rendering the ink permanent until removal is desired. The polyelectrolyte shell of the microparticles is designed such that the polyelectrolytes are labile when exposed to a certain type and/or intensity of external energy. The external energy source is non-invasive and does not require dermal abrasion to remove the ink. Upon exposure to the external energy source the microparticle shells break down or otherwise rupture such that the chromophore is released. The chromophore is designed to be readily taken up by the body for removal by the body through natural processes.

**[0052]** The compositions and methods disclosed herein provide several advantages over standard tattoos and their removal. Standard tattoos are made using unregulated pigments of undisclosed nature which, once implanted, are in direct contact with living tissue for the recipient's life, even if no longer visible at the tissue marking site. The disclosed tattoo inks can reduce short- and long-term health risks associated with standard tattoo pigments. For example, in contrast to tattoo inks derived from heavy metals, the microparticles are inert when implanted in tissue. A course of many treatments to remove a standard tattoo is not always successful, yet it is time-consuming and expensive, may expose the tissue to a damaging amount of radiation, requires guesswork and experimentation on the part of the practitioner, and, in the case of multicolored tattoos, may require multiple lasers. Through use of the tattoo ink disclosed herein, tissue marking removal treatments can become essentially 100% effective and the associated costs of removal in terms of time (such as length of treatment course) and/or money can be reduced compared to standard tattoo removal treatment.

**Removable Tattoo Inks**

**[0053]** Aspects of the present disclosure relate to removable tissue marking compositions, such as microparticles for use in removable tattoo inks. Thus, disclosed is a removable tissue marking, such as tattoo ink. The removable tissue marking composition includes colored microparticles that comprise an internal core,

comprising one or more bio-absorbable chromophores, and an outer shell. The disclosed microparticles typically have a diameter of about 0.5 μm - 10 μm, but may be smaller or larger as long as the microparticles can be implanted into a tissue to provide a tissue marking. They can be spherical or any other shape.

**[0054]** The outer shell of a disclosed microparticle comprises alternating layers of oppositely charged polyelectrolytes. Polyelectrolytes are defined as polymers composed of repeating electrolyte groups that readily dissociate in an aqueous environment, giving the polyelectrolyte an overall negative (polyanion) or positive (polycation) charge. In certain example embodiments, the microparticles comprise between 2 and 16 alternating layers of polyelectrolytes. In certain example embodiments, the positively charged polyelectrolytes are selected from the group consisting of poly(allylamine hydrochloride) (PAH), poly(diallyldimethylammonium chloride) (PDADMAC), poly(ethylenimine (PEI), and poly-L-arginine. In certain example embodiments, the negatively charged polyelectrolytes are selected from the group consisting of polystyrene sulfonate (PSS), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), heparin, and poly-L-glutamic acid.

**[0055]** In certain example embodiments, the microparticles comprise at least one layer of polyallylamine hydrochloride (PAH) and at least one layer of polystyrene sulfonate (PSS). In some embodiments, the outer shell, comprises between about 2 and about 16 layers of polystyrene sulfonate (PSS) and polyallylamine hydrochloride (PAH) for example about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 layers of PSS and PAH. In some embodiments, the PSS and PAH layers alternate. In some examples, the inner layer comprises PSS. In some examples, the outer layer comprises PSS. In some examples, the inner layer comprises PAH. In some examples, the outer layer comprises PAH. In some embodiments, the microparticle further comprises a coating over the outer shell, such as polystyrene, for example high molecular weight polystyrene.

**[0056]** The outer shell is configured to maintain structural integrity during the implantation process, but labile upon exposure to certain types or intensities of externally applied energy sources, such as electromagnetic radiation, ultraviolet energy, infrared energy, ultrasonic energy, laser light, or radio frequency energy.

**[0057]** In some embodiments, the outer shell and/or coating is substantially visibly transparent such that the chromophores are detectable through the outer shell

and/or coating. In some embodiments, negatively-charged chromophores are mixed with PSS, and then layered with PAH, thereby resulting in an opaque microparticle. In some embodiments, positively-charged chromophores are mixed with PAH, and then layered with PSS, thereby resulting in an opaque microparticle. In some embodiments, negatively-charged chromophores are mixed with negatively-charged polyelectrolytes during layer formation. In some embodiments, positively-charged chromophores are mixed with positively-charged polyelectrolytes during layer formation. The resultant microparticle shell is comprised of layers of oppositely charged polyelectrolytes, with each layer being mixed with chromophores having the same charge as the polyelectrolyte layer. The opposing polyelectrolyte layers are held together due to electrostatic attraction.

**[0058]** In some embodiments, negative-charged chromophores (such as black and white chromophores) form a negatively-charged layer of the microparticle shell, to which a positively-charged layer of a polyelectrolyte, such as PAH, interacts due to electrostatic attraction. In some embodiments, positively-charged chromophores form a positively-charged layer of the microparticle shell, to which a negatively-charged layer of a polyelectrolyte, such as PSS, interacts due to electrostatic attraction.

**[0059]** In some embodiments, the outer shell and/or coating of the microparticles includes specific absorption components that absorb in a particular spectral region, such as infrared or near-infrared, visible, ultraviolet, or radio frequency. The choice of material allows the microparticles to be broken down or ruptured by an energy source, such as from laser light or ultraviolet light, thereby releasing the chromophores.

**[0060]** Examples of useful infrared- or near-infrared-absorbing materials include polymers, polyelectrolytes, small molecules, DNA, protein, and biomolecules.

**[0061]** In some embodiments, the chromophores range in size from 1 – 100 nanometers, for example about 1.0 nm, 2.0 nm, 3.0 nm, 4.0 nm, 5.0 nm, 6.0 nm, 7.0 nm, 8.0 nm, 9.0 nm, 10.0 nm, 11.0 nm, 12.0 nm, 13.0 nm, 14.0 nm, 15.0 nm, 20.0 nm, 25.0 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm or 100 nm, such as between about 1.0 nm and about 5 nm, about 3.0 nm and about 15.0 nm, about 5.0 nm and about 20.0 nm, about 3.0 nm and about 50.0 nm, about 5.0 nm and about 70.0 nm and about 50.0 nm and about 100 nm in size.

**[0062]** In some embodiments, the microparticles range in size from 0.1 - 100 μm, for example about 0.1 μm, 0.2 μm, 0.3 μm, 0.4 μm, 0.5 μm, 0.6 μm, 0.7 μm, 0.8 μm, 0.9 μm, 1.0 μm, 2.0 μm, 3.0 μm, 4.0 μm, 5.0 μm, 6.0 μm, 7.0 μm, 8.0 μm, 9.0 μm,

10.0 μm, 11.0 μm, 12.0 μm, 13.0 μm, 14.0 μm, 15.0 μm, 20.0 μm, 25.0 μm, 30 μm, 40 μm, 50 μm, 60 μm, 70 μm, 80 μm, 90 μm or 100 μm, such as between about 0.1 μm and about 5 μm, about 3.0 μm and about 15.0 μm, about 5.0 μm and about 20.0 μm, about 0.5 μm and about 50.0 μm, about 5.0 μm and about 70.0 μm and about 50.0 μm and about 100 μm in size. In specific embodiments, the microparticle is from about 10 μm to about 30.0 μm in size.

**[0063]** In some embodiments, the microparticles are suspended in a liquid carrier, wetting agent, emulsifier or surfactant, for example, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, polyoxyl 40 stearate, alcohol, water, or glycerin, or any combination thereof, to facilitate implantation of the ink into the tissue of a subject.

**[0064]** The disclosed removable tattoo inks include one or more chromophores encapsulated within the outer shell. The chromophore can be of any colored material that has the properties of being bio-absorbable to the human, or animal body. Generally speaking, the chromophores useful as tattoo ink include stains, dyes, colored drugs and proteins, and other materials including, but not limited to, those approved by the FDA for use within the body. Chromophores may be mixed in combinations before or during inner core formation, so that it may only be necessary to select a small number of different chromophores to obtain a broad range of colors for various tattoo purposes. For example, the pure chromophores can be mixed to form intermediate colors and shades. Thus, combinations of two or more chromophores can be mixed to form desired colors and shades, and then encapsulated to form microparticles. Additionally or alternatively, different colored microparticles can be mixed together to form a colored mixture. In one non-limiting example, blue microparticles may be mixed with red microparticles to form a purple tattoo ink mixture.

**[0065]** Useful bio-absorbable chromophores include: nanoparticles, drugs and dyes such as titanium dioxide, iron oxide, gold nanoparticles, quantum dots, rifampin (red), β-carotene (orange), tetracycline (yellow), indocyanine green (such as Cardio-Green®), Evan's blue, methylene blue; soluble inorganic salts such as copper sulfate (green or blue), $Cu(NH_3)^{2+}$ (dark blue), $MnO_4$ (purple), $NiCl_2$ (green), $CrO_4$ (yellow), $Cr^2O7^{2-}$ (orange); proteins such as rhodopsin (purple and yellow forms) and green fluorescent protein (fluoresces green under blue light); and any of the Food and Drug Administration (FDA) approved dyes used commonly in foods, pharmaceutical preparations, medical devices, or cosmetics, such as the well-characterized sodium

salts FD&C Blue No. 1 (Brilliant Blue FCF), FD&C Green No. 3 (Fast Green FCF), FD&C Red No. 3 (Erythrosine), FD&C Red No. 40 (ALLURA® Red AC), FD&C Yellow No. 5 (Tartrazine), and FD&C Yellow No. 6 (Sunset Yellow FCF). Additional FDA approved dyes and colored drugs are described in the Code of Federal Regulations (CFR) for Food and Drugs (see Title 21 of CFR chapter 1, parts 1-99). Bio-absorbable chromophores for use in the disclosed compositions and methods are generally water-soluble at physiological pH, although fat-soluble chromophores (such as β-carotene) will also work if they are rapidly flushed from tissue, or digestible or metabolizable through enzymatic pathways (such as methylene blue, which is rapidly metabolized by mitochondrial reductases, and proteins which are digested by proteases). In some embodiments, the chromophore comprises a Food and Drug Administration (FDA)-approved dye. In some embodiments, the chromophore is selected from the group consisting of phthalocyanine dyes, cyanine dyes, and pyrylium dyes. In some embodiments, the chromophore comprises carbon black. In some embodiments, the chromophore is selected from the group consisting of rifampin, β-carotene, tetracycline, indocyanine green, Evan's blue, and methylene blue. In some embodiments, the chromophore is selected from the group consisting of FD&C Blue No. 2, FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6. In some embodiments, the chromophore comprises a soluble inorganic salt selected from the group consisting of copper sulfate, $Cu(NH_3)^{2+}$, $MnO_4$, $NiCl_2$, $CrO_4$, and $Cr^2O7^{2-}$.

**[0066]** The tattoo inks of the present invention can be used as tissue marking pigments for cosmetic, identification, and other purposes. For example, the disclosed microparticles can be suspended in a liquid carrier, wetting agent, emulsifier or surfactant, for example, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, polyoxyl 40 stearate, alcohol, water, and/or glycerin, to form a tissue marking ink in the same manner as standard tattoo pigments.

**[0067]** The removable tattoo inks disclosed herein can be implanted into skin or similar superficial tissue with an electromagnetic coil tattooing machine (such as that disclosed in U.S. Pat. No. 4,159,659); a rotary permanent cosmetics application machine (such as that disclosed in U.S. Pat. No. 5,472,449); or with any manual tattooing device (such as the sterile, single-use device marketed by Softap Inc., San Leandro, Calif.). Alternatively, the inks can be implanted using a non-invasive method, for example, as described in U.S. Pat. No. 5,445,611.

**[0068]** Tissue markings in skin must be properly placed to provide permanent markings. Skin is composed of the outermost epidermis, generated by the constantly dividing stratum basale, and the underlying dermis. Dermal cells, such as fibroblasts, mast cells, and others, which do not generally replicate, are located within a resilient proteinaceous matrix. It is the upper dermis, below the stratum basale, into which the microparticles are implanted to provide a tissue marking (such as a tattoo). After implantations, microparticles in the dermis form part of a permanent tissue marking if they are phagocytosed by dermal cells or if they remain in the extracellular matrix.

**[0069]** In addition to skin, microparticles of the present invention can be implanted into a wide variety of living tissues comprising relatively stationary, infrequently-replicating cells. For example, the microparticles can be implanted into the internal surfaces of the body that are contiguous with the external skin, including, but not limited to, the inner surfaces of the mouth and lips, the gums and tongue, inner surfaces of the eyelid, and the tissues lining internal body passages (such as the nasal, ear, anal, urethral, and vaginal passages, and the gastrointestinal tract). Other tissues that can be marked include the tissues of and/or under the fingernails and toenails, the dentin of the teeth, and the colored iris and white sclera of the eye.

**[0070]** As a result of their versatility, the microparticles can be used to produce a wide variety of cosmetic tissue markings including decorative artistic tattoos that are removable and revisable; cosmetic makeup that is permanent as long as the wearer desires it; revisable corrective and reconstructive pigmentation as an adjunct to plastic surgery and to address other cosmetic problems, for example, to correct blotchy skin pigmentation or to mask thinning hair by adding pigment to the scalp; and reversible addition of pigment to small or large areas of the body purely for cosmetic reasons, for example, to create the look of a tan without exposure to ultraviolet rays.

**[0071]** In addition to marking skin, the microparticles can be used to produce new cosmetic markings in other tissues. It is especially important that these new types of markings are removable to allow risk-free experimentation. For example, the microparticles can be implanted into areas of externally visible non-skin tissue, which are important to human appearance. Colored microparticles can be added to the cornea or to the colored iris of the eye, for example, to change apparent eye color. White microparticles which are highly light-scattering can be implanted into the dentin and/or sclera, for example, to whiten the teeth and/or eyes. Colored microparticles can be added to the tissue of and/or under the fingernails and/or

toenails, for example, to create solid colors, patterns, or designs for decorative purposes. Identification markings made with the microparticles can be changed, updated, and/or removed. In some cases, selectively detectable (such as normally invisible) microparticles may be advantageous. Some examples of markings to fill identification needs include markings to assist tracking bodily sites of medical interest in external and superficial internal tissue, for example, marking a radiation therapy field on the skin, or marking a colon polyp in the intestine which can subsequently be monitored endoscopically; identification markings for humans, for example, emergency information regarding an individual's medical history, "dog-tags" on military personnel, and identification markings on newborn babies to ensure no hospital mix-ups; and identification markings for animals (such as wild animals, livestock, sport/show animals, and pets), for example, information markings for the return of lost pets.

**Methods of Making Removable Tattoo Inks**

**[0072]** Disclosed herein is a method of making removable tattoo ink. A disclosed method includes forming a solid internal core. In certain example embodiments, the solid internal core has a diameter of 0.1 μm - 10 μm. The solid internal core may be formed using known methods in the art. Materials used to form the internal core should be capable of subsequent removal of the core material, for example by chelation, after formation of an outer shell of the internal core material. In certain example embodiments, the solid internal core is, comprised of calcium carbonate ($CaCO_3$).

**[0073]** In some embodiments, forming the internal core comprises mixing in solution the $CaCl_2$ and $Na_2CO_3$, to form the internal core comprising $CaCO_3$ and then removing the residual NaCl. For example, a solution of between about 0.1 – 10 M $CaCl_2$ (such as 0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 0.6 M, 0.7 M, 0.8 M, 0.9 M, 1.0 M, 1.5 M, 2.0 M, 2.5 M, 3.0 M, 3.5 M, 4.0 M, 4.5 M, 5.0 M, 6.0 M, 7.0 M, 8.0 M, 9.0 M or 10 M or anywhere in between) is mixed with a solution of between about 0.1 – 10 M $Na_2CO_3$ (such as about 0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 0.6 M, 0.7 M, 0.8 M, 0.9 M, 1.0 M, 1.5 M, 2.0 M, 2.5 M, 3.0 M, 3.5 M, 4.0 M, 4.5 M, 5.0 M, 6.0 M, 7.0 M, 8.0 M, 9.0 M or 10 M or anywhere in between) and stirred for a period of time sufficient for the formation of microparticles containing $CaCO_3$, for example between 10 seconds and 20 minutes (such as about 10 seconds, 20 seconds, 30 seconds, 40 seconds, 50 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes,

7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, or 20 minutes, or anywhere in between), although the solution can be stirred longer. In some embodiments, the formed microparticles are washed to remove the residual NaCl, for example between about 1 and 10 times; although in certain applications, the microparticles can be washed more than 10 times. In between each wash, the microparticles can be centrifuged to pellet the microparticles and facilitate washes. In some examples, the microparticles are washed with deionized water and then air dried.

**[0074]** The internal core is then coated with alternating layers of oppositely charged polyelectrolytes to form an outer shell. In certain example embodiments, the alternating layers of oppositely charged electrolytes may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 alternating layers of oppositely charged electrolytes. In certain example embodiments, the charged polyelectrolytes are selected from the group consisting of poly(alylamine hydrochloride) (PAH), polystyrene sulfonate (PSS), poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid. In certain example embodiments, the outer shell comprises at least one layer of PAH. In certain example embodiments, the outer shell comprises at least one layer of PSS. In certain other example embodiments, the outer shell comprise alternating layers of PSS and PAH.

**[0075]** In some embodiments, coating the solid internal core with at least one layer of polystyrene sulfonate comprises contacting the solid internal core with a solution comprising polystyrene sulfonate (PSS), for example contacting the $CaCO_3$ with a solution of about 0.1 to about 10 mg/mL PSS (such as about 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL, 0.9 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, 3.5 mg/mL, 4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 6.0 mg/mL, 7.0 mg/mL, 8.0 mg/mL, 9.0 mg/mL or 10 mg/mL, or anywhere in between) from about 30 seconds to about 60 minutes (such as about 30 seconds, 40 seconds, 50 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, or anywhere in between), although longer times can be used. In some embodiments, coating the

internal core with at least one layer of polyallylamine hydrochloride comprises contacting the internal core comprising $CaCO_3$ with a solution comprising polyallylamine hydrochloride (PAH), for example contacting the $CaCO_3$ with a solution of about 0.1 to about 10 mg/mL PAH (such as about 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL, 0.9 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, 3.5 mg/mL, 4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 6.0 mg/mL, 7.0 mg/mL, 8.0 mg/mL, 9.0 mg/mL or 10 mg/mL or anywhere in between) from about 30 seconds to about 60 minutes (such as about 30 seconds, 40 seconds, 50 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, or anywhere in between), although longer times can be used. This can be alternated, to create a microparticle with multiple layers of PSS and PAH.

**[0076]** After forming the outer shell the solid internal core is removed. In certain example embodiments, the solid internal core is removed using a metal chelation agent such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA) and the like. Removal of the internal solid core creates a microparticle formed by the outer shell and defining a hollow inner core.

**[0077]** The hollow microparticles are then loaded with one or more bio-absorable chromophores. Example bio-absorbable chromophores include, but are not limited to, titanium dioxide, iron oxide, gold nanoparticles, quantum dots, FD&C Blue No. 2, FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, FD&C Yellow No. 6, natural dyes, or Spirulina. The bio-absorbable chromophores may be loaded into the hollow particles using any suitable means known in the art so long as the structural integrity of the outer shell is made. In one example embodiment, the one or more chromophores are loaded osmotically. For example, the microparticles may be suspended in a solution comprising the one or more dyes to be loaded under conditions sufficient to allow osmotic loading of the one or more chromophores into the hollow microparticles. For embodiments comprising more than one chromophore to be loaded, the chromophores may be loaded sequentially. The microparticles may be placed in a first solution for loading of a first chromophore and then placed in a second solution for loading of a second chromophore. The chromophores may be loaded at a concentration in the range of 10 mg/mL to 300 mg/mL (such as about 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL,

50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL, 175 mg/mL, 200 mg/mL, 250 mg/mL or 300 mg/mL, or anywhere in between) is agitated on a shaker table for about 60 minutes to 150 minutes (such as about 60 minutes, 70 minutes, 80 minutes, 90 minutes, 100 minutes, 110 minutes, 120 minutes, 130 minutes, 140 minutes, or 150 minutes, or anywhere in between), although longer times can be used, allowing for the osmotic loading of the one or more bio-absorbable dyes into the internal cavity of the hollow microparticles.

**[0078]** In certain example embodiments, the surface of the dye-loaded microparticles is coated in a layer of silica, by hydrolyzing tetraethyl orthosilicate in ethanol and water basic solution, to prevent leakage of the one or more chromophores from the loaded microparticles.

**[0079]** In certain example embodiments, the polyelectrolytes may be exposed to heat to thermally condense the polyelectrolyte layers in order to reduce leakage of the one or more chromophores from the loaded microparticles. For example, the loaded microparticles may be heated to approximately 70°C for a period of time sufficient to facilitate thermal condensation of the polyelectrolytes, for example, between 10 minutes and 1 hour (such as about 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, or anywhere in between), although longer times can be used.

**[0080]** In certain example embodiments, the shells of the microparticles are made using a layer-by-layer core synthesis procedure, by mixing the chromophores with the polyelectrolytes during formation of the alternating layers of oppositely charged polyelectrolytes. In particular, black and/or white chromophores are added to the polyelectrolytes during formation of the alternating layers of oppositely charged polyelectrolytes. In certain example embodiments, negatively-charged black and/or white chromophores are mixed with the negatively-charged polyelectrolyte, and then the mixed layer is applied to the core. Thereafter, another layer of an oppositely charged polyelectrolyte is applied to the mixed layer. In certain example embodiments, negatively-charged black and/or white chromophores are mixed with negatively-charged PSS, and the chromophore layer is applied to the core. Thereafter, positively-charged PAH is absorbed on top of the chromophore layer due to electrostatic attraction. In this way, alternating layers of chromophores and PAH can be formed. In certain example embodiments, the shell is no longer translucent, but instead is opaque due to the inclusion of the chromophores in the shell of the

microparticles. After the shell containing the black and/or white chromophores is made, the hollow core of the microparticles are loaded with either the black and/or white chromophores.

**[0081]** In certain example embodiments, the shells of the microparticles are made using a layer-by-layer core synthesis procedure, by adding a layer of negatively-charged chromophores and a layer of positively-charged polyelectrolytes. In particular, the shells of the microparticles are made using a layer-by-layer core synthesis procedure, by adding a layer of negatively-charged black and/or white chromophores and a layer of positively-charged polyelectrolytes. In certain example embodiments, a layer of negatively-charged black and/or white chromophores interacts with a layer of positively-charged PAH. The number of alternating layers of chromophores and polyelectrolytes may vary from 2 to 16 layers. In this way, alternating layers of chromophores and PAH can be used to form the shell of the microparticles. In certain example embodiments, the shell is no longer translucent, but instead is opaque due to the inclusion of the chromophores in the shell of the microparticles. After the shell containing the black and/or white chromophores is made, the hollow core of the microparticles are loaded with either the black and/or white chromophores.

**[0082]** In certain example embodiments, the shells of the microparticles are made using a layer-by-layer core synthesis procedure, by adding a layer of positively-charged chromophores and a layer of negatively-charged polyelectrolytes. In certain example embodiments, a layer of positively-charged chromophores interacts with a layer of negatively-charged PSS. The number of alternating layers of chromophores and polyelectrolytes may vary from 2 to 16 layers. In this way, alternating layers of chromophores and PSS can be used to form the shell of the microparticles. In certain example embodiments, the shell is no longer translucent, but instead is opaque due to the inclusion of the chromophores in the shell of the microparticles. After the shell containing the chromophores is made, the hollow core of the microparticles are loaded with chromophores.

**[0083]** After formation of chromophore-loaded microparticles, the microparticles may be added to an application formulation suitable for use in applying the tattoo ink. In certain example embodiments the application formulation is an aqueous formulation comprising at least 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17

mg/mL, 18 mg/mL, 19 mg/mL, or 20 mg/mL of loaded microparticles. In certain example embodiments, the microparticles are freeze-dried to form a powder. The powder may then be resuspended in the application formulations. In certain example embodiments, the application formulation may comprise a 1:1 mixture of glycerine and isopropyl alcohol. In certain example embodiments, the powder is added at 15% to 80% w/v of the application solution. The application solution of "ink" may then be applied using standard tattoo application techniques known in the art.

**Methods of Tattoo Removal**

**[0084]** The tattooed or marked tissue of a subject that has been tattooed or marked with one or more of the tattoo inks comprising the microparticles of the current disclosure, can be removed by the application of external energy sources, including, but not limited to ultrasonic energy, laser light operating in the near infrared, radio frequency ablation, radio frequency cryoablation, or ultraviolet light. By way of example, ultrasonic energy is applied to the site of the tattoo to be removed and the ultrasonic energy ruptures the microparticles present in the tattoo thereby releasing the contents of the microparticles, which are absorbed by the body.

**[0085]** The ultrasonic energy is applied using an external source, for example, using a commercially available ultrasound machine, such as available from Dynatronics, such as a Dynatron 360 at a specific or variable intensity and for a controlled length of time. The ultrasonic energy can be administered in one or several pulses. For example, the ultrasonic energy can be administered in one or several sessions, such as sessions separated by minutes, days, weeks or even months, depending on such factors as the size of the tattoo for removal. In some embodiments, an area of a subject's tissue, such as an area of skin of the subject, is treated with ultrasonic energy between about 0.5 MHz and about 5 MHz at a power of between about 0.5 $W/cm^2$ to about 10 $W/cm^2$ for a period of about 1 minute to about 60 minutes. For example, ultrasonic energy can be applied to the tissue of the subject that is between about 0.5 MHz and about 5.0 MHz, such as about 0.5 MHz, 0.6 MHz, 0.7 MHz, 0.8 MHz, 0.9 MHz, 1.0 MHz, 1.1 MHz, 1.2 MHz, 1.3 MHz, 1.4 MHz, 1.5 MHz, 2.0 MHz, 2.5 MHz, 3.0 MHz, 3.5 MHz, 4.0 MHz, 4.5 MHz, or 5.0 MHz, such as between about 0.5 MHz and about 2.0 MHz, between about 1.5 MHz and about 3.0 MHz, between about 0.5 MHz and about 4.0 MHz, or between about 2.5 MHz and about 5.0 MHz. The ultrasonic energy can be applied over a specific area, with a

power dispersed over that area, for example with a power of between about 0.5 $W/cm^2$ to about 10 $W/cm^2$, such as about 0.5 $W/cm^2$, 0.6 $W/cm^2$, 0.7 $W/cm^2$, 0.8 $W/cm^2$, 0.9 $W/cm^2$, 1.0 $W/cm^2$, 1.1 $W/cm^2$, 1.2 $W/cm^2$, 1.3 $W/cm^2$, 1.4 $W/cm^2$, 1.5 $W/cm^2$, 2.0 $W/cm^2$, 2.5 $W/cm^2$, 3.0 $W/cm^2$, 3.5 $W/cm^2$, 4.0 $W/cm^2$, 4.5 $W/cm^2$, 5.0 $W/cm^2$, 5.5 $W/cm^2$, 6.0 $W/cm^2$, 6.5 $W/cm^2$, 7.0 $W/cm^2$, 7.5 $W/cm^2$, 8.0 $W/cm^2$, 8.5 $W/cm^2$, 9.0 $W/cm^2$, 9.5 $W/cm^2$, or 10.0 $W/cm^2$, such as between about 0.5 $W/cm^2$ and about 4.0 $W/cm^2$, between about 3.5 $W/cm^2$ and about 6.0 $W/cm^2$, between about 6.0 $W/cm^2$ and about 9.0 $W/cm^2$, or between about 2.5 $W/cm^2$ and about 7.0 $W/cm^2$. Depending on the energy of ultrasonic energy being applied (e.g. the more power the less time), ultrasonic energy can be applied to the tattoo area from about 1 minute to about 60 minutes, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 60 minutes. Such application can be continuous or as one or more pulses, for example to minimize heating and possible damage to the tissue of the subject. Adjustments to the duration, power and frequency of the ultrasonic energy may be made by the user, such as by the practitioner or technician. Cells in the tissue may or may not be ruptured concomitantly, depending on the amount of energy applied and the pulse length in which it is delivered; after application of ultrasonic energy, chromophore dispersal occurs through physiological processes in both cases and the marking is removed from the tissue. The total systemic dose of the released chromophores (stains, dyes, drugs, nanoparticles or proteins) is generally low following a removal treatment.

**[0086]** In general, the total amount of ultrasonic energy necessary to remove tissue markings of the invention can be reduced compared to standard laser therapy to remove standard tattoos because the electromagnetic absorption and/or structural properties of the microparticles are carefully chosen in advance with removal in mind. This reduction means less secondary damage is incurred by surrounding cells, and patient pain is reduced.

**[0087]** Some patients may desire partial removal of a tissue marking which may also be achieved by ultrasonic energy. Incomplete removal can be achieved, for example, by reducing the intensity of the ultrasonic energy to affect only a fraction of microparticles, or by only treating certain areas of the tissue marking. It may be desirable, for example, to reduce the size of the marking (such as to thin a cosmetic eyebrow or eyeliner); to remove a portion of a marking including a smaller mark, symbol, text, or identifying information (such as to remove a name from a vow

tattoo); to reduce the color-intensity of a marking (such as to lighten a dark lipliner); or to transform the appearance of the tissue marking (such as to create a decorative light-on-dark pattern within a previously solid dark tissue marking).

**[0088]** In the event that a new tissue marking is desired to replace an existing marking, ultrasonic energy is used to remove all or part of the original marking. Colored microparticles are then implanted into the tissue. The process could be used to update marks (such as bar codes), symbols, text, or identifying information, for example, to change a phone number and/or address marking on a pet after a move; to rework or refresh the appearance of the remaining tissue marking, for example, to add details to an artistic tattoo after regions have been removed to reduce the tattoo size; or to replace completely the original marking with a new tissue marking.

## EXAMPLES

**[0089]** The following examples demonstrate the manufacture, use and removal of the tattoo inks of the present disclosure.

**[0090]** The disclosed tattoo ink compositions were synthesized after several prototype iterations resulting in the current ink design, which consists of transparent, translucent, or opaque ink-filled microparticles that are 25 microns in diameter, equal in size and consistency to the current conventional permanent tattoo inks, which are made of heavy metals. The relative size of the particle in the dermis makes the ink permanent because macrophage cells are unable to remove the particle from the dermis because it is too large for the macrophage to remove to the lymphatic system.

**[0091]** The disclosed tattoo ink is prepared by (1) forming an internal core of calcium carbonate ($CaCO_3$), from mixing in solution $CaCl_2$ and $Na_2CO_3$, and then removing the residual NaCl; (2) coating the internal core of calcium carbonate ($CaCO_3$) with alternating layers of polyelectrolytes, for example, using layer-by-layer deposition of at least one layer of polystyrene sulfonate (PSS) and at least one layer of polyallylamine hydrochloride (PAH); (3) dissolving the calcium carbonate ($CaCO_3$) core with a metal chelation agent, such as EDTA, EGTA, and the like, thereby creating a hollow microparticle; (4) osmotically loading of the hollow microparticles with one or more bio-absorbable chromophores; and (5) entrapping the loaded dye in the microparticles using heat. The microparticles are then resuspended in a liquid carrier, such as water, ethanol, isopropanol, glycerin, witch hazel, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, or polyoxyl 40 stearate to a thickness and concentration sufficient for use as a tattoo ink.

**[0092]** An alternative method of preparing the tattoo ink includes the steps of: (1) forming an internal core of calcium carbonate ($CaCO_3$), from mixing in solution $CaCl_2$ and $Na_2CO_3$, and then removing the residual NaCl; (2) coating the internal core of calcium carbonate ($CaCO_3$) with alternating layers of polyelectrolytes, for example, using layer-by-layer deposition of at least one layer of polystyrene sulfonate (PSS) and at least one layer of polyallylamine hydrochloride (PAH); (3) dissolving the calcium carbonate ($CaCO_3$) core with a metal chelation agent, such as EDTA, EGTA, and the like, thereby creating a hollow microparticle; (4) osmotically loading of the hollow microparticles with one or more bio-absorbable chromophores; and (5) entrapping the loaded dye in the microparticles by adding a layer of silica coating to the microparticles to prevent leakage of the dye. The microparticles are then resuspended in a liquid carrier, such as water, ethanol, isopropanol, glycerin, witch hazel, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, or polyoxyl 40 stearate to a thickness and concentration sufficient for use as a tattoo ink.

**[0093]** Another alternative method of preparing the tattoo ink includes the steps of: (1) forming an internal core of calcium carbonate ($CaCO_3$), from mixing in solution $CaCl_2$ and $Na_2CO_3$, and then removing the residual NaCl; (2) mixing black and/or white chromophores with polystyrene sulfonate (PSS); (3) coating the internal core of calcium carbonate ($CaCO_3$) with alternating layers of polyelectrolytes, for example, using layer-by-layer deposition of at least one layer of the chromophore/polystyrene sulfonate (PSS) from step (2) above, and at least one layer of polyallylamine hydrochloride (PAH); (4) dissolving the calcium carbonate ($CaCO_3$) core with a metal chelation agent, such as EDTA, EGTA, and the like, thereby creating a hollow microparticle; (5) osmotically loading of the hollow microparticles with one or more bio-absorbable chromophores; and (6) entrapping the loaded dye in the microparticles by adding a layer of silica coating to the microparticles to prevent leakage of the dye. The microparticles are then resuspended in a liquid carrier, such as water, ethanol, isopropanol, glycerin, witch hazel, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, or polyoxyl 40 stearate to a thickness and concentration sufficient for use as a tattoo ink.

**[0094]** Another alternative method of preparing the tattoo ink includes the steps of: (1) forming an internal core of calcium carbonate ($CaCO_3$), from mixing in solution $CaCl_2$ and $Na_2CO_3$, and then removing the residual NaCl; (2) coating the internal core of calcium carbonate ($CaCO_3$) with alternating layers of negatively-

charged chromophores and positively-charged polyelectrolytes, for example, using layer-by-layer deposition of at least one layer of black and/or white chromophores, and at least one layer of polyallylamine hydrochloride (PAH); (3) dissolving the calcium carbonate ($CaCO_3$) core with a metal chelation agent, such as EDTA, EGTA, and the like, thereby creating a hollow microparticle; (4) osmotically loading of the hollow microparticles with one or more bio-absorbable black and/or white chromophores; and (5) entrapping the loaded dye in the microparticles by adding a layer of silica coating to the microparticles to prevent leakage of the dye. The microparticles are then resuspended in a liquid carrier, such as water, ethanol, isopropanol, glycerin, witch hazel, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, or polyoxyl 40 stearate to a thickness and concentration sufficient for use as a tattoo ink.

**[0095]** Another alternative method of preparing the tattoo ink includes the steps of: (1) forming an internal core of calcium carbonate ($CaCO_3$), from mixing in solution $CaCl_2$ and $Na_2CO_3$, and then removing the residual NaCl; (2) coating the internal core of calcium carbonate ($CaCO_3$) with alternating layers of positively-charged chromophores and negatively-charged polyelectrolytes, for example, using layer-by-layer deposition of at least one layer of positively-charged chromophores, and at least one layer of polystyrene sulfonate (PSS); (3) dissolving the calcium carbonate ($CaCO_3$) core with a metal chelation agent, such as EDTA, EGTA, and the like, thereby creating a hollow microparticle; (4) osmotically loading of the hollow microparticles with one or more bio-absorbable chromophores; and (5) entrapping the loaded dye in the microparticles by adding a layer of silica coating to the microparticles to prevent leakage of the dye. The microparticles are then resuspended in a liquid carrier, such as water, ethanol, isopropanol, glycerin, witch hazel, polysorbate, PEG, propylene glycol, polyoxyl 8 stearate, or polyoxyl 40 stearate to a thickness and concentration sufficient for use as a tattoo ink.

**[0096]** The tattoo ink is synthesized with a combination of bio-absorbable chromophores. The bio-absorbable chromophores may include, but are not limited to: nanoparticles, drugs and dyes such as titanium dioxide, iron oxide, gold nanoparticles, quantum dots, rifampin (red), β-carotene (orange), tetracycline (yellow), indocyanine green (such as Cardio-Green®), Evan's blue, methylene blue; soluble inorganic salts such as copper sulfate (green or blue), $Cu(NH_3)^{2+}$ (dark blue), $MnO_4$ (purple), $NiCl_2$ (green), $CrO_4$ (yellow), $Cr^2O7^{2-}$ (orange); proteins such as rhodopsin (purple and

yellow forms) and green fluorescent protein (fluoresces green under blue light); and any of the Food and Drug Administration (FDA) approved dyes used commonly in foods, pharmaceutical preparations, medical devices, or cosmetics, such as the well-characterized sodium salts FD&C Blue No. 1 (Brilliant Blue FCF), FD&C Green No. 3 (Fast Green FCF), FD&C Red No. 3 (Erythrosine), FD&C Red No. 40 (ALLURA® Red AC), FD&C Yellow No. 5 (Tartrazine), and FD&C Yellow No. 6 (Sunset Yellow FCF). Bio-absorbable chromophores for use in the disclosed compositions are generally water-soluble at physiological pH. The bio-absorbable chromophores may comprise a Food and Drug Administration (FDA)-approved dye. Further, the chromophores may be selected from the group consisting of phthalocyanine dyes, cyanine dyes, and pyrylium dyes. In some embodiments, the chromophores comprise carbon black. In some embodiments, the chromophores are selected from the group consisting of rifampin, β-carotene, tetracycline, indocyanine green, Evan's blue, and methylene blue. In some embodiments, the chromophore is selected from the group consisting of FD&C Blue No. 2, FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6. In some embodiments, the chromophores comprise a soluble inorganic salt selected from the group consisting of copper sulfate, $Cu(NH_3)^{2+}$, $MnO_4$, $NiCl_2$, $CrO_4$, and $Cr^2O7^{2-}$.

**[0097]** After synthesis, the chromophore-filled microparticles are resuspended in a liquid carrier and the UltraInk™ tattoo ink is ready for delivery using conventional tattoo needles and electric tattoo machines.

**[0098]** If an individual desires removal of the tattoo, external energy, such as thermal, sonic (including ultrasonic, audible, and subsonic), light (including laser light, infrared light, or ultraviolet light), or radio frequency (including diathermy, ablation and cryoablation), is capable of removing the tattoo by mechanical lysis of the microparticles into smaller particles thereby releasing the chromophores, that are then reabsorbed, along with the smaller microparticle fragments, by the body by virtue of being bio-absorbable.

**[0099]** After tattoo removal, the skin of the human or animal returns to normal in appearance, texture, and color.

**[0100]** If an individual desires to maintain the tattoo for a longer period, UltraInk™ may also be used to re-tattoo the original design.

**[0101]** A detailed protocol to create and synthesize UltraInk using chromophores with a wide range of encapsulation layers is disclosed herein. The synthesis of

UltraInk™ is performed safely in a standard laboratory. UltraInk™ provides an option to conventional permanent tattoo inks providing future consumers with an alternative that may be permanent or removable, which has tremendous advantages to meet the continued demand for tattoos in the younger population, and the documented significant rate of regret and demand for tattoo removal in the maturing population.

**Materials and Methods:**

**[0102]** The design for the removable tattoo inks disclosed herein is based on a microparticle structure that is between 0.1 µm - 100 µm in diameter using biodegradable materials. The amounts that were used are found in Table 1. At this size, the microparticle is easily applicable with a sterile needle, but too large to diffuse through the dermal layer, ultimately shielding the dye from the body to prevent degradation.

| **Materials** | **Amount** |
|---|---|
| **$CaCl_2$** | 15 mL (0.33 M) |
| **$Na_2CO_3$** | 15 mL (0.33 M) |
| **NaCl** | 250 mL (0.5 M) |
| **PAH** | 5 mg/mL |
| **PSS** | 5 mg/mL |
| **EDTA** | 45 mL (0.2 M) |
| **Food coloring** | 200 mg/mL |

Table 1. Reagents and equipment used for the synthesis of removable tattoo ink.

**Method of Core Synthesis:**

$$CaCl_2 + Na_2CO_3 \rightarrow 2NaCl + CaCO_3$$

Calculations:

$$0.33\ \frac{mole}{L} CaCl_2 \times 110.98\ \frac{g}{mol} \times 0.015\ L = 0.54935\ g\ CaCl_2$$

(~0.54935 g $CaCl_2$ added to 15 mL $dH_2O$ to make a 0.33 M $CaCl_2$ solution).

$$0.33\ \frac{mol}{L} Na_2CO_3 \times 105.99\ \frac{g}{mol} \times 0.015\ L = 0.52465\ g\ Na_2CO_3$$

(~ 0.52465 g $Na_2CO_3$ added to 15 mL $dH_2O$ to make a 0.33 M $Na_2CO_3$ solution).

**[0103]** A 0.33 M $CaCl_2$ solution was added to a 0.33 M $Na_2CO_3$ solution while stirring vigorously for approximately 30 seconds. The solution immediately turned a milky white upon mixing, indicative of the formation of calcium carbonate ($CaCO_3$) microparticles. The solution was allowed to sit at room temperature for approximately 15 minutes. The 15-minute wait time was adapted from Petrov, A. I., et al. (2005). "Protein-calcium carbonate coprecipitation: A tool for protein encapsulation." Biotechnology Progress 21(3): 918-925. The precipitate was then filtered off, washed with $dH_2O$ and air dried. Figure 2 depicts the formation of the calcium carbonate ($CaCO_3$) microparticles.

**Shell (PAH/PSS) Preparation:**

Calculations:

$$0.5\ \frac{mol}{L}\ NaCl \times 58.44\ \frac{g}{mol} \times 0.250\ L\ = 7.305\ g\ NaCl$$

(~ 7.305 g NaCl added to 250 mL $dH_2O$ to make a 0.5 M NaCl solution).

$$0.05\ \frac{mol}{L}\ NaCl \times 58.44\ \frac{g}{mol} \times 0.250\ L\ = 0.7305\ g\ NaCl$$

(~ 0.7305 g NaCl added to 250 mL $dH_2O$ to make a 0.05 M NaCl solution).

**[0104]** Polyelectrolyte layers were deposited onto the calcium carbonate ($CaCO_3$) microparticles (prepared as described in the Method of Core Synthesis) by incubating the $CaCO_3$ microparticles (5% w/v) in solutions of poly(allylamine hydrochloride) (PAH, M.W. = 120,000-200,000 Daltons) or polystyrene sulfonate (PSS, M.W. = 70,000 Daltons) at a concentration of 5 mg/mL in 0.5 M NaCl. During incubation, samples were gently agitated on a shaker table for approximately 30 minutes. To remove excess polyelectrolyte, samples were centrifuged (1500 rpm, 5 minutes), the supernatant was removed, and samples were resuspended in $dH_2O$. The microparticles were then sonicated in a bath sonicator for approximately two (2) minutes to remove any aggregation. The samples are then washed two (2) additional times to remove excess polyelectrolyte. This procedure was then repeated with the alternate polyelectrolyte. In this particular example, PSS was used in the first deposition step and PAH was used in the last deposition step. Figure 3 depicts the shell formation.

**Hollow Microparticle Preparation:**

Calculations:

$$0.2\ \frac{mol}{L}\ EDTA \times 292.24\ \frac{g}{mol} \times 0.045\ L\ =\ 2.63\ g\ EDTA$$

(~ 2.63 g EDTA added to 45 mL $dH_2O$ to make a 0.2 M EDTA solution).

$$0.5\ M\ NaOH \times 40.0\ \frac{g}{mol} \times 0.045\ L\ =\ 0.9\ g\ NaOH$$

(0.9 g NaOH added to 45 mL 0.2 M EDTA solution to dissolve EDTA).

**[0105]** After the final polyelectrolyte layer number was achieved, the $CaCO_3$ core was dissolved by resuspending in a 0.2 M EDTA solution at pH ~7 (pH adjusted with NaOH) and gently agitated for 15 minutes. Centrifugation (1500 rpm, 10 min) and washing with $dH_2O$ were used to remove the dissolved core (repeated 5x), thus creating hollow microparticles. Figure 4 depicts the formation of the hollow microparticles.

**Method of Dye Loading:**

**[0106]** After the removal of the $CaCO_3$ core, and thus the formation of a hollow microparticle, dye was loaded into the hollow core by resuspension in FD&C Blue 1, FD&C Blue 2, Natural Dye (obtained from Color Maker) or Spirulina (obtained from Color Maker) at a concentration of 200 mg/mL. Samples were gently agitated on a shaker table for 2 hours, allowing for osmotically driven loading of dye into the internal cavity of the hollow microparticle. After agitation, samples were heated to 70 °C for 30 minutes to allow for thermal condensation, entrapping the loaded dye permanently. Figure 5 depicts the dye-loaded microparticles.

A **Modified Protocol** to create and synthesize UltraInk™ using chromophores with a wide range of encapsulation layers is disclosed.

**Materials and Methods:**

**[0107]** The design for the removable tattoo inks disclosed herein is based on a microparticle structure that is between 0.1 µm - 100 µm in diameter using biodegradable materials. The amounts that were used are found in Table 2. At this size, the microparticle is easily applicable with a sterile needle, but too large to

diffuse through the dermal layer, ultimately shielding the dye from the body to prevent degradation.

| Materials | Amount |
|---|---|
| **$CaCl_2$** | 15 mL (0.33 M) |
| **$Na_2CO_3$** | 15 mL (0.33 M) |
| **NaCl** | 1 L (0.2 M) |
| **PAH** | 5 mg/mL |
| **PSS** | 5 mg/mL |
| **EDTA** | 45 mL (0.2 M) |
| **Food coloring** | 2.5 g/L |

Table 2. Reagents and equipment used for the modified synthesis of removable tattoo ink.

**Modified Core Synthesis:**

$$CaCl_2 + Na_2CO_3 \rightarrow 2NaCl + CaCO_3$$

Calculations:

$$0.33\ \frac{mole}{L} CaCl_2 \times 110.98\ \frac{g}{mol} \times 0.200\ L = 7.32468\ g\ CaCl_2$$

$$0.33\ \frac{mol}{L} Na_2CO_3 \times 105.99\ \frac{g}{mol} \times 0.200\ L = 6.99534\ g\ Na_2CO_3$$

(~0.52465 g $Na_2CO_3$ added to 15 mL $dH_2O$ to make a 0.33 M $Na_2CO_3$ solution).

**[0108]** A 0.33 M $CaCl_2$ solution was added to a 0.33 M $Na_2CO_3$ solution which contained 2.5 g/L of Red 40 dye while stirring vigorously for ~30 seconds. The solution immediately turned a red milky white upon mixing, indicative of the formation of calcium carbonate ($CaCO_3$) microparticles. The solution was allowed to sit at room temperature for ~15 minutes. The 15 minute wait time was adapted from: Petrov, A. I., et al. (2005). "Protein-calcium carbonate coprecipitation: A tool for protein encapsulation." Biotechnology Progress 21(3): 918-925. The precipitate is then filtered off, washed with $dH_2O$ three times. Figure 7 depicts the formation of the calcium carbonate ($CaCO_3$) microparticles.

**Modified Shell (PAH/PSS) Preparation:**

Calculations:

$$0.2\ \frac{mol}{L}\ NaCl \times 58.44\ \frac{g}{mol} \times 1.0\ L\ = 11.688\ g\ NaCl$$

(~ 11.688 g NaCl added to 1 L $dH_2O$ to make a 0.2 M NaCl solution).

**[0109]** Polyelectrolyte layers were deposited onto $CaCO_3$ microparticles (preparation shown in **Modified Core Synthesis**) by incubating $CaCO_3$ microparticles (5% w/v) (ex. 1.5 g per 30 mL = 1.5g/30mL x 100 = 5% w/v) in solutions of poly(allylamine hydrochloride) (PAH, M.W. = 120,000-200,000 Daltons) or polystyrene sulfonate (PSS, M.W. = 70,000 Daltons) at a concentration of 2 mg/mL in 0.5 M NaCl. The first layer deposited is PSS, as it interacts most favorably with the core. During incubation, samples were gently agitated on a shaker table for ~15 minutes. To remove excess polyelectrolyte, samples were centrifuged (2000 rpm, 2 minutes), the supernatant was removed, and samples were resuspended in $dH_2O$. The samples are then washed two additional times to remove excess polyelectrolyte. This procedure was then repeated with the alternate polyelectrolyte. In certain example embodiments, PSS was used in the first deposition step and PAH was used in the last deposition step.

**Modified Hollow Microparticle Preparation:**

Calculations:

$$0.2\ \frac{mol}{L}\ EDTA \times 292.24\ \frac{g}{mol} \times 1.0\ L\ = 58.448\ g\ EDTA$$

(~2.63 g EDTA added to 1 $dH_2O$ to make a 0.2 M EDTA solution).

$$0.5\ M\ NaOH \times 40.0\ \frac{g}{mol} \times 1.0\ L\ =\ 20\ g\ NaOH$$

(0.9 g NaOH added to 45 mL 1 EDTA solution to dissolve EDTA).

**[0110]** The EDTA solution was made by adding dry EDTA to $dH_2O$ while stirring on a stir plate. The NaOH was then added and allowed to dissolve. The pH was measured and adjusted with NaOH to arrive at a final pH between 6.8-7.2.

**[0111]** After the final polyelectrolyte layer number was achieved, the $CaCO_3$ core was dissolved by resuspending in 30 mL of EDTA solution and gently agitating for

30 minutes on the shaker table. Each tube was occasionally opened during this 30 minute period to allow for the release of gases resulting from the reaction of the core with the EDTA solution. After 30 minutes, the samples were centrifuged and the supernatant was removed and replaced with fresh EDTA. This was repeated five times. After the fifth treatment, the hollow capsules were washed three times with $dH_2O$. Figure 8 depicts the formation of the hollow microparticles.

**Modified Method of Dye Loading:**

**[0112]** After the removal of the $CaCO_3$ core and thus the formation of a hollow microcapsule, dye was loaded into the hollow core by resuspension in the color of choice. Capsules were dispersed in 142 g/L of Red 40 concentrated solution and stirred overnight, allowing for osmotically driven loading of dye into the internal cavity of the hollow microparticles. After overnight stirring, capsules were washed with $dH_2O$ three times and isopropanol three times. A layer of silica coating was deposited on the microparticle surface by hydrolyzing Tetraethyl orthosilicate in ethanol and water basic solution to prevent dye molecule leaking. Figure 9 depicts the dye-loaded microparticles formed from the modified synthesis protocol.

**Dye Leakage Study:**

**[0113]** To determine if leakage is an issue from the microparticles (post-loading), the loaded microparticles were suspended in deionized water and allowed to equilibrate at room temperature. Daily for two (2) weeks, samples were spun at 1500 rpm for 5 minutes and 500 μL of the supernatant was collected. A plate reader was then used to determine the presence of free dye in the supernatant, which would indicate the loss of dye from the loaded microparticles. As shown in Figure 6, no significant color loss was indicated for 2-10 polyelectrolyte bilayers.

**Tattoo Ink Production:**

**[0114]** To produce ink thick enough for tattooing, dye-loaded microparticles were freeze-dried in deionized water for two (2) days to produce an ink powder. This powder was then re-suspended in a 1:1 mixture of glycerine and isopropyl alcohol (IPA) at a concentration of 40% w/v. If the suspension is too thick, additional increments of glycerine and IPA may be added until the desired thickness is achieved. The concentration should reside between 30% and 40% w/v to achieve the desired tattoo color richness.

**[0115]** Various modifications and variations of the described methods and compositions of the disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it will be understood that it is capable of further modifications and that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known customary practice within the art to which the invention pertains and may be applied to the essential features herein before set forth.

**[0116]** All publications, patents, and patent applications mentioned herein are incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. In the event of there being a difference between definitions set forth in this application and those in documents incorporated herein by reference, the definitions set forth herein control.

What is claimed is:

1. A method of making a removable tattoo ink, comprising:

forming a solid core;

coating the solid core with at least two layers of oppositely charged polyelectrolytes;

removing the solid core to create microparticles with a hollow core; and

loading one or more dyes into the hollow core of the microparticles to generate dye-loaded microparticles.

2. The method of claim 1, further comprising depositing a silica coating on the surface of the dye-loaded microparticles.

3. The method of claim 1 or 2, further comprising freeze drying the dye-loaded microparticles to form a powder.

4. The method of claim 3, wherein the powder is re-suspended in a tattoo application solution.

5. The method of claim 4, wherein the powder is 15% to 80% w/v of the tattoo application solution.

6. The method of claim 5, wherein the tattoo application solution is a 1:1 mixture of glycerine and isopropyl alcohol.

7. The method of any one of claims 1 to 6, wherein loading the one or more dyes comprises suspending the microparticles in a dye loading solution comprising the one or more dyes.

8. The method of any one of claims 1 to 7, wherein the microparticles have a diameter of approximately 0.5 μm to 100 μm.

9. The method of any one of claims 1 to 8, where the microparticles have a diameter of approximately 25 μm.

10. The method of any one of claims 1 to 9, wherein the solid core comprises calcium carbonate.

11. The method of any one of claims 1 to 10, wherein forming the solid core comprises mixing in solution $CaCl_2$ and $Na_2CO_3$.

12. The method of any one of claims 1 to 11, wherein the oppositely charged polyelectrolytes are labile upon application of an external energy source.

13. The method of claim 12, wherein the external energy source is electromagnetic radiation, ultrasonic energy, laser light operating in a near infrared range, ultraviolet light, a radio frequency sufficient to cause radio frequency diathermy, radio frequency ablation, or radio frequency cryoablation.

14. The method of any one of claims 1 to 13, wherein the oppositely charged polyelectrolytes comprise between 2 and 16 alternating layers of poly(allylamine hydrochloride) (PAH) and polystyrene sulfonate (PSS).

15. The method of any one of claims 1 to 13, wherein the oppositely charged polyelectrolytes comprise between 2 and 16 alternating layers of one or more of poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid.

16. The method of any one of claims 1 to 13, wherein the oppositely charged polyelectrolytes comprise polymers containing azobenzene moieties.

17. The method of claim 16, wherein the azobenzene moieties comprise Brilliant Yellow (BY), or poly{1-4[4-(3-carboxy-4-hydroxyphenylazo) benzenesulfonamido]-1,2-ethanediyl sodium salt} (PAZO).

18. A method of making a removable tattoo ink, comprising:

forming a solid core;

mixing one or more chromophores with a polyelectrolyte;

coating the solid core with at least two layers of oppositely charged polyelectrolytes, wherein at least one polyelectrolyte layer comprises chromophores;

removing the solid core to create microparticles with a hollow core; and

loading one or more dyes into the hollow core of the microparticles to generate dye-loaded microparticles.

19. A method of making a removable tattoo ink, comprising:

forming a solid core;

coating the solid core with alternating layers of negatively-charged chromophores and positively-charged polyelectrolytes;

removing the solid core to create microparticles with a hollow core; and

loading one or more dyes into the hollow core of the microparticles to generate dye-loaded microparticles.

20. The method of claim 18 or 19, further comprising a silica coating on the surface of the dye-loaded microparticles.

21. The method of any one of claims 18 to 20, further comprising freeze drying the dye-loaded microparticles to form a powder.

22. The method of claim 21, wherein the powder is re-suspended in a tattoo application solution.

23. The method of claim 22, wherein the powder is 15% to 80% w/v of the tattoo application solution.

24. The method of claim 23, wherein the tattoo application solution is a 1:1 mixture of glycerine and isopropyl alcohol.

25. The method of any one of claims 18 to 24, wherein loading the one or more dyes comprises suspending the microparticles in a dye loading solution comprising the one or more dyes.

26. The method of any one of claims 18 to 25, wherein the microparticles have a diameter of approximately 0.5 μm to 100 μm.

27. The method of any one of claims 18 to 26, where the microparticles have a diameter of approximately 25 μm.

28. The method of any one of claims 18 to 27, wherein the solid core comprises calcium carbonate.

29. The method of any one of claims 18 to 28, wherein forming the solid core comprises mixing in solution $CaCl_2$ and $Na_2CO_3$.

30. The method of any one of claims 18 to 29, wherein the oppositely charged polyelectrolytes are labile upon application of an external energy source.

31. The method of claim 30, wherein the external energy source is electromagnetic radiation, ultrasonic energy, laser light operating in a near infrared range, ultraviolet light, a radio frequency sufficient to cause radio frequency diathermy, radio frequency ablation, or radio frequency cryoablation.

32. The method of any one of claims 18 to 31, wherein the oppositely charged polyelectrolytes comprise between 2 and 16 alternating layers of poly(allylamine hydrochloride) (PAH) and polystyrene sulfonate (PSS).

33. The method of any one of claims 19 to 31, wherein the positively-charged polyelectrolytes comprise between 2 and 16 alternating layers of poly(allylamine hydrochloride) (PAH).

34. The method of any one of claims 19 to 31, wherein the negatively-charged chromophores comprise between 2 and 16 alternating layers of black and/or white chromophores.

35. The method of any one of claims 18 to 31, wherein the oppositely charged polyelectrolytes comprise between 2 and 16 alternating layers of one or more of poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid.

36. The method of any one of claims 18 to 31, wherein the oppositely charged polyelectrolytes comprise polymers containing azobenzene moieties.

37. The method of claim 36, wherein the azobenzene moieties comprise Brilliant Yellow (BY), or poly{1-4[4-(3-carboxy-4-hydroxyphenylazo) benzenesulfonamido]-1,2-ethanediyl sodium salt} (PAZO).

38. A removable tattoo ink made by the method of any one of claims 1 to 37.

39. A removable tattoo ink, comprising colored microparticles, wherein the microparticles, comprise:

an internal core, comprising one or more bio-absorbable chromophores, and

an outer shell, comprising at least two layers of oppositely charged polyelectrolytes, wherein the outer shell is rupturable by application of an external energy source.

40. The removable tattoo ink of claim 39, further comprising a silica coating on the surface of the microparticles.

41. A removable tattoo ink of claim 39 or 40, wherein the outer shell comprises at least one layer of polystyrene sulfonate (PSS) and at least one layer of polyallylamine hydrochloride (PAH).

42. A removable tattoo ink of claim 39 or 40, wherein the outer shell comprises between 2 and 16 alternating layers of poly(allylamine hydrochloride) (PAH) and polystyrene sulfonate (PSS).

43. A removable tattoo ink of claim 39 or 40, wherein the outer shell comprises between 2 and 16 layers of one or more of poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid.

44. The removable tattoo ink of any one of claims 39 to 43, wherein the bio-absorbable chromophores are selected from the group consisting of titanium dioxide, iron oxide, gold nanoparticles, quantum dots, FD&C Blue No. 2, FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6.

45. The removable tattoo ink of any one of claims 39 to 44, wherein the external energy source is electromagnetic radiation or ultrasonic energy.

46. The removable tattoo ink of any one of claims 39 to 44, wherein the external energy source is laser light operating in the near infrared.

47. The removable tattoo ink of any one of claims 39 to 44, wherein the external energy source is ultraviolet light.

48. The removable tattoo ink of any one of claims 39 to 44, wherein the external energy source is radio frequency diathermy, radio frequency ablation or radio frequency cryoablation.

49. A removable tattoo ink, comprising colored microparticles, wherein the microparticles, comprise:

an internal core, comprising one or more bio-absorbable chromophores, and

an outer shell, comprising between 2 and 16 layers of one or more of poly(diallyldimethylammonium chloride) (PDADMAC), poly(acrylic acid) (PAA), poly(vinyl sulfate) (PVS), dextran sulfate (DS), poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), heparin, poly-L-arginine, or poly-L-glutamic acid, wherein the outer shell is rupturable by application of an external energy source.

50. The removable tattoo ink of claim 49, further comprising a layer of silica coating on the surface of the microparticles.

51. The removable tattoo ink of any one of claims 49 to 50, wherein the external energy source is electromagnetic radiation or ultrasonic energy.

52. The removable tattoo ink of any one of claims 49 to 50, wherein the external energy source is laser light operating in the near infrared.

53. The removable tattoo ink of any one of claims 49 to 50, wherein the external energy source is ultraviolet light.

54. The removable tattoo ink of any one of claims 49 to 50, wherein the external energy source is radio frequency diathermy, radio frequency ablation or radio frequency cryoablation.

55. The removable tattoo ink of any one of claims 49 to 54, wherein the bio-absorbable chromophores are selected from the group consisting of titanium dioxide, iron oxide, gold nanoparticles, quantum dots, FD&C Blue No. 2, FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6.

56. A removable tattoo ink, comprising:

forming a solid core;

mixing one or more chromophores with a polyelectrolyte;

coating the solid core with at least two layers of oppositely charged polyelectrolytes, wherein at least one polyelectrolyte layer comprises chromophores;

removing the solid core to create microparticles with a hollow core; and

loading one or more dyes into the hollow core of the microparticles to generate dye-loaded microparticles.

57. The method of claim 56, further comprising a silica coating on the surface of the dye-loaded microparticles.

58. A removable tattoo ink, comprising:

forming a solid core;

coating the solid core with at least two alternating layers of negatively-charged chromophores and positively-charged polyelectrolytes;

removing the solid core to create microparticles with a hollow core; and

loading one or more dyes into the hollow core of the microparticles to generate dye-loaded microparticles.

59. The method of claim 58, further comprising a silica coating on the surface of the dye-loaded microparticles.

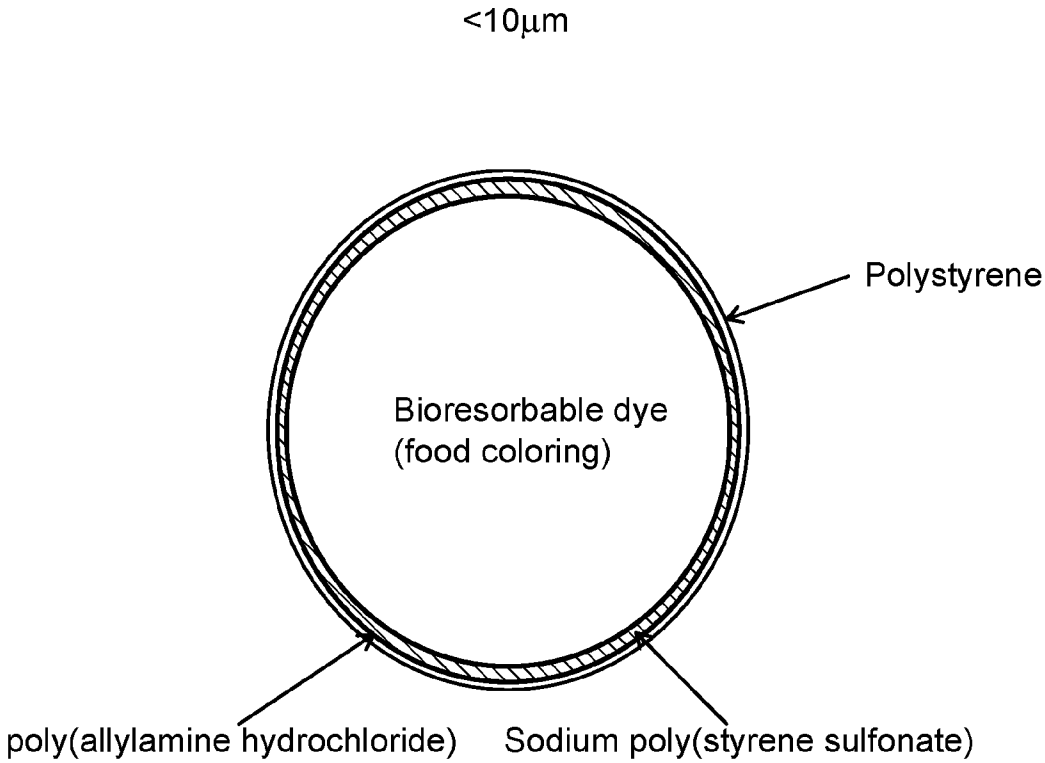
<10μm
Polystyrene
Bioresorbable dye
(food coloring)
poly(allylamine hydrochloride)
Sodium poly(styrene sulfonate)

FIGURE. 1

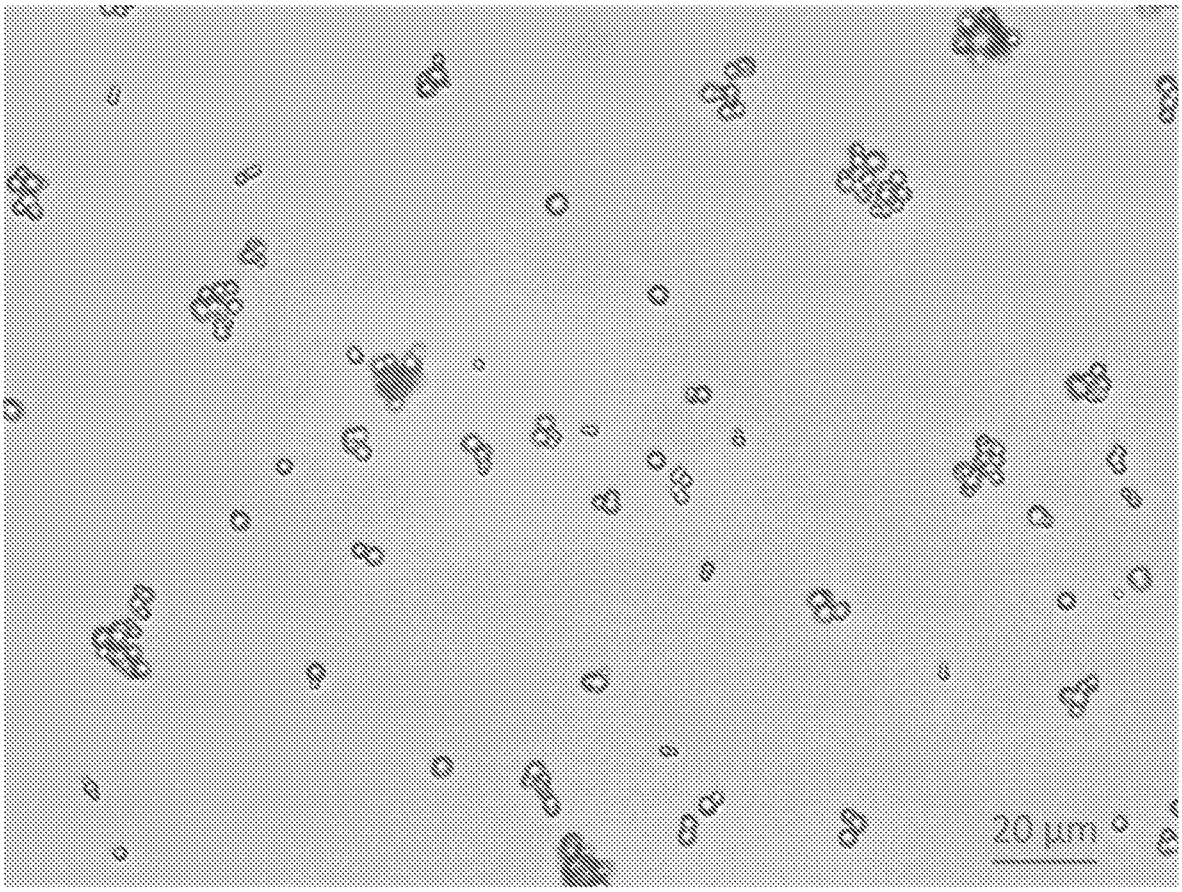


FIGURE 2

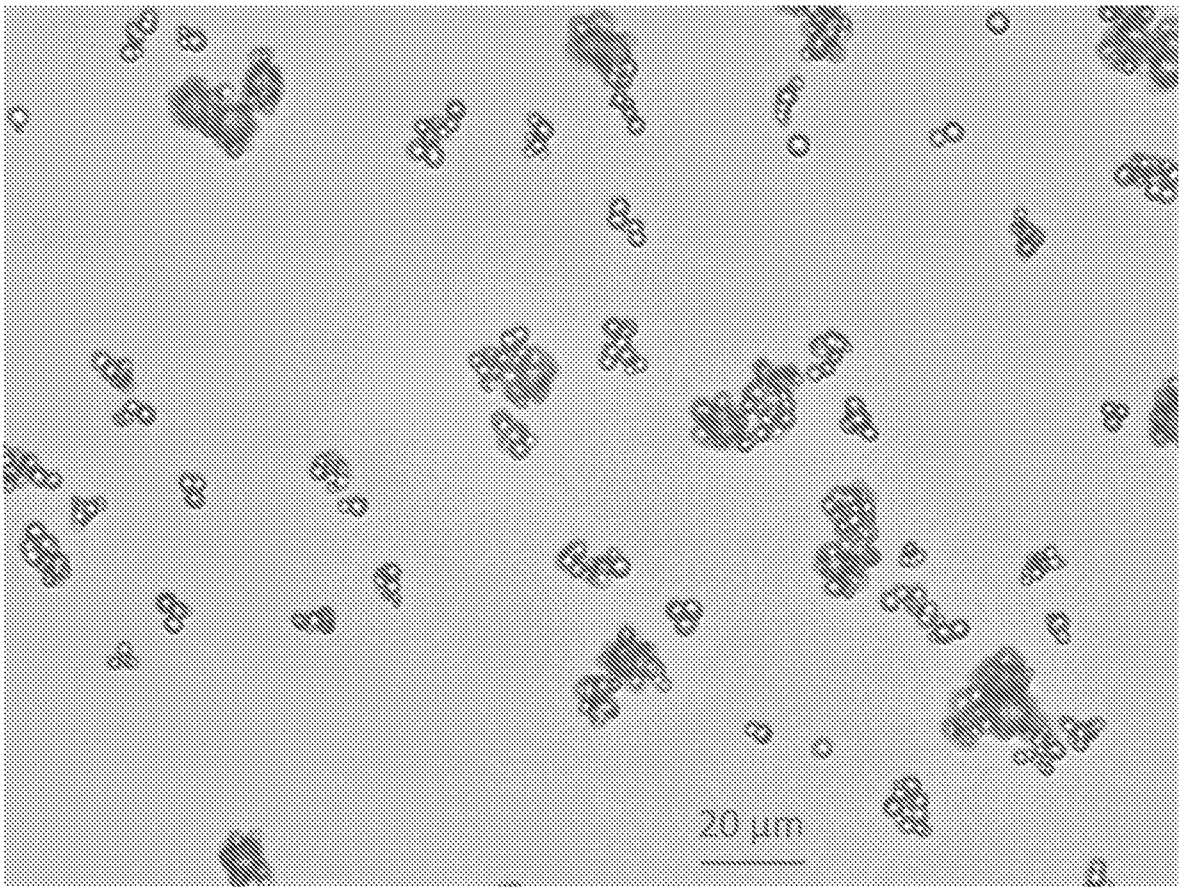


FIGURE 3

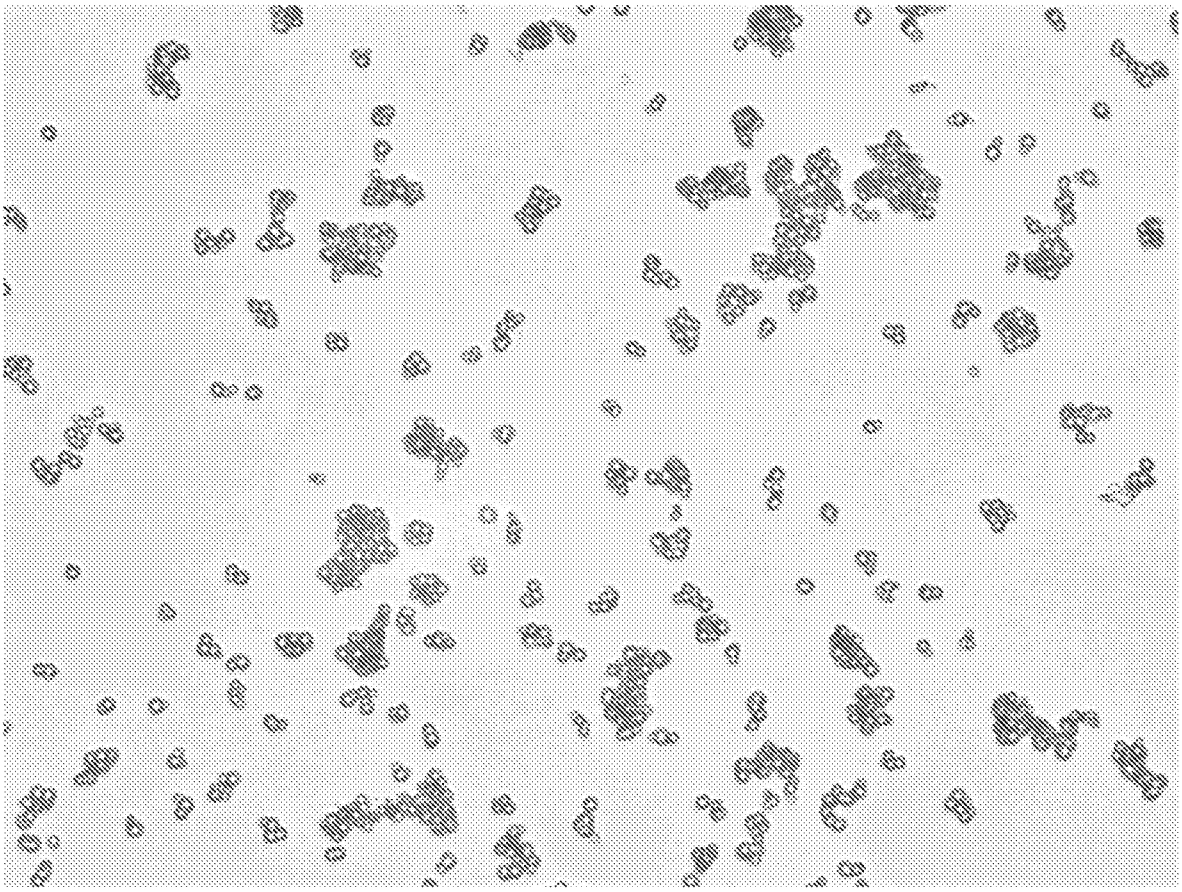

FIGURE 4

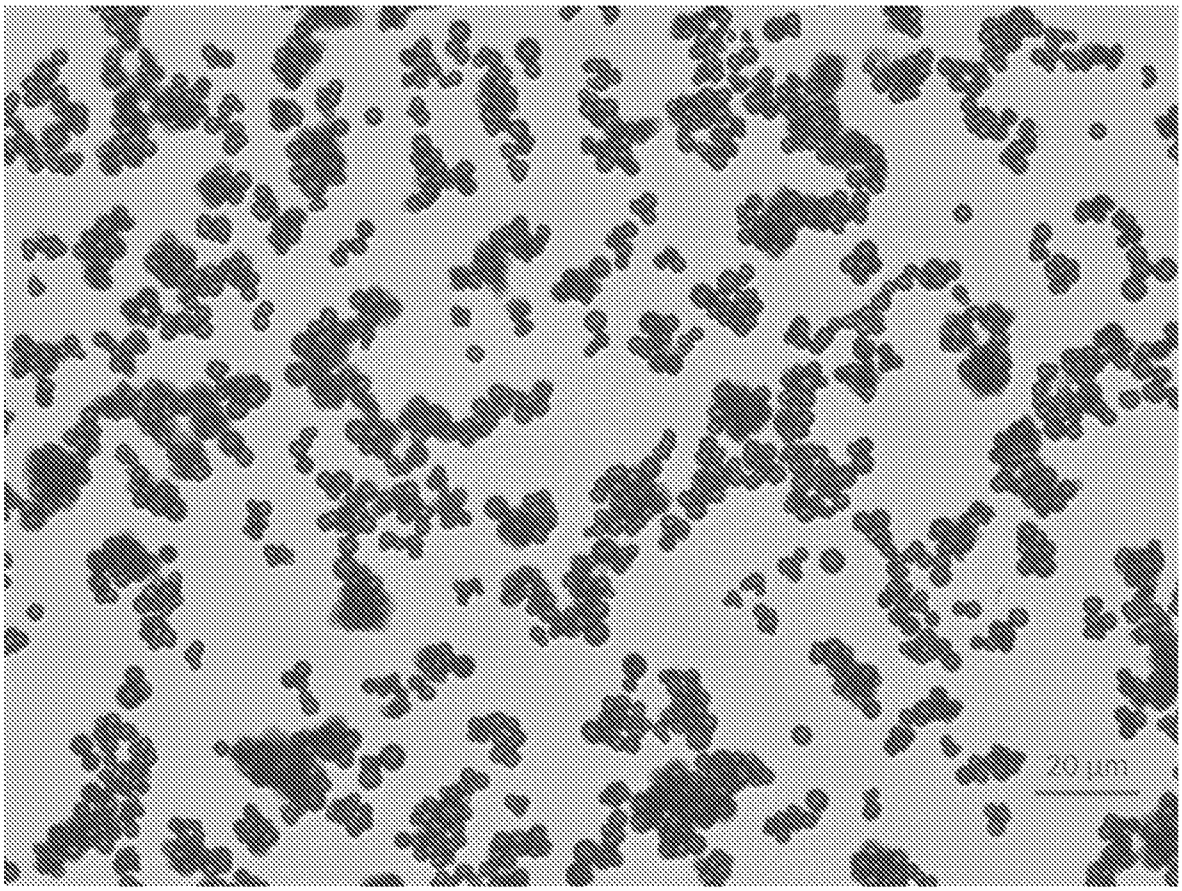


FIGURE 5

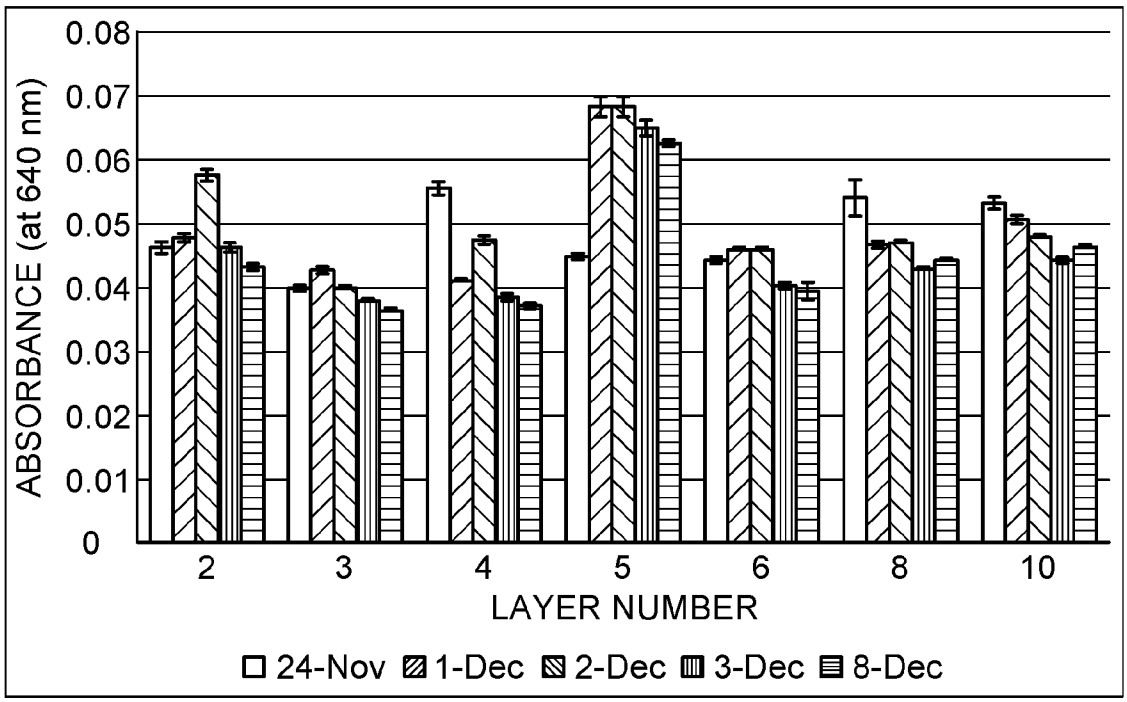
ABSORBANCE (at 640 nm)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2
3
4
5
6
8
10
LAYER NUMBER
24-Nov
1-Dec
2-Dec
3-Dec
8-Dec

FIGURE. 6

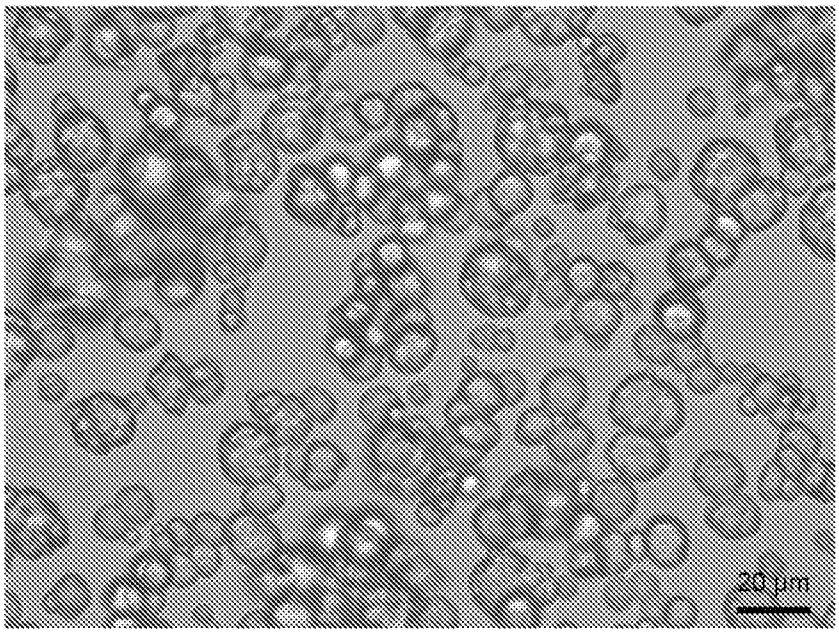


FIGURE 7

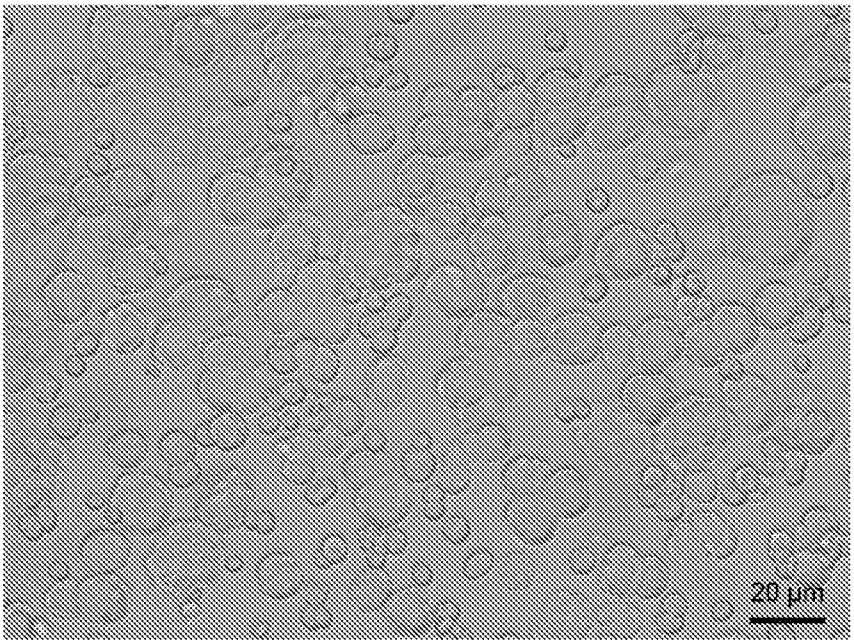


FIGURE 8

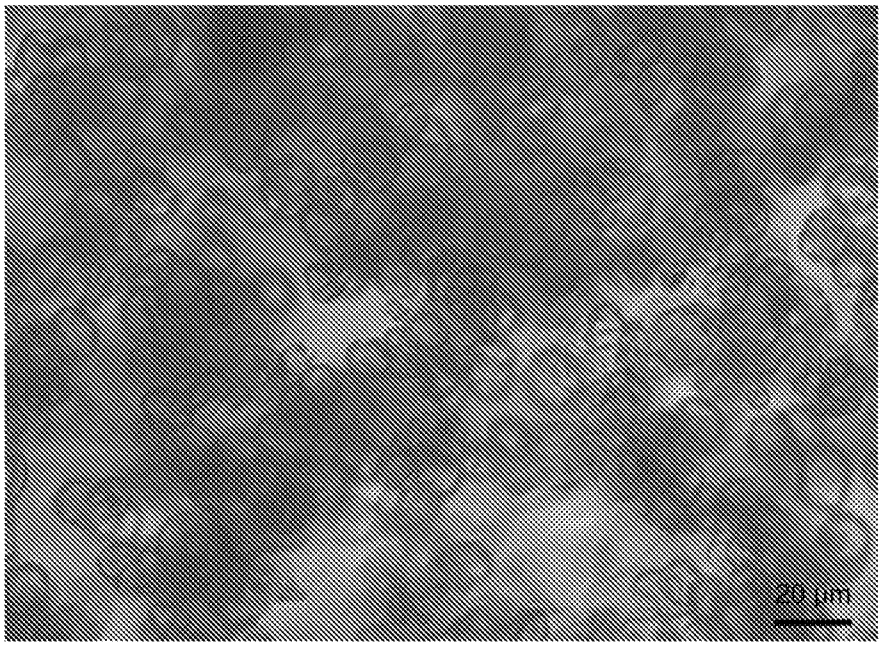


FIGURE 9

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/026683

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 8/11; A61K 8/81; A61Q 1/02; C08K 5/41; C09D 141/00 (2017.01)
CPC - A61K 8/11; A61K 8/81; A61Q 1/025; C08K 5/41; C09D 141/00 (2017.02)

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)
See Search History document

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)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | US 2015/0265508 A1 (ULTRA INK LLC) 24 September 2015 (24.09.2015) entire document | 1, 19, 39, 41, 42, 58<br>---<br>2-4, 18, 20, 40, 43, 56, 57, 59 |
| X<br>---<br>Y | US 6,013,122 A (KLITZMAN et al) 11 January 2000 (11.01.2000) entire document | 49, 51-53<br>---<br>43, 50, 54 |
| Y | US 2007/0107625 A1 (ANDERSON et al) 17 May 2007 (17.05.2007) entire document | 2, 18, 20, 40, 50, 54, 56, 57, 59 |
| Y | US 2013/0302425 A1 (OHTAKE et al) 14 November 2013 (14.11.2013) entire document | 3, 4 |

☐ Further documents are listed in the continuation of Box C. ☐ 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 |
|---|---|
| 06 June 2017 | 28 JUN 2017 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, VA 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Blaine R. Copenheaver<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/026683

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

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

3. ☒ Claims Nos.: 7-17, 21-38, 44-48, 55
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

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

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

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

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

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

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.