Title: HAIR SEQUESTERED TRACER FOR PROSTHESIS LEAK DETECTION

Abstract: There is provided use of a tracer for sequestering in hair for detecting leakage of a prosthesis. Also provided is use of a tracer for sequestering in hair for detecting the time of leakage of a prosthesis. Also provided is use of a tracer for sequestering in hair for detecting the severity of leakage of a prosthesis. A filler composition for a prosthesis comprising a tracer that is sequestered in the hair is provided. Additionally, a prosthesis comprising a filler composition, the filler composition comprising a tracer that is sequestered in the hair is provided. Methods for detecting leakage from a prosthesis are also provided. Methods often include a) treating a hair sample from a subject having a prosthesis to extract a tracer, thereby obtaining an extract; and b) testing the extract for the tracer, wherein the presence of the tracer or a metabolite thereof in the extract is indicative of a leakage from the prosthesis. Kits for submitting a test for prosthesis leakage are also provided.
Filler Compositions For A Prosthesis, Uses thereof, Prostheses Containing Filler Compositions, Methods For Detecting Leaks In Prostheses And Kits Therefor

Technical Field

This invention relates to the field of prostheses and fillers for prostheses. In particular, this invention relates to the compositions that are enveloped or contained within prostheses their uses and methods for detecting leakage of those compositions from the prostheses.

Background

There are many known prostheses and fillers for prostheses and many are described in US patents 5,545,220; 5,658,330; 6,074,421; and 6,099,565. Methods for making these fillers and prostheses are also described by these patents.

Many prostheses leak. Leaking of a prosthesis can cause problems to the subject in whom the prosthesis resides. Means for assessing leakage of a prosthesis include mammography, computed tomography (CT) scanning, ultrasonography, and nuclear magnetic resonance imaging (MRI). Mammography can cause leakage of a prosthesis. Frequent CT scans are discouraged in order to reduce exposure to radiation. The sensitivity of ultrasonography is low, having a range of 32% and 74% accuracy. MRI evaluation is inhibited by poor accessibility to diagnostic equipment, in terms of both financial restrictions and regional availability of necessary equipment. (Brown et al., (1997) Rupture of silicone-gel breast implants: causes, sequelae, and diagnosis, Lancet, 350: 1531-1537).

International Patent Publication WO 2005/039450 describes prostheses containing a rupture indicator. The prostheses described include an external envelope of medical grade elastomer containing a fluid material and a biologically compatible chemical indicator for indicating rupture of the prosthesis, and an internal envelope of medical grade elastomer disposed within the external envelope, the internal envelope containing an implant filling material. This publication also describes methods for detecting rupture of a prosthesis, which includes surgically implanting a prosthesis containing a biologically compatible chemical indicator in a location of a patient body in need of the prosthesis; and detecting a change in a bodily secretion, peripheral blood, or locally around the prosthesis for indication of leaking out of the indicator from the prosthesis. The bodily excretion or secretion that can be used, according to WO 2005/039450, for the detection of prosthesis rupture includes materials such as urine, saliva, perspiration and feces. The changes include a presence of the chemical indicator or metabolized product thereof in the bodily excretion, secretion or peripheral blood, an odor from the indicator in the bodily excretion or secretion, a color change of at least one of
the body's excretions or secretions, and a change in sensation or taste caused by the presence of the indicator in the bodily secretion. Local skin color change and local x-ray opacity change is also described as indicative of leakage.

Lykissa et al. in "Total Platinum Concentration and Platinum Oxidation States in Body Fluids, Tissue, and Explants from Women Exposed to Silicon and Saline Breast Implants by IC-ICPMS" (Anal. Chem 2006, 78, 2925-2933) describe that chromatography-inductively coupled plasma-mass spectrometry was used to determine the total platinum concentration and platinum oxidation states in samples from women exposed to silicone and saline breast implants. Samples included the following: whole blood, urine, hair, nails, perspiration, brain tissue, breast milk, and explants. This report shows that women exposed to silicone breast implants have platinum levels that exceed that of the general population. The study is described as being useful for risk assessment of platinum exposure in women that have had, or currently have, silicone or saline breast implants.

Tanaka et al. in "Absorption, distribution and excretion of 14C-levofloxacin after single oral administration in albino and pigmented rats: binding characteristics of levofloxacin-related radioactivity to melanin in vivo" (J. Pharm Pharmacol. 2004 Apr; 56(4):463-469) describe that after a single oral administration of (14)C-levofloxacin at a dose of 20 mg kg(-1) under non-fasting conditions, the absorption, distribution and excretion of radioactivity were studied in albino and pigmented rats. Good penetration of radioactivity into tissues was indicated by higher concentrations in most tissues compared with serum and there were no quantitative differences in the distribution of radioactivity between albino and pigmented rats except for melanin-containing tissues such as the uveal tract of eyes and hair follicles. There was selective and strong binding of drug-related radioactivity to these tissues in pigmented rats.

Kosuge et al. in "Time course of appearance of ofloxacin in human scalp hair after oral administration" (Ther Drug Monit. 1995 Feb; 17(1): 101-3) describe that the time course of appearance of antimicrobial ofloxacin (OFLX) in human scalp hair was monitored in three healthy male volunteers after the oral administration of 100 mg OFLX three times daily for 2 consecutive days. Hair samples were collected from each subject by plucking several strands of frontal hair every day from 1 till 16 days after administration. A single hair was dissolved in 1 M NaOH to extract OFLX by chloroform, and the drug was measured by high-performance liquid chromatography and fluorescence detection. OFLX started to appear in the hair 1 to 3 days after administration and reached the maximal level approximately 4 to 9 days, remaining
at almost the same level thereafter. This finding suggests the slow transfer of OFLX from hair follicle cells to hair matrix may be due to the slow dissociation of OFLX from bound melanin.


Health concerns associated with silicone implant leakage, including inflammation and scarification of the tissue surrounding the implant, as well as possible systemic immune responses, ultimately led to a Food and Drug Administration (FDA) silicone breast implant ban in 1992. This ban was lifted in 2005 and the FDA recommended that patients who have silicone implants undergo MRI every two years in order to detect leakage.

**Summary**

An illustrative embodiment of this invention is based, in part, on the elucidation of compounds that are suitable for detection close to the time a leak in a prosthesis occurs and for an extended period of time thereafter. Another illustrative embodiment of this invention is based, in part, on non-invasive methods of detecting the suitable compounds. Another
illustrative embodiment of this invention is based, in part, on providing easily available
detection methods. The detection methods may be provided, in part, in kits.

In an illustrative embodiment of the present invention, there is provided a filler composition for a prosthesis comprising a tracer that is sequestered in the hair.

In another illustrative embodiment of the present invention, there is provided a filler composition described herein wherein the tracer is selected from the group consisting of:
quinolines, steroids, tricyclic antidepressants, aminoglycosides, beta-2 adrenergic receptor agonists, beta blockers and phthalates.

In another illustrative embodiment of the present invention, there is provided a filler composition described herein wherein the tracer is selected from the group consisting of:
chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol mesylate, salmeterol, formoterol, bumberterol, dichloroisoprenaline, practolol, pronethaolol, alpenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamine, clozapine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methyllecgonine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, triazolobendiazepine, ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethy1 metabolites of tricyclics.

In another illustrative embodiment of the present invention, there is provided a filler composition described herein wherein the tracer is selected from the group consisting of:
chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol
mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, and butoxamine.

In another illustrative embodiment of the present invention, there is provided a filler composition described herein wherein the tracer is ofloxacin.

In another illustrative embodiment of the present invention, there is provided a prosthesis comprising a filler composition, the filler composition comprising a tracer that is sequestered in the hair.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the tracer is selected from the group consisting of: quinolines, steroids, tricyclic antidepressants, aminoglycosides, beta-2 adrenergic receptor agonists, beta blockers and phthalates.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamines, cloazepine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methylecgonine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, triazolobendiazepine, ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethyl metabolites of tricyclics.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC.
17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol, mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alpenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, and butoxamine.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the tracer is ofloxacin.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the prosthesis is selected from the group consisting of breast, buttock, calf, arm, penile, testicular, facial, pectoral, brow, nose, chin, cheek, lip, triceps and biceps.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the prosthesis is selected from the group consisting of breast, buttock, chin, cheek, pectoral and testicular.

In another illustrative embodiment of the present invention, there is provided a prosthesis described herein wherein the prosthesis is a breast implant.

A method for detecting leakage from a prosthesis comprising a filler comprising a tracer, the method comprising, a) treating a hair sample from a subject having the prosthesis to extract a tracer, thereby obtaining an extract; and b) testing the extract for the tracer, wherein the presence of the tracer or a metabolite thereof in the extract is indicative of a leakage from the prosthesis.

In another illustrative embodiment of the present invention, there is provided a method for detecting leakage from a prosthesis comprising a filler comprising a tracer, the method comprising, a) obtaining a hair sample from a subject having the prosthesis; b) treating the hair sample to extract a tracer thereby obtaining an extract; and c) testing the extract for the tracer, wherein the presence of the tracer or a metabolite thereof in the extract is indicative of leakage from the prosthesis.
In another illustrative embodiment of the present invention, there is provided a method described herein wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levaterol, terbutaline, pirbuterol, procaterol, metaproteuronil, fenoterol, bitolterol mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamine, clozapine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinaine, meprobamate, methylecgonine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, triazolobenzodiazepine, ofloxacin-N-oxide, desmethyl-oxofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethyl metabolites of tricyclics.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levaterol, terbutaline, pirbuterol, procaterol, metaproteuronil, fenoterol, bitolterol mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, and butoxamine.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the tracer is ofloxacin.
In another illustrative embodiment of the present invention, there is provided a method described herein wherein the treating comprises base extraction followed by solid phase extraction.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the base extraction is sodium hydroxide extraction.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the solid phase extraction comprises cation exchange.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the extract is tested using mass spectrometry, Enzyme-linked immunosorbent assay (ELISA), Fluorescence spectroscopy, ultraviolet (UV) spectrometry, nuclear magnetic resonance (NMR), gas chromatography (GC), liquid chromatography (LC), medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), mass spectrometry (MS), tandem MS (MS/MS), LC-MS, GC-MS, HPCL-MS/MS, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS.

In another illustrative embodiment of the present invention, there is provided a method described herein wherein the extract is tested using mass spectrometry.

In another illustrative embodiment of the present invention, there is provided a kit for submitting a test for prosthesis leakage, the kit comprising: a) a container for holding an extracted hair; and b) instructions for hair extraction and delivery of the container.

In another illustrative embodiment of the present invention, there is provided a kit described herein further comprising a tool for extracting a hair for providing an extracted hair; and another illustrative embodiment of the present invention, there is provided a kit described herein further comprising a questionnaire.

In another illustrative embodiment of the present invention, there is provided a kit described herein wherein the questionnaire comprises an envelope for delivery of the container.

In another illustrative embodiment of the present invention, there is provide a use of a tracer for sequestering in hair for detecting leakage of a prosthesis.

In another illustrative embodiment of the present invention, there is provide a use of a tracer for sequestering in hair for detecting the time of leakage of a prosthesis.

In another illustrative embodiment of the present invention, there is provide a use of a tracer for sequestering in hair for detecting the severity of leakage of a prosthesis.
**Brief Description of the Drawings**

Figure 1 is a mass spectra of ciprofloxaxin standard using MS.
Figure 2 is a mass spectra of ciprofloxaxin standard using MS/MS.
Figure 3 is a mass spectra of a NaOH digest of a hair sample, pretreated with ciprofloxaxin using MS.
Figure 4 is a mass spectra of a NaOH digest of a hair sample, pretreated with ciprofloxaxin using MS, zoomed into a region of interest.
Figure 5 is a mass spectra of a NaOH digest of a hair sample pretreated with ciprofloxaxin using MS/MS.
Figure 6 is a mass spectra of a NaOH digest of a hair sample with no ciprofloxaxin using MS.
Figure 7 is a mass spectra of a NaOH digest of a hair sample with no ciprofloxaxin using MS, zoomed into a region of interest.
Figure 8 is a mass spectra of a NaOH digest of a hair sample with no ciprofloxaxin using MS/MS.

**Detailed Description**

Filler compositions for a prosthesis are well known in the art. Typical fillers include silicone, silicone based polymers and saline solutions as well as many others. Fillers of the present invention will comprise any filler composition currently used, known, discovered or invented in the future that also comprise a tracer that is sequestered in the hair.

A marker is a compound that can be identified in a tissue or bodily fluid, such as hair, blood, urine, or perspiration if the tissue or bodily fluid has come into contact with the marker. Many marker compounds are known to a person of skill in the art of markers and include members of the quinoline family, steroid family, phtalate family, heavy metals, radio-active compounds, fluorescent compounds and dyes. Other markers include food stuffs such as capsaicin and garlic. Markers for use in the present invention are termed tracers. Some markers are not suitable for use as tracers in the present invention. Tracers, which are markers suitable for use in the present invention, have the following characteristics: sequestered in the hair; a suitable safety profile; suitable stability; suitable affinity for melanin; and suitable solubility.

Compounds that are known to be sequestered in the hair and are markers include, without limitation: Florafur, codeine, alprazolam, amphetamine, norsteroids, anabolic steroids,
endogenous steroids, corticosteroids, anhydroecgonine methyl ester, barbiturate, benzodiazepines, benzoylecgonine, benzphetamine, beta 2 adrenergic agonists, beta blockers, buprenorphine, caffeine, carbamazepine, chloroquine, ciprofloxacin, clenbuteral, clenbuterol, clonazepam, clozapine, cocaethylene, cocaine and metabolites, d-amphetamine, dehydroepiandrosterone (DHEA), dextromoramide, dextropropoxyphene, digoxin, dimethylamphetamine, doxepin, N-methyl-D-aspartic acid (MDMA), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), ephedrine, ethyl glucuronide, exogenous steroids, fatty acid ethyl esters, fenethylline, fentanyl, flunitrazepam, fluoxetine, furancarboxylic acid, gamma-hydroxybutyric acid (GHB), haloperidol, heroin, terbinafine, lidocaine, lorazepam, meperidine, meprobamate, methadone, methamphetamine, methylecgonine, monodesethylchloroquine, morphine, nandrolone, nicotine, cotinine, norbuprenorphine, norcocaine, nordiazepam, norfloxacin, norpropoxyphene, ofloxacin, opiates, oxazepam, phencyclidine, phenobarbital, phenytoin, pholcodine, prednison, selegiline metabolites, stanozolol, temafloxacin, testosteron, tetrahydrocannabinol, thyroxine, triazolobendiazepine, and tricyclic antidepressants. Of these, the following are unsuitable as tracers for use in the present invention: endogenous steroids including DHEA and testosterone, furancarboxylic acid, thyroxine, ethyl glucuronide and fatty acid ethyl esters because they are normally present in the body; caffeine, cotinine and nicotine because they are common in the environment; Floraflur because of high toxicity; carbamazepine because it may render oral contraceptives ineffective; phenytoin because it is teratogenic; fluoxetine because it is too commonly used;amphetamine, buprenorphine cocaethylene (psychoactive) cocaine, d-amphetamine, dextromoramide, dimethylamphetamine, MDMA, GHB, heroin, methamphetamine, norcocaine (active metabolite), and phencyclidine because they are controlled substances or have psychoactive effects; codeine, norsteroids, anabolic stanozolol, barbiturate, benzodiazepines, digoxin, fentanyl, flunitrazepam, haloperidol, lidocaine, methadone, meperidine, morphine, nandrolone, norbuprenorphine, nordiazepam, norpropoxyphene, oxazepam, phenobarbital, and stanozolol. The following are non-limiting examples that may be suitable as tracers for use in the present invention: tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamine, beta 2 adrenergic agonists, beta blockers, chloroquine, ciprofloxacin, clenbuteral, clenbuterol, clozapine, clonazepam, clonidine, corticosteroids, dextropropoxyphene, doxepin, EDDP, ephedrine exogenous steroids, fenethylline, terbinafine, meprobamate, methylecgonine, monodesethylchloroquine,
norfloxacin, pholcodine, prednisone, ofloxacin, selegiline, temafloxacin, tetrahydrocannabinol, tricyclic antidepressants, and triazolobendiazepine.

Compound known to have a suitable safety profile have no acute undesirable effects and are markers include: Vitamin C, dihydrotestosterone, pyridoxine, ergocalciferol, vitamin A, cholecalciferol, niacin, thiamine, riboflavin, ketoconazole, benzophenone, norsteroids, codeine, benzodiazipines, caffeine, carbamazepine, cotinine, DHEA and endogenous steroids, ethyl glucuronide and fatty acid ethyl esters, flunitrazepam, fluoxetine, furancarboxylic acid, nandrolone, nicotine, nordiazepam, oxazepam, stanozolol, testosterone, thyroxine tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamines, beta 2 adrenergic agonists, beta blockers, chloroquine, ciprofloxacin, clenbuteral, clenbuterol, cloazepine, clonazepam, clozapine, corticosteroids, dextropropoxyphene, doxepin, EDDP, ephedrine, exogenous steroids, prednisone, fenethylline, terbinafine, meprobamate, methylecgonine, monodesethylchloroquine, norfloxacin, pholcodine, ofloxacin, selegiline, temafloxacin, tetrahydrocannabinol, tricyclic antidepressants, triazolobendiazepine. Of these, the following are unsuitable as tracers for use in the present invention: ketoconazole and benzophenone because they are present in over-the-counter shampoo and sunscreen; nicotine and cotinine because they are common in the environment; vitamin C, dihydrotestosterone, pyridoxine, ergocalciferol, vitamin A, cholecalciferol, niacin, thiamine, riboflavin and caffeine because they are commonly found in food; DHEA, testosterone and endogenous steroids, thyroxine, furancarboxylic acid, ethyl glucuronide and fatty acid ethyl esters because they are naturally found in the body; norsteroids, codeine, benzodiazipines, carbamazepine, flunitrazepam, fluoxetine, nandrolone, nordiazepam, oxazepam, and stanozolol. The following are non-limiting examples that may be suitable as tracers for use in the present invention: tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamines, beta 2 adrenergic agonists, beta blockers, chloroquine, ciprofloxacin, clenbuteral, clenbuterol, clonazepam, clozapine, exogenous steroids including corticosteroids and prednisone, dextropropoxyphene, doxepin, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methylecgonine, monodesethylchloroquine, norfloxacin, pholcodine, ofloxacin, selegiline, temafloxacin, tetrahydrocannabinol, tricyclic antidepressants, and triazolobendiazepine.

Compound known to have an affinity for melanin and are markers include: Aminoglycosides, fluoroquinolones, chlorpromazine and other phenothiazines, clenbuterol, salbutamol, nor-testosterone, trenbolone, diethylilbestrol, chloroquine, haloperidol, trimipramine, desipramine, sulpiride, benzophenone, cocaine, opioides, imipramine,
clomipramine, amitryptiline, quinine, iron, nickel, lead, copper. Of these, the following are unsuitable as tracers for use in the present invention: lead and cocaine for toxic or psychoactive effects; iron, nickel, and quinine because they are common in the environment and tonic water (quinine); nor-testosterone, trenbolone, chlorpromazine and other phenothiazines, sulpiride, haloperidol, and benzophenone. The following are non-limiting examples that may be suitable as tracers for use in the present invention: aminoglycosides, fluoroquinolones, clembuterol, salbutamol chloroquine, trimipramine, desipramine, imipramine, clomipramine, oflaxacin, and amitryptiline.

Compounds known to have suitable stability and are markers include: Perhexilene (2-6 days), riluzole (12 hrs), lomustine (metabolites 16-48 hr.) clopidogrel (platelet bound 11 days), progesterone (25-50 hrs), amphotericin B (15 days elimination; 24 hrs from serum), azelastine (22 hrs), cinacalcet (30-40 hrs), benzodiazepines (40-50 hrs.), tiludronate (50 hrs), flurazepam (metabolite 47-100 hrs), donepezil (70 hrs), ciprofloxacin (4 hrs), norfloxacin (3-4 hrs), and cotinine (15-20 hrs). Of these, the following are unsuitable as tracers for use in the present invention: Perhexilene and lomustine because they have a small therapeutic window or are toxic, progesterone, cinacalcet, benzodiazepines, flurazepam, and cotinine. The following are non-limiting examples that may be suitable as tracers for use in the present invention: riluzole, clopidogrel, azelastine, donepezil, ciprofloxacin, ofloxacin, and norfloxacin.

Compounds known to have suitable solubility and are markers include: Quinine (500 mg/L), ciprofloxacin (1.1 mg/L) THC, hydrocodone, melphalan (0.1g/100mL), codeine, urea, ethinamate (1500 mg/L), ethotoin (5280 mg/L), fenoldopam (4000 mg/L). Of these, the following are unsuitable as tracers for use in the present invention: fenoldopam, because it has a short half life; urea, because it is naturally present in the body; quinine, hydrocodone, melphalan, and codeine. The following are non-limiting examples that may be suitable as tracers for use in the present invention: ciprofloxacin, ethotoin, THC, ofloxacin and ethinamate.

Markers that are unsuitable for use in the present invention are: 1) compounds that cause moderate to severe toxicity, side effects or birth defects; 2) compounds that are common in the environment or are commonly prescribed; 3) compounds that are known to be quickly excreted or degraded in vivo without sequestering in the hair; 4) compounds that would interfere with silicone polymerization or stability of polymerized silicone; and 5) compounds that are not pharmacologically systemic (i.e., serum soluble and tissue permeable).
Compounds that may be useful as tracers in the present invention provide compounds that may be detected very soon after a leak occurs and that may be detected long after a leak has occurred. Suitable tracers may be cleared from a biological system via typical metabolic clearance through bodily fluids such as urine, feces, perspiration and blood. Clearance of the tracer via a typical metabolic clearance allows detection of a tracer in a bodily fluid within hours of a prosthesis leak occurring. Suitable tracers may also sequester in the hair. By being able to sequester in the hair, suitable tracers are detectable long after a prosthesis leak occurs. Provided that the appropriate part of the hair is not cut off, the tracer may be detected in the hair many years after the leak has occurred and long after the tracer would have been cleared via a typical metabolic route. Many suitable tracers are first able to be detected in the hair from 1 to 3 days after the leakage and are detectable in the hair thereafter. Tracers suitable for use in this invention exhibit properties amenable to a mass-spectrometry-based hair analysis protocol. Such properties include: 1) stability throughout the extraction process; 2) presence of an ionizable group; and 3) mass fingerprint that is distinguishable from all other compounds present in a typical hair sample.

Under normal washing conditions, some compounds and drugs may be detected in hair years after ingestion. Conversely, the harsh conditions used in cosmetic bleaching or dying of hair are known to reduce the concentration of some compounds and drugs in hair by 40 to 60 percent (Jurado et al. "Influence of the cosmetic treatment of hair on drug testing"; Int J Legal Med. 1997;110(3):159-63). This decrease however, is insufficient to reduce the concentration of a marker to levels below the limit of detection of a mass spectrometry-based hair analysis protocol. Alternatively, individuals wishing to cosmetically treat their hair could submit a sample prior to each treatment or alternatively submit an untreated hair sample, such as a pubic hair sample. Cosmetic treatment also does not appreciably increase the susceptibility of hair to environmental contamination (Skopp, et al. "On cosmetically treated hair—aspects and pitfalls of interpretation"; Forensic Sci Int. 1997 Jan 17;84(l-3):43-52).

Specific examples of tracers for use in the present invention include, without limitation: chloroquine, quinoline, ofloxacin, norfloxacin, tetracycline, ciprofloxacin, AM-1 155, OPC-17116, Q-35, fluorescein, hydrocortisone, dexamethasone, prednisone. The tricyclic antidepressants imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, and doxepin. The aminoglycosides amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin and spectinomycin. The beta-2 adrenergic receptor...
agonists clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol mesylate, salmeterol, formoterol, and bambuterol. The beta blockers dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, Tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamine, clozapine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methylecgonine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, and triazolobendiazepine. Some of the metabolites of the foregoing list, which may also be suitable for use as a tracer or be tested for as indicative of prosthesis leakage may include, without limitation: ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethyl metabolites of tricyclics.

Tracers of the present invention may be mixed with typical filler compositions known to a person of skill in the art of prostheses thereby producing fillers comprising tracers. Fillers comprising tracers may then be used to prepare prostheses. Methods of making prostheses are known to a person of skill in the art of prostheses. There are many different sorts of prostheses, including breast, buttock, calf, arm, penile, testicular, facial, pectoral, brow, nose, chin, cheek, lip, triceps and biceps. Prostheses like these and others may be provided with fillers comprising tracers.

Some prostheses are provided with compartments. Each compartment may be provided with tracer or particular compartments may be provided with tracer while some are not provided with a tracer. Furthermore, some compartments may be provided with filler and some compartments may not be provided with filler. The filler in a particular compartment may or may not be mixed with tracer. The tracer in a particular compartment may or may not be mixed with filler. In some illustrative embodiments, prostheses are provided with an exterior membrane and an interior membrane such that the tracer is provided between the two membranes and the filler is provided in inside the interior membrane. The filler provided inside the interior membrane may or may not be mixed with filler. The tracer between the interior and exterior membrane may or may not be mixed with filler.

The amount of tracer in a single prosthesis is a therapeutically suitable dose. As such, the amount of tracer to be included in a prosthesis is determined, in part, by the characteristics of the subject receiving the prosthesis and the particular tracer being used. A therapeutically
suitable amount of a tracer may vary according to factors such as the age, sex, and weight of the subject, and the ability of the compound to elicit a desired response in the subject. A therapeutically suitable amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. For any particular subject, specific quantities of the tracer may be adjusted according to the individual need and the professional judgement of the person administering or supervising the introduction of the prosthesis. Amount ranges set forth herein are exemplary only and do not limit the quantities that may be selected by medical practitioners. The amount of tracer in the filler may vary according to factors such as the disease state, age, sex, and weight of the subject as well as size and type of the prosthesis.

In general, tracers for use in the invention should be used without causing substantial toxicity. Toxicity of tracers for use in the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population).

Some tracers for use in the present invention have been studied in detail with respect to their therapeutically suitable doses. For example ofloxacin is tolerated at levels of 200-400 mg every 12 hours for 3-14 days and a single dose up to 2500mg/kg in mice is not lethal. Ciprofloxacin is often prescribed at 500-1000 mg every 24 hours for 3-14 days. Norfloxacin is often prescribed at 400 mg every 12 hours for 3-28 days and also at a single dose of 800mg. Hydrocortisone is known to be lethal in mammals at a dose of 6ml/kg and is often prescribed at a dosage of 20-25 mg/day. Dexamethasone is often prescribed in range of 1-2 mg/kg/day to a maximal level of 4-5 mg/kg in patients with shock. Fluoroscein has a LD50 of 4700 mg/kg (oral dose) in mice and is often injected at about 7mg/kg in patients for retinal imaging. Chloroquine has a LD50 of 500 mg/kg via oral route in mice and is often prescribed at a total dose of 1500 mg given over 48 hours for treatment of malaria.

Methods of implanting prostheses into a subject are well known to a person of skill in the art. As used herein, a "subject" may be a human, non-human primate, mammal, domestic pet, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc. It is common for more than one prosthesis to be implanted in a single subject. In such cases, it is possible to select prostheses that contain different tracers. By selecting different tracers for each of the prostheses it is possible to determine which prosthesis is leaking by identifying the tracer that is detected.
Methods for detecting leakage from a prosthesis comprise a) obtaining a sample from a subject having the prosthesis; b) treating the sample to extract a tracer thereby obtaining an extract; and c) testing the extract for the tracer or a metabolite thereof. The presence of the tracer or a metabolite thereof in the extract is indicative of a leakage from the prosthesis.

Metabolites include, but are not limited to, compounds formed through the endogenous processes of demethylation, oxidation, and/or hydroxylation of parent compounds or their metabolites.

A sample may be a bodily fluid obtained from the subject, such as blood, feces, urine, or perspiration or may be a tissue such as hair or a tissue sample from close to the location of the prosthesis in the subject. Methods of obtaining such samples are known to a person of skill in the art. In the particular example when the sample is a hair from the subject, the hair should ideally be removed from the subject by plucking such that the root of the hair is obtained.

Hair growth typically occurs at a rate of approximately 1 cm per month for an average person's scalp. The growth rate of a subject's hair can be used to provide information regarding the timing of a leakage. For example, if the sample of hair is cut into measured segments (e.g. 1 cm, 0.5 cm, 0.25 cm or 0.1 cm) and the measuring is achieved by measuring the distance from the root of the hair, then the time that has elapsed since a leakage event may be determined based on which measured segment of hair provides an extract that is positive for the tracer. This may provide information regarding whether or not a particular activity is causing or exacerbates a leak in a prosthesis. For example, if the subject knows that a particular activity occurred at two particular times in the last month and the tracer is only found in the hair at two measured segments occurring at 0.5 cm and 1 cm from the root, then it is possible that this activity is causing or exacerbating the prosthesis leak. Alternatively, this information may provide information that a medical practitioner may use to prescribe a treatment course based on the timing of the leak. This information also permits a medical practitioner to prescribe the tracer for an alternative treatment (i.e. using the tracer for a therapy unrelated to the prosthesis) and based on the timing of the alternative treatment and the location of the tracer in the hair, a potential false positive may be identified as a false positive.

Once a sample has been obtained from a subject, the sample is treated to provide an extract. A typical hair sample may be treated by cutting the hair into measured segments, as described above, ordering and recording the order of the hair segments and subjecting each measured segment to a base-digestion (e.g. sodium hydroxide). Following base digestion, solid
phase extraction on a cation exchange column or cartridge is performed and the eluate from the column or cartridge is provided as the extract.

The extract is then tested for the presence of the tracer or metabolites thereof. Possible testing techniques include, without limitation, Enzyme-linked immunosorbent assay (ELISA), Fluorescence spectroscopy, ultraviolet (UV) spectrometry, nuclear magnetic resonance (NMR), gas chromatography (GC), liquid chromatography (LC, medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), mass spectrometry (MS), tandem MS (MS/MS), LC-MS, GC-MS, HPCL-MS/MS, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS.

Tracers and their metabolites may be detected in hair at amounts that represent their relative concentration in the sample and because deposition of these compounds in hair is achieved over time in accordance with hair growth, both leakage/rupture severity and duration may be determined depending on the quantity of tracer or metabolite thereof detected. As a non-limiting example, the presence of the tracer ofloxacin or norfloxacin may be detected by respective spectrophotometry excitation and emission at 290nm and 460nm. As another non-limiting example, the presence of the tracer fluoroquinolone may be detected by reverse phase HPLC-MS/MS.

HPLC-based approaches may lead to false positive detection if related molecules of similar size and characteristics are present in the sample. This leads to less accurate results if the person has been taking medications or exposed to compounds of similar properties in the environment. False-positive results may complicate identification of the tracer molecule in samples with high confidence, making it difficult to take action when leakage is suspected.

Fluorescence-based approaches to determine the presence of the tracer compound may result in false-positive results based on the presence of molecules with similar fluorescent properties. Additionally, fluorescence-based approaches may have a reduced sensitivity due to background fluorescence under baseline conditions. Detection by this technique would require a leakage event releasing levels of tracer high enough to overcome the background fluorescence. This could result in false-negatives for people with small leakage events or event which leak very slowly over a long period of time.

Mass spectrometry-based detection allows for highly accurate detection of low levels of molecule in the tested solution (eg. ofloxacin in the range of 1 ng/L of blood; 0.3 ug/L of urine). Mass spectrometry also allows confirmation of the specific molecular size of the target compound, reducing likelihood of false-positive test results, from related molecules. Mass
spectrometry can also detect the relative abundance of the specific molecule in the solution at very low levels, without overlap from similar compounds or environmental contaminants. This higher level of specificity increases the confidence in test results, allowing for confirmation of leakage events.

A tracer may be used for detecting leakage of a prosthesis. Tracers may be used for detecting the time of leakage of a prosthesis. Alternatively, tracers may be used for detecting the severity of leakage of a prosthesis. The tracer, in addition to being useful as a marker, may also be used for reducing the possibility that infection or serious infection of a subject occurs after leakage of a prosthesis. For example, if the tracer is also an antibiotic, then the antibiotic may act to combat infection, if the leakage is caused, for example, by trauma to the subject in the area of the prosthesis, or a bacterial or fungal contaminant is introduced to the implant during production or implantation. Alternatively, the tracer may have a different alternative therapeutic use and may be suitable for specific application in specific prostheses to be used for specific applications.

Many subjects may not have easy accessibility to suitable resources for testing an extract. Alternatively, a subject may not want to take the time to visit a testing facility. In such cases, a kit for submitting a test for prosthesis leakage may be provided to the subject. The kit may include: a) a container for holding a sample; and b) instructions for obtaining a sample and delivery of the container.

The container for holding the sample may comprise a variety of known containers, but should be provided so that until the sample is received in the container, the container is kept uncontaminated or sterile. The container should be resealable so that once the sample is received in the container, possible further contamination is reduced or prevented. The container may also be large enough to permit a full sample to be contained within a single container. A plurality of containers may be provided in the kit so that the subject may provide more than one sample, either at the same time or at various intervals throughout time, such as every week, every month, every year, or as often as desired.

The instructions for obtaining a sample will provide details of how to obtain a sample so that possible contamination of the sample does not occur or is minimized. Furthermore, the instructions will provide minimum quantities that are required. The container may have markings that provide guidance for the subject when trying to determine is a sample is of a sufficient quantity. The instructions for obtaining a sample will also provide maximum time limits by which a sample must be obtained and then delivered to the testing facility. The
delivery location or address of the testing facility to which a sample should be delivered will also be provided in the instructions. The delivery location may be a remote location from which the testing facility picks up samples.

Some kits may be provided with tools for aiding a subject to obtain a sample. For example, sterile tweezers may be provided for extracting a hair to be provided as a sample. Rubber or latex gloves may be provided also.

Some kits may be provided with a questionnaire. The questionnaire may be generic or specific to a particular tracer or prosthesis. The questionnaire is provided so that a subject may complete the questionnaire in order to provide the testing facility with information, such as medication history of the subject, which may identify a false positive result. Alternatively, if the subject would like specific information regarding a particular time period or a particular prosthesis, the questionnaire may provide information for the testing facility to examine a particular section of the sample or to treat the sample using a particular method or test the extract using a particular method.

Tools for aiding the subject to deliver the sample may also be provided, such as envelopes or pre-printed labels. A questionnaire may be provided such that is capable of folding into a holder suitable for holding the container and delivery of the container with the sample.

Various alternative embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

**Prophetic Examples**

1) **Processing of hair samples to detect leakage-tracer molecule or metabolites**

Hair samples received for tracer analysis could be processed as follows:

1. Segmentation. Hair strands (single or multiple) are observed under magnification to determine the scalp-proximal or root end. The length of the hair is measured and recorded. The strands are left whole or cut into fragments starting at various points from the scalp-proximal end. Cut fragments may be 5 to 10 mm in length but may be smaller or larger in size as required. The position of fragments relative to the whole hair length is recorded and tracked throughout processing.

2. The hair or fragments are cleaned of external contamination. In some cases washing may not be required. For example, whole hair or hair fragments are washed in 0.1% w/v
sodium dodecylsulfate (SDS) in distilled water for 10 minutes with agitation followed by 1 to 3 rinses with distilled water. Other reagents that could be used to wash the sample include, but are not limited to: isopropanol; methanol; dichloromethane; acetone; distilled water mixed with acetone; distilled water containing any number from one or more of variety of detergents; and distilled water containing metal chelators (e.g., EDTA).

3. The hair or fragments are digested to release the tracer. For example, whole hair or hair fragments are digested for 30 minutes at 80°C in 1 volume (e.g., 0.5 mL) 1 N sodium hydroxide (NaOH) prepared in distilled water. Other digestion conditions could include, but are not limited to: enzymatic digestion with proteinase K, a glucuronidase/arylsulfatase mixture or other enzymes; methanol extraction for several hours (5-24 hrs) with or without ultrasonic bath; aqueous extraction in acid (0.01 N - 0.5 N HCl) or phosphate buffer (pH 6 - 8); and extraction with acidic urea and thioglycolate.

4. The digestion reaction are neutralized or otherwise stopped. For example, the digestion reaction is allowed to cool and then neutralized with 1 volume (e.g., 0.5 mL) 1 N hydrochloric acid (HCl) prepared in distilled water.

5. The digested sample is prepared for extraction of the tracer. In some methods, a preparation step before extraction is not required. For example, 2 volumes (e.g., 2 mL) of 50 mM phosphate buffer pH 8 prepared in distilled water are added to the neutralized digestion reaction.

6. The tracer is extracted using an appropriate solvent, hi some cases solvent extraction is not required. For example, the tracer is extracted using 10 volumes (e.g., 5 mL) of chloroform for 20 minutes with agitation. The organic and aqueous phases may be separated by centrifugation at 1,700xg for 5 minutes, hi this example, the organic phase (chloroform) may be recovered for further analysis. Other extraction buffers include, without limitation, organic or aqueous based solvents optimal for the selected tracer including: n-hexane; acetonitrile; acetone; and dichloromethane.

7. The tracer is concentrated or the solvent removed in order to dissolve the tracer in an instrument-compatible solvent. Alternatively, the sample is applied directly without changing the extraction solvent. For example, the organic phase is evaporated under a nitrogen stream at room temperature.

8. The tracer sample is dissolved in a solvent and analyzed by mass spectroscopy. In some cases, samples of the tracer include an internal marker for quantification and analyzed by mass spectroscopy. The sample is applied directly to a mass spectrometer. In some cases, the
sample is further purified by gas or liquid phase chromatography or other means of purification/sample clean-up before mass spectroscopic analysis. For example, the dried tracer sample is dissolved in 1 volume (e.g., 0.5 mL) 0.1% formic acid prepared in distilled water to which 1 volume (e.g., 0.5 mL) acetonitrile is added.

9. The appearance of mass peaks indicative of the tracer (i.e., a mass spectra 'fingerprint' of the tracer) is compared to a known spectra of the tracer, a library of hair matrix samples (tracer-negative) prepared from a representative population set and a library of tracer-positive samples within a representative hair matrix sample. Comparison to solutions of known tracer concentration allow accurate quantitation of tracer levels in the tested samples.

The representative sets used in the comparison are compiled from hair of a similar pigment and cosmetic treatment (e.g., artificial hair dyes, permanent solution, peroxide or others). For example, a quantity of the prepared sample solution (e.g., 40 microlitres) is injected directly into a Nanospray ionization chamber for MS or tandem MS/MS analysis or other in-line or off-line tandem MS instrumentation methods.

10. A report of the tracer concentration per unit protein mass of hair is then prepared for dissemination to the requester. The mass of the hair is determined by protein analysis methods that may include, but are not limited to: UV spectroscopy, Bradford or similar protein assay, determination of ionized amino acid parent compounds by mass spectroscopy.

2) **Ofloxacin and norfloxacin as leakage/rupture indicating components of silicone breast implants**

Either ofloxacin or norfloxacin, each prepared in a pharmacologically acceptable carrier including but not limited to 0.9% sodium chloride injection, 5% dextrose injection, 5% dextrose and 0.9% sodium chloride injection, 5% sodium bicarbonate injection, 5% dextrose, 0.45% sodium chloride, and 0.15% potassium chloride injection; 1/6 M sodium lactate injection, is infused within the silicone filler material during breast implant fabrication or filling. The carrier permits ofloxacin/norfloxacin stability, and also permits ofloxacin/norfloxacin diffusion concurrent with silicone diffusion upon induced or spontaneous rupture of the shell of the implant. The implant shell is created or coated so as to limit diffusion of the fluoroquinolone derivative contained within it. The mass of either ofloxacin or norfloxacin within each finished silicone/fluoroquinolone-derivative-filled implant may range between 10mg and 2000mg such that complete release of either ofloxacin or norfloxacin does not result in serum concentrations of either of the two fluoroquinolone derivatives being above
clinically acceptable levels. In the event that two implants are to be surgically implanted within the same subject, the first implant contains ofloxacin within its filler material, and the second implant contains norfloxacin within its filler material. Using different fluoroquinolone derivatives in each of two implants allows the discernment of which implant is subject to leakage, should it occur.

Following implantation, monitoring for ruptures or leaks is then performed at regular intervals to facilitate early detection. Any fluoroquinolone derivatives that have leaked from the breast implant, indicating a loss of implant integrity, are detected by their accumulation within patient hair. Patient's hair samples, obtained from the vertex region of the scalp, are cut into 5-10 mm long sections. Order and orientation of the hair samples to the scalp is recorded. Individual hair samples are then subjected to base-digestion followed by solid phase extraction on a strong cation exchange column or cartridge. Eluate from the column is then analyzed for potential fluoroquinolone derivatives by reverse phase high pressure liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Alternately, presence of ofloxacin and norfloxacin can be detected in the HPLC eluate by respective spectrophotometric excitation and emission at 290nm and 460nm. Because fluoroquinolone derivatives are detected in hair at amounts that represent their relative concentration in serum, and because deposition of these compounds in hair is "recorded" over time in accordance with hair growth, both leakage/rupture severity and duration, respectively, can be determined.

Following MS/MS collection, positive detection of ofloxacin or norfloxacin in hair is determined as any detectable level above baseline established on samples lacking ofloxacin or samples from a population of subjects with intact implants containing ofloxacin or norfloxacin. The concentration of ofloxacin or norfloxacin present per mg of hair is calculated based on comparison to spectra collected from solutions of known ofloxacin and norfloxacin concentration. The concentration level of ofloxacin or norfloxacin detected can then be used to estimate levels of leakage from ruptured implants. Any levels of ofloxacin or norfloxacin detected in isolated hair above environmental levels is classified as a positive result for implant leakage requiring intervention by a medical professional.

3) Animal Implant Model.

Small-rodent animals, such as mice or rats, will be used in the following experiment as model for subglandular breast implants in humans. Animals are surgically implanted with a subcutaneous, fluid-filled silicone implant distal to their thoracic spine. Implants are 2 ml in
volume for mice or 5 ml in volume for rats. Ofloxacin is added to the filler of some of the implants, at 2500mg/kg animal weight, during implant filling. A total of 25 animals are used in the experiment, five animals per group, assigned randomly. The groups are treated as follows: 1. intact implants containing ofloxacin, 2. mechanically weakened implants containing ofloxacin, 3. intact implants without ofloxacin, 4. mechanically weakened implants without ofloxacin, 5. animals undergoing surgery but receiving no implants (sham). Implants are mechanically weakened, introducing a small leak, to simulate leakage events that would occur during the life of a silicone implant in a human subject. Mechanical weakening of the implants is applied following filling, but prior to implantation with a tool that ensures each implant receives consistent mechanical wear.

Over a two year timecourse, once a month animal hair or whiskers are plucked from all animals, taking note of the date of isolation and orientation of the hair. Hair from all mice are digested and extracted in parallel, by a technician blinded to the treatment condition, as outlined above. Extracted compounds are assayed via LC/MS/MS to quantify tracer molecule levels in the hairs the animals. An internal standard of isotope-labeled ofloxacin are added to each sample to allow for quantification of ofloxacin concentration in each hair sample fragment. Quantified levels of ofloxacin in the animal's hair are statistically compared between the experimental groups. Animals are then sacrificed and implants are assayed for total volume loss and inspected under a dissecting microscope for puncture severity.

4) **Marker Detection in Hair.**

Sixteen human female volunteers between 18 and 45 years of age are recruited to participate in the experiment. Pregnant women, women without hair, and women who have taken any fluoroquinolone antibiotic in the past 4 years are disqualified from participating in the study. Volunteers are randomly assigned to four experimental groups. 1) 400 mg oral ofloxacin; 2) 40 mg oral ofloxacin; 3) 4 mg oral ofloxacin; 4) 0 mg oral ofloxacin. Dosages reflect the amount of ofloxacin that would enter the bloodstream of an individual with a subglandular silicone breast implant with ofloxacin added as a tracer in the case of a rupture (400 mg), leak (40 mg) or small leak (4 mg). Both participants and researchers are blinded to experimental conditions during the study. Hair samples are collected every 2 days for the first 8 days, then every month for the first year, and every 4 months for the second year. Samples are kept in a sterile container with the date of preparation noted.
Hairs are cut, recording the distance and orientation from the scalp, and digested as outlined above. Following extraction, compounds are assayed by LC/MS/MS as previously described. Isotope-labeled ofloxacin are added to each sample as an internal control to allow quantification of ofloxacin concentration in each hair sample and the location of the tracer along the growing hair (distance from scalp).

**Laboratory Examples**

1) **Mass Spectrometry Analysis of Hair Samples with and without Tracer**

Hair was obtained by plucking individual hairs from the vertex region of the scalp with sterile tweezers and collecting the sample in a sterile microfuge tube from three male and one female human volunteers, 21 - 35 years of age, who had no history of taking fluoroquinolone antibiotics in the last year.

Hair from human volunteers was cut into 5 mm long fragments, discarding the root-end, washed for 10 minutes in 0.1% sodium dodecylsulfate (in distilled water) with agitation, and rinsed a further three times with distilled water. Washed hair fragments were then pre-treated with distilled water with and without ciprofloxacin (10 mg/mL) for 16 hrs at room temperature. The hair fragments were then rinsed three times with distilled water and processed for mass spectroscopy by digestion in 0.5 mL 1 N sodium hydroxide for 30 minutes at 80°C. The digestion reaction was neutralized with 0.5 mL 1 N hydrochloric acid and then 1 mL of 50 mM phosphate buffer (pH 8) was added to the mixture. Chloroform (5 mL) was added to the mixture and the suspension was agitated at room temperature for 20 minutes followed by centrifugation at 1,700 g for five minutes to separate the organic and aqueous phases. The organic phase was then recovered and dried under a nitrogen stream at room temperature or evaporated at low pressure. The residue was dissolved in 0.5 mL 0.5% formic acid prepared in distilled water to which 0.5 mL acetonitrile was added. This solution was then applied to a mass spectrometer instrument for MS or MS/MS analysis. In parallel, a small quantity of pure ciprofloxacin was diluted in 0.5 mL NaOH and processed as described above as a standard.

As can be seen in mass spectra 1 and 2 (Figures 1 and 2), the predicted 332.3 and 314.5 peaks were observed in the ciprofloxacin standard. MS/MS analysis of the standard revealed peaks revealing loss of H_2O(314.0) CO_2 (288.2), and F and the piperazine ring (245.1) from the parent compound (332.0). Spectra 3(Figure 3) shows hair pretreated with ciprofloxacin (Turiel E, et al. "Study of the evolution and degradation products of ciprofloxacin and oxolinic acid in river water samples by HPLC-UV/MS/MS-MS" *J Environ Monit*. 2005 Mar;7(3): 189-95). The parent compound peak 332.3 is not visible until the region of interest is expanded (Spectra4 in
Figure 4). MS/MS filtering of the 332.3 region reveals peaks corresponding to the expected analytes and parent compound (245.0, 288.0, 314.0, and 332.0) (Spectra 5; Figure 5). As can be seen in the control hair sample not pretreated with ciprofloxacin, a similar peak to the parent compound (332.2) is visible, but the expected analytes (245.0, 288.0, and 314.0) are not visible (Spectra 6, 7 and 8 in Figures 6, 7 and 8 respectively).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings of this invention that changes and modification may be made thereto without departing from the spirit or scope of the appended claims. All patents, patent applications and publications referred to herein are hereby incorporated by reference.
What is claimed is:

1. A filler composition for a prosthesis comprising a tracer that is sequestered in the hair.

2. The filler of claim 1 wherein the tracer is selected from the group consisting of: quinolines, steroids, tricyclic antidepressants, aminoglycosides, beta-2 adrenergic receptor agonists, beta blockers and phthalates.

3. The filler of claim 1 wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol mesylate, salmeterol, formoterol, bumberterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepinicolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, beta xolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol, alprazolam, anhydroegonine methyl ester, benzphetamines, cloazepine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methylcgongine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, triazolobendiazepine, ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethyl metabolites of tricyclics.

4. The filler of claim 1 wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol
mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, and butoxamine.

5. The filler of claim 1 wherein the tracer is ofloxacin.

6. A prosthesis comprising a filler composition, the filler composition comprising a tracer that is sequestered in the hair.

7. The prosthesis of claim 6 wherein the tracer is selected from the group consisting of: quinolines, steroids, tricyclic antidepressants, aminoglycosides, beta-2 adrenergic receptor agonists, beta blockers and phthalates.

8. The prosthesis of claim 6 wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM 1155, OPC 17116, Q 35, fluoroscein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clenbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol, alprazolam, anhydroecgonine methyl ester, benzphetamine, clozapine, clonazepam, clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate, methylczongine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol, triazolobendiazepine, ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine, bisdesethylchloroquine, and desmethyl metabolites of tricyclics.

9. The prosthesis of claim 6 wherein the tracer is selected from the group consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM
1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine, desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline, protriptyline, dothiepin hydrochloride, doxepin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, hygromycin, spectinomycin, clonbuterol, salbutamol, levalbuterol, terbutaline, pirbuterol, procaterol, metaproterenol, fenoterol, bitolterol, mesylate, salmeterol, formoterol, bambuterol, dichloroisoprenaline, practolol, pronethaolol, alprenolol, carteolol, levobunolol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, carvedilol, celiprolol, labetalol, and butoxamine.

10. The prosthesis of claim 6 wherein the tracer is ofloxacin.

11. The prosthesis of any one of claims 6 to 10 wherein the prosthesis is selected from the group consisting of breast, buttock, calf, arm, penile, testicular, facial, pectoral, brow, nose, chin, cheek, lip, triceps and biceps.

12. The prosthesis of any one of claims 6 to 10 wherein the prosthesis is selected from the group consisting of breast, buttock, chin, cheek, pectoral and testicular.

13. The prosthesis of any one of claims 6 to 10 wherein the prosthesis is a breast implant.

14. A method for detecting leakage from a prosthesis comprising a filler comprising a tracer, the method comprising,

   a) treating a hair sample from a subject having a prosthesis to extract a tracer, thereby obtaining an extract; and

   b) testing the extract for the tracer,

   wherein the presence of the tracer or a metabolite thereof in the extract is indicative of a leakage from the prosthesis.

15. A method for detecting leakage from a prosthesis comprising a filler comprising a tracer, the method comprising,

   a) obtaining a hair sample from a subject having the prosthesis;
b) treating the hair sample to extract a tracer thereby obtaining an
extract; and

c) testing the extract for the tracer,
wherein the presence of the tracer or a metabolite thereof in the extract is
indicative of leakage from the prosthesis.

16. The method of claim 14 or 15 wherein the tracer is selected from the group
consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM
1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine,
desipramine, trimipramine, clomipramine, lofepramine, amitriptyline, nortriptyline,
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pindolol, propranolol, sotalol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, esmolol,
metoprolol, nebivolol, carvedilol, celiprolol, labetalol, butoxamine, tetrahydrocannabinol,
alprazolam, anhydroecgonine methyl ester, benzphetamine, clozapine, clonazepam,
clozapine, dextropropoxyphene, EDDP, ephedrine, fenethylline, terbinafine, meprobamate,
methylecgonine, monodesethylchloroquine, pholcodine, selegiline, tetrahydrocannabinol,
triazolobendiazepine, ofloxacin-N-oxide, desmethyl-ofloxacin, desethylchloroquine,
bisdesethylchloroquine, and desethyl metabolites of tricyclics.

17. The method of claim 14 or 15 wherein the tracer is selected from the group
consisting of: chloroquine, quinoline, ofloxacin, norfloxacin, temafloxacin, ciprofloxacin, AM
1155, OPC 17116, Q 35, fluorescein, hydrocortisone, dexamethasone, prednisone, imipramine,
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18. The method of claim 14 or 15 wherein the tracer is ofloxacin.

19. The method of any one of claims 14 to 18 wherein the treating comprises base extraction followed by solid phase extraction.

20. The method of claim 19 wherein the base extraction is sodium hydroxide extraction.

21. The method of claim 19 or 20 wherein the solid phase extraction comprises cation exchange.

22. The method of any one of claims 14 to 21 wherein the extract is tested using mass spectrometry, Enzyme-linked immunosorbent assay (ELISA), Fluorescence spectroscopy, ultraviolet (UV) spectrometry, nuclear magnetic resonance (NMR), gas chromatography (GC), liquid chromatography (LC, medium pressure liquid chromatography (MPLC), high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), mass spectrometry (MS), tandem MS (MS/MS), LC-MS, GC-MS, HPCL-MS/MS, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS.

23. The method of any one of claims 14 to 21 wherein the extract is tested using mass spectrometry.

24. A kit for submitting a test for prosthesis leakage, the kit comprising:
   a) a container for holding an extracted hair; and
   b) instructions for hair extraction and delivery of the container.

25. The kit of claim 24 further comprising a tool for extracting a hair for providing an extracted hair;

26. The kit of claim 24 or 25 further comprising a questionnaire.
27. The kit of claim 26 wherein the questionnaire comprises an envelope for delivery of the container.

28. Use of a tracer for sequestering in hair for detecting leakage of a prosthesis.

29. Use of a tracer for sequestering in hair for detecting the time of leakage of a prosthesis.

30. Use of a tracer for sequestering in hair for detecting the severity of leakage of a prosthesis.
Figure 6

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

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 17 August 2007 (17-08-2007)

Date of mailing of the international search report: 29 August 2007 (29-08-2007)

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