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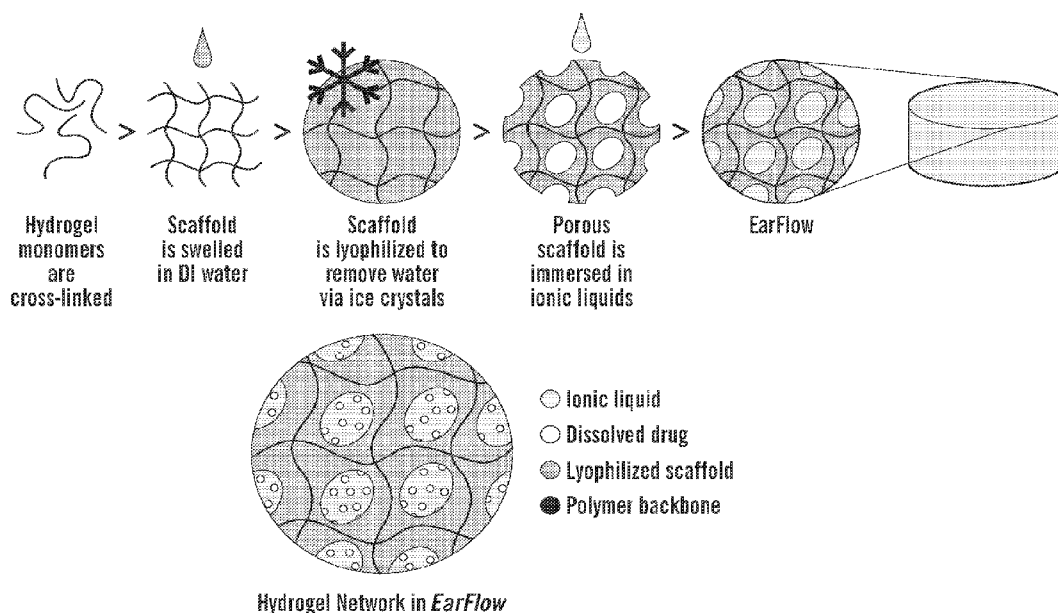


FIG. 6

(57) Abstract: The technology described herein is directed to compositions, kits, combination, and hydrogels comprising a hydrogel scaffold and at least one of a loading liquid (e.g., an ionic liquid) and a drug. Further described herein are methods of treating diseases, e.g., ear diseases by administering the compositions and hydrogels described herein.



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HYDROGEL DEVICES FOR DRUG DELIVERY TO THE EAR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/195,319 filed June 1, 2021, the contents of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0002] The technology described herein relates to methods and compositions for drug delivery, e.g., topical delivery and/or delivery in the ear.

BACKGROUND

[0003] Many ear diseases require surgical intervention or systemic medication because no topical approach is available. For example, the tympanic membrane in the ear is near-impermeable to most drugs. This makes direct delivery to the middle ear without injection or surgery largely ineffective. Where topical medications do exist, they usually require frequent dosing and are difficult for patients to apply at home, particularly when the patients are children. This results in heavy use of antibiotics and frequent resort to surgical intervention. Failure to take these aggressive treatment options carries significant risks of permanent hearing loss, and for children, learning and language development delays. A less invasive approach that provides effective drug delivery into the ear is needed.

SUMMARY

[0004] Described herein are compositions for drug delivery, particularly for use in the ear, that provide a surprising ability to deliver drugs to and through surfaces in the ear such as the tympanic membrane (TM). These compositions and methods provide sustained drug delivery without resorting to invasive measures to perforate the TM or risking the health of young patients with systemic antibiotics. This reduces healthcare expenses caused by in-clinic procedures and the systemic costs of furthering antibiotic resistance, and this also minimizes the risk of antibiotic resistance and recurrent infections.

[0005] In one aspect of any of the embodiments, described herein is a composition comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid. In some embodiments of any of the aspects, the at least one loading liquid comprises at least one ionic liquid.

[0006] In one aspect of any of the embodiments, described herein is a kit or combination comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid. In some embodiments of any of the aspects, the at least one loading liquid comprises at least one ionic liquid.

[0007] In some embodiments of any of the aspects, the at least one ionic liquid has a cationic component and an anionic component; the cationic component is selected from the group consisting

of benzyl pyridinium, benzyl dimethyl dodecyl ammonium, a choline cation, phosphonium, tetraalkylphosphonium, and benzethonium; and the anionic component is selected from the group consisting of bistriflimide, a geranate anion, oleate, 2-octenoic acid, hexanoate, dodecyldimethyl ammonia propane sulfonate, N-Lauryl sarconsinate, and geraniolate. In some embodiments of any of the aspects, the at least one ionic liquid comprises choline and geranic acid (CAGE), choline and hexenoic acid (CAHA), or choline and 2-octenoic acid. In some embodiments of any of the aspects, the at least one ionic liquid comprises choline and geranic acid (CAGE) or choline and hexenoic acid (CAHA).

[0008] In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 30 μ L of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 40 μ L of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 200 μ L of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 1000 μ L of the at least one ionic liquid.

[0009] In some embodiments of any of the aspects, the one or more drugs are selected from: antibiotics; steroids; growth factors; biologics; viral vectors; polypeptides; nucleic acid molecules; polysaccharides; anesthetics; and external, middle or inner ear disease therapeutics. In some embodiments of any of the aspects, the one or more drugs comprise at least one antibiotic. In some embodiments of any of the aspects, the one or more drugs comprise at least one steroid. In some embodiments of any of the aspects, the one or more drugs comprise at least one antibiotic and steroid. In some embodiments of any of the aspects, the one or more drugs are selected from dexamethasone and ciprofloxacin. In some embodiments of any of the aspects, the one or more drugs comprise dexamethasone and ciprofloxacin.

[0010] In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 1 mg/mL of dexamethasone. In some embodiments of any of the aspects, the composition, kit, or combination comprises 100-400 mg/mL of dexamethasone. In some embodiments of any of the aspects, the composition, kit, or combination comprises 200 mg/mL of dexamethasone. In some embodiments of any of the aspects, the composition, kit, or combination comprises at least 1 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the composition, kit, or combination comprises 100-500 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the composition, kit, or combination comprises 250 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the composition, kit, or combination comprises 200 mg/mL of ciprofloxacin.

[0011] In some embodiments of any of the aspects, the hydrogel scaffold comprises, consists of, or consists essentially of one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-

co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer. In some embodiments of any of the aspects, the at least one polyacrylate and/or at least one polymethacrylate comprises, consists of, or consists essentially of one or more of: poly(2-hydroxyethyl methacrylate) (pHEMA), poly(acrylic acid) (PAA), poly(methyl methacrylate) (PMMA), hydroxyethyl acrylate (HEA), poly(2-hydroxyethyl acrylate) (pHEA), polyacrylamide (PAM), poly(acrylonitrile-co-acrylic acid) (PANCAA), gelatin methacrylate (GelMA), hyaluronic acid methacrylate (MeHA); poly(epoxy methacrylate) (pEMA), poly(acryl amide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylamido glycolic acid), poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), poly(ethyl acrylate) (PEA), and poly(ethyl methacrylate) (PEMA). In some embodiments of any of the aspects, the at least one protein comprises, consists of, or consists essentially of one or more of: collagen, gelatin, and silk fibroin. In some embodiments of any of the aspects, the at least one polysaccharide comprises, consists of, or consists essentially of one or more of: hyaluronic acid, glycosaminoglycans, agarose, alginate, and chitosan. In some embodiments of any of the aspects, the hydrogel scaffold comprises, consists of, or consists essentially of poly(2-hydroxyethyl methacrylate) (pHEMA).

[0012] In some embodiments of any of the aspects, the composition, kit, or combination further comprises at least one hydrogel crosslinker. In some embodiments of any of the aspects, the at least one hydrogel crosslinker comprises at least one of: ethylene glycol dimethacrylate (EGDMA), N,N'-methylenebisacrylamide (MBAA-crosslinker), ammonium persulfate (APS-initiator), tetramethylethylenediamine (TEMEDA-catalyst), and Fe⁺³ (ionic cross linker). In some embodiments of any of the aspects, the hydrogel scaffold does not comprise a crosslinker. In some embodiments of any of the aspects, the composition does not comprise a crosslinker.

[0013] In some embodiments of any of the aspects, the composition, kit, or combination further comprises a radical initiator or photoinitiator. In some embodiments of any of the aspects, the radical initiator or photoinitiator is azobisisobutyronitrile (AIBN).

[0014] In some embodiments of any of the aspects, the hydrogel scaffold is not formulated with water.

[0015] In some embodiments of any of the aspects, the composition has a size no greater than 12 mm by 12 mm by 12 mm. In some embodiments of any of the aspects, the composition has a size no greater than 8 mm by 8 mm by 8 mm. In some embodiments of any of the aspects, the composition has a size of at least 8 mm by 8 mm by 7.5 mm. In some embodiments of any of the aspects, the composition has a size of at least 5.4 mm by 5.4 mm by 2.7 mm.

[0016] In one aspect of any of the embodiments, described herein is a method of drug delivery, the method comprising administering, to a patient in need of a drug, a composition described herein, wherein the composition comprises the drug. In one aspect of any of the embodiments, described herein is a composition as described herein, comprising a drug, for use in a method of drug delivery, the method comprising administering the composition to a patient in need of the drug. In some

embodiments of any of the aspects, the composition is administered by placing the composition in contact with the ear, the outer ear, ear canal, tympanic membrane, oval window, round window membrane, eustachian tube, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.

[0017] In one aspect of any of the embodiments, described herein is a method of treating an ear condition or disease, the method comprising administering to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient a composition as described herein, optionally wherein the composition comprises a drug. In one aspect of any of the embodiments, described herein is a composition as described herein, for use in a method of treating an ear condition or disease, the method comprising administering to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient a composition as described herein, optionally wherein the composition comprises a drug.

[0018] In some embodiments of any of the aspects, the administration comprises circumferential application or contact with the skin of the ear canal. In some embodiments of any of the aspects, the administration comprises contact with the auricular concha and pinna.

[0019] In some embodiments of any of the aspects, the ear condition or disease is selected from the group consisting of: otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss. In some embodiments of any of the aspects, the ear condition or disease is otitis media.

[0020] In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the tympanic membrane, oval window, round window membrane, eustachian tube or ear canal. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the tympanic membrane. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the oval window. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the window membrane. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the eustachian tube. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the ear canal.

[0021] In some embodiments of any of the aspects, the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, oval window or the round window membrane.

[0022] In some embodiments of any of the aspects, the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the round window, and/or the oval window.

[0023] In some embodiments of any of the aspects, the ear condition or disease is otitis externa. In some embodiments of any of the aspects, the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.

[0024] In some embodiments of any of the aspects, the composition is left in the ear for at least 2 days. In some embodiments of any of the aspects, the composition is left in the ear for at least 3 days. In some embodiments of any of the aspects, the composition is left in the ear for at least 5 days. In some embodiments of any of the aspects, the composition is left in the ear for at least 7 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 10 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 28 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 12 months. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 7 days. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 10 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 10 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 28 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 12 months.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Fig. 1 depicts a schematic of otitis media. During otitis media, the middle ear cavity is filled

with fluid, and that the Eustachian tube is narrowed, making fluid drainage challenging.

[0026] Fig. 2 depicts the trilaminar structure of the tympanic membrane (TM). Importantly the top stratum corneum layer is tightly packed, making the TM effectively impermeable to most therapeutic molecules.

[0027] Fig. 3 depicts a schematic of the EarFlow device. Placing EarFlow on the tympanic membrane will allow steroids and antibiotics to flow into the middle ear space to treat otitis media.

[0028] Fig. 4 depicts a summary of existing technologies. Existing technologies are all deficient in one of the six key specifications: efficacy, non-invasiveness, low cytotoxicity, low impact on hearing,

localized delivery, and convenience.

[0029] Fig. 5 depicts a finite element model of the tympanic membrane (TM) from the Puria group [1] illustrating the extent of motion on the TM surface. Heatmap legend represents total displacement in meters.

[0030] Fig. 6 depicts the proposed method of fabricating EarFlow. From starting monomers, the hydrogel was crosslinked, biopsy punched as discs, and swelled in deionized water. The hydrogel was then lyophilized to create porosity in the scaffold, and then immersed in ionic liquid to achieve the final structure.

[0031] Fig. 7 depicts the drug eluting mechanism of EarFlow. As the scaffold is placed on the tympanic membrane (TM), it is anticipated that the ionic liquid will elute out of the scaffold and across the TM.

[0032] Figs. 8A-8B depict the effect of EarFlow on sound-induced motion of the tympanic membrane (TM). (Fig. 8A) Cylindrical device on the TM model. The model was based on the Puria group's finite element model in O'Connor et al. [1]. Modelling the cylindrical device on the TM allowed the computation of hearing outcomes, such as the middle ear gain (MEG). (Fig. 8B) MEG is a metric to measure the ability of sound conduction. Here, MEG across the human hearing range (0.1-20 kHz) was plotted. The data was computed from Puria group's finite element model used O'Connor et al. [1].

[0033] Figs. 9A-9C depict tables of design specifications.

[0034] Figs. 10A-10B depict a summary of design specifications of modelling EarFlow's impact on middle ear gain (MEG) when placed on the tympanic membrane.

[0035] Fig. 11 depicts a summary of design specifications of modelling diffusion across the tympanic membrane.

[0036] Fig. 12 depicts a table of polymer solution ratio, including monomers of hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and MilliQ water.

[0037] Fig. 13 depicts a Franz diffusion cell setup. Figure from Kim et al. [2].

[0038] Fig. 14 depicts a table of a range of parameters to test in in silico transtympanic diffusion model and outcomes to measure.

[0039] Fig. 15 depicts a model of the tympanic membrane layers. Layers were assigned the skin material or the fat material.

[0040] Fig. 16 depicts a model of the tympanic membrane and middle ear space from COMSOL Multiphysics. The middle ear space was modelled to have 50% water.

[0041] Fig. 17 depicts a table of values of parameters used in the tympanic membrane diffusion model.

[0042] Fig. 18 depicts a graph of ionic liquid uptake for 4 mm diameter hydrogels. Hydrogels formulated without water (1A, 2A) had significantly higher IL uptake rate than those formulated with water (1B, 2B). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

[0043] Fig. 19 depicts a graph of ionic liquid uptake for 8 mm diameter hydrogels. Hydrogels formulated without the crosslinker (1A) had significantly greater uptake rate than those formulated

with the crosslinker (2A) across all ionic liquids tested. Hydrogels swelled with more than 40 μL of ionic liquids, fulfilling the design specification. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

[0044] Fig. 20 depicts the concentration of dexamethasone with EarFlow loaded with ionic liquid-dexamethasone (IL-Dex) and with EarFlow loaded with saline-dexamethasone (PBS-Dex). EarFlow loaded with IL-Dex show higher dexamethasone concentrations than EarFlow loaded with PBS-Dex throughout most time points and in tissue layers, indicating that ILs are potentially more efficacious at drug delivery.

[0045] Figs. 21A-21C depict graphs of human dermal fibroblast proliferation in the presence of EarFlow. Fibroblasts cultured with EarFlow hydrogels swelled with saline showed comparable cell counts to control groups. 1A+PBS: 1A hydrogel swelled with saline; 1A+IL: 1A hydrogel swelled with ionic liquid; 2A+PBS: 2A hydrogel swelled with saline; 2A+IL: 2A hydrogel swelled with ionic liquid. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

[0046] Figs. 22A-22C depict graphs of human epidermal keratinocyte proliferation in the presence of EarFlow. At Day 1, the differences in cell counts amongst groups were not statistically significant. At Day 8, the differences in cell counts amongst the control and experimental groups became more significant, but the cells still proliferated in the presence of EarFlow. 1A+PBS: 1A hydrogel swelled with phosphate buffered saline (PBS); 1A+IL: 1A hydrogel swelled with ionic liquid (IL); 2A+PBS: 2A hydrogel swelled with PBS; 2A+IL: 2A hydrogel swelled with IL. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

[0047] Figs. 23A-23B depict graphs of EarFlow toxicity to human dermal fibroblasts and human epidermal keratinocytes. EarFlow hydrogels swelled with saline showed comparable cell counts as control groups when tested with both keratinocytes and fibroblasts, indicating low cytotoxicity to both cell types. In particular, EarFlow swelled with ionic liquids did not appear to be significantly cytotoxic to fibroblasts. (Fig. 23A) Cytotoxicity to human dermal fibroblasts. (Fig. 23B) Cytotoxicity to human epidermal keratinocytes. 1A+PBS: 1A hydrogel swelled with phosphate buffered saline (PBS); 1A+IL: 1A hydrogel swelled with ionic liquid (IL); 2A+PBS: 2A hydrogel swelled with PBS; 2A+IL: 2A hydrogel swelled with IL. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

[0048] Fig. 24 depicts EarFlow adhesion to tissues. EarFlow adhered to porcine skin both when the tissue was vertical (as in the case of the tympanic membrane) and when the tissue was 180° horizontal from the original placement position, with the hydrogel pointing downward.

[0049] Figs. 25A-25C depict human epidermal keratinocyte adhesion to EarFlow. Across all time points, there were significantly fewer cells attached to EarFlow compared to the control group, indicating that EarFlow fulfilled the design specification of low cell adhesion. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

[0050] Figs. 26A-26E depict keratinocyte adhesion to EarFlow gels and to well plates. At Day 7, cells continued to proliferate in the well plates but did not adhere to EarFlow, indicating that EarFlow fulfilled the design specification of low cell adhesion.

[0051] Fig. 27 depicts EarFlow placement on the tympanic membrane (TM). In less than one minute, the 8 mm EarFlow device was placed successfully on porcine skin through a 3D printed, anatomically accurate ear canal model as an in vitro model of the TM and ear canal.

[0052] Figs. 28A-28B depicts a comparison of middle ear gain (MEG) with varying device radii. (Fig. 28A) The device radius was varied from 2-2.75 mm, in 0.25 mm increments. Across most frequencies (0.1-10 kHz), MEG did not vary significantly based on the different radii, and the cylinders maintained MEG above 90% of the baseline value. (Fig. 28B) At around 10 kHz, MEG became more affected by changes in device size. Specifically, MEG worsened with increasing device sizes at high frequencies.

[0053] Figs. 29A-29B depicts a comparison of middle ear gain (MEG) with varying device geometries. (Fig. 29A) The device geometry was varied between the cylinder, cube, and hemisphere. Across most frequencies (0.1-10 kHz), MEG did not vary significantly based on the different geometries, and the cylinders maintained MEG above 90% of the baseline value. (Fig. 29B) At high frequencies, the cylinder had the strongest negative impact on MEG and the cube had the lowest impact.

[0054] Figs. 30A-30B depict a comparison of middle ear gain (MEG) with varying device locations. (Fig. 30A) Across most frequencies, MEG did not vary significantly based on the location of the device. (Fig. 30B) Beginning around 10 kHz, the varying locations started to display detectable differences in MEG. Specifically, the anterior-superior location demonstrated the most drastic negative changes in MEG; the posterior-inferior region showed the least negative changes.

[0055] Fig. 31 depicts a comparison of middle ear gain (MEG) with varying device densities. Across the range of densities tested (1000-2000 kg/m³), MEG did not vary significantly from one another (all displayed by the same line) and did not decrease significantly below 90% of baseline MEG for most frequencies.

[0056] Figs. 32A-32B depicts diffusion throughout the tympanic membrane (TM). (Fig. 32A) In less than 10 seconds, the TM reached a drug concentration greater than the desired minimum inhibitory concentration. (Fig. 32B) Quantification of drug concentration at the medial (bottom) layer of the TM showed that the desired concentration was reached in less than 10 seconds.

[0057] Figs. 33A-33B depict diffusion throughout the middle ear space. (Fig. 33A) At 30 minutes, the entire middle ear cavity, as modelled by the large rectangle, reached a drug concentration greater than the necessary concentration. (Fig. 33B) Quantification of drug concentration at the medial (bottom) edge of the middle ear space showed that the necessary concentration was reached at around 12 minutes.

- [0058] Figs. 34A-34B depict analysis of design specifications achievement for EarFlow
- [0059] Fig. 35 depicts a cross section of histologically processed healthy tympanic membrane with hematoxylin and eosin staining [4]. Light color indicates extracellular material and darker color indicates cell nuclei. Number labels on lines indicate the different layers and their measured lengths (see Fig. 36).
- [0060] Fig. 36 depicts a table of the thickness of the tympanic membrane (TM) and its constituent layers as measured in the representative image of a haematoxylin & eosin-stained healthy TM (Fig. 35).
- [0061] Fig. 37 depicts ionic liquids (ILs) in transtympanic drug delivery.
- [0062] Fig. 38 depicts a schematic design factor summary.
- [0063] Fig. 39 depicts a table of design specifications.
- [0064] Fig. 40 depicts a schematic of the build process for one exemplary embodiment of EarFlow.
- [0065] Fig. 41 depicts pictures of the influence of water and polymer content on hydrogel properties.
- [0066] Fig. 42 depicts a table of design specification metrics.
- [0067] Fig. 43 depicts a graph demonstrating that ILs are effective in transporting drugs across the tympanic membrane.
- [0068] Fig. 44 depicts a graph of EarFlow mediated drug delivery across intact tissues. Dexamethasone delivery amounts are shown for the time periods and tissues indicated.

DETAILED DESCRIPTION

[0069] As demonstrated herein, the inventors have found that hydrogels are able to provide desirable drug delivery kinetics in the ear, without causing detrimental interference with hearing. Accordingly, described herein is a composition comprising: at least one drug and at least one hydrogel scaffold. In one aspect of any of the embodiments, described herein is a composition comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid. In some embodiments of any of the aspects, the hydrogel scaffold is in, or is provided in, the form of a hydrogel. These compositions are referred to at times herein as “EarFlow.”

[0070] As used herein, “hydrogel” refers to a substance that is formed when an organic polymer (natural or synthetic) is crosslinked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. As used herein, “hydrogel scaffold” refers to the polymer component of the hydrogel. Exemplary polymers are described below herein. The hydrogel scaffold can be lyophilized, dehydrated, or anhydrated, e.g., as in a kit for hydration before use. The hydrogel scaffold can be hydrated, e.g., providing a gel. When hydrated, the composition (e.g., the hydrogel) can further comprise water, a solution where water is the solvent, or a mixture comprising water.

[0071] Exemplary hydrogel scaffolds include polyacrylates, polymethacrylates, proteins, and polysaccharides. Hydrogel scaffolds are known in the art and include, but are not limited to the hydrogel scaffolds described in *Hydrogels*, Kirk-Othmer Encyclopedia of Chemical Technology, 4th Edition, vol. 7, pp. 783-807, John Wiley and Sons, New York; and Majee, S. B. , (Ed.). (2016). *Emerging Concepts in Analysis and Applications of Hydrogels*. IntechOpen. doi.org/10.5772/61692; the contents of which are incorporated herein by reference in their entirety.

[0072] In some embodiments of any of the aspects, the hydrogel scaffold comprises one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer. In some embodiments of any of the aspects, the hydrogel scaffold consists of one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer. In some embodiments of any of the aspects, the hydrogel scaffold comprises, consists of, or consists essentially of one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer. In some embodiments of any of the aspects, the hydrogel scaffold consists of at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), or poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer.

[0073] In some embodiments of any of the aspects, the at least one polyacrylate and/or at least one polymethacrylate comprises, consists of, or consists essentially of one or more of: poly(2-hydroxyethyl methacrylate) (pHEMA), poly(acrylic acid) (PAA), poly(methyl methacrylate) (PMMA), hydroxyethyl acrylate (HEA), poly(2-hydroxyethyl acrylate) (pHEA), polyacrylamide (PAM), poly(acrylonitrile-co-acrylic acid) (PANCAA), gelatin methacrylate (GelMA), hyaluronic acid methacrylate (MeHA); poly(epoxy methacrylate) (pEMA), poly(acryl amide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylamido glycolic acid), poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), poly(ethyl acrylate) (PEA), and poly(ethyl methacrylate) (PEMA). Further discussion and examples of acrylate-based hydrogel scaffolds can be found at Serrano-Aroca, Á. , & Deb, S. , (Eds.). (2020). *Acrylate Polymers for Advanced Applications*. IntechOpen. doi.org/10.5772/intechopen.77563, the contents of which are incorporated by reference herein in its entirety.

[0074] In some embodiments of any of the aspects, the hydrogel scaffold comprises poly(2-hydroxyethyl methacrylate) (pHEMA). In some embodiments of any of the aspects, the hydrogel scaffold consists of poly(2-hydroxyethyl methacrylate) (pHEMA). In some embodiments of any of the aspects, the hydrogel scaffold consists essentially of poly(2-hydroxyethyl methacrylate) (pHEMA).

[0075] In some embodiments of any of the aspects, the at least one protein of a hydrogel scaffold comprises, consists of, or consists essentially of one or more of: collagen, gelatin, and silk fibroin.

[0076] In some embodiments of any of the aspects, the at least one protein of a hydrogel scaffold comprises, consists of, or consists essentially of one or more of: hyaluronic acid, glycosaminoglycans, agarose, alginate, and chitosan.

[0077] In some embodiments of any of the aspects, the hydrogel scaffold can comprise one type of hydrogel scaffold molecule. In some embodiments of any of the aspects, the hydrogel scaffold can comprise two or more types of hydrogel scaffold molecule, e.g., two polyacrylates, or a polyacrylate and a protein.

[0078] In some embodiments of any of the aspects, the composition, kit, or combination further comprises at least one hydrogel crosslinker. Hydrogel crosslinkers are molecules that can form a three-dimensional network when reacted under suitable conditions with an appropriate hydrogel polymer, thereby promoting and/or leading to gelation of the polymer and hydrogel crosslinker into a hydrogel. Hydrogel crosslinkers are known in the art and include but are not limited to: ethylene glycol dimethacrylate (EGDMA), N,N'-methylenebisacrylamide (MBAA-crosslinker), ammonium persulfate (APS-initiator), tetramethylethylenediamine (TEMEDA-catalyst), and Fe³⁺ (ionic cross linker). In some embodiments of any of the aspects, the the composition, kit, or combination does not comprise a hydrogel crosslinker.

[0079] In some embodiments of any of the aspects, the composition, kit, or combination further comprises at least one hydrogel initiator, e.g., a radical initiator or a photoinitiator. Hydrogel initiators are molecules or energy sources that promote polymerization and/or cross-linking of hydrogel scaffolds, e.g., by providing radical species that promoter or initiate cross-linking reactions between a cross-linker and a hydrogel scaffold. Hydrogel initiators are known in the art and include but are not limited to: radical initiators such as halogen molecules, azo compounds and organic and inorganic peroxides such as Benzoyl peroxide, di- t-Butyl peroxide; and photoinitiators such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO). In some embodiments of any of the aspects, the radical initiator or photoinitiator comprises, consists of, or consists essentially of azobisisobutyronitrile (AIBN).

[0080] In some embodiments of any of the aspects, the composition further comprises a loading liquid, e.g., a liquid that is not pure water. The loading liquid can provide a means of loading a drug into the hydrogel composition and/or providing a fluid environment in the hydrogel composition that varies from pure water in one or more respects (e.g., pH, salt concentration, etc). Such fluid environments can improve drug solubility, retention, half-life, and/or delivery kinetics. Exemplary loading liquids include saline, other salt solutions, sugar solutions, pH buffered solvents, ionic liquids, organic solvents, mixtures or combinations of any of the foregoing.

[0081] In some embodiments of any of the aspects, the loading liquid can be the solution where water is the solvent, or the mixture comprising water that hydrates the hydrogel scaffold. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is not formulated with water.

[0082] In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 60% water by w/w. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 50% water by w/w. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 40% water by w/w. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 30% water by w/w. In some embodiments of any of the 20% water by w/w. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 10% water by w/w. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 5% water by w/w.

[0083] In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 60% water by w/v. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 50% water by w/v. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 40% water by w/v. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 30% water by w/v. In some embodiments of any of the 20% water by w/v. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 10% water by w/v. In some embodiments of any of the aspects, the hydrogel scaffold or hydrogel is formulated with a liquid that comprises no more than 5% water by w/v.

[0084] In some embodiments of any of the aspects, the composition or kit described herein further comprises at least one ionic liquid.

[0085] The term "ionic liquids (ILs)" as used herein refers to organic salts or mixtures of organic salts which are in liquid state at room temperature. This class of solvents has been shown to be useful in a variety of fields, including in industrial processing, catalysis, pharmaceuticals, and electrochemistry. The ionic liquids contain at least one anionic and at least one cationic component. Ionic liquids can comprise an additional hydrogen bond donor (i.e. any molecule that can provide an -OH or an -NH group), examples include but are not limited to alcohols, fatty acids, and amines. The at least one anionic and at least one cationic component may be present in any molar ratio. Exemplary molar ratios (cation:anion) include but are not limited to 1 : 1, 1:2, 2: 1, 1 :3, 3: 1, 2:3, 3:2, and ranges between these ratios. For further discussion of ionic liquids, see, e.g., Hough, et al, "The third

evolution of ionic liquids: active pharmaceutical ingredients", New Journal of Chemistry, 31 : 1429 (2007) and Xu, et al., "Ionic Liquids: Ion Mobilities, Glass Temperatures, and Fragilities", Journal of Physical Chemistry B, 107(25): 6170-6178 (2003); each of which is incorporated by reference herein in its entirety. In some embodiments of any of the aspects, the ionic liquid or solvent exists as a liquid below 100 °C. In some embodiments of any of the aspects, the ionic liquid or solvent exists as a liquid at room temperature.

[0086] The cation of an IL described herein can be a cation comprising a quaternary ammonium. A quaternary ammonium is a positively charged polyatomic ion of the structure NR_4^+ , each R independently being an alkyl group or an aryl group.

[0087] In some embodiments of any of the aspects, the cation has a molar mass equal to or greater than choline, e.g., a molar mass equal to or greater than 104.1708 g/mol. In some embodiments of any of the aspects, the cation has a molar mass greater than choline, e.g., a molar mass equal greater than 104.1708 g/mol.

[0088] In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl, alkane, alkene, or aryl. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl, alkane, or alkene. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkane or alkene. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 10 carbon atoms in length, e.g., no more than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 carbon atoms in length. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 12 carbon atoms in length. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 15 carbon atoms in length. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 20 carbon atoms in length.

[0089] In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 10 carbon atoms, e.g., no more than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 12 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 15 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises a carbon chain of no more than 20 carbon atoms.

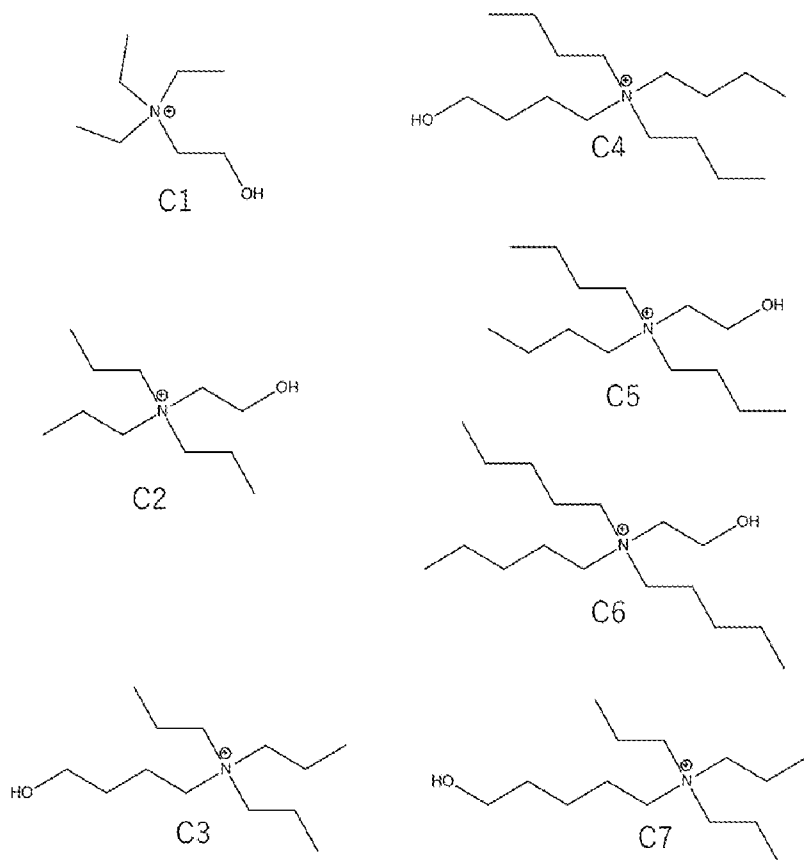
[0090] In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl group of no more than 10 carbon atoms, e.g., no more

than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl group of no more than 12 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl group of no more than 15 carbon atoms. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkyl group of no more than 20 carbon atoms.

[0091] In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an alkane, alkene, aryl, heteroaryl, alkyl, or heteroalkyl. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an unsubstituted alkane, unsubstituted alkene, unsubstituted aryl, unsubstituted heteroaryl, unsubstituted alkyl, or unsubstituted heteroalkyl. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an unsubstituted alkane. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises an unsubstituted alkene. In some embodiments of any of the aspects, each R group of the quaternary ammonium independently comprises one or more substituent groups.

[0092] In some embodiments of any of the aspects, at least one R group of the quaternary ammonium comprises a hydroxy group. In some embodiments of any of the aspects, one R group of the quaternary ammonium comprises a hydroxy group. In some embodiments of any of the aspects, only one R group of the quaternary ammonium comprises a hydroxy group.

[0093] Exemplary, non-limiting cations can include choline and any of the cations designated C1-C7 which are defined by structure below.



[0094] Further non-limiting examples of cations include the following:

1-(hydroxymethyl)-1-methylpyrrolidin-1-ium
 1-(2-hydroxyethyl)-1-methylpyrrolidin-1-ium
 1-ethyl-1-(3-hydroxypropyl)pyrrolidin-1-ium
 1-(3-hydroxypropyl)-1-methylpyrrolidin-1-ium
 1-(4-hydroxybutyl)-1-methylpyrrolidin-1-ium
 1-ethyl-1-(4-hydroxybutyl)pyrrolidin-1-ium
 1-(4-hydroxybutyl)-1-propylpyrrolidin-1-ium
 1-(5-hydroxypentyl)-1-propylpyrrolidin-1-ium
 1-ethyl-1-(5-hydroxypentyl)pyrrolidin-1-ium
 1-(5-hydroxypentyl)-1-methylpyrrolidin-1-ium
 1-(hydroxymethyl)-1-methylpiperidin-1-ium
 1-(2-hydroxyethyl)-1-methylpiperidin-1-ium
 1-ethyl-1-(2-hydroxyethyl)piperidin-1-ium
 1-ethyl-1-(3-hydroxypropyl)piperidin-1-ium
 1-(3-hydroxypropyl)-1-propylpiperidin-1-ium
 1-(3-hydroxypropyl)-1-methylpiperidin-1-ium

1-(4-hydroxybutyl)-1-methylpiperidin-1-ium
1-ethyl-1-(4-hydroxybutyl)piperidin-1-ium
1-(4-hydroxybutyl)-1-propylpiperidin-1-ium
1-butyl-1-(5-hydroxypentyl)piperidin-1-ium
1-(5-hydroxypentyl)-1-propylpiperidin-1-ium
1-ethyl-1-(5-hydroxypentyl)piperidin-1-ium
1-(5-hydroxypentyl)-1-methylpiperidin-1-ium
3-ethyl-1-methyl-1H-imidazol-3-ium
1-methyl-3-propyl-1H-imidazol-3-ium
3-butyl-1-methyl-1H-imidazol-3-ium
1-methyl-3-pentyl-1H-imidazol-3-ium
1,2-dimethyl-3-pentyl-1H-imidazol-3-ium
3-butyl-1,2-dimethyl-1H-imidazol-3-ium
1,2-dimethyl-3-propyl-1H-imidazol-3-ium
3-(hydroxymethyl)-1,2-dimethyl-1H-imidazol-3-ium
3-(2-hydroxyethyl)-1,2-dimethyl-1H-imidazol-3-ium
3-(3-hydroxypropyl)-1,2-dimethyl-1H-imidazol-3-ium
3-(4-hydroxybutyl)-1,2-dimethyl-1H-imidazol-3-ium
3-(5-hydroxypentyl)-1,2-dimethyl-1H-imidazol-3-ium
3-(5-hydroxypentyl)-1-methyl-1H-imidazol-3-ium
3-(4-hydroxybutyl)-1-methyl-1H-imidazol-3-ium
3-(3-hydroxypropyl)-1-methyl-1H-imidazol-3-ium
3-(2-hydroxyethyl)-1-methyl-1H-imidazol-3-ium
3-(hydroxymethyl)-1,2,4,5-tetramethyl-1H-imidazol-3-ium
3-(2-hydroxyethyl)-1,2,4,5-tetramethyl-1H-imidazol-3-ium
3-(3-hydroxypropyl)-1,2,4,5-tetramethyl-1H-imidazol-3-ium
3-(4-hydroxybutyl)-1,2,4,5-tetramethyl-1H-imidazol-3-ium
3-(5-hydroxypentyl)-1,2,4,5-tetramethyl-1H-imidazol-3-ium
1-(5-hydroxypentyl)pyridin-1-ium
1-(4-hydroxybutyl)pyridin-1-ium
1-(3-hydroxypropyl)pyridin-1-ium
1-(2-hydroxyethyl)pyridin-1-ium
1-(hydroxymethyl)pyridin-1-ium
1-hydroxypyridin-1-ium
(hydroxymethyl)trimethylphosphonium
triethyl(hydroxymethyl)phosphonium

triethyl(2-hydroxyethyl)phosphonium
 (2-hydroxyethyl)tripropylphosphonium
 (3-hydroxypropyl)tripropylphosphonium
 tributyl(3-hydroxypropyl)phosphonium
 (3-hydroxypropyl)tripentylphosphonium
 (4-hydroxybutyl)tripentylphosphonium
 (5-hydroxypentyl)tripentylphosphonium

[0095] In some embodiments of any of the aspects, the cation is choline, C1, C6, and/or C7. In some embodiments of any of the aspects, the cation is C1, C6, and/or C7. In some embodiments of any of the aspects, the cation is choline.

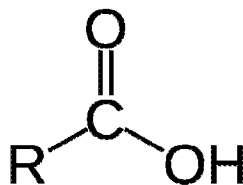
[0096] In some embodiments of any of the aspects, the cation is benzyl pyridinium, benzyl dimethyl dodecyl ammonium, a choline cation, phosphonium, tetraalkylphosphonium, or benzethonium.

[0097] In some embodiments of any of the aspects, the anion is bistriflimide, a geranate anion, oleate, 2-octenoic acid, hexanoate, dodecyldimethyl ammonia propane sulfonate, N-Lauryl sarconsinate, or geraniolate.

[0098] In some embodiments of any of the aspects, the cation is benzyl pyridinium, benzyl dimethyl dodecyl ammonium, a choline cation, phosphonium, tetraalkylphosphonium, or benzethonium; and the anion is bistriflimide, a geranate anion, oleate, 2-octenoic acid, hexanoate, dodecyldimethyl ammonia propane sulfonate, N-Lauryl sarconsinate, or geraniolate.

[0099] In some embodiments of any of the aspects, the anion of an IL described herein comprises, consists of, or consists essentially of a carboxylic acid. In some embodiments, the anion comprises one carboxylic acid group.

[00100] A carboxylic acid is a compound having the structure of Formula I, wherein R can be any group.



Formula I

[00101] Generally, the anion is $R-X^-$, where X is CO_2^- , SO_3^- , OSO_3^{2-} or OPO_3^{2-} ; and R is optionally substituted C_1 - C_{10} alkyl, optionally substituted C_2 - C_{10} alkenyl, or optionally substituted C_2 - C_{10} alkynyl, optionally substituted aryl, or optionally substituted heteroaryl.

[00102] In some embodiments, R is an optionally substituted linear or branched C_1 - C_9 alkyl. For example, R is a C_1 - C_9 alkyl optionally substituted with 1, 2, 3, 4, 5 or 6 substituents independently

selected from the group consisting of C₁-C₃alkyl, hydroxy (OH), halogen, oxo (=O), carboxy (CO₂), cyano (CN) and aryl. In some embodiments, R is a C₁-C₆alkyl optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of C₁-C₃alkyl, hydroxy, carboxy and phenyl. Preferably, R is a C₁-C₅alkyl, optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of methyl, ethyl, hydroxyl, carboxy, and phenyl. Exemplary alkyls for R include, but are not limited to, methyl, carboxymethyl, hydroxymethyl, ethyl, 1-hydroxyethyl, 2-phenylethyl, propyl, prop-2-yl, 1-methylpropyl, 2-methylpropyl, 3-carboxypropyl, 2,3-dicarboxymethyl-2-hydroxypropyl, butyl, pentyl, 1,2,3,4,5-pentahydroxypentyl, hexyl, 2-ethylhexyl and nonyl.

[00103] In some embodiments, R is an optionally substituted linear or branched C₂-C₈alkenyl. For example, R is a C₂-C₉alkenyl optionally substituted with 1, 2, 3, 4, 5 or 6 substituents independently selected from the group consisting of C₁-C₃alkyl, hydroxy, halogen, oxo, carboxy, cyano and aryl. In some embodiments, R is a C₂-C₆alkenyl optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of C₁-C₃alkyl, hydroxy, carboxy and phenyl. Preferably, R is a C₁-C₅alkenyl, optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of methyl, ethyl, hydroxyl, carboxy, and phenyl. Exemplary alkenyls for R include, but are not limited to, ethenyl, 2-carboxyethenyl, 1-methylpropenyl and 2-methylpropenyl.

[00104] In some embodiments, R is an optionally substituted aryl or heteroaryl. For example, R is an aryl or heteroaryl optionally substituted with 1, 2, 3, 4, 5 or 6 substituents independently selected from the group consisting of C₁-C₃alkyl, hydroxy, halogen, oxo, carboxy, cyano and aryl. In some embodiments, R is an aryl optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of C₁-C₃alkyl, hydroxy, carboxy and phenyl. Preferably R is a phenyl substituted with 1, 2 or 3 substituents independently selected from the group consisting of methyl, ethyl, hydroxyl, carboxy, and phenyl. Exemplary aryls for R include, but are not limited to, phenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, dihydroxyphenyl, trihydroxyphenyl, 3,4,5-trihydroxyphenyl, and 1,1-biphen-4-yl.

[00105] In some embodiments, X is CO₂⁻ and R is methyl, carboxymethyl, hydroxymethyl, ethyl, 1-hydroxyethyl, 2-phenylethyl, propyl, prop-2-yl, 1-methylpropyl, 2-methylpropyl, 3-carboxypropyl, 2,3-dicarboxymethyl-2-hydroxypropyl, butyl, pentyl, 1,2,3,4,5-pentahydroxypentyl, hexyl, 2-ethylhexyl, nonyl, ethenyl, 2-carboxyethenyl, 1-methylpropenyl, 2-methylpropenyl, 3,4,5-trihydroxyphenyl, or 1,1-biphen-4-yl. In some other embodiments, X is OSO₃⁻ and R is methyl, carboxymethyl, hydroxymethyl, ethyl, 1-hydroxyethyl, 2-phenylethyl, propyl, prop-2-yl, 1-methylpropyl, 2-methylpropyl, 3-carboxypropyl, 2,3-dicarboxymethyl-2-hydroxypropyl, butyl, pentyl, 1,2,3,4,5-pentahydroxypentyl, hexyl, 2-ethylhexyl, nonyl, ethenyl, 2-carboxyethenyl, 1-

methylpropenyl, 2-methylpropenyl, 3,4,5-trihydroxyphenyl, or 1,1-biphen-4-yl. In yet some other embodiments, X is OPO_3^{2-} or SO_3^- and R is 2-hydroxyphenyl, 3-hydroxyphenyl or 4-hydroxyphenyl.

[00106] The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., $\text{C}_1\text{-C}_{10}$ means one to ten carbons). An alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An “alkenyl” is an unsaturated alkyl group is one having one or more double bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), and the higher homologs and isomers.

[00107] The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term “heteroaryl” refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term “heteroaryl” includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Exemplary aryl and heteroaryl groups include, but are not limited to, phenyl, 4-nitrophenyl, 1-naphthyl, 2-naphthyl, biphenyl, 4-biphenyl, pyrrole, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, pyrazole, 3-pyrazolyl, imidazole, imidazolyl, 2-imidazolyl, 4-imidazolyl, benzimidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, thiazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, pyridine, 2-pyridyl, naphthyridinyl, 3-pyridyl, 4-pyridyl, benzophenonepyridyl, pyridazinyl, pyrazinyl, 2-pyrimidyl, 4-pyrimidyl, pyrimidinyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, indolyl, 5-indolyl, quinoline, quinolinyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, 6-

quinolyl, furan, furyl or furanyl, thiophene, thiophenyl or thienyl, diphenylether, diphenylamine, and the like.

[00108] The term “optionally substituted” means that the specified group or moiety is unsubstituted or is substituted with one or more (typically 1, 2, 3, 4, 5 or 6 substituents) independently selected from the group of substituents listed below in the definition for “substituents” or otherwise specified. The term “substituents” refers to a group “substituted” on a substituted group at any atom of the substituted group. Suitable substituents include, without limitation, halogen, hydroxy, caboxy, oxo, nitro, haloalkyl, alkyl, alkenyl, alkynyl, alkaryl, aryl, heteroaryl, cyclyl, heterocyclyl, aralkyl, alkoxy, aryloxy, amino, acylamino, alkylcarbanoyl, arylcarbanoyl, aminoalkyl, alkoxycarbonyl, carboxy, hydroxyalkyl, alkanesulfonyl, arenesulfonyl, alkanesulfonamido, arenesulfonamido, aralkylsulfonamido, alkylcarbonyl, acyloxy, cyano or ureido. In some cases, two substituents, together with the carbons to which they are attached to can form a ring.

[00109] In some embodiments of any of the aspects, the anion is an alkane. In some embodiments of any of the aspects, the anion is an alkene. In some embodiments of any of the aspects, the anion comprises a single carboxyl group. In some embodiments of any of the aspects, the carbon chain of the carboxylic acid comprises one or more substituent groups. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises one or more substituent groups, wherein each substituent group comprises at least one carbon atom. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises one or more substituent groups, wherein at least one substituent group comprises a methyl group. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises two substituent groups, wherein each substituent group comprises at least one carbon atom. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises two substituent groups, wherein one substituent group comprises a methyl group. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises two substituent groups, wherein each substituent group comprises a methyl group.

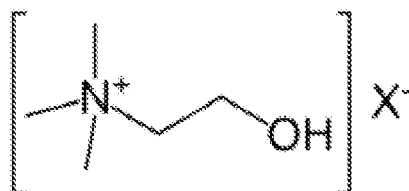
[00110] In some embodiments of any of the aspects, the anion is an unsubstituted alkane. In some embodiments of any of the aspects, the anion is an unsubstituted alkene. In some embodiments of any of the aspects, the carbon chain backbone of the carboxylic acid comprises one or more substituent groups. In some embodiments of any of the aspects, the carbon chain of the carboxylic acid comprises one or more substituent groups, wherein each substituent group comprises at least one carbon atom. In some embodiments of any of the aspects, the carbon chain of the carboxylic acid comprises one or more substituent groups, wherein each substituent group is alkyl, aryl, heteroalkyl, heteroaryl, alkane, or alkene. In some embodiments of any of the aspects, the carbon chain of the carboxylic acid comprises one or more substituent groups, wherein each substituent group is

unsubstituted alkyl, unsubstituted aryl, unsubstituted heteroalkyl, unsubstituted heteroaryl, unsubstituted alkane, or unsubstituted alkene.

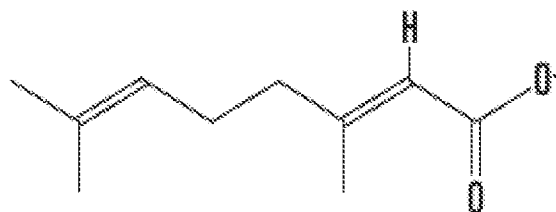
[00111] Exemplary carboxylic acids can include, but are not limited to lactic acid; glycolic acid; malonic acid; maleic acid; glutaric acid; citric acid; gluconic acid; adipic acid; octenoic acid; octanoic acid; Glycolic acid; Propanoic acid; Isobutyric acid; Butyric acid; Gallic acid; Decanoic Acid; 3,3-dimethylacrylic acid; 2-Ethylhexyl sulfate; 4-hydroxybenzenesulfonic acid; Isovaleric acid; Hydrocinnamic acid; Phenylphosphoric acid; Biphenyl-3-carboxylic acid; Geranic Acid; Citronellic Acid; (9Z)-octadec-9-enoic acid; (9Z,12Z)-octadeca-9,12-dienoic acid; (R)-5-(1,2-dithiolan-3-yl)pentanoic acid; Hexenoic Acid; Hexanoic Acid; 3-methylbutanoic acid; Nonanedioic Acid; Pentanoic acid; 2-hydroxyoctanoic acid; (E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid; 2-ethylhexyl sulfate; 2-(dimethylamino)ethanol; 8-hydroxycapric acid; 2-methylpropanoic acid; Ascorbic Acid; Butanoic acid; Salicylic Acid; Hydroxyl(phenyl)acetic acid; sodium ethylhexyl sulfate; hydroxybenzenesulfonic acid; acid; 4-phenolsulfonic acid;

[00112] In some embodiments of any of the aspects, the anion is gerante or geranic acid.

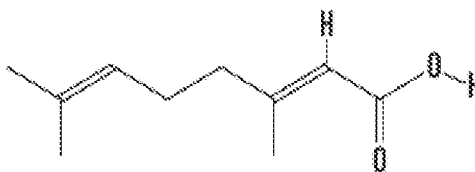
[00113] In some embodiments of any of the aspects, the ionic liquid is CAGE. CAGE is an ionic liquid comprising the cation choline (see, e.g., Formula I) and the anion geranate or geranic acid (see, e.g., Formula II and III). Preparation of CAGE can be, e.g., as described in International Patent Publication WO 2015/066647; which is incorporated by reference herein in its entirety, or as described in the examples herein.



Formula I



Formula II



Formula III

[00114] In some embodiments of any of the aspects, the anion of CAGE comprises geranate and/or geranic acid. In some embodiments of any of the aspects, the anion comprises geranate. In some embodiments of any of the aspects, the anion comprises geranic acid.

[00115] In some embodiments of any of the aspects, the anion of the IL comprises hexenoic acid. In some embodiments of any of the aspects, the ionic liquid is CAHA. CAHA is an ionic liquid comprising the cation choline (see, e.g., Formula I) and the anion hexenoic acid.

[00116] In some embodiments of any of the aspects, the anion of the IL comprises 2-octenoic acid. In some embodiments of any of the aspects, the ionic liquid is choline and 2-octenoic acid.

[00117] In some embodiments of any of the aspects, the IL comprises the combination of any cation described herein and any anion described herein.

[00118] In some embodiments of any of the aspects, the IL is at a concentration of at least 0.01% w/v. In some embodiments of any of the aspects, the IL is at a concentration of at least 0.05% w/v. In some embodiments of any of the aspects, the IL is at a concentration of at least 0.1% w/v. In some embodiments of any of the aspects, the IL is at a concentration of at least 0.2% w/v, at least 0.3% w/v, at least 0.4% w/v, at least 0.5% w/v, at least 1% w/v or greater. In some embodiments of any of the aspects, the IL is at a concentration of from about 0.01% w/v to about 1% w/v. In some embodiments of any of the aspects, the IL is at a concentration of from 0.01% w/v to 1% w/v. In some embodiments of any of the aspects, the IL is at a concentration of from about 0.05% w/v to about 0.5% w/v. In some embodiments of any of the aspects, the IL is at a concentration of from 0.05% w/v to 0.5% w/v.

[00119] In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w. In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w in water. In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w in saline or a physiologically compatible buffer.

[00120] In some embodiments of any of the aspects, the IL is at a concentration of from about 5% w/w to about 75% w/w. In some embodiments of any of the aspects, the IL is at a concentration of from 5% w/w to 75% w/w. In some embodiments of any of the aspects, the IL is at a concentration of from about 5% w/w to about 75% w/w in water, saline or a physiologically compatible buffer. In some embodiments of any of the aspects, the IL is at a concentration of from 5% w/w to 75% w/w in water, saline or a physiologically compatible buffer.

[00121] In some embodiments of any of the aspects, the IL is at a concentration of at least about 0.1 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of at least 0.1 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from about 10 % w/w to about 70 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from 10 % w/w to 70 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from about 30 % w/w to about 50 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from 30 % w/w to 40 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from about 30 % w/w to about 50 % w/w. In some embodiments of any of the aspects, the IL is at a concentration of from 30 % w/w to 40 % w/w.

[00122] In some embodiments of any of the aspects, the % w/w concentration of the IL is % w/w concentration in water, saline, or a physiologically compatible buffer.

[00123] In some embodiments of any of the aspects, the IL is 100% by w/w or w/v.

[00124] In some embodiments, the IL is an anhydrous salt, e.g., an ionic liquid not diluted or dissolved in water. In some embodiments, the IL is provided as an aqueous solution.

[00125] In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w and has a ratio of cation:anion of at least 1:3. In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w in water and has a ratio of cation:anion of at least 1:3. In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w and has a ratio of cation:anion of 1:3 or 1:4. In some embodiments of any of the aspects, the IL is at a concentration of at least 25% w/w in water and has a ratio of cation:anion of 1:3 or 1:4. In some embodiments of any of the aspects, the IL is a gel, or a shear-thinning Newtonian gel.

[00126] In some embodiments of any of the aspects, the IL is at a concentration of 40-60% w/w in water and has a ratio of cation:anion ratio of 1:1 to 1:4. In some embodiments of any of the aspects, the IL is at a concentration of 50% w/w in water and has a ratio of cation:anion ratio of 1:2.

[00127] In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 10:1 to about 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 10:1 to 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 5:1 to about 1:5. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 5:1 to 1:5. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 2:1 to about 1:4. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 2:1 to 1:4. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 2:1 to about 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 2:1 to 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 2:1 to about 1:2. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 2:1 to 1:2. In some embodiments of any of the aspects, the IL has a ratio of cation:anion such that there is a greater amount of anion, e.g., a ratio of less than 1:1.

In some embodiments of any of the aspects, the IL has a ratio of cation:anion such that there is an excess of anion. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 1:1 to about 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 1:1 to 1:10. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 1:1 to about 1:4. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 1:1 to 1:4. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 1:1 to about 1:3. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 1:1 to 1:3. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from about 1:1 to about 1:2. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of from 1:1 to 1:2. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of about 1:1, 1:2, 1:3, or 1:4. In some embodiments of any of the aspects, the IL has a ratio of cation:anion of 1:1, 1:2, 1:3, or 1:4. Without wishing to be constrained by theory, compositions with higher amounts of anion relative to cation display greater hydrophobicity.

[00128] In some embodiments of any of the aspects, the IL is at a concentration of at least 20 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least about 20 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least 25 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least about 25 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least 50 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least about 50 mM. In some embodiments of any of the aspects, the IL is at a concentration of at least 100 mM, 500 mM, 1 M, 2 M, 3 M or greater. In some embodiments of any of the aspects, the IL is at a concentration of at least about 100 mM, 500 mM, 1 M, 2 M, 3 M or greater.

[00129] In some embodiments of any of the aspects, the IL is at a concentration of from about 50 mM to about 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from 50 mM to 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from about 500 mM to about 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from 500 mM to 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from about 1 M to about 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from 1 M to 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from about 2 M to about 4 M. In some embodiments of any of the aspects, the IL is at a concentration of from 2 M to 4 M.

[00130] In some embodiments of any of the aspects, the IL concentration in the composition or formulation is about 0.1 mM to 20 mM. In some embodiments of any of the aspects, the IL concentration in the composition or formulation is about 0.5 mM to 20 mM, 0.5 mM to 18 mM, 0.5 mM to 16 mM, 0.5 mM to 14 mM, 0.5 mM to 12 mM, 0.5 mM to 10 mM, 0.5 mM to 8 mM, 1 mM to

20 mM, 1 mM to 18 mM, 1 mM to 16 mM, 1 mM to 14 mM, 1 mM to 12 mM, 1 mM to 10 mM, 1 mM to 8 mM, 2 mM to 20 mM, 2 mM to 18 mM, 2 mM to 16 mM, 2 mM to 14 mM, 2 mM to 12 mM, 2 mM to 10 mM, 2 mM to 8 mM, 4 mM to 20 mM, 4 mM to 18 mM, 4 mM to 16 mM, 4 mM to 14 mM, 4 mM to 12 mM, 4 mM to 10 mM, 4 mM to 8 mM, 6 mM to 20 mM, 6 mM to 18 mM, 6 mM to 16 mM, 6 mM to 14 mM, 6 mM to 12 mM, 6 mM to 10 mM, 6 mM to 8 mM, 8 mM to 20 mM, 8 mM to 18 mM, 8 mM to 16 mM, 8 mM to 14 mM, 8 mM to 12 mM, 8 mM to 10 mM, 10 mM to 20 mM, 10 mM to 18 mM, 10 mM to 16 mM, 10 mM to 14 mM, 10 mM to 12 mM, 12 mM to 20 mM, 12 mM to 18 mM, 12 mM to 16 mM, 12 mM to 14 mM, 14 mM to 20 mM, 14 mM to 18 mM, 14 mM to 16 mM, 16 mM to 20 mM, 16 mM to 18 mM, or 18 mM to 20 mM. In some embodiments of any of the aspects, the IL concentration in the composition or formulation is about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM or about 20 mM.

[00131] In some embodiments of any of the aspects, a composition, kit, or combination described herein can comprise one, two, three, or more of any of the loading liquids described herein. In some embodiments of any of the aspects, a composition, kit, or combination described herein can comprise one, two, three, or more of any of the ionic liquids described herein.

[00132] It is specifically contemplated that a composition, kit, or combination described herein can comprise one, two, three, or more of any of the types of components described herein, e.g., active agents (i.e. drugs), hydrogel scaffolds and/or loading liquids (e.g., saline and/or ILs). For example, a composition can comprise a mixture, solution, combination, or emulsion of two or more different ionic liquids, and/or a mixture, solution, combination, or emulsion of two or more different hydrogel scaffolds, and/or a mixture, solution, combination, or emulsion of two or more different active compounds.

[00133] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

- at least one hydrogel scaffold;
- at least one loading liquid; and
- at least one drug.

[00134] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

- at least one hydrogel scaffold;
- at least one ionic liquid; and
- at least one drug.

[00135] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water; and
at least one drug.

[00136] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water;
at least one loading liquid; and
at least one drug.

[00137] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water;
at least one ionic liquid; and
at least one drug.

[00138] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
at least one hydrogel crosslinker;
at least one loading liquid; and
at least one drug.

[00139] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
at least one hydrogel crosslinker;
at least one ionic liquid; and
at least one drug.

[00140] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water;
at least one hydrogel crosslinker; and
at least one drug.

[00141] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;

water;
at least one hydrogel crosslinker;
at least one ionic liquid; and
at least one drug.

[00142] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
at least one hydrogel crosslinker;
at least one hydrogel initiator;
at least one loading liquid; and
at least one drug.

[00143] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water;
at least one hydrogel crosslinker;
at least one hydrogel initiator; and
at least one drug.

[00144] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
water;
at least one hydrogel crosslinker;
at least one hydrogel initiator;
at least one ionic liquid; and
at least one drug.

[00145] In one aspect of any of the embodiments, described herein is a hydrogel composition comprising:

at least one hydrogel scaffold;
at least one hydrogel crosslinker;
at least one hydrogel initiator;
at least one ionic liquid; and
at least one drug.

[00146] The compositions described herein are contemplated for placement within the ear, specifically within the outer or middle ear.

[00147] The outer ear contains the ear canal, which ranges from 3-8 mm in diameter and 3-8 cm in length. When the composition is for placement in contact with the ear canal epithelium, the composition or hydrogel has a fully hydrated/swelled size and shape of a rectangle, cube, cylinder, cone, sphere, dome, pyramid, disc, tetrahedron, prism, or any other shape of variable dimensions. In some embodiments of any of the aspects, when the composition is for placement in contact with the ear canal epithelium, the composition or hydrogel has a fully hydrated/swelled size and shape of a rectangle, cube, cylinder, cone, sphere, dome, pyramid, disc, tetrahedron, or prism.

[00148] The middle ear begins where the outer ear terminates at the tympanic membrane. The tympanic membrane is roughly 10 mm x 9 mm in diameter, for a total surface area of approximately 90 mm². On the medial side of the tympanic membrane is found the tympanic cavity, and on the medial side of the tympanic cavity are found the oval window and the round window. Babies and children can have a tympanic membrane with slightly smaller dimensions, although the tympanic membrane typically does not change in size significantly after birth. Microtia and other congenital deformities can cause a smaller ear canal and tympanic membrane. Additionally, surgery to correct medical conditions, such as cholesteatoma, can also result in a narrowed ear canal. These conditions can further increase the need for EarFlow, as it can be challenging to administer ear drop formulations regularly through small, narrow ear canals. The EarFlow device can be placed on or in contact with one or more of the ear canal, tympanic membrane, the tympanic cavity, the round window, the oval window, or the Eustachian tubes.

[00149] In some embodiments of any of the aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 1 mm x at least 1 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 8 mm x at least 7 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 9 mm x at least 8 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a face of 8-10 mm x 7-9 mm.

[00150] In some embodiments of any of the aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled shape of a cylinder, a disk, pyramid, a cone, a hemisphere, a polyhedron, a cube, a shape created by introducing a curve or angle into any of the preceding shapes, or a shape created by rounding one or more of the edges of one of the preceding shapes. In some embodiments of any of the

aspects, when the composition is for placement in contact with the round window or oval window, the composition or hydrogel has a fully hydrated/swelled shape of a cylinder or disk.

[00151] In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 1 mm x at least 1 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 8 mm x at least 7 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a surface face of at least 9 mm x at least 8 mm. In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled size and shape such that it has a face of 8-10 mm x 7-9 mm.

[00152] In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled shape of a cylinder, a disk, a pyramid, a cone, a hemisphere, a polyhedron, a cube, a shape created by introducing a curve or angle into any of the preceding shapes, or a shape created by rounding one or more of the edges of one of the preceding shapes. In some embodiments of any of the aspects, when the composition is for placement in contact with the tympanic membrane, the composition or hydrogel has a fully hydrated/swelled shape of a cylinder or a disk.

[00153] A cone has a base formed by a closed curve, a pyramid has a polygonal base, and a circular cone has a base which is a circle.

[00154] It should be noted that in reference to a composition or hydrogel surface, “surface” refers to an outer edge or face of the composition or hydrogel and is understood to have a topography of a plane which can have curvature. In some embodiments of any of the aspects, the surface is a plane. In some embodiments of any of the aspects, the surface is curved or has multiple curves.

[00155] In some embodiments of any of the aspects, the composition or hydrogel has a fully hydrated/swelled size no greater than 12 mm by 12 mm by 12 mm. In some embodiments of any of the aspects, the composition or hydrogel has a fully hydrated/swelled size no greater than 8 mm by 8 mm by 8 mm. In some embodiments of any of the aspects, the composition or hydrogel has a fully hydrated/swelled size of at least 8 mm by 8 mm by 7.5 mm. In some embodiments of any of the aspects, the composition or hydrogel has a fully hydrated/swelled size of at least 5.4 mm by 5.4 mm by 2.7 mm.

[00156] The size of a dehydrated or partially dehydrated hydrogel composition, when fully hydrated or swelled (or vice versa) is readily calculated, e.g., based on known rates or swelling for different hydrogel scaffolds.

[00157] In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises at least 30 μL of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises at least 40 μL of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises at least 200 μL of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises at least 1000 μL of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises 30-1000 μL of the at least one ionic liquid. In some embodiments of any of the aspects, the composition, kit, combination, or hydrogel comprises 200-1000 μL of the at least one ionic liquid.

[00158] In some embodiments of any of the aspects, the composition, kit, or combination comprises a further active agent or ingredient, e.g., a drug, e.g., a drug for a middle ear condition or disease. As used herein, an “active compound” or “active agent” is any agent which will exert an effect on a target cell or organism. The terms “compound” and “agent” refer to any entity which is normally not present or not present at the levels being administered and/or provided to a cell, tissue or subject. An agent can be selected from a group comprising: chemicals; small organic or inorganic molecules; signaling molecules; nucleic acid sequences; nucleic acid analogues; proteins; peptides; enzymes; aptamers; peptidomimetic, peptide derivative, peptide analogs, antibodies; intrabodies; biological macromolecules, extracts made from biological materials such as bacteria, plants, fungi, or animal cells or tissues; naturally occurring or synthetic compositions or functional fragments thereof. In some embodiments, the agent is any chemical, entity or moiety, including without limitation synthetic and naturally-occurring non-proteinaceous entities. Agents can be known to have a desired activity and/or property, or agents can be selected from a library of diverse compounds.

[00159] As used herein, the term “small molecule” refers to a chemical agent which can include, but is not limited to, a peptide, a peptidomimetic, an amino acid, an amino acid analog, a polynucleotide, a polynucleotide analog, an aptamer, a nucleotide, a nucleotide analog, an organic or inorganic compound (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[00160] In some embodiments of any of the aspects, the active compound can be a therapeutic compound or drug, e.g., an agent or compound which is therapeutically effective for the treatment of at least one condition in a subject. Therapeutic compounds are known in the art for a variety of conditions, see, e.g., the database available on the world wide web at [drugs.com](https://www.drugs.com) or the catalog of

FDA-approved compounds available on the world wide web at catalog.data.gov/dataset/drugsfda-database; each of which is incorporated by reference herein in its entirety.

[00161] In some embodiments of any of the aspects, the one or more drugs are selected from: antibiotics; steroids; growth factors; biologics; viral vectors; polypeptides; nucleic acid molecules; polysaccharides; anesthetics; analgesics; and external, middle or inner ear disease therapeutics. Exemplary such drugs are known in the art, including drugs suitable for ear diseases or conditions described herein.

[00162] As used herein, “antibiotic” refers to any chemical or biological agent with therapeutic usefulness in the inhibition of bacterial cell growth or in killing bacteria, e.g., those that are bactericidal or bacteriostatic. Categories of antibiotics can include, but are not limited to those that target the bacterial cell wall (e.g., penicillins, cephalosporins), those that target the bacterial cell membrane (e.g., polymyxins), those that target bacterial enzymes (e.g., rifamycins, lipiarmycins, quinolones, sulfonamides), protein synthesis inhibitors (e.g., macrolides, lincosamides, and tetracyclines), aminoglycosides, cyclic lipopeptides, glycyclines, oxazolidinones, beta-lactams, and lipiarmycins. Exemplary, non-limiting antibiotics include penicillin, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, flucloxacillin, ampicillin, amoxicillin, pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin, epicillin, cabenicillin, ticarcillin, temocillin, mezlocillin, piperacillin, azolocillin, clavulanic acid, sulbactam, tazobactam, cafadroxil, cephalixin, cefalotin, cefapirin, cefazolin, cefradine, cefaclor, cefonicid, cefprozil, cefuroxime, loracarbef, cefmetazole, cefotetan, cefoxitin, cefotiam, cefdinir, cefixime, cefotaxime, ceftiofur, cefepime, cefiderocol, cefpodoxime, ceftazidime, latamoxef, ceftriaxone, cefoperazone, cefepime, cefiderocol, cefpodoxime, ceftiofur, ceftazidime, ceftriaxone, cefoperazone, ceftazidime, latamoxef, cefepime, cefiderocol, cefpodoxime, rifampicin, rifabutin, rifapentine, rifamixin, fidaxomicin, ciproflaxacin, moxifloxacin, levofloxacin, sulfafurazole, azithromycin, clarithromycin, erythromycin, fidaxomicin, spiramycin, telithromycin, lincomycin, clindamycin, pirlimycin, tetracycline, eravacycline, sarecycline, omadacycline, doxycycline, kanamycin, tobramycin, gentamicin, neomycin, streptomycin, vancomycin, tigecycline, linezolid, posizolid, tedizolid, radezolid, cycloserine, contezolid, and daptomycin. One of skill in the art can readily identify an antibiotic agent of use *e.g.* see *Antibiotics in Laboratory Medicine*, Victor Lorian (ed.) Wolters Kluwer; and *Antibiotics Manual*, David Schlossberg and Rafik Samuel, John Wiley and Sons (2017); each of which is incorporated by reference herein in its entirety. In some embodiments of any of the aspects, the at least one antibiotic is ciprofloxacin, amoxillin, neomycin, polymyxin, colistin, or ofloxacin.

[00163] As used herein, the term “steroid” refers to a chemical substance comprising three cyclohexane rings and a cyclopentane ring. The rings are arranged to form tetracyclic cyclopentaphenanthrene, i.e. gonane. In some embodiments, the steroid is a corticosteroid.

[00164] As used herein, the term “corticosteroid” refers to a class of steroid hormones that are produced in the adrenal cortex or produced synthetically. Corticosteroids are involved in a wide range

of physiologic systems such as stress response, immune response and regulation of inflammation, carbohydrate metabolism, protein catabolism, blood electrolyte levels, and behavior. Corticosteroids are generally grouped into four classes, based on chemical structure. Group A corticosteroids (short to medium acting glucocorticoids) include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, and prednisone. Group B corticosteroids include triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, and halcinonide. Group C corticosteroids include betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, and fluocortolone. Group D corticosteroids include hydrocortisone-17-butyrate, hydrocortisone-17-valerate, acemetasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate, and fluprednidene acetate. Non-limiting examples of corticosteroids include, aldosterone, beclomethasone, beclomethasone dipropionate, betamethasone, betamethasone-21-phosphate disodium, betamethasone valerate, budesonide, clobetasol, clobetasol propionate, clobetasone butyrate, clocortolone pivalate, cortisol, cortisteron, cortisone, deflazacort, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, diflorasone diacetate, dihydrocortison, flucinonide, fludrocortisones acetate, flumethasone, flunisolide, flucinolone acetonide, fluticasone furate, fluticasone propionate, halcinonide, halpmetasone, hydrocortisone, hydrocortisone acetate, hydrocortisone succinate, 16 α -hydroxyprednisolone, isoflupredone acetate, medrysone, methylprednisolone, prednacinolone, prednicarbate, prednisolone, prednisolone acetate, prednisolone sodium succinate, prednisone, triamcinolone, triamcinolone, and triamcinolone diacetate. As used herein, the term “corticosteroid” can include, but is not limited to, the following generic and brand name corticosteroids: cortisone (CORTONE™ ACETATE™, ADRESON™, ALTESONA™, CORTELAN™, CORTISTAB™, CORTISYL™, CORTOGEN™, CORTONE™, SCHEROSON™); dexamethasone-oral (DECADRON-ORAL™, DEXAMETH™, DEXONE™, HEXADROL-ORAL™, DEXAMETHASONE™ INTENSOL™, DEXONE 0.5™, DEXONE 0.75™, DEXONE 1.5™, DEXONE 4™); hydrocortisone-oral (CORTEF™, HYDROCORTONE™); hydrocortisone cypionate (CORTEF ORAL SUSPENSION™); methylprednisolone-oral (MEDROL-ORAL™); prednisolone-oral (PRELONE™, DELTA-CORTEF™, PEDIAPRED™, ADNISOLONE™, CORTALONE™, DELTACORTRIL™, DELTASOLONE™, DELTASTAB™, DI-ADRESON F™, ENCORTOLONE™, HYDROCORTANCYL™, MEDISOLONE™, METICORTELONE™, OPREDSONE™, PANAAFCORTELONE™, PRECORTISYL™, PRENISOLONA™, SCHERISOLONA™, SCHERISOLONE™); prednisone (DELTASONE™, LIQUID PRED™, METICORTEN™, ORASONE 1™, ORASONE 5™, ORASONE 10™, ORASONE 20™, ORASONE 50™, PREDNICEN-M™, PREDNISONE INTENSOL™, STERAPRED™, STERAPRED DS™, ADASONE™, CARTANCYL™, COLISONE™,

CORDROL™, CORTAN™, DACORTIN™, DECORTIN™, DECORTISYL™, DELCORTIN™, DELLACORT™, DELTADOME™, DELTACORTENET™, DELTISONA™, DIADRESON™, ECONOSONE™, ENCORTON™, FERNISONE™, NISONA™, NOVOPREDNISONE™, PANAFECORT™, PANASOL™, PARACORT™, PARMENISON™, PEHACORT™, PREDELIN™, PREDNICORT™, PREDNICOT™, PREDNIDIB™, PREDNIMENT™, RECTODELT™, ULTRACORTEN™, WINPRED™); triamcinoloneoral (KENACORT™, ARISTOCORT™, ATOLONE™, SHOLOG A™, TRAMACORT-D™, TRI-MED™, TRIAMCOT™, TRISTOPLEX™, TRYLONE D™, U-TRI-LONE™). Methods of synthesizing steroids and corticosteroids are well known in the art and such compounds are also commercially available.

[00165] In some embodiments of any of the aspects, the at least one steroid is dexamethasone, hydrocortisone, methylprednisolone, or betamethasone.

[00166] Anesthetics are known in the art and can include, by way of non-limiting example, benzocaine, butamben, dibucaine, lidocaine, oxybuprocaine, pramoxine, proxymetacaine, and tetracaine. Pain relief agents or analgesics are known in the art and can include, by way of non-limiting example, acetaminophen, NSAIDs (e.g., aspirin, ibuprofen, naproxen), COX-2 inhibitors (e.g., rofecoxib, celecoxib, etoricoxib), opioids (e.g., codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, tramadol, venlafaxine, tapentadol, cannabinoids, opioid potentiators (e.g., hydroxyzine, promethazine, carisoprodol, or tripeleminamine), adjuvant analgesics (e.g., orphenadrine, mexiletine, pregabalin, gabapentin, cyclobenzaprine, hyoscine (scopolamine)), carbamazepine, and gabapentinoids.

[00167] In some embodiments of any of the aspects, the at least one anesthetic is lidocaine, tetracaine, bupivacaine, benzocaine, proparacaine, or oxybuprocaine.

[00168] External ear or outer ear therapeutics are known in the art and include but are not limited to antiseptics such as betadine, gentian violet, hydrogen peroxide, and the like.

[00169] Middle ear therapeutics are known in the art and include but are not limited to anti-inflammatory agents and monoclonal antibodies (such as the anti-IL-5 monoclonal antibody mepolizumab for treatment of eosinophilic otitis media). Anti-inflammatory agents are known in the art. Exemplary anti-inflammatories include, but are not limited to, non-steroidal anti-inflammatory drugs (NSAIDs - such as aspirin, ibuprofen, or naproxen); steroids such as corticosteroids, including glucocorticoids (e.g., cortisol, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, and beclomethasone); methotrexate; sulfasalazine; leflunomide; anti-TNF medications; cyclophosphamide; pro-resolving drugs; mycophenolate; and the like. In some embodiments, the anti-inflammatory agent can be a steroid (e.g., a corticosteroid or glucocorticoid).

[00170] Inner ear therapeutics are known in the art and include but are not limited to steroids, brain-derived nerve growth factor (BDNF), gentamycin, and gene therapy molecules including viral vectors.

[00171] Exemplary drugs for certain conditions described herein are provided in Table 1. In addition, small molecules, biologics, and gene therapies are contemplated for use in treatment of each of the diseases and conditions described herein.

[00172] Table 1

Disease or condition	Exemplary Drugs
Otitis media	Antibiotics, e.g., ciprofloxacin, amoxillin, neomycin, polymyxin, colistin, ofloxacin
Sensorineural hearing loss	Steroids, e.g., corticosteroids (e.g., dexamethasone)
Otitis externa; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa;	Antimicrobials or antibiotics (e.g., acetic acid, aminoglycosides, polymyxin B, and quinolones); corticosteroids.
Myringitis	Antimicrobial, antiviral, antibiotics, analgesics, antiinflammatories
Mucosalization of the ear drum, ear canal or mastoid bowl	Antibiotics
Cancer	Chemotherapeutics, immunotherapies
Trauma related hearing loss	Corticosteroids, gene therapies, or small molecule drugs (e.g., D-methionine, N—acetylcysteine, Sodium thiosulfate, Ebsele) (for further discussion see Ren et al. Front. Cell. Neurosci 2019 13:323 and Crowon et al. Otol. Neurotol. 2017 38:792-803; the contents of which are incorporated by reference herein in their entirety).
Labyrinthitis	Corticosteroids, antivirals, antibiotics, diphenhydramine, lorazepam
Vestibular neuritis	Meclizine, lorazepam, prochlorperazine, corticosteroids (e.g., prednisone)
Vertigo	Prochlorperazine, antihistamines
Tympanic membrane perforation	Antibiotic (e.g., ofloxacin)
Genetic forms of hearing loss	Gene therapy

Meniere's disease	Meclizine, diazepam, lorazepam, promethazine, prochlorperazine, ondansetron, steroids, gentamicin
Vestibular hypofunction	Meclizine, diazepam, lorazepam, promethazine, prochlorperazine
Conductive hearing loss	Antimicrobials, antibiotics, antifungals
Age and noise related hearing loss	Corticosteroids, gene therapies, or small molecule drugs (e.g., D-methionine, N—acetylcysteine, Sodium thiosulfate, Ebsele)
Infection	Antimicrobials, antibiotics, antifungals
Inflammation	Anti-inflammatories

[00173] In some embodiments of any of the aspects, the one or more drugs comprise at least one antibiotic. In some embodiments of any of the aspects, the one or more drugs comprise at least one steroid. In some embodiments of any of the aspects, the one or more drugs comprise at least one antibiotic at at least one steroid.

[00174] In some embodiments of any of the aspects, the one or more drugs are selected from dexamethasone and ciprofloxacin. In some embodiments of any of the aspects, the one or more drugs comprise dexamethasone and ciprofloxacin.

[00175] In some embodiments of any of the aspects, the steroid is at least 1 mg/mL of steroid. In some embodiments of any of the aspects, the steroid is at least 10 mg/mL of steroid. In some embodiments of any of the aspects, the steroid is at least 100 mg/mL of steroid. In some embodiments of any of the aspects, the steroid is 100-400 mg/mL of steroid. In some embodiments of any of the aspects, the steroid is 200 mg/mL of steroid.

[00176] In some embodiments of any of the aspects, the dexamethasone is at least 1 mg/mL of dexamethasone. In some embodiments of any of the aspects, the dexamethasone is at least 10 mg/mL of dexamethasone. In some embodiments of any of the aspects, the dexamethasone is at least 100 mg/mL of dexamethasone. In some embodiments of any of the aspects, the dexamethasone is 100-400 mg/mL of dexamethasone. In some embodiments of any of the aspects, the dexamethasone is 200 mg/mL of dexamethasone.

[00177] In some embodiments of any of the aspects, the antibiotic is at least 1 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is at least 10 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is at least 100 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is at least 200 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is at least 300 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is 100-500 mg/mL of antibiotic.

In some embodiments of any of the aspects, the antibiotic is 250 mg/mL of antibiotic. In some embodiments of any of the aspects, the antibiotic is 200 mg/mL of antibiotic.

[00178] In some embodiments of any of the aspects, the ciprofloxacin is at least 1 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is at least 10 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is at least 100 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is at least 200 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is at least 300 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is 100-500 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is 250 mg/mL of ciprofloxacin. In some embodiments of any of the aspects, the ciprofloxacin is 200 mg/mL of ciprofloxacin.

[00179] In one aspect of any of the embodiments, described herein is a kit or combination comprising at least one hydrogel scaffold and at least one of: at least one loading liquid (e.g., an ionic liquid) and at least one drug. The compositions described herein can be packaged and provided to users/medical personnel as fully hydrated hydrogels loaded with drugs (and optionally ionic liquid(s)), fully ready for placement. Alternatively, the compositions described herein can be provided as dehydrated, partially dehydrate, or lyophilized hydrogel scaffolds, such that the user/medical personnel can hydrate the hydrogel scaffold and/or load the drugs (and optionally ionic liquid(s)) before use. This second approach can provide more flexibility for users to determine drug identity(ies) and doses, and/or improve shelf-life and costs of transport/storage. It is particularly contemplated that drugs and/or loading liquids (e.g., ionic liquids) can be added to the hydrogel compositions after the hydrogels are placed within or upon the subject.

[00180] In one aspect, described herein is a kit comprising a composition or combination as described herein. A kit is any manufacture (e.g., a package or container) comprising at least one article, e.g., the hydrogel scaffold and/or loading liquid (e.g., ionic liquid) and/or drug described herein, the manufacture being promoted, distributed, or sold as a unit for performing the methods described herein.

[00181] The kits described herein can optionally comprise additional components useful for performing the methods described herein. By way of example, the kit can comprise one or more items for placement of the hydrogel composition in or on the subject or an instructional material which describes performance of a method as described herein, and the like. Additionally, the kit may comprise an instruction leaflet and/or may provide information as to the relevance of the obtained results. Instructions can be printed on a separate leaflet, on the device, or on the packaging of the kit itself.

[00182] The compositions, kits, and combinations described herein are shown to provide improved drug delivery kinetics. Accordingly, described herein is a method of drug delivery, the

method comprising administering, to a patient in need of a drug, a composition described herein, wherein the composition comprises the drug. The composition can comprise the drug before or after administration, e.g., a hydrogel as described herein can be administered and the drug then loaded onto/into the hydrogel or the drug can be loaded before administration. In some embodiments of any of the aspects, the composition is administered by placing the composition in contact with the tympanic membrane, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.

[00183] The compositions, kits, and combinations described herein are shown to provide improved drug delivery kinetics to the ear, without detrimental interference with the patient's hearing. Accordingly, described herein is a method of treating an ear condition or disease, the method comprising administering to the ear of a patient the composition of any of the preceding claims. The composition can be administered to the ear canal or middle ear (e.g., tympanic membrane, oval window membrane, or round window membrane). In some embodiments of any of the aspects, the composition is administered by placing it in contact with a surface of the ear canal or middle ear (e.g., tympanic membrane, oval window membrane, or round window membrane). In some embodiments of any of the aspects, the composition is administered to the ear canal, tympanic membrane, or round window membrane. In some embodiments of any of the aspects, the composition is administered to the ear canal or tympanic membrane. In some embodiments of any of the aspects, the composition is administered by placing it in contact with a surface of the ear canal, tympanic membrane, or round window membrane. In some embodiments of any of the aspects, the composition is administered by placing it in contact with a surface of the ear canal or tympanic membrane.

[00184] An ear condition or disease is a disease or condition affecting one or more tissues or structures of the ear. In some embodiments of any of the aspects, the ear condition or disease is selected from the group consisting of: otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss.

[00185] In some embodiments of any of the aspects, the ear condition or disease is otitis media. In some embodiments of any of the aspects, the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane or the round window membrane. In some embodiments of any of the aspects, the ear condition or disease is otitis media or sudden sensorineural hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane or the round window membrane. In some embodiments of any of the

aspects, the ear condition or disease is otitis media or sudden sensorineural hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane. In some embodiments of any of the aspects, the ear condition or disease is otitis media and the composition is administered by placing the composition in contact with the tympanic membrane.

[00186] In some embodiments of any of the aspects, the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the oval window membrane, or the round window membrane.

[00187] In some embodiments of any of the aspects, the ear condition or disease is otitis externa. In some embodiments of any of the aspects, the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.

[00188] In some embodiments of any of the aspects, the composition is left in the ear for at least 2 days. In some embodiments of any of the aspects, the composition is left in the ear for at least 2 days.

[00189] In some embodiments of any of the aspects, the composition is left in the ear for at least 3 days. In some embodiments of any of the aspects, the composition is left in the ear for at least 5 days.

[00190] In some embodiments of any of the aspects, the composition is left in the ear for at least 7 days.

[00191] In some embodiments of any of the aspects, the composition is left in the ear for no more than 7 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 10 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 28 days. In some embodiments of any of the aspects, the composition is left in the ear for no more than 12 days. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 18 months.

[00192] In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 7 days. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 10 days. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 28 days. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 12 months. In some embodiments of any of the aspects, the composition is physically removed from the ear after at least 18 months.

[00193] In some embodiments of any of the aspects, the composition is physically removed from the ear within 7 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 10 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 28 days. In some embodiments of any of the aspects, the composition is physically removed from the ear within 12 months. In some embodiments of any of the aspects, the composition is physically removed from the ear within 18 months.

[00194] In some embodiments, the methods described herein relate to treating a subject having or diagnosed as having a condition described herein, e.g. otitis media with a composition described herein. Subjects having such conditions, e.g., otitis media, can be identified by a physician using current methods of diagnosing the relevant condition. For example, symptoms and/or complications of otitis media which characterize these conditions and aid in diagnosis are well known in the art and include but are not limited to, hearing difficulties, loss of balance, fluid in the ear, fever, and ear pain. Tests that may aid in a diagnosis of, e.g. otitis media include, but are not limited to, visual inspection of the ear with an otoscope or pneumatic otoscope, tympanometry, or a hearing test. A family history of otitis media, or exposure to risk factors for otitis media (e.g. contact with cigarette smoke or having a recent cold) can also aid in determining if a subject is likely to have otitis media or in making a diagnosis of otitis media.

[00195] The compositions and methods described herein can be administered to a subject having or diagnosed as having a condition described herein. In some embodiments, the methods described herein comprise administering an effective amount of compositions described herein, e.g. a drug to a subject in order to alleviate a symptom of a disease or condition described herein. As used herein, "alleviating a symptom" is ameliorating any condition or symptom associated with the disease. As compared with an equivalent untreated control, such reduction is by at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, 99% or more as measured by any standard technique. A variety of means for administering the compositions described herein to subjects are known to those of skill in the art.

[00196] The term "effective amount" as used herein refers to the amount of a drug or composition needed to alleviate at least one or more symptom of the disease or disorder, and it relates to a sufficient amount of pharmacological composition to provide the desired effect. The term "therapeutically effective amount" therefore refers to an amount of a drug or composition that is sufficient to provide a particular effect when administered to a typical subject. An effective amount as used herein, in various contexts, would also include an amount sufficient to delay the development of a symptom of the disease, alter the course of a symptom disease (for example but not limited to, slowing the progression of a symptom of the disease), or reverse a symptom of the disease. Thus, it is not generally practicable to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" can be determined by one of ordinary skill in the art using only routine experimentation.

[00197] Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index

and can be expressed as the ratio LD50/ED50. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the active ingredient, which achieves a half-maximal inhibition of symptoms) as determined in cell culture, or in an appropriate animal model. Levels in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay, *e.g.*, assay for the level of an infectious organism, among others. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

[00198] Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the minimal effective dose and/or maximal tolerated dose. The dosage can vary depending upon the dosage form employed and the route of administration utilized. A therapeutically effective dose can be estimated initially from cell culture assays. Also, a dose can be formulated in animal models to achieve a dosage range between the minimal effective dose and the maximal tolerated dose. The effects of any particular dosage can be monitored by a suitable bioassay, *e.g.*, assay for tumor growth and/or size among others. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

[00199] In some embodiments, the technology described herein relates to a pharmaceutical composition comprising a composition or combination as described herein, and optionally a pharmaceutically acceptable carrier. In some embodiments, the active ingredients of the pharmaceutical composition comprise a hydrogel scaffold and at least one of a loading liquid(s), and/or drug(s) as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist essentially of a hydrogel scaffold and at least one of a loading liquid(s), and/or drug(s) as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist of a hydrogel scaffold and at least one of a loading liquid(s), and/or drug(s) as described herein. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. Some non-limiting examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG);

(12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C₂-C₁₂ alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein. In some embodiments, the carrier inhibits the degradation of the active agent as described herein.

[00200] In some embodiments of any of the aspects, the hydrogel scaffold and at least one of a loading liquid(s), and/or drug(s) described herein is administered as a monotherapy, e.g., another treatment for the disease or condition is not administered to the subject.

[00201] In some embodiments of any of the aspects, the methods described herein can further comprise administering a second agent and/or treatment to the subject, e.g. as part of a combinatorial therapy. For example, a patient can be further administered oral or systemic antibiotics, analgesics, or anti-nausea medications.

[00202] In certain embodiments, an effective dose of a composition as described herein can be administered to a patient once. In certain embodiments, an effective dose of a composition as described herein can be administered to a patient repeatedly. In some embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis.

[00203] Treatment according to the methods described herein can reduce levels of a marker or symptom of a condition, e.g. levels of an infectious organism, reduction in hearing ability, inflammation, or obstruction of the ear by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 % or at least 90% or more.

[00204] The dosage of a composition as described herein can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. With respect to duration and frequency of treatment, it is typical for skilled clinicians to monitor subjects in order to determine when the treatment is providing therapeutic benefit, and to determine whether to increase or decrease dosage, increase or decrease administration frequency, discontinue treatment, resume treatment, or make other alterations to the treatment regimen. The dosing schedule can vary from once a week to daily depending on a number of clinical factors, such as the subject's sensitivity to the drug(s). The desired dose or amount of activation can be administered at one time or divided into subdoses, e.g., 2-4 subdoses and administered over a period of time, e.g., at appropriate intervals through the day or other appropriate schedule. In some embodiments, administration can be chronic, e.g., one or more doses and/or treatments daily over a period of weeks or months. Examples of dosing and/or treatment

schedules are administration daily, weekly, monthly, or yearly over a period of 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, or more.

[00205] The dosage ranges for the administration of compositions, according to the methods described herein depend upon, for example, the form of the drug, its potency, and the extent to which symptoms, markers, or indicators of a condition described herein are desired to be reduced, for example the percentage reduction desired for inflammation or infection or the extent to which, for example, improvements in hearing are desired to be induced. Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication.

[00206] The efficacy of a composition or drug in, e.g. the treatment of a condition described herein, or to induce a response as described herein can be determined by the skilled clinician. However, a treatment is considered "effective treatment," as the term is used herein, if one or more of the signs or symptoms of a condition described herein are altered in a beneficial manner, other clinically accepted symptoms are improved, or even ameliorated, or a desired response is induced e.g., by at least 10% following treatment according to the methods described herein. Efficacy can be assessed, for example, by measuring a marker, indicator, symptom, and/or the incidence of a condition treated according to the methods described herein or any other measurable parameter appropriate, e.g. level of an infectious organism, degree or extent of inflammation, degree or extent of pain, and reduction or improvement in hearing ability. Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization, or need for medical interventions (i.e., progression of the disease is halted). Methods of measuring these indicators are known to those of skill in the art and/or are described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human or an animal) and includes: (1) inhibiting the disease, e.g., preventing a worsening of symptoms (e.g. pain or inflammation); or (2) relieving the severity of the disease, e.g., causing regression of symptoms. An effective amount for the treatment of a disease means that amount which, when administered to a subject in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators of a condition or desired response, (e.g. level of an infectious organism, degree or extent of inflammation, degree or extent of pain, and reduction or improvement in hearing ability). It is well within the ability of one skilled in the art to monitor efficacy of administration and/or treatment by measuring any one of such parameters, or any combination of parameters. Efficacy can be assessed in animal models of a condition described herein, for example treatment of otitis media. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant change in a marker is observed, e.g. fluid buildup, fluid discharge, inflammation, or infection levels.

[00207] A combination as described can comprise a hydrogel scaffold in combination with one or more of: at least one loading liquid (e.g., at least one ionic liquid), water, and at least one drug. As used herein, “in combination with” refers to two or more substances being present in the same formulation in any molecular or physical arrangement, e.g. in an admixture, in a solution, in a mixture, in a suspension, in a colloid, in an emulsion. The formulation can be a homogeneous or heterogenous mixture. In some embodiments of any of the aspects, the active compound(s) can be comprised by a superstructure, e.g., nanoparticles, liposomes, vectors, cells, scaffolds, or the like, said superstructure is which in solution, mixture, admixture, suspension, etc., with the IL.

[00208] In one respect, the present invention relates to the herein described compositions, methods, and respective component(s) thereof, as essential to the technology, yet open to the inclusion of unspecified elements, essential or not ("comprising). In some embodiments of any of the aspects, other elements to be included in the description of the composition, method or respective component thereof are limited to those that do not materially affect the basic and novel characteristic(s) of the technology (e.g., the composition, method, or respective component thereof “consists essentially of” the elements described herein). This applies equally to steps within a described method as well as compositions and components therein. In other embodiments of any of the aspects, the compositions, methods, and respective components thereof, described herein are intended to be exclusive of any element not deemed an essential element to the component, composition or method (e.g., the composition, method, or respective component thereof “consists of” the elements described herein). This applies equally to steps within a described method as well as compositions and components therein.

[00209] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. 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. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[00210] The terms “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease by a statistically significant amount. In some embodiments, “reduce,” “reduction” or “decrease” or “inhibit” typically means a decrease by at least 10% as compared to a reference level (e.g. the absence of a given treatment or agent) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least

about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% , or more. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as compared to a reference level. A decrease can be preferably down to a level accepted as within the range of normal for an individual without a given disorder.

[00211] The terms “increased”, “increase”, “enhance”, or “activate” are all used herein to mean an increase by a statically significant amount. In some embodiments, the terms “increased”, “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level. In the context of a marker or symptom, an “increase” is a statistically significant increase in such level.

[00212] As used herein, a "subject" means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, “individual,” “patient” and “subject” are used interchangeably herein.

[00213] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of a disease or condition. A subject can be male or female.

[00214] A “subject in need” of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at risk of developing that condition.

[00215] As used herein, the terms “protein” and “polypeptide” are used interchangeably herein to designate a series of amino acid residues, connected to each other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms "protein", and "polypeptide" refer to a polymer of amino acids, including modified amino acids (e.g., phosphorylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and “polypeptide” are often used in reference to relatively large polypeptides, whereas the term "peptide"

is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to a gene product and fragments thereof.

[00216] As used herein, the term "nucleic acid" or "nucleic acid sequence" refers to any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double-stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable DNA can include, e.g., genomic DNA or cDNA. Suitable RNA can include, e.g., mRNA.

[00217] In some of the aspects described herein, a nucleic acid sequence encoding a given polypeptide as described herein, or any module thereof, is operably linked to a vector. In some of the aspects described herein, a gene therapy comprises a vector. The term "vector", as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term "vector" encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

[00218] In some embodiments of any of the aspects, the vector is recombinant, e.g., it comprises sequences originating from at least two different sources. In some embodiments of any of the aspects, the vector comprises sequences originating from at least two different species. In some embodiments of any of the aspects, the vector comprises sequences originating from at least two different genes, e.g., it comprises a fusion protein or a nucleic acid encoding an expression product which is operably linked to at least one non-native (e.g., heterologous) genetic control element (e.g., a promoter, suppressor, activator, enhancer, response element, or the like).

[00219] In some embodiments of any of the aspects, the vector or nucleic acid described herein is codon-optimized, e.g., the native or wild-type sequence of the nucleic acid sequence has been altered or engineered to include alternative codons such that altered or engineered nucleic acid encodes the same polypeptide expression product as the native/wild-type sequence, but will be transcribed and/or translated at an improved efficiency in a desired expression system. In some embodiments of any of the aspects, the expression system is an organism other than the source of the native/wild-type sequence (or a cell obtained from such organism). In some embodiments of any of the aspects, the vector and/or nucleic acid sequence described herein is codon-optimized for expression in a mammal or mammalian cell, e.g., a mouse, a murine cell, or a human cell. In some embodiments of any of the aspects, the vector and/or nucleic acid sequence described herein is codon-optimized for expression in a human cell. In some embodiments of any of the aspects, the vector and/or nucleic acid sequence

described herein is codon-optimized for expression in a yeast or yeast cell. In some embodiments of any of the aspects, the vector and/or nucleic acid sequence described herein is codon-optimized for expression in a bacterial cell. In some embodiments of any of the aspects, the vector and/or nucleic acid sequence described herein is codon-optimized for expression in an *E. coli* cell.

[00220] As used herein, the term "expression vector" refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification.

[00221] As used herein, the term "viral vector" refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain the nucleic acid encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring any nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[00222] As used herein, the terms "treat," "treatment," "treating," or "amelioration" refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition associated with a disease or disorder. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder associated with a condition. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a disease is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term "treatment" of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment).

[00223] In some embodiments of any of the aspects, described herein is a prophylactic method of treatment. As used herein "prophylactic" refers to the timing and intent of a treatment relative to a disease or symptom, that is, the treatment is administered prior to clinical detection or diagnosis of that particular disease or symptom in order to protect the patient from the disease or symptom. Prophylactic treatment can encompass a reduction in the severity or speed of onset of the disease or symptom, or contribute to faster recovery from the disease or symptom. Accordingly, the methods

described herein can be prophylactic relative to permanent hearing loss, total hearing loss, hearing loss, or further hearing loss. In some embodiments of any of the aspects, prophylactic treatment is not prevention of all symptoms or signs of a disease.

[00224] As used herein, the term “pharmaceutical composition” refers to the active agent in combination with a pharmaceutically acceptable carrier e.g. a carrier commonly used in the pharmaceutical industry. The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a carrier other than water. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a cream, emulsion, gel, liposome, nanoparticle, and/or ointment. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be an artificial or engineered carrier, e.g., a carrier that the active ingredient would not be found to occur in nature.

[00225] As used herein, the term “nanoparticle” refers to particles that are on the order of about 1 to 1,000 nanometers in diameter or width. The term “nanoparticle” includes nanospheres; nanorods; nanoshells; and nanoprisms; these nanoparticles may be part of a nanonetwork. The term “nanoparticles” also encompasses liposomes and lipid particles having the size of a nanoparticle. Exemplary nanoparticles include lipid nanoparticles or ferritin nanoparticles. Lipid nanoparticles can comprise multiple components, including, e.g., ionizable lipids (such as MC3, DLin-MC3-DMA, ALC-0315, or SM-102), pegylated lipids (such as PEG2000-C-DMG, PEG2000-DMG, ALC-0159), phospholipids (such as DSPC), and cholesterol.

[00226] Exemplary liposomes can comprise, e.g., DSPC, DPPC, DSPG, Cholesterol, hydrogenated soy phosphatidylcholine, soy phosphatidyl choline, methoxypolyethylene glycol (mPEG-DSPE) phosphatidyl choline (PC), phosphatidyl glycerol (PG), distearoylphosphatidylcholine, and combinations thereof.

[00227] As used herein, the term “administering,” refers to the placement of a compound as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent at a desired site. Pharmaceutical compositions comprising the compounds disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject. In some embodiments, administration comprises physical human activity, e.g., an injection, act of ingestion, an act of application, and/or manipulation of a delivery device or machine. Such activity can be performed, e.g., by a medical professional and/or the subject being treated.

[00228] As used herein, “contacting” refers to any suitable means for delivering, or exposing, an agent to at least one cell. Exemplary delivery methods include, but are not limited to, direct delivery

to cell culture medium, perfusion, injection, or other delivery method well known to one skilled in the art. In some embodiments, contacting comprises physical human activity, e.g., an injection; an act of dispensing, mixing, and/or decanting; and/or manipulation of a delivery device or machine.

[00229] The term “statistically significant” or “significantly” refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[00230] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean $\pm 1\%$.

[00231] As used herein, the term “comprising” means that other elements can also be present in addition to the defined elements presented. The use of “comprising” indicates inclusion rather than limitation.

[00232] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[00233] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00234] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, "e.g." is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

[00235] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[00236] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and

is not intended to limit the scope of the present invention, which is defined solely by the claims. Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 20th Edition, published by Merck Sharp & Dohme Corp., 2018 (ISBN 0911910190, 978-0911910421); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), W. W. Norton & Company, 2016 (ISBN 0815345054, 978-0815345053); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties.

[00237] One of skill in the art can readily identify a chemotherapeutic agent of use (*e.g.* see Physicians' Cancer Chemotherapy Drug Manual 2014, Edward Chu, Vincent T. DeVita Jr., Jones & Bartlett Learning; Principles of Cancer Therapy, Chapter 85 in Harrison's Principles of Internal Medicine, 18th edition; Therapeutic Targeting of Cancer Cells: Era of Molecularly Targeted Agents and Cancer Pharmacology, Chs. 28-29 in Abeloff's Clinical Oncology, 2013 Elsevier; and Fischer D S (ed): The Cancer Chemotherapy Handbook, 4th ed. St. Louis, Mosby-Year Book, 2003).

[00238] In some embodiments of any of the aspects, the disclosure described herein does not concern a process for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

[00239] In all embodiments where a composition described herein is placed in the ear, the composition can be place, administered, or provided via minimally invasive methods and/or involves only a minor intervention.

[00240] Other terms are defined herein within the description of the various aspects of the invention.

[00241] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00242] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[00243] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[00244] In some embodiments, the present technology may be defined in any of the following numbered paragraphs:

1. A composition for transtympanic drug delivery, the composition comprising an ionic liquid and one or more drugs.
2. The composition of any of the preceding paragraphs, wherein the ionic liquid is choline and geranic acid (CAGE).

3. The composition of any of the preceding paragraphs, wherein the one or more drugs are selected from dexamethasone and ciproflaxin.
4. The composition of any of the preceding paragraphs, wherein the ionic liquid and one or more drugs are in a hydrogel scaffold.
5. The composition of any of the preceding paragraphs, wherein the hydrogel scaffold comprises, consists of, or consists essentially of one or more of: poly(2-hydroxyethyl methacrylate) (pHEMA); poly(acrylic acid) (PAA); and poly(methyl methacrylate) (PMMA).
6. The composition of any of the preceding paragraphs, wherein the hydrogel scaffold comprises, consists of, or consists essentially of poly(2-hydroxyethyl methacrylate) (pHEMA).
7. A method of treating an ear condition or disease, the method comprising administering to the outer ear of a patient the composition of any of the preceding paragraphs.
8. The method of any of the preceding paragraphs, wherein the ear condition or disease is otitis media.
9. The method of any of the preceding paragraphs, wherein the composition is administered by placing the composition in contact with the tympanic membrane.

[00245] In some embodiments, the present technology may be defined in any of the following numbered paragraphs:

1. A composition comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid.
2. The composition of paragraph 1, wherein the at least one loading liquid comprises at least one ionic liquid.
3. A kit or combination comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid.
4. The kit or combination of paragraph 3, wherein the at least one loading liquid comprises at least one ionic liquid.
5. The composition, kit, or combination of any one of the preceding paragraphs, wherein the at least one ionic liquid has a cationic component and an anionic component; and
 wherein the cationic component is selected from the group consisting of benzyl pyridinium, benzyl dimethyl dodecyl ammonium, a choline cation, phosphonium, tetraalkylphosphonium, and benzethonium; and
 wherein the anionic component is selected from the group consisting of bistriflimide, a geranate anion, oleate, 2-octenoic acid, hexanoate, dodecyldimethyl ammonia propane sulfonate, N-Lauryl sarconsinate, and geraniolate,
6. The composition, kit, or combination of any one of the preceding paragraphs, wherein the at least one ionic liquid comprises choline and geranic acid (CAGE), choline and hexenoic acid (CAHA), or choline and 2-octenoic acid.

7. The composition, kit, or combination of any one of the preceding paragraphs, wherein the at least one ionic liquid comprises choline and geranic acid (CAGE) or choline and hexenoic acid (CAHA).
8. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 30 μL of the at least one ionic liquid.
9. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 40 μL of ionic liquid.
10. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 200 μL of ionic liquid.
11. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 1000 μL of ionic liquid.
12. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs are selected from: antibiotics; steroids; growth factors; biologics; viral vectors; polypeptides; nucleic acid molecules; polysaccharides; anesthetics; and external, middle or inner ear disease therapeutics.
13. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs comprise at least one antibiotic.
14. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs comprise at least one steroid.
15. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs comprise at least one antibiotic and steroid.
16. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs are selected from dexamethasone and ciprofloxacin.
17. The composition, kit, or combination of any one of the preceding paragraphs, wherein the one or more drugs comprise dexamethasone and ciprofloxacin.
18. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 1 mg/mL of dexamethasone.
19. The composition, kit, or combination of any one of the preceding paragraphs, comprising 100-400 mg/mL of dexamethasone.
20. The composition, kit, or combination of any one of the preceding paragraphs, comprising 200 mg/mL of dexamethasone.
21. The composition, kit, or combination of any one of the preceding paragraphs, comprising at least 1 mg/mL of ciprofloxacin.
22. The composition, kit, or combination of any one of the preceding paragraphs, comprising 100-500 mg/mL of ciprofloxacin.
23. The composition, kit, or combination of any one of the preceding paragraphs, comprising 250 mg/mL of ciprofloxacin.

24. The composition, kit, or combination of any one of the preceding paragraphs, comprising 200 mg/mL of ciprofloxacin.
25. The composition, kit, or combination of any one of the preceding paragraphs, wherein the hydrogel scaffold comprises, consists of, or consists essentially of one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer.
26. The composition, kit, or combination of paragraph 25, wherein the at least one polyacrylate and/or at least one polymethacrylate comprises, consists of, or consists essentially of one or more of: poly(2-hydroxyethyl methacrylate) (pHEMA), poly(acrylic acid) (PAA), poly(methyl methacrylate) (PMMA), hydroxyethyl acrylate (HEA), poly(2-hydroxyethyl acrylate) (pHEA), polyacrylamide (PAM), poly(acrylonitrile-co-acrylic acid) (PANCAA), gelatin methacrylate (GelMA), hyaluronic acid methacrylate (MeHA); poly(epoxy methacrylate) (pEMA), poly(acryl amide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylamido glycolic acid), poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), poly(ethyl acrylate) (PEA), and poly(ethyl methacrylate) (PEMA).
27. The composition, kit, or combination of paragraph 25, wherein the at least one protein comprises, consists of, or consists essentially of one or more of: collagen, gelatin, and silk fibroin.
28. The composition, kit, or combination of paragraph 25, wherein the at least one polysaccharide comprises, consists of, or consists essentially of one or more of: hyaluronic acid, glycosaminoglycans, agarose, alginate, and chitosan.
29. The composition, kit, or combination of any one of the preceding paragraphs, wherein the hydrogel scaffold comprises, consists of, or consists essentially of poly(2-hydroxyethyl methacrylate) (pHEMA).
30. The composition, kit, or combination of any one of the preceding paragraphs, further comprising at least one hydrogel crosslinker.
31. The composition, kit, or combination of paragraph 31, wherein the at least one hydrogel crosslinker comprises at least one of: ethylene glycol dimethacrylate (EGDMA), N,N'-methylenebisacrylamide (MBAA-crosslinker), ammonium persulfate (APS-initiator), tetramethylethylenediamine (TEMEDA-catalyst), and Fe⁺³ (ionic cross linker).
32. The composition, kit, or combination of any one of the preceding paragraphs, wherein the hydrogel scaffold does not comprise a crosslinker.
33. The composition, kit, or combination of any one of the preceding paragraphs, further comprising a radical initiator or photoinitiator.
34. The composition, kit, or combination of paragraph 34, wherein the radical initiator or photoinitiator is azobisisobutyronitrile (AIBN).

35. The composition, kit, or combination of any one of the preceding paragraphs, wherein the hydrogel scaffold is not formulated with water.
36. The composition, kit, or combination of any one of the preceding paragraphs, wherein the composition has a size no greater than 12 mm by 12 mm by 12 mm.
37. The composition, kit, or combination of any one of the preceding paragraphs, wherein the composition has a size no greater than 8 mm by 8 mm by 8 mm.
38. The composition, kit, or combination of any one of the preceding paragraphs, wherein the composition has a size of at least 8 mm by 8 mm by 7.5 mm.
39. The composition, kit, or combination of any one of the preceding paragraphs, wherein the composition has a size of at least 5.4 mm by 5.4 mm by 2.7 mm.
40. A method of drug delivery, the method comprising administering, to a patient in need of a drug, the composition of any of the preceding paragraphs, wherein the composition comprises the drug.
41. The method of paragraph 41, wherein the composition is administered by placing the composition in contact with the ear, the outer ear, ear canal, tympanic membrane, oval window, round window membrane, eustachian tube, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.
42. A method of treating an ear condition or disease, the method comprising administering to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient the composition of any of the preceding paragraphs.
43. The method of any one of the preceding paragraphs, wherein the administration comprises circumferential application or contact with the skin of the ear canal.
44. The method of any one of the preceding paragraphs, wherein the administration comprises contact with the auricular concha and pinna.
45. The method of any one of the preceding paragraphs, wherein the ear condition or disease is selected from the group consisting of:
otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss.
46. The method of any one of the preceding paragraphs, wherein the ear condition or disease is otitis media.
47. The method of any one of the preceding paragraphs, wherein the composition is administered by placing the composition in contact with the tympanic membrane, oval window, round window membrane, eustachian tube or ear canal.

48. The method of any one of paragraphs 42-47, wherein the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, oval window or the round window membrane.
49. The method of any one of paragraphs 42-47, wherein the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the round window, and/or the oval window.
50. The method of any one of the preceding paragraphs, wherein the ear condition or disease is otitis externa.
51. The method of any one of paragraphs 42-47, wherein the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.
52. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for at least 2 days.
53. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for at least 3 days.
54. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for at least 5 days.
55. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for at least 7 days.
56. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for no more than 10 days.
57. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for no more than 28 days.
58. The method of any one of the preceding paragraphs, wherein the composition is left in the ear for no more than 12 months.
59. The method of any one of the preceding paragraphs, wherein the composition is physically removed from the ear after at least 7 days.
60. The method of any one of the preceding paragraphs, wherein the composition is physically removed from the ear after at least 10 days.
61. The method of any one of the preceding paragraphs, wherein the composition is physically removed from the ear within 10 days.
62. The method of any one of the preceding paragraphs, wherein the composition is physically removed from the ear within 28 days.
63. The method of any one of the preceding paragraphs, wherein the composition is physically removed from the ear within 12 months.

64. The composition of any one of paragraphs 1-40, for use in a method of drug delivery.
65. The composition of paragraph 64, wherein the method comprises administering the by placing the composition in contact with the ear, the outer ear, ear canal, tympanic membrane, oval window, round window membrane, eustachian tube, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.
66. The composition of any one of paragraphs 1-40, for use in a method of treating an ear condition or disease, the method comprising administering the composition to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient.
67. The composition of any one of paragraphs 64-66, wherein the administration comprises circumferential application or contact with the skin of the ear canal.
68. The composition of any one of paragraphs 64-67, wherein the administration comprises contact with the auricular concha and pinna.
69. The composition of any one of paragraphs 64-68, wherein the ear condition or disease is selected from the group consisting of:
otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss.
70. The composition of any one of paragraphs 64-69, wherein the ear condition or disease is otitis media.
71. The composition of any one of paragraphs 64-70, wherein the composition is administered by placing the composition in contact with the tympanic membrane, oval window, round window membrane, eustachian tube or ear canal.
72. The composition of any one of paragraphs 69-71, wherein the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, oval window or the round window membrane.
73. The composition of any one of paragraphs 69-71, wherein the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the round window, and/or the oval window.
74. The composition of any one of paragraphs 69-71, wherein the ear condition or disease is otitis externa.
75. The composition of any one of paragraphs 69-71, wherein the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.

76. The composition of any one of paragraphs 64-75, wherein the composition is left in the ear for at least 2 days.
77. The composition of any one of paragraphs 64-76, wherein the composition is left in the ear for at least 3 days.
78. The composition of any one of paragraphs 64-77, wherein the composition is left in the ear for at least 5 days.
79. The composition of any one of paragraphs 64-78, wherein the composition is left in the ear for at least 7 days.
80. The composition of any one of paragraphs 64-79, wherein the composition is left in the ear for no more than 10 days.
81. The composition of any one of paragraphs 64-80, wherein the composition is left in the ear for no more than 28 days.
82. The composition of any one of paragraphs 64-81, wherein the composition is left in the ear for no more than 12 months.
83. The composition of any one of paragraphs 64-82, wherein the composition is physically removed from the ear after at least 7 days.
84. The composition of any one of paragraphs 64-83, wherein the composition is physically removed from the ear after at least 10 days.
85. The composition of any one of paragraphs 64-84, wherein the composition is physically removed from the ear within 10 days.
86. The composition of any one of paragraphs 64-85, wherein the composition is physically removed from the ear within 28 days.
87. The composition of any one of paragraphs 64-86, wherein the composition is physically removed from the ear within 12 months.

[00246] The technology described herein is further illustrated by the included examples which in no way should be construed as being further limiting.

EXAMPLES

[00247] *Example 1: EarFlow : a Transtympanic Drug Delivery Device Towards Treating Otitis Media*

[00248] Otitis media (OM) is a group of afflictions that affects the middle ear, also known as middle ear infections. Around 80% of children will have at least one episode of acute OM, and 80-90% of children will have at least one episode of OM with effusion before school age. Treating OM requires the delivery of antibiotics to the middle ear space. However, despite the prevalence of OM, existing solutions are not ideal. Oral antibiotics are often multi-day, multi-dose regimes with systemic

side effects, making patient compliance challenging and increasing the prevalence of antibiotic resistance. Antibiotic eardrops often have low efficacy due to the barrier presented by the tympanic membrane (TM), commonly known as the eardrum, which separates the middle ear space from the environment.

[00249] This report presents EarFlow, a novel, non-invasive, sustained drug delivery device to be placed on the TM to elute antibiotics and steroids into the middle ear space. EarFlow is a hydrogel device loaded with ionic liquids containing ciprofloxacin and dexamethasone, which will be co-delivered to treat OM. EarFlow can be successfully manufactured from hydrogels and hold a large volume of antibiotic- and steroid-loaded ionic liquids. *Ex vivo* diffusion studies demonstrated that EarFlow is able to effectively deliver dexamethasone across intact membranes and into the middle ear space throughout 7 days, indicating EarFlow's potential in clearing OM. In addition, EarFlow showed low cytotoxicity and cell adhesion, allowing for healing of the TM. Finally, EarFlow can be placed on the TM through the human ear canal in less than 10 minutes, indicating that it can be placed in an office setting, eliminating the need for lengthy, invasive procedures.

[00250] EarFlow represents a novel approach to treating OM, reducing the side effects associated with systemic antibiotic administration, the ineffectiveness of existing localized solutions, and the invasiveness of tympanostomy tube placement. Importantly, EarFlow is a localized delivery platform for loading other therapeutic molecules applicable to treating other hearing afflictions and beyond.

[00251] Background

[00252] Otitis media (OM) is a group of afflictions affecting the middle ear, including acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM). OM is characterized by excess fluid in the middle ear space caused by infectious pathogens, impairing tympanic membrane (TM) vibration and therefore hearing levels (Fig. 1). Symptoms include a painful, infected ear, often with hearing loss, fever, and vomiting [5]. AOM is the most common indication for antibiotic prescriptions to children, with direct and indirect costs totaling near \$3 billion in 1995 in the US [6]. Around 80% of children will have at least one episode of AOM, and 80-90% of children will have at least one episode of OME before school age [5]. As OM commonly occurs in young children, without treatment, OM can lead to hearing loss, which can lead to further challenges in learning and language development. In terms of management, the CDC reports that around 66% of people aged 0-19 years will receive recommended first-line antibiotics for AOM [7]. Worryingly, researchers report that 94% of prescriptions for OM exceed the recommended five day treatment duration [8], which raises serious concerns about the possibility of the contributions of OM antibiotic prescription to the antibiotic resistance challenge.

[00253] Treatment of OM focuses on two goals: 1) reduction of symptoms and 2) minimization of recurrence. Existing treatments include 1) oral administration of antibiotics, 2) antibiotic ear drops (otic drops), 3) tympanostomy tube insertion, and 4) intratympanic injections [9]. Despite the

pervasiveness of OM, none of the existing solutions are ideal. Systemic delivery of antibiotics increases the incidence of antibiotic resistance and causes severe systemic side effects such as nausea, vomiting, and medication intolerance [9, 10]. Antibiotic ear drops have low efficacy due to their inability to reach the middle ear space in the presence of an intact TM [10]. Additionally, the placement of tympanostomy tubes and intratympanic injections are invasive procedures that may lead to post-surgical complications [11, 12] or create perforations in the TM; maintaining an intact TM is important for retaining normal hearing.

A localized and efficacious drug delivery method is necessary to avoid these complications. The TM is a trilaminar structure that separates the outer ear and the middle ear and serves as a protective barrier for pathogens (Fig. 1). Importantly, the TM is responsible for sound conduction to the ossicular chain. Ideally, one could directly apply antibiotics in a non-invasive fashion through the external auditory canal (EAC) such that the solution diffuses across the TM to the middle ear space. However, the TM is a near-impermeable membrane to most drugs. This is largely due to its lateral-facing stratum corneum (SC) in the epidermal epithelium layer, which is composed of densely packed corneocytes [13], a type of keratinocyte, surrounded by a lipid bilayer, often described as a brick-and-mortar-like structure (Fig. 2). In addition, the lamina propria layer contains fibroblasts and collagen, creating the fiber structure [13].

Thus, transtympanic drug delivery will require the development of a novel, non-invasive, and efficacious mechanism to permeate the TM. Such an approach should sustain drug delivery without resorting to invasive measures to perforate the TM or risking the health of young patients with systemic antibiotics. This could not only reduce healthcare expenses caused by in-clinic procedures and the systemic costs of furthering antibiotic resistance, but also minimize the risk of antibiotic resistance and recurrent infections. Described herein is a novel drug delivery device, EarFlow, to be placed on the TM, which utilizes drug-loaded ionic liquids (ILs) to non-invasively deliver relevant antibiotics to treat OM (Fig. 3). ILs are a class of compounds composed of bulky cations and anions that are liquid at room temperature, and they have been shown to enhance transdermal drug delivery for a variety of therapeutic molecules [14].

[00254] EarFlow represents the first-of-its-kind device in potentially achieving non-invasive and sustained drug delivery into the middle ear. While the device outlined below focuses on delivering ciprofloxacin and dexamethasone, the platform can be easily tuned to deliver other therapeutic molecules, such as other antibiotics or steroids, growth factors, or therapies for inner ear diseases.

[00255] Users and Impact

[00256] There are three primary aims of the project described herein: 1) modelling EarFlow on the TM in silico, 2) modelling drug diffusion across the TM in silico, and 3) fabricate the EarFlow device and evaluate its drug eluting abilities.

[00257] First, in modelling EarFlow on the TM in silico, it was aimed to create a finite element (FE) model platform that is useful for researchers and ENT surgeons to predict the potential impact of devices on the TM on sound conduction. Currently, hearing research is heavily reliant on animal models, like chinchillas, despite the fact that these animal models often have different TM properties compared to humans. For example, chinchilla TMs are usually 10 times thinner than human TMs [15], which makes the translation of drug delivery results in animal models to humans difficult. By modeling the impact of a device on a physiologically accurate model of the human TM, hearing responses in humans can be predicted more efficiently and cost-effectively. Given that there is currently no drug delivery device in development for placement on the TM, this aim will help elucidate the effects of mass and TM placement location of the device on conductive hearing loss, which could be a useful tool for researchers studying hearing diseases and the impact of other TM devices on sound conduction.

[00258] The second aim of this project focuses on modelling diffusion across the TM using FE modelling. Despite the benefits of localized drug delivery across the TM, there is little research on computational models of transport and diffusion across the TM. In addition, intact TMs are difficult to obtain and store, often degrading within 48 hours at room temperature [15]. Thus, successfully modelling transport phenomena across the stratum corneum in the TM permits hearing researchers and drug delivery researchers to better evaluate the potential of drug delivery across the TM in silico prior to in vitro or

in vivo validation. Furthermore, the SC is present elsewhere in the body—notably, as the top layer of the human epidermis. Thus, creating a generalized diffusion model across the SC may be applicable for other drug delivery applications that benefit researchers, such as transdermal drug delivery.

[00259] Finally, creating EarFlow represents a novel method of drug delivery for OM. In terms of the broader impact of a drug delivery device placed on the TM, OM primarily affects children and is one of the most common afflictions that physicians treat in young patients. Given this unique user group, special considerations must be taken to ensure patient adherence and to prevent long-term side effects. As such, the proposed non-invasive drug delivery device, EarFlow, would improve upon existing solutions in that it minimizes patient adherence issues (compared to oral antibiotics courses administered daily for at least a week and other invasive procedures) and would prevent systemic exposure to antibiotics, which in turn decreases the incidence of antibiotic resistance. The EarFlow platform can also be easily tuned to load other therapeutics to treat other hearing afflictions. Thus, EarFlow could have profound global health implications.

[00260] Background

[00261] Treating Otitis Media

[00262] Oral Administration of Antibiotics. Oral administration of antibiotics is one of the most common routes of treatment of OM. The World Health Organization defines antibiotics as medicines

used to treat or prevent bacterial infections [16]. Depending on patient symptoms, age, and other underlying conditions (e.g. known allergies and previous infections), different antibiotics may be prescribed. Antibiotics are often given proportional to body weight, and most treatments courses are multiday (7

to 10 days long) and multidose [6], making patient compliance particularly challenging for young children. While non-invasive, oral administration requires a higher dosage than localized delivery to counteract the reduced bioavailability of drugs due to clearance of the drug after systemic drug administration. One study showed that following oral administration of amoxicillin, a first line antibiotic for OM, 15-35% of children have no detectable drug in their middle ear fluid, indicating ineffective delivery [9]. In addition, prescribed dosages of antibiotics vary widely depending on weight, and higher dosages of antibiotics are related to higher incidence and severity of side effects [10]. Oral administration may increase the incidence of multi-drug resistance and may decrease the effectiveness of later treatments

with antibiotics [9]. Particularly, for OME, which is characterized by biofilm presence, oral administration is not nearly as effective as localized administration, given that eliminating biofilms requires a concentration of antibiotics magnitudes higher than what is required to eliminate planktonic bacteria [9].

[00263] Antibiotic Otic Drops (Ear Drops). Because the barrier posed by the intact TM impairs the ability of small molecule drugs to diffuse across the TM [15], topical antibiotic drops containing ciprofloxacin are ineffective for OM (without the presence of a tympanostomy tube) and are not usually indicated [10]. These antibiotic drops are generally used for otitis externa, otherwise known as ear canal infections [17].

[00264] Tympanostomy Tubes. Tympanostomy tubes, also known as ventilation tubes, may be implanted in the TM to equalize pressure between the external environment and the middle ear space. Additionally, they can allow the drainage of infected fluids and provide a route for antibiotic delivery. The insertion of tympanostomy tubes is an invasive procedure frequently conducted under general anesthesia. Furthermore, many tympanostomy tubes fail, either becoming obstructed [11], falling out prematurely, causing additional scar formation, or otorrhea and biofilm formation [12]. In addition, the removal of tympanostomy tubes can cause a permanent perforation in the TM, which results in conductive hearing loss and recurrent infections.

[00265] Iontophoresis Coupled with Tympanostomy Tubes. Iontophoresis is the process of applying a small electrical current to aid the movement of ions across a membrane [18]. Very recently, iontophoresis has been used as a novel technology for drug delivery across the TM in the Tula tympanostomy tube system [19]. Iontophoresis is used to deliver local anesthesia to numb the TM, which then allows tympanostomy tube placement in children in an office setting. This is in

contrast to general anesthesia (the standard procedure for tympanostomy tube placement), which must be conducted in the operating room. Iontophoresis is advantageous compared to conventional tympanostomy tube placement due to its non-invasiveness. However, it is unclear the extent to which the drug traverses across the TM. In addition, this approach has only been utilized to deliver local anesthesia for tympanostomy tube placement but has not been validated for the delivery of antibiotics and steroids. Given that this approach requires an external device continuously applying electric current to allow membrane permeation, iontophoresis is likely not suitable for the purpose of sustaining drug delivery over 7 days to treat OM, as the usage of an external device will be bulky and inconvenient for the patient's daily activities. Finally, this technology still utilizes tympanostomy tubes as the primary treatment method, which, as discussed previously, has surgical complications—including scar tissue formation, perforation of the TM, and biofilm formation.

[00266] **Transtympanic Injections.** Transtympanic injections are narrow gauge needle injections performed through the TM to directly deliver drugs to the middle ear space. While this delivers a localized, high concentration of drug to the middle ear space, injections are invasive and must be done under local anesthesia. Surgical complications include pain, vertigo, tinnitus, and chronic TM perforations [20, 9]. The perforation of the TM is undesirable, as an intact TM is required to conduct sound successfully and to serve as a barrier to external pathogens.

[00267] **Hydrogel Drug Delivery.** As one of the technologies to treat OM, Yang et al. developed a hydrogel formulation that crosslinks in vivo, with chemical permeation enhancers placed on the TM to deliver ciprofloxacin to the middle ear. This single-dose, non-invasive approach allowed sustained drug delivery over 7 days and had been shown to effectively clear OM with no recurrence in chinchilla disease models [15]. However, the usage of chemical permeation enhancers disrupted the SC layer of the TM and was shown to be cytotoxic to in vitro cell cultures. The application of the hydrogel on TM also decreased hearing by up to 15 dB, likely due to the mass of the hydrogel impairing TM motion, though hearing is expected to return to normal once the hydrogel fully degrades. There have been no studies conducted on these devices regarding the long-term effects of a damaged SC layer.

[00268] A summary of these approaches can be found in Fig. 4. These approaches all lack one or two of the critical parameters required for the ideal treatment of OM. EarFlow, the proposed drug delivery device, will aim to address the existing shortcomings in all six parameters. All parameters are treated as necessary to address in the solution.

[00269] **Literature on Middle Ear Models.** Computational models of the acousto-mechanical behavior of the middle ear include both mathematical models and FE models. A mathematical model by Fay et al. [21] captured both experimentally measured geometries of the TM and estimations of the TM's material properties. The model was shown to be acoustically accurate over the entire human

hearing frequency range (20 Hz–20 kHz) and coupled the displacement of the TM to the acoustics of the ear canal.

Compared to purely mathematical models, FE models may confer improved complexity of the TM and achieve further multiphysics coupling. Gan et al. [22] created a 3D FE model of the TM that was acoustically, structurally, and fluid coupled, representing the standards of a TM model. Further work by O'Connor et al. improved the anatomical accuracy of FE models by using a CT scan of the human TM, making it a suitable model for the purposes of this project.

[00270] In addition to mathematical and FE models, in vitro measurements of the TM have complemented FE models by providing key information on where to place EarFlow. Cheng et al.'s model [23] revealed that the anterior-superior quadrant of the TM had the fewest motions relative to other regions during sound conduction, which is helpful in informing an ideal device location. This literature review compared the strengths and limitations of several existing mathematical and FE models. Key findings include that 1) the degree of TM motion varies depending on the region of TM analyzed, and 2) increased TM perforation size increases hearing loss. It was determined that a suitable FE model for this project's purpose should be acoustically and structurally coupled, while also maintain anatomical accuracy. Thus, the Puria group's FE model of the TM and middle ear [1] (Fig. 5) will provide the

optimal baseline model to build upon for this project, from which we can obtain relevant data, such as middle ear gain and TM motion. The Puria group, based at MEE hospital, has provided this COMSOL model file to our group for modelling experiments on the impact of device placement and TM modification on sound conduction.

[00271] Diffusion Across the Tympanic Membrane. Diffusion is a well-explored topic both mathematically and computationally. However, there is no FE model focused on diffusion across the complete TM. That said, there are a number of research studies on diffusion across the stratum corneum of other tissues, which is thought to be the primary barrier for diffusion across the TM due to its brick-and-mortar structure. Existing models of diffusion across the TM include mathematical models [24], geometric models [25], and FE models [26, 27]. Bhatt et al. created and published results on a mathematical SC diffusion model, which utilized the first principles of diffusion—Fick's first and second law. As one of the earlier SC diffusion models, the model demonstrated strengths in comparing in silico results to in vitro results, although it utilized several unrealistic assumptions, including homogenous TM material properties. In contrast to the mathematical models, Wang et al. [25] built a geometric model

(greater in complexity) and included several additional parameters for the chemistry of the transported species, including four representative permeants and their associated mass transfer coefficients, lipid and corneocyte diffusivities, and partition coefficients. The paper also distinguished between the intercellular and intracellular pathway, and its findings revealed that contrary to prior knowledge and

assumptions in the field, permeant diffusion did not necessarily always involve intracellular pathways. Therefore, investigating diffusion through intercellular pathways can be relevant to certain material and therapeutic systems for drug delivery.

[00272] Compared to the earlier mathematical and geometric models, FE models, including work conducted by Barbero and Frasc, have increased complexity and eliminate some assumptions made in prior models [26]. In this work, the researchers created a heat flow model in ANSYS to model diffusion, and importantly, recapitulated the geometric features of SC by generating the FE mesh based on the mouse SC [26]. The authors built upon this work in their paper in 2006 by varying the diffusion coefficient in the intracellular and transcellular pathways [27]. As in Wang et al., this SC diffusion model also supported the existence and utility of a transcellular diffusion pathway. This literature review helped identify several key parameters necessary in this project's FE diffusion model. Such key parameters can be broadly divided into TM structural parameters (e.g. lipid size and thickness, corneocyte size and thickness, SC thickness) and the parameters of the transported molecules (e.g. diffusivity, permeability, partition coefficient). Importantly, this literature review also helped inform the design decision of using an FE model due to its amenability to rapid prototyping of varying parameters and accurate capturing of geometric parameters. The literature review additionally informed the decision of using a 2D representation of the TM instead of a 3D representation, as a 2D representation was shown to be adequately comparable to a 3D representation for a tissue with the same trilaminar structure throughout its depth dimension [26]. Finally, the literature review revealed tradeoffs and limitations of the models and demonstrated that it is ideal to compare the model outputs to in vitro or in vivo data.

[00273] Tympanic Membrane Physiology. During OM, the TM experiences several changes, becoming thicker, inflamed, and opaque [28]. In addition, the middle ear space can also become filled with fluid, unable to be drained out by the Eustachian tube due to inflammation and dysfunction [5]. These changes are important to note for developing an accurate diffusion model that illustrates the state of the middle ear space during OM.

[00274] EarFlow : Design and Development

[00275] Design and Fabrication of EarFlow. Based on the literature review, it was determined that an ideal drug delivery mechanism for treating OM must be non-invasive, localized, and efficacious, while sustaining therapeutic drug delivery for at least 7 days. The proposed device utilized a combination of a hydrogel and ionic liquid solutions (ILs) in order to achieve sustained and non-invasive drug delivery to treat OM.

[00276] To fabricate the device, a hydrogel scaffold was synthesized from biocompatible precursor monomers. In the selection of hydrogel scaffold material, it was critical to consider several properties of the resultant hydrogel, including biocompatibility, degradation rate, and cell adhesion.

Primarily, the major design criteria in choosing this hydrogel was low propensity for cell adhesion, as cell adhesion to the device may prevent keratinocyte migration during TM healing and cause changes in the device properties. In addition, the hydrogel material should be selected to prevent tissue or scar tissue formation around the device. The search of a hydrogel material was narrowed down to the polyacrylate family,

which has been commonly used in the context of biological implants [29]. Several polyacrylate hydrogel materials were considered in this process, including poly(acrylic acid), poly(methyl methacrylate), and poly(2-hydroxyethyl methacrylate).

[00277] Ultimately, poly(2-hydroxyethyl methacrylate) (pHEMA) was selected for EarFlow prototype devices due to its biocompatibility, lack of degradation, and bioinertness, as demonstrated in previous research [30, 31]. pHEMA is distinguished by its lack of hydrolyzable and enzymatically degradable bonds, making it suitable for ensuring stability by lack of degradation over the treatment period. Importantly, pHEMA has been shown to be biocompatible in several in vivo applications requiring bioinertness, including drug-eluting contact lenses [30, 31] and other drug delivery mechanisms to the eye [32].

[00278] A lyophilization approach was used to introduce pores to the scaffold. Following the synthesis of the hydrogel to create an interconnected mesh network between the monomers, the hydrogel was swelled in deionized water. The scaffold was then lyophilized, which sublimated ice crystals out of the scaffold, resulting in pores in the scaffold. The lyophilized scaffold was immersed in ILs to uptake the IL solutions and create the final device, containing ciprofloxacin and dexamethasone, each at a concentration of 200 mg/mL. This approach is summarized in Fig. 6. The IL and drug-containing device can then be placed on the TM, through which ILs can be readily eluted across the TM and deliver ciprofloxacin and dexamethasone across the TM (Fig. 7).

[00279] In considering the global, cultural, social, and economic factors around OM, it was noted that OM is a globally prevalent issue. Thus, the design of a non-invasive drug delivery device that can be placed on the TM without surgical procedures will both improve the accessibility of OM treatment and decrease the cost of treatment. With respect to public health and safety, the creation of a localized, non-invasive drug delivery device (as opposed to existing systemic oral antibiotic delivery) will likely reduce the incidence of antibiotic resistance. Compared to invasive treatments, such as tympanostomy tube insertions, EarFlow represents a safer approach, reducing the possibility of surgical complications.

[00280] Design of COMSOL Models for Assessing Sound Conduction and Diffusion. There was interest in optimizing EarFlow through two primary objectives: 1) building upon an existing FE model to include new parameters and assess how varying the parameters of the device (e.g. geometry, size, weight, placement location) may impact hearing, and 2) designing a basic FE model for drug diffusion through the TM into the middle ear space. Towards the design process, the specifications

and metrics of success for these objectives are as follows:

1. Design simple drug delivery device models of EarFlow using computer-aided design (CAD) software with varying sizes, geometries, and weights, to be placed on a geometrically, mechanically, and acoustically accurate TM and middle ear model for sound transmission.
2. Analyze middle ear gain (MEG) between sound pressure in the environment and pressure in the vestibule of the cochlea as a proxy for hearing outcomes with varying device properties as compared to normal, unimpaired MEG.
3. Create a 2D or 3D FE model of drug diffusion across the TM, with particular accuracy focused on modelling the three layers of the TM.

[00281] Modelling the Impact of EarFlow Placement on the TM to Sound Conduction. The project was aimed to elucidate the impact of drug delivery devices on conductive hearing by augmenting existing FE models of the TM. Building upon the Puria group's FE model [1], the inventors designed and built setups in COMSOL to model the impact of parameters, including 1) device geometry, 2) device location, 3) device size, and 4) device mass on hearing. This aim required building simple CAD models of drug delivery devices in Autodesk Fusion with varying geometries, such as cylinders and hemispheres (Fig. 8A). The effects on hearing were computed using the acoustics module available in COMSOL. Specifically, the output data of middle ear gain (MEG) (Fig. 8B) was analyzed. MEG is a suitable proxy measure for the ear's hearing transfer function; the more the MEG decreases from the baseline, the worse sound conduction and therefore hearing will likely be for the patient.

[00282] The minimal inhibitory content (MIC) of ciprofloxacin in the middle ear to clear otitis media is 0.1–0.5 $\mu\text{g/mL}$ for *Haemophilus influenzae* [15] and 4 $\mu\text{g/mL}$ for *Streptococcus pneumoniae* [33]. To clear OM, the middle ear concentration of ciprofloxacin must be maintained above the higher MIC of the two pathogens; thus, the composition must maintain a minimum MIC of at least 4 $\mu\text{g/mL}$. Our calculations show that this delivery rate is possible with 40 μL of IL loaded at 250 mg/mL and placed on the TM. Geometrically, this volume can be contained in a cube with 3.4 mm side length, a cylinder with 2.5 mm radius and 2 mm height, or a hemisphere with 2.7 mm radius. Given that the cross-sectional areas of these geometries are no larger than 50% of the TM surface area (which can be approximated as a circle of around 10 mm radius), these would be feasible devices to model on the TM. Upon this, several additional device sizes were tested in COMSOL to analyze the tradeoffs between different geometries. While there exist FE models of the TM and middle ear, the current lack of drug delivery devices placed on the TM means that the effect of device mass on the TM to sound conduction is not extensively researched. Given that drug delivery devices on the TM may significantly improve the existing landscape of drug delivery for various hearing disorders, this computational model will help elucidate the design considerations of drug delivery devices for

placement on a delicate tissue like the TM, making sustained, effective, and non-invasive drug delivery a reality.

[00283] Modelling Diffusion Across the Tympanic Membrane. Using COMSOL, a 2D model of diffusion across the TM was built. Histologically processed and stained TM images were used as a reference for creating a physiologically accurate model, including relevant corneocyte sizes and thicknesses of the three layers (epithelium, lamina propria, mucosal epithelium). Importantly, this model recapitulated these three layers of the TM and thus may be more accurate in describing transport phenomena across the TM compared to existing models, which only focused on the SC.

[00284] The model was computed in COMSOL to allow for analysis of how Earflow 's properties may affect diffusion in the TM. Based on existing literature, it was determined that an ideal diffusion model would include the following diffusion- and transport-related parameters: diffusion coefficient, partition coefficient between lipid and corneocyte phase, hydrophilicity and lipophilicity of drug compound (solute), and size of drug compounds. While not all of these parameters were available in the COMSOL module used, those available were incorporated in the model. The geometric parameters that needed to be defined include the following: thickness of corneocytes, cross-sectional length of corneocytes, and thickness of the lipid phase. Transport across the TM is not extensively researched, despite the clear potential of non-invasive transtympanic drug delivery. As such, this model will help elucidate factors relevant to transport across the TM and guide design principles in drug diffusion across the TM.

[00285] Design Specifications

[00286] Design Specifications for EarFlow. Below are the quantitative and qualitative specifications for the physical device, EarFlow. These specifications are summarized in Figs. 9A-9C.

[00287] Device Manufacturing

- Device size. The human TM has a diameter of approximately 8-10 mm. Based on discussions with ear, nose, and throat (ENT) surgeons at Mass Eye and Ear hospital, to minimize the effect of the device on hearing, the device should ideally occupy no more than 70% of the surface area of the TM. EarFlow should ideally thus have a radius of less than 4 mm and a diameter of less than 8 mm. The size of the device was controlled by using 4-8 mm diameter biopsy punches to punch out hydrogel disks.
- Porosity of the scaffold. To ensure that the scaffold would be able to hold the necessary volume of IL, the hydrogel scaffold must be porous, and ideally at least 80% porous. This porosity was achieved via lyophilization of the scaffold and was measured using either the swelling ratio of the hydrogel or SEM images of the lyophilized scaffold, specifically measuring the pore size and pore distribution in the scaffold. If this porosity cannot be achieved, a PHEMA monomer with a lower molecular weight would be selected to decrease the chain length in the crosslinked hydrogel, and the hydrogel would be resynthesized with the new monomer.

[00288] Drug Delivery and Release

- Loading concentrations of drugs in the IL. Based on a literature review of MIC of pathogens in the middle ear as well as calculations considering the optimal device volume to minimize impact on hearing, it was determined that the IL should be loaded with 250 mg/mL of ciprofloxacin. This amount was carefully measured using an electronic balance and pipettes.
- Total volume of IL held in device. Based on a literature review of the MIC of pathogens in OM and in consideration of the maximum loading concentration of antibiotics in the IL used, it was calculated that a minimum of 40 μ L of IL should be loaded in the drug delivery device to sustain one course of antibiotic delivery. This volume was assessed in hydrogel-IL uptake studies evaluating the percent swelling of hydrogels that are placed in IL solutions.
- Sustained delivery timeframe. The device should sustain drug delivery for 7 days to clear OM, as existing oral antibiotic courses are usually given in 7-day doses to clear OM entirely and prevent recurrent infections. This specification was measured in vitro using a Franz diffusion chamber system to ensure that the device is continuously eluting drugs over 7 days. Specifically, the concentration of ciprofloxacin in the acceptor chamber each day was quantified using UV spectrophotometry. If drug delivery cannot be sustained over 7 days, a higher loading concentration of ILs may be used.
- Device degradation rate. Given that the degradation of hydrogel scaffolds may significantly affect their physical properties (including the drug release profile), the device should not show any sign of degradation within 7 days (the length of which the device is placed on the TM). Minimizing degradation was achieved by fabricating the scaffold with pHEMA, a bioinert polymer that is resistant to hydrolyzation or enzymatic degradation due to its carbon-carbon backbone.

[00289] Cytotoxicity and Cell Adhesion Considerations

- Cytotoxicity of ILs. To ensure the biocompatibility of the device, the carrier solution (IL) should not be toxic to cells on the TM, which are primarily fibroblasts and keratinocytes. To minimize cytotoxicity, ILs were made from biocompatible starting materials. The cytotoxicity of ILs was measured by an in vitro cell viability analysis of both fibroblasts and keratinocytes cultured with the IL. Specifically, if the cell viability was maintained above 90%, the IL can be considered to be of low cytotoxicity. If the cell viability was below this threshold, the IL starting materials would be reassessed and reselected to synthesize a new IL. The new IL would then undergo cytotoxicity testing.
- Cytotoxicity of the hydrogel device. Again, to ensure the biocompatibility of the device, the EarFlow hydrogel scaffold should not be toxic to cells on the TM. The hydrogel material, pHEMA, was carefully selected based on previous literature review confirming the biocompatibility of pHEMA in vivo. The cytotoxicity of the device was measured by an in vitro cell viability analysis of both fibroblasts and keratinocytes cultured with the device. Specifically, if the cell viability can be maintained above 90%, the device can be considered to be of low cytotoxicity. If the cell viability was

below this threshold, the hydrogel material would be reassessed, and a new scaffold material would be selected.

[00290] Clinical Considerations

- **Hearing threshold changes.** Based on literature review and consultation with ENT surgeons, a significant change in hearing was defined as a loss of hearing of more than 15 dB; this is also the value at which existing solutions, such as hydrogels, had been shown to affect hearing [15]. Given that the goal of EarFlow is to improve upon current state of the art, EarFlow aimed to minimally affect hearing, specifically by no more than 15 dB. This change in hearing was measured by modelling the device on an acoustically and mechanically-coupled COMSOL model, which output the middle ear gain (MEG) as a proxy for hearing changes. Based on literature review, it was determined that an output MEG that was below 90% of the baseline MEG should be considered a significant change in hearing. If EarFlow placement caused a decrease below 90% of the baseline MEG, the device size would be accordingly decreased to decrease the load on the TM. Based on literature review and COMSOL simulations, it was also determined that the anterior-superior quadrant of the TM was an ideal location to place the device, as this was the location in which there was the lowest sound-induced motion when the TM was subjected to sound pressure waves.
- **Easy to place into and remove from the EAC.** Ease of device placement could allow ENT surgeons to place the device into the EAC in an office setting and eliminate the need of anaesthetics for young patients, which will improve patient experience and compliance. The prototyped device was placed in a 3D printed human EAC model and assessed for feasibility of placement. The aim of this specification was to have the device placed in under 10 minutes, based on literature review of existing in-office procedures [19]. If the prototyped device was deemed not to be easy to place or took more than 10 minutes to place, physical components (e.g. handles) may be added to the device to help place the device in the EAC.
- **Device adhesion to TM.** In order for drug delivery to be sustained, the device must adhere to the TM throughout the treatment period without falling out. To test this, the device was vertically and horizontally placed on porcine skin and whether it dislodged or fell off was assessed.
- **Cell adhesion.** To ensure that the device does not integrate into the TM and can be easily removed from the TM after treatment, the device must be bioinert and have low cell adhesion. In addition, keratinocytes constantly migrate radially outward on the TM from the umbo. Thus, to ensure that the device does not affect keratinocyte migration, the device must not allow for a high amount of cell attachment. This was measured by cell adhesion studies, specifically by placing EarFlow adjacent to cell cultures of human keratinocytes. After 7 days, the devices were treated with a Live/Dead stain (BioVision) and imaged using confocal microscopy to determine if any cell adhesion has occurred. If there were more than 10% of keratinocytes attached to the device, the hydrogel material would be

reassessed, and a new hydrogel synthesis protocol would be selected. Additionally, non-adhesive coatings may be considered for usage.

[00291] Design Specifications for Modelling EarFlow on the TM

[00292] Below are the qualitative specifications of the FE model of EarFlow on the TM, which are summarized in Figs. 10A-10B.

- Anatomically accurate representation of the human middle ear. To allow for maximum translatability of in silico results, the FE model would ideally be an anatomically accurate representation of the human middle ear. This includes accuracy in both the thickness and the area of the TM. Specifically, the literature-reported value of the mean TM thickness is between 80–100 μm [34]. The literature-reported value of the TM diameter is around 8-10 mm [34]. In addition, the TM should be conically shaped, and have a Young's modulus between 10–300 MPa, consistent with previously reported values of the Young's modulus of the human TM [35, 36]. The Puria group's FE model was used to model EarFlow in silico, as it is generated from a micro-CT scan of the human middle ear, making it anatomically accurate and suitable for analyzing the changes in human sound conduction when EarFlow is placed.
- Acoustically and mechanically coupled. The FE model should have both acoustic (e.g. sound conduction) and mechanical properties defined (e.g. TM motion), and the two components should be coupled to allow accurate representation and data output based on EarFlow placement. To fulfill this, the Puria group's FE model was used as a basis of the middle ear, as it is an acoustically and mechanically coupled model of the middle ear.
- Amenable to changes in device placement, geometry, density, and size. The model allowed the placement of EarFlow at different locations on the TM. The device size, geometry, and density were amenable to changes in the FE platform to allow the modelling of variations in these parameters.
- Output data representative of sound conduction. To allow for the analysis of the impact of EarFlow on the TM, the FE model should generate data that is representative of the capacity of sound conduction based on the device placement. This was achieved as the MEG, a proxy of sound conduction, can be calculated from the data generated from the model

[00293] Design Specifications for Modelling Modelling Diffusion across the TM

[00294] To accurately represent diffusion across the TM, the model should satisfy the following qualitative specifications, which are summarized in Fig. 11.

- Recapitulate the geometry of the TM. To accurately represent diffusion across the TM, the TM geometry must be accurately depicted. This includes depicting approximately accurate thickness of each of the three layers in the TM. To do so, a histologically stained image of a healthy TM (not shown) was used to measure the proportion that each layer of the TM constitutes in the total TM thickness. The FE model was built based on the measured proportions of the different layers.

- Allow user to model and test different drug compounds by modifying various properties. To model different drug compounds, the FE model should include parameters that can be modified to reflect varying drug properties, specifically the diffusion coefficient. The model should allow the diffusion coefficient to be varied between $1e^{-5} - 1e^{-11} \text{ m}^2_{\text{s}}$, which is a relevant range to test as determined by prior literature review [27].
- Output diffusion data. To allow the analysis of drug diffusion across the TM, the model should output diffusion data.

[00295] Materials and Methods

[00296] Fabricating and Testing EarFlow

[00297] Ionic Liquid Synthesis and Characterization

[00298] Ionic Liquid Synthesis. Choline-hexenoic acid IL (CAHA) was synthesized at a 1:2 ratio from choline bicarbonate and hexenoic acid (Sigma-Aldrich). A hot plate was set to 52°C with the stirrer function on. A round bottom flask was clamped to sit level in an oil bath on the hot plate. 20 g of hexenoic acid crystals were weighed out, dissolved in minimal Milli-Q water, and added to a 500 mL round bottom flask with a stir bar. 18.09 g of choline bicarbonate was added dropwise to the round bottom flask to satisfy the desired molar ratio. The mixture was left to synthesize overnight. The resulting synthesized mixture was removed from heat the next day and placed on a rotary evaporator for two hours to remove water, with the water bath set at 50°C and the vacuum at 150 mbar. The dried IL was poured into a petri dish (with the stir bar removed) and was then placed in a vacuum oven for 48 hours at 60°C. Once the IL was oven dried, it was transferred to a 20 mL glass vial for characterization.

[00299] NMR Analysis. One-dimensional ^1H nuclear magnetic resonance (1D ^1H NMR) was used to confirm the identity of the synthesized IL.

[00300] Drug Loading into Ionic Liquids. Dexamethasone powder (>99%, Sigma-Aldrich) was dissolved in IL to achieve a concentration of 200 mg/mL (IL-Dex). Ciprofloxacin powder (> 99%, Sigma-Aldrich) was dissolved in IL to achieve a concentration of 200 mg/mL (IL-Cip). IL-Dex and IL-Cip solutions were heated to 80°C in an oven for an hour and then mixed to allow for even distribution of the drug in IL.

[00301] Fabrication of EarFlow

[00302] Hydrogel Fabrication and Processing. Four polymer solutions were made from backbone monomer 2-hydroxyethyl methacrylate (HEMA) (Sigma-Aldrich) and crosslinker ethylene glycol dimethacrylate (EGDMA) (Sigma-Aldrich), with monomer ratios as detailed in Fig. 12. MilliQ water (MilliporeSigma) was added at 20% of the polymer volume to ensure that the polymer mass content in each solution remained the same. 0.93 wt% of radical initiator azobisisobutyronitrile (AIBN) was added to each polymer solution. After the addition of AIBN, the solutions were mixed well and

pipetted into silicone molds. The molds were then placed in an oven and cured at 70°C for 3 hours. See Fig. 41.

[00303] Hydrogel Swelling. After polymerization, the pHEMA gels were immersed in MilliQ water for one week to fully swell the gels and remove unreacted residues.

[00304] Hydrogel Lyophilization and Reswelling. After swelling, pHEMA hydrogel sheets were cut into disks using a 4 mm or 8 mm diameter biopsy punch, and were then frozen at -80°C overnight. The hydrogels were then freeze dried in a lyophilizer (SP Scientific) for three days to allow for complete drying and create a porous network in the hydrogels. Masses of the lyophilized hydrogels were measured with a high-resolution balance. Lyophilized hydrogels were re-swelled in IL, IL-Cip, or IL-Dex for one week. Weights of reswelled hydrogels were then measured.

[00305] Characterization of EarFlow

[00306] Characterization of Ionic Liquid Uptake in Hydrogels. The swelling ratio q was determined as the ratio between the weight of the hydrogel swelled in IL and the weight of the unswelled (lyophilized), crosslinked hydrogel.

$$q = \frac{W_s}{W_l} \quad (5.1)$$

Where W_l is the weight of the lyophilized, unswelled hydrogel and W_s is the weight of the hydrogel swelled in IL. The swelling percentage was determined as:

$$\% = \frac{W_s - W_l}{W_l} \quad (5.2)$$

3 samples were measured for hydrogels made from each polymer solution, in combination with three IL samples tested (IL, IL-Cip, IL-Dex), and the values were reported as means $\pm$ standard deviation. Two-way Analysis of Variance (ANOVA) was conducted with Prism 9 (GraphPad) to determine the effects of hydrogel formulation and ionic liquid on the swelling ratio.

[00307] In vitro Drug Release Profile

[00308] Custom 9 mm diameter Franz diffusion cells mounted with porcine skin were used to create a diffusion setup as an in vitro mimic of the TM and middle ear space (Fig. 13). Porcine skin was utilized due to the limited availability of TMs. Porcine skin conductivity was measured prior to usage to ensure the intactness of the tissue and ultimately to ensure that there is no leakage. The acceptor chamber of the Franz diffusion cell was filled with 1.5 mL of PBS solution to mimic the middle ear space volume. The 8 mm radius IL-Dex-hydrogel scaffold or IL-Cip-hydrogel scaffold was placed in the donor compartment and in contact with porcine skin ($n=3$). Control groups included hydrogels swelled with PBS-Dex and PBS-Cip solutions at equivalent concentrations ($n=3$), as well as a negative control group of the hydrogel scaffold swelled with only PBS ($n=1$). At time points 1, 2, 3, 4, hours and 1, 3, 5 days,

100 μ L of media from the acceptor well media was pipetted into a scintillation vial containing 3 mL of 50% methanol and 50% PBS (MeOH/PBS) solution. At Day 7, the porcine skin stratum corneum, epidermis, dermis layers and the acceptor chamber fluid were transferred to scintillation vials containing 3 mL of MeOH/PBS. Using previously established calibration curves for ciprofloxacin and dexamethasone, UV spectrophotometry was used to quantify the amount of ciprofloxacin and dexamethasone present at each time point and on the porcine skin with the Synergy H1 Plate Reader (BioTek). Specifically, ciprofloxacin absorbance was measured at 275 nm and dexamethasone absorbance was measured at 241 nm. Two-way ANOVA was conducted on the concentrations of drug in each group using Prism 9 (GraphPad). Results were plotted as mean $\pm$ standard deviation.

[00309] Cell Proliferation and Cell Toxicity. Human dermal fibroblasts (HDFs) and human epidermal keratinocytes (HEKs) were seeded at 20,000 and 25,000 cells/well respectively in 24-well plates and allowed to attach to the wells for 24 hours. IL-hydrogel constructs were then placed onto transwell membrane inserts to be in contact with the cell media for 7 days. Cell media was changed every 2-3 days.

An MTS Assay (Promega) was used to assess cell proliferation on 1, 3, 7, and 14 days following device placement. A CytoTox-Fluor colorimetric assay (Promega) was used to assess potential cytotoxicity of hydrogels in each group after 1 hour of cell attachment. Briefly, both assays were conducted following the manufacturers' protocols, and 100 μ L of cell culture media was transferred from the 24-well plates to appropriate 96-well plates at the measurement timepoints. 96-well plates were then read using the Synergy H1 Plate Reader (BioTek). Results from the four experimental groups were compared to the two control groups using one-Way ANOVA. The experimental and control groups were as follows, with triplicates for each group.

1. Control group 1: Cells cultured on the well plate with no transwell membrane insert
2. Control group 2: Cells cultured on the well plate with transwell membrane insert placed
3. Experimental group 1: Hydrogel 1A swelled with PBS (1A+PBS)
4. Experimental group 2: Hydrogel 1A swelled with IL (1A+IL)
5. Experimental group 3: Hydrogel 2A swelled with PBS (2A+PBS)
6. Experimental group 4: Hydrogel 2A swelled with IL (2A+IL)

[00310] Cell Adhesion. HEKs were cultured and seeded with the pHEMA-IL hydrogel discs at 25,000 cells/well. The cells and hydrogel discs were incubated together at 37°C for 24 hours to allow for cell attachment to the devices. An MTS Assay (Promega) was used to assess cell proliferation on Day 1, 4, 7 and compare cell counts amongst the groups. On Day 7, the gels were transferred to a separate 48-well plate, and the original plate with cells was stained with the Live-Dead cell staining kit (BioVision). The cells and gels were then imaged under a confocal microscope (Leica) to assess

cell growth on the cell culture well plastic and cell adhesion onto the device. Images were then processed in Fiji [37]. Results amongst groups were compared using one-way ANOVA in Prism (GraphPad). The experimental and control groups were as follows, with six replicates for each group.

1. Control group 1: Cells cultured in the plate with no hydrogel
2. Experimental group 1: Hydrogel 1A swelled with PBS (1A+PBS)
3. Experimental group 2: Hydrogel 1A swelled with IL (1A+IL)
4. Experimental group 3: Hydrogel 2A swelled with PBS (2A+PBS)
5. Experimental group 4: Hydrogel 2A swelled with IL (2A+IL)

[00311] Adhesion to Tissues. EarFlow devices were placed on porcine skin both vertically and horizontally (upside down), and device adhesion to the tissue was qualitatively assessed.

[00312] Assessing Ease of Placement of EarFlow into the Ear Canal. A 3D Transcanal Endoscopic Ear Surgery (TEES) simulator (model courtesy of Dr. Samuel Barber [3]) was 3D printed to include an anatomically accurate and to-scale ear canal model, and porcine skin was mounted on the model to mimic the TM. EarFlow was then placed into the EAC model using alligator forceps, and the ease of placement was assessed. The amount of time required to place EarFlow on the porcine skin was recorded. Videos of EarFlow placement were recorded using an endoscope.

[00313] Modelling EarFlow 's Impact on Hearing

[00314] Device Modelling with Varying Parameters

[00315] To assess the potential effects of device placement on the TM on conductive hearing loss, the devices were modelled on an existing middle ear model [1] using COMSOL Multiphysics. To model the baseline drug delivery device parameters, EarFlow was modelled as a new geometry component in COMSOL as solid cylinders of 2 mm radius and 2 mm height, with a density of 1120 kg/m³ to match a typical hydrogel polymer density. Device parameters were then varied with values specified in Fig. 14, with only one parameter varied at a time. When one parameter was varied, the other parameters were held at baseline values: a cylinder of 2 mm radius, located in the anterior-superior region, and with a density of 1120 kg/m³ (selected to be representative of hydrogel molecular weight). This type of testing method did not account for the potential interactions between the variables; this method assumed that the interaction effects among device size, device geometry, device density, and device location are low.

[00316] Varying Device Size. A cylinder was extruded in COMSOL to model EarFlow and placed on the TM's lateral (outward facing) surface in the middle ear FE model from the Puria group. The radius of the device was varied from 2 mm to 2.75 mm in 0.25 mm increments, and the height of the cylinder was fixed at 2 mm; additionally, the density of the device was fixed at 1120 kg/m³, and the device location was fixed at the anterior-superior quadrant of the TM. The study was then computed with each cylinder size.

[00317] Varying Device Geometry. Three device geometries (cylinder, hemisphere, cube) were extruded and each placed on the middle ear FE model. To ensure that the resultant middle ear gain is not affected by the size or weight of the device, the device density was fixed at 1120 kg/m^3 and the device volume was fixed at around 0.025 cm^3 (25 mm^3) for all three geometries.

[00318] Varying Device Location. To analyze the effects of the device placement location on hearing, the device location was varied and placed in one of the four quadrants of the TM (anterior-inferior, anterior-superior, posterior-inferior, posterior-superior). The device size, density, and geometry were fixed.

[00319] Varying Device Density. To analyze the effects of the device density and therefore mass on hearing, the device density was varied from 1000 to 2000 kg/m^3 in increments of 200 kg/m^3 with the device size and location fixed at 2 mm radius and in the anterior-superior region, respectively.

[00320] Analysis of Middle Ear Gain

[00321] To quantify the changes in hearing with the varying device parameters, middle ear gain (MEG), defined as the cochlear-vestibule pressure (PCV) divided by the average sound pressure over a plane in the ear canal near the TM [1], was computed from the output data of the COMSOL model. Cochlear-vestibule pressure was obtained in the “Results-Derived Values” tab in COMSOL, through which the surface average pressure was generated from the “Average Surface Average” tab. Under “Expressions”, normal stress was added via the tab “Component1 – Solid Mechanics – Stress – Normal Stress”. The derived values were then evaluated. To obtain the surface pressure, data was directly exported from “Surface Average 2”. The MEG for the model with the device was then graphed on a logarithmic y-axis in MATLAB, with hearing frequencies ranging from 0.1 to 20 kHz on a logarithmic x-axis. This was compared with the MEG of the baseline model and 90% of the MEG of the baseline model.

[00322] Modelling Diffusion

[00323] Building the Diffusion Model. A 2D simple diffusion model was created based on an image of a histologically stained healthy TM and the relative proportion of each layer. The total TM thickness was $85 \text{ }\mu\text{m}$. The stratum corneum (SC) was modelled as $9 \text{ }\mu\text{m} \times 4 \text{ }\mu\text{m}$ rectangles with $1 \text{ }\mu\text{m}$ spacing between the rectangles for a total thickness of $20 \text{ }\mu\text{m}$. The lamina propria layer was modelled as two rectangles (one for the radial collagen layer and one for the circular collagen layer) for a total thickness of $40 \text{ }\mu\text{m}$. The mucosal epithelium layer was modelled as a rectangle with a total thickness of $25 \text{ }\mu\text{m}$ (this was selected to be slightly higher than the proportion of mucosal epithelium).

[00324] The SC corneocytes and mucosal epithelium layers were defined with the built-in material fat. The SC lipids and the lamina propria layer were defined with the built-in material skin (Fig. 15).

In addition, below the TM layers, a 2D model of the middle ear space was generated with rectangles

to represent the receptor sink in which diffusion would occur (Fig. 16). The sink was modelled to have 50% water as a mimic of actual physiological conditions in OM.

[00325] Given that there were no consistent literature reported values used for the permeability and diffusion coefficients of tissues, the lowest permeability values and diffusion values reported in the literature [38, 27] were used as the slowest case diffusion estimates. The values of relevant parameters are detailed in (Fig. 17). With these values, the COMSOL physics modules Transport of Diluted Species in

Porous Media and Brinkman Equations were added and coupled to allow the computation of the model.

[00326] Computing Diffusion. The TM model was computed in COMSOL under the time-dependent diffusion study for a time range of 0 to 30 minutes. The resulting figures and data for solute concentration

throughout the model were exported. A concentration of at least 4 µg/mL (the MIC of ciprofloxacin against middle ear pathogens) at the outlet and throughout the middle ear space is necessary to clear OM. 4 µg/mL is equivalent to 4e-6 g/mL. The molar mass of ciprofloxacin is 331.346 g/mol. Thus, the molar concentration of ciprofloxacin that is necessary in the middle ear space is:

$$4 \times 10^{-6} \frac{g}{mL} \times \frac{1}{331.346} \frac{mol}{g} \times \frac{1}{1e-6} \frac{mL}{m^3} = 0.012 \frac{mol}{m^3}$$

The time-dependent solute concentration achieved in the diffusion model was compared with the above value.

[00327] Results

[00328] Characterization of EarFlow

[00329] Characterization of Ionic Liquid Uptake in Hydrogels. Using four different hydrogels (the formulations of which are detailed in Fig. 12) immersed in either IL, IL-Cip, or IL-Dex, the swelling ratio q of each polymer solution was calculated, compared, and shown in Fig. 18 for the 4 mm lyophilized PHEMA hydrogels. The hydrogels are also shown in Fig. 41. Interestingly, the two gels formulated without water (1A, 2A) had significantly greater uptake rate than those with water (1B, 2B) across all three ILs tested. Specifically, both 1A and 2A hydrogels swelled to more than 1.5 times their original weight on average. Based on this data, hydrogels 1A and 2A (synthesized without water) were selected for conducting further characterization testing due to their high IL uptake rate.

[00330] 4mm hydrogels can swell 1.2-1.7x their original weight in IL, holding 10µL of IL, with highest swelling occurring in 1A and 2A (synthesized without water).

[00331] The densities of choline-based ILs have been previously reported to range from 0.88-1.54 g/mL [39]. Assuming that the density of CAHA IL is around 1 g/mL, and noting that most of the 4

mm lyophilized hydrogels had a weight of around 10-15 mg, this indicated that around 10 mg, or 10 μL of IL was uptaken by each lyophilized hydrogel. As stated in the MIC calculations, the device should be able to hold a minimum of 40 μL of IL. This indicated that quadrupling the size of current gels (around 40 mm^3) to volume of 160 mm^3 , or 0.160 cm^3 , would allow the gels to hold 40 μL of IL.

[00332] Accounting for this new volume necessary for the gels to hold 40 μL of IL, this volume can be held by a cylinder of 8 mm diameter and 3.5 mm height. The calculation of this volume is given below.

$$4^2\pi \times 3.5 = 175.9\text{mm}^3 \quad (6.1)$$

[00333] To confirm the above calculations, swelling ratio testing was conducted with 8 mm diameter hydrogels (8 mm hydrogels). Results demonstrated that the 8 mm hydrogels can swell to 2.5-3.5 times their original weight (around 50 mg) in IL, with the greatest swelling occurring in 1A when it was swelled in pure IL (Fig. 19). The weight of the 1A hydrogel was 51.9 ± 8.5 mg. Swelling to 2.5 times its original weight, the 1A hydrogel was thus able to hold 50-100 mg of IL. This confirmed that the 8 mm hydrogel device was porous enough to hold more than 40 μL of IL, even when considering the higher bound of IL density. This new size and volume of hydrogel was used in the in vitro drug release studies to hold the necessary volume of IL.

[00334] 1A hydrogel was optimal, among the tested formulations, for swelling. 1A hydrogel original weight is around 51.9 ± 8.5 mg. 1A hydrogel can swell to 2.5x its original weight in IL. 8mm hydrogels can swell to 2.5-3.5x their original weight (around 50 mg) in IL, with highest swelling occurring in 1A (synthesized without crosslinker) and in pure IL.

[00335] In vitro Drug Release Profile

[00336] In vitro diffusion studies demonstrated that IL-Dex loaded in EarFlow successfully permeated the porcine tissue, as indicated by the high concentration of dexamethasone in the epidermis and dermis layers of the porcine skin as well as in the acceptor chamber at Day 7 (Fig. 20). As there was a detectable concentration of dexamethasone across all time-points, it was shown that EarFlow was able to sustain dexamethasone delivery over 7 days, indicating that treatment with EarFlow can clear OM effectively.

[00337] In addition, diffusion studies demonstrated that EarFlow loaded with IL-Dex delivered a higher amount of dexamethasone than EarFlow loaded with PBS-Dex over the same period of time. This is shown by the higher concentration of dexamethasone at Day 1, 3, 5, in the epidermis and dermis layers of the tissue, and in the acceptor chamber at Day 7 when delivered by EarFlow loaded with IL-Dex (Fig. 20).

[00338] Cell Proliferation. With the MTS cell proliferation assay, it was shown that at Day 1 and Day 7, there was some difference in fibroblast proliferation (as measured by cell count) between IL

groups

and the control groups (Figs. 21A-21C). However, while the overall cell counts appeared to be decreasing in all groups over time, the fibroblasts did indeed still proliferate in the presence of the EarFlow scaffold even at Day 7 (Fig. 21C). The cell proliferation assay on keratinocytes showed that there were differences in cell counts between hydrogels loaded with ionic liquids and the control groups (Figs. 22A-22C). However, these differences did not appear to be statistically significant at Day 1

(Fig. 22A). At Day 3 and Day 8, the differences in cell counts between the control and experimental groups became more significant, but the cells were still proliferating up to Day 8 (Fig. 22C), which is promising as we therefore could expect the cells to continue to proliferate after EarFlow is removed from the TM.

[00339] Cell Toxicity. Cell toxicity assays with EarFlow pHEMA hydrogel scaffolds demonstrated that the EarFlow hydrogel material itself had low toxicity to fibroblasts and keratinocytes (Figs. 23A-23B). Specifically, referring back to the design goal of 90% of cell viability, EarFlow hydrogels swelled with either saline or ionic liquids achieved at least 90% of the cell counts of control groups and did not appear to be significantly cytotoxic to fibroblasts (Fig. 23A). With respect to keratinocyte cytotoxicity, the 1A hydrogel and IL combination showed lower cell counts compared to the controls, while the 2A hydrogel and IL combination showed comparable cell counts to the controls (Fig. 23B). In addition, lyophilized hydrogels swelled with saline showed cell counts comparable to control groups for both fibroblasts and keratinocytes. This is promising, as the type of hydrogel and the type of ionic liquid used could be modified and tested in additional combinations to mediate cell toxicity.

[00340] EarFlow provided > 90% fibroblast survival, > 90% keratinocyte survival for 2A + IL, and < 90% keratinocyte survival for 1A+IL.

[00341] EarFlow Adhesion to Tissues. EarFlow was able to adhere to porcine tissues physically, both when the tissue was vertical (to mimic the TM) and when the tissue was placed at a 180° angle (Fig. 24).

[00342] Cell Adhesion. Comparing the control group of keratinocytes seeded on cell culture plastic wells with the experimental groups, which measured cells that adhered to gels, it was shown that across all time points (Day 1, Day 4, Day 7), there were significantly fewer cells attached to the gels themselves than to the cell culture wells (Figs. 25A-25C). Specifically, at Day 7, the experimental groups (gels) on average had $4.1 \pm 0.27\%$ of cells of the control group. This data demonstrated that the design specification of less than 10% of keratinocytes adhering to the gels was fulfilled, indicating that EarFlow is bioinert and will not integrate into the TM over the time scale of treatment, permitting effective removal of the device at the end of the treatment period.

[00343] Confocal images of cells and gels stained with the live-dead cell assay at Day 7 confirmed that across all experimental groups, there was little to no cell adhesion to the gels themselves, as shown by the low levels of green "Live" staining on the gels (Figs. 26A-26E). As a comparison, there were cells proliferating in the control group (Fig. 26A) and on the well plates themselves (Figs. 26B-26E).

[00344] Ease of Placement of EarFlow. EarFlow was able to be placed onto porcine skin through the 3D human EAC model in less than 1 minute, indicating the ease of placement of the device. Successful EarFlow placement on the tissue is shown in Fig. 27. As device placement can be completed in under 10 minutes, noninvasive placement of EarFlow in a clinic setting in an awake patient is feasible.

[00345] COMSOL Modelling of EarFlow on the Middle Ear

[00346] The Effect of Device Size on Middle Ear Gain. Results demonstrated that loading on the TM with additional mass did indeed decrease the MEG in most cases (Figs. 28A-28B). Based on conversations with physicians, it was determined that a decrease below 90% of the baseline MEG would be considered a significant effect on hearing. The results showed the raw data with 90% of the baseline subtracted; positive MEG values indicated improved sound conduction, whereas negative MEG values indicated worsened sound conduction, and a value near zero indicated no significant changes compared to 90% of the baseline. Cylinders of radius 2, 2.25, and 2.5 mm maintained MEG above 90% of the baseline value at most frequencies (with the exception of at 20 kHz). The cylinder of radius 2.75 mm, however, showed decrease in MEG below the 90% baseline at additional frequencies (0.1 kHz and 7 kHz) compared to the other sizes tested. This data confirmed the technical specification choice of maintaining the device size below or equal to a radius of 4 mm.

[00347] The Effect of Device Geometry on Middle Ear Gain. Results demonstrated that across the range of frequencies important for human hearing, the device geometry did not have any significant variations compared to one another, and all three device geometries resulted in MEG above than 90% of the baseline MEG (Figs. 29A-29B). Because the variations in geometry did not significantly impact sound conduction, in vitro and ex vivo studies were conducted with the cylindrical device geometry due to its ease of manufacturing with standard biopsy punches.

[00348] The Effect of Device Location on Middle Ear Gain. Results demonstrated that across most hearing frequencies, MEG did not vary significantly based on the location of the device on the TM (Figs. 30A-30B). However, towards higher frequencies (above 10 kHz), the different locations began to impact MEG. For example, at around 18 kHz, devices placed at the posterior-superior quadrant unexpectedly improved MEG above baseline values.

[00349] The Effect of Device Density on Middle Ear Gain. Results demonstrated that within the density range of 1000-2000 kg/m³, the resulting MEG did not vary significantly from one another and did not decrease below 90% of baseline MEG for most frequencies (Fig. 31).

[00350] COMSOL Modelling of Diffusion across the Tympanic Membrane

[00351] As detailed above, a COMSOL model with the relevant TM parameters and diffusion parameters was generated to allow the modelling of diffusion across the TM. Notably, literature reported values of permeability, porosity, and diffusion coefficients for human tissues often had a large range of variations. Thus, conservative estimates were used to model all aspects; in other words, parameters were assumed to take on the values that would allow the slowest possible permeation. These values used are detailed above.

[00352] Figs. 32A-32B shows that with the given parameters, the desired drug concentration (i.e. MIC of cipro oxacin against *S. pneumoniae*) of 4 µg/mL, or 0.012 mol/m³ (detailed calculations above) was achieved throughout the entire tympanic membrane starting 30 seconds after the diffusion of modelled drug molecules, which is a very reasonable timeframe of initial delivery. This indicated that EarFlow could be promising for the successful delivery of antibiotics over time, potentially satisfying the design specification of sustained drug delivery over 7 days (see above).

[00353] Extending this computation method to the entire middle ear space, Figs. 33A-33B showed that the entire middle ear space reaches the necessary drug concentration at around 12 minutes, a similarly reasonable timeframe for beginning to treat OM. That said, it is important that the drug delivery is sustained for not only the initial 30 seconds but throughout a 7-day period to effectively clear OM. Thus, rather than simply placing ILs on the surface of the TM for 30 seconds, the creation of an IL-holding device is necessary to sustain the delivery.

[00354] Given the in vitro diffusion results using Franz diffusion cells above, it is believed that this concentration will be further increased in the middle ear space over time. As the device holds approximately 8 µg total of ciprofloxacin (0.040 mL of IL loaded at 200 mg/mL), it is expected that further drug elution from the reservoir-like device to replenish any drugs that may be depleted over the course of treatment. Thus, it is expected that the concentration of ciprofloxacin in the middle ear space to exceed the MIC of the relevant bacterial strains (e.g. *H. influenzae* and *S. pneumoniae*) for OM for at least 7 days, effectively treating OM in a localized fashion.

[00355] Discussion

[00356] Results

[00357] Otitis media (OM) is caused by infectious pathogens in the middle ear, causing pain, impaired hearing, and potentially, delayed language development in children. Despite its prevalence, existing solutions are ineffective, involve systemic antibiotic delivery, or require invasive procedures|all of which have considerable side effects and potential long-term complications. An ideal solution for treating OM should non-invasively deliver antibiotics and/or steroids into the middle ear space for a sustained period (around 7 days) to clear pathogens.

[00358] This project details EarFlow, a non-invasive drug delivery device to be placed on the tympanic membrane (TM) for treating OM.

[00359] Achievement of Design Specifications

[00360] Figs. 34A-34B detail the design specifications for EarFlow previously described above and indicates whether these design specifications have been achieved. Each of these specifications are discussed in detail below.

[00361] COMSOL Modelling. The design specifications for the COMSOL modelling of EarFlow were achieved by using a biologically accurate and acousto-mechanical coupled middle ear model from the Puria group [1] and successfully varying the device parameters of interest in the relevant range in COMSOL.

[00362] The design specifications for COMSOL modelling of drug diffusion were achieved by building a TM model that recapitulated the biological structure and adding the relevant diffusion modules in COMSOL. The built diffusion model allowed for variations of the diffusion coefficients in the desired range, such that all design specifications were fulfilled.

[00363] Device Manufacturing

- Device size. The device radius can be varied from 2 mm to 4 mm, achieving the specification of radius less than 4 mm. However, based on the hydrogel-IL uptake data, the 4 mm radius device was necessary to hold 40 μ L of ionic liquids..
- Porosity of scaffold. The porosity of the scaffold was not evaluated due to time constraints and access to SEM imaging facility. Ideally, the porosity of the scaffold can be measured in future iterations of the process. However, results from IL uptake studies suggested that the hydrogel scaffolds have sufficient porosity to hold the necessary volume of IL (40 μ L).

[00364] Drug Delivery and Release

- Loading concentrations of drugs in the IL. ILs synthesized were able to be loaded with ciprofloxacin at a concentration of 250 mg/mL, fulfilling the design specification.
- Total volume of IL held in device. Results from the hydrogel-IL uptake studies demonstrated that the 8 mm lyophilized hydrogel scaffolds successfully swelled to up to 250% their original weight and were able to hold more than 40 μ L of ionic liquids.
- Sustained delivery timeframe. EarFlow loaded with IL-Dex was able to deliver dexamethasone across intact porcine skin and into the acceptor chamber (the middle ear space mimic), sustaining delivery over 7 days. Importantly, EarFlow loaded with IL-Dex demonstrated a higher delivery rate than the control group (EarFlow loaded with PBS-Dex). It is anticipated that results for ciprofloxacin delivery should show similar improvement in drug transport with ILs compared to PBS.
- Device degradation rate. Minimizing degradation was achieved by fabricating the scaffold with pHEMA, a bioinert polymer that is resistant to hydrolysis or enzymatic degradation.

[00365] Cytotoxicity

- Cytotoxicity of ILs. Fibroblasts cultured with the hydrogel device swelled in ILs had cell counts that were not statistically significantly different from control groups, indicating low cytotoxicity to

fibroblasts. However, keratinocyte cell numbers showed a statistically significant decrease when cultured with the pHEMA hydrogel swelled in ILs compared to control groups. In addition, cell proliferation studies indicated that both fibroblast and keratinocyte cell counts are lower when cultured with ionic liquids,

indicating that cells still proliferated, but at a slower rate in the presence of ILs.

- Cytotoxicity of the hydrogel. Fibroblasts and keratinocytes cultured with the pHEMA hydrogel swelled in saline had cell counts that were not statistically significantly different from control groups, indicating low cytotoxicity of the hydrogel, and achieving the design specification.

[00366] Clinical Considerations

- Hearing threshold changes. While hearing threshold changes when EarFlow is placed on the TM were not evaluated in in vivo settings, COMSOL modelling of the device suggested that placement of the device should not have an effect on hearing to a significant extent. Hearing changes will be evaluated in in vivo models in the future.
- Easy to place into and remove from the EAC. Using a 3D printed EAC model, it was validated that EarFlow can be placed into the EAC in less than 1 minute. Future studies will continue to assess this ease of placement in consultation with ENT surgeons.
- Device adhesion to TM. EarFlow was able to adhere to porcine skin both when the porcine skin was held vertical (with the tissue perpendicular to the ground) and when it was horizontal (upside down), indicating that the device will adhere to the TM to sustain treatment over time.
- Cell adhesion. Cell adhesion assays demonstrated that compared to control group cell counts, less than 10% of keratinocytes adhered to EarFlow itself at the end of the 7-day timeframe, fulfilling the design specification and indicating that the device should not integrate into the TM. Specifically, experimental groups on average had around 4.1 ± 0.26% of cells of the control group at Day 7.

[00367] Comparison to Existing Technologies

[00368] The existing approaches for treating OM were analyzed above, and all approaches lack at least one of the desired parameters required for the ideal treatment of OM identified via discussions with ENT surgeons, namely efficacy, non-invasiveness, low cytotoxicity, low impact on hearing, localized treatment, and convenience. EarFlow addresses all of these critical parameters. Cellular assays demonstrated that while ILs held by EarFlow may have some low cytotoxicity, cells still proliferated in their presence, unlike with other chemical permeation enhancers [15]. Importantly, the pHEMA hydrogel scaffold itself did not have significant toxicity to the cells. There were limitations to the 2D cell culture model that could be mediated in an in vivo model. However, these in vitro studies represent a good approximation of potential cytotoxic effects to the two major cell lines that would be in contact with EarFlow.

[00369] In vitro diffusion studies demonstrated that EarFlow is able to sustain drug delivery of dexamethasone throughout a 7-day timeframe, comparable to current drug delivery standards. As a

sustained drug delivery device designed to be placed on TM, EarFlow is thus able to effectively deliver drugs non-invasively and locally, preventing any systemic exposure to antibiotics and a affiliated side effects.

[00370] Based on COMSOL modelling results, it is anticipated that the placement of EarFlow on the TM will have a low impact on the patient's hearing.

[00371] Finally, validation with a 3D anatomically accurate EAC model demonstrated that EarFlow can be easily and quickly placed on the TM in an office setting without the need for general anaesthesia, indicating practical feasibility of device placement and removal. In vitro cell adhesion assays corroborated that EarFlow remains bioinert, preventing any tissue or scar formation around the device and allowing it to be easily removed after the treatment period. Overall, these early designs and studies with the novel EarFlow device are promising towards providing a practical and effective solution for treating OM.

[00372] Future Directions

[00373] First, based on the initial analysis of IL uptake by the lyophilized pHEMA hydrogel, it was identified that the hydrogels were able to swell to around 150% of their lyophilized weight in IL and thus can hold a considerable volume of IL. However, the 2 mm radius hydrogel discs were only able to hold around 10 μ L of IL instead of 40 μ L of IL due to their low lyophilized volume and weight. Based on calculations, it is expected that the 4 mm radius device should hold at least 40 μ L of IL; thus, the 4 mm radius devices were utilized in diffusion studies. If the larger radius devices were utilized for treatment, the effect of placing a slightly larger device on the TM on hearing changes should also be evaluated in in silico and in vivo studies.

[00374] The quantification of ciprofloxacin concentration is more challenging than that of dexamethasone concentration: while dexamethasone concentration can be quantified by UV-Vis spectroscopy, ciprofloxacin concentration quantification requires high performance liquid chromatography or liquid chromatography-mass spectrometry. While this project has not yet assessed the delivery of ciprofloxacin, the dexamethasone diffusion studies demonstrated promising results. It is anticipated that these results will be similarly encouraging for ciprofloxacin, which will be investigated in future studies. Importantly, EarFlow loaded with IL-Dex demonstrated a higher delivery rate than the control group (EarFlow loaded with PBS-Dex). These studies would benefit from additional replicates in each experiment group to increase statistical significance.

[00375] In addition, though the ex vivo diffusion studies demonstrated promising results, it is important to note that the thickness of porcine skin (around 3-4 mm) tested in this study is much greater than the thickness of human TM tissues (around 0.08-0.1 mm); TM tissues were not utilized in this study due to the limited supply of the tissue and challenges harvesting the tissue in COVID-19 times. This indicated that the diffusion data presented in this study may be a lower bound estimate of the actual amount of drug that can diffuse into the middle ear space in 7 days given a thinner TM

tissue in vivo. Thus, future diffusion studies will ideally be conducted in in vivo animal models, which were not pursued during the duration of this project due to the time and costs of establishing such models. Validating EarFlow in animal models will also allow more robust and realistic assessment of the cytotoxicity of the device, as one can utilize histological staining to assess TM intactness after the placement of EarFlow.

[00376] Additionally, any potential systemic effects of the device can be studied in vivo. Based on the results from cellular assays, it appeared that the ILs have some cytotoxicity or may slow down cell growth. In addition, based on the fibroblast and keratinocyte proliferation data, even the control groups demonstrated a visible decrease in cell numbers by Day 7. This indicates that cell loss may have occurred due to experimental conditions, such as frequent media changes that could have washed away the cells. In addition, the cytotoxicity assay is only suitable for assessing cytotoxicity for up to 1 hour of cell seeding rather than long-term cytotoxicity. It would be important to repeat cell proliferation and toxicity assays to increase statistical power. It is important to note that the in vitro cellular assays were conducted with a monolayer of cells seeded on cell culture plastic, which is expected to be less robust than the actual stratum corneum of the TM, a multi-layer tissue. With a stratum corneum type barrier, some of the cytotoxicity will likely be mediated by the barrier structure, and thus the cytotoxicity of the IL may be less relevant long-term than the in vitro studies would suggest. In addition, given the regenerative capacity of cells in the TM [13], it is hypothesized that even if EarFlow poses some cytotoxicity, as long as there remains some number of cells of each relevant cell population on the TM (which will likely be the case), cells could regenerate once the device is removed after the 7-day treatment period. Future studies could also compare the toxicity of ILs to chemical permeability enhancers currently utilized in drug delivery and are known to be cytotoxic in vitro, such as limonene [15]. Additional combinations of IL formulations and hydrogel formulations may also be explored and evaluated for their cytotoxicity.

[00377] Towards the COMSOL modelling work of this project to determine potential impacts of EarFlow on hearing, the modelling of EarFlow on sound-induced motion of the TM assumed that there were little to no interaction effects of the parameters tested. Future COMSOL modelling could vary several parameters concurrently to assess potential interactions between EarFlow device size, geometry, location, and density. In addition, with respect to the drug diffusion modelling, the COMSOL diffusion modules utilized were able to specify the diffusion coefficients of the solutes, but did not allow additional definitions of specification of the solute properties, such as solute size or hydrophobicity. For future iterations of the COMSOL diffusion model, it would be ideal to incorporate more specific properties to the drug solute to have a more accurate model. Additionally, given that the geometry of the TM was modelled based on measurements from a histologically stained image of the TM, it is important to note that there are significant patient-to-patient variability and

variability in OM conditions that may cause varying TM and TM sub-layer thicknesses; thus, the COMSOL model is an approximation of the actual TM geometry in a particular patient.

[00378] Additional critical future studies include continuing assessment of the feasibility and ease of placing EarFlow through the EAC based on ENT surgeons' feedback and modifying the design of the device as necessary. Additionally, as EarFlow would primarily be placed in children, understanding potential discomfort and movement affiliated with device placement will be critical for device adoption. Towards the validation pathways of the device, it would be important to establish a sterilization protocol for the device and to understand the costs of producing the device as well. It would also be interesting to investigate the regulatory pathway of EarFlow, as it would likely represent a combination product of device and drug.

[00379] Impact

[00380] OM is a globally prevalent issue, with the incidence of acute OM being 10.85% globally, translating to 709 million cases per year (figures from 2005 [40]). In 2006, it was estimated that the US medical spending on OM was \$2.8 billion [41]. 51% of global cases occurs in those under age ve [40]. Given that OM impairs hearing and causes signi cant pain and inflammation, it is imperative to treat OM efficaciously, locally, and non-invasively to prevent any delays in learning and language development and to reduce the incidence of long-term effects.

[00381] EarFlow represents a promising novel method for drug delivery to treat OM. With its usage of ILs as the drug delivery vehicle, EarFlow has the ability to non-invasively and locally deliver ciprofloxacin and dexamethasone to the middle ear space in a sustained manner, representing a promising method towards reducing systemic side effects of antibiotics administration and bypassing the need for invasive surgical procedures, which could significantly improve patient treatment experience and potentially reduce the incidence of antibiotic resistance. This will decrease the number of tympanostomy tubes that need to be placed each year (the most common surgery for US children [42]), which is currently about 6.26 million per year in the US alone [43], ultimately reducing scar tissue, chronic TM perforations, as well as biofilms/otorrhea experienced by patients following tympanostomy tube insertion. This will also reduce the number of children that need to undergo general anesthesia to receive tympanostomy tubes, lowering healthcare system costs as well. Finally, the IL-hydrogel platform could also be customized to load other therapeutics to treat additional ear and hearing ailments or other in afflictions requiring localized drug delivery.

[00382] Measurements of the Thickness of Healthy Tympanic Membrane Layers.

[00383] The human TM generally has an average thickness of 80-100 μm across its surface [34], being thicker in some areas (particularly near the umbo and annulus) and thinner between those regions. A representative image of a healthy TM (Fig. 35) [4] was used to measure the approximate proportion that each layer represents in the full TM. Each layer's thickness was measured in Fiji [37]. From these measurements, it was possible to identify the approximate dimensions that should be

given to each layer in COMSOL. In the COMSOL model, the TM has a thickness of 85 μm , and each layer takes the approximate relative thicknesses as reported in Fig. 36.

[00384] Calculations of Minimal Inhibitory Concentration and Necessary Device Geometries

[00385] The minimal inhibitory concentration (MIC) of ciprofloxacin in the middle ear during otitis media is 0.1-0.5 $\mu\text{g/mL}$ for *Haemophilus influenzae* [15] and 4 $\mu\text{g/mL}$ for *Streptococcus pneumoniae* [33]. To clear OM, the middle ear concentration of ciprofloxacin must be kept above the higher MIC of the two; thus, it is necessary to maintain a minimum concentration of 4 $\mu\text{g/mL}$.

[00386] Approximating the middle ear space volume as 1.5-2 mL and if the middle ear space drug concentration needs to be maintained above 4 $\mu\text{g/mL}$, then, at least 8 μg of ciprofloxacin should be delivered into the middle ear to maintain this concentration.

[00387] Taking a more conservative assumption to account for larger middle ear volumes, it is assumed that a minimum of 10 μg of ciprofloxacin must be maintained in the middle ear. Given that ciprofloxacin can be loaded in the IL at a concentration up to 250 mg/mL , and assuming a transfer rate of 0.1% (though this transfer rate could be higher or lower), or 250 $\mu\text{g/mL}$, it was concluded that 40 μL of IL is required to contain 10 μg of ciprofloxacin within the device. 40 μL is equivalent to 0.040 mL, or 0.040 cm^3 , which is a reasonable volume of IL to be held in the device.

[00388] Accounting for this new volume necessary for the gels to hold 40 μL of ionic liquids, this volume can be achieved by a cylinder of 4 mm radius and 3.5 mm height.

$$4^2\pi \times 3.5 = 175.9\text{mm}^3 \quad (\text{B.1})$$

[00389] This new size and volume of gel will be used in the ex vivo drug release studies.

[00390] This volume can be contained in a cylinder of 2.5 mm radius and 2 mm height.

$$2.5^2\pi \times 2 = 39.27\text{mm}^3 \quad (\text{B.2})$$

[00391] This volume can also be contained a cube of 3.4 mm side length.

$$3.4^3 = 39.3\text{mm}^3 \quad (\text{B.3})$$

[00392] Finally, this volume can also be contained in a hemisphere of 2.7 mm radius.

$$2.7^3 \times \pi \times 4 \div 3 \div 2 = 41.2\text{mm}^3 \quad (\text{B.4})$$

[00393] Initial uptake studies conducted with hydrogel cylinders of 4 mm radius and 3.5 mm height demonstrated that the hydrogels were able to uptake ILs of the same weight as the gel (around 10 mg). Thus, assuming that the density of IL is 1 g/mL , and noting that most of the lyophilized hydrogels of 4 mm radius and 3.5 mm had a weight of around 10-15 mg, this indicated that around 10 mg, or 10 μL of IL was uptaken by each gel. This indicated that quadrupling the size of gels (currently around 40 mm^3) to a volume of 0.160 cm^3 , or 160 mm^3 would allow the gels to hold 40 μL of IL.

[00394] As stated above, accounting for this new volume necessary for the gels to hold 40 μL of ionic liquids, this volume can be achieved by a cylinder of 4 mm radius and 3.5 mm height.

$$4^2\pi \times 3.5 = 175.9\text{mm}^3 \quad (\text{B.5})$$

[00395] Given that the human TM has a radius of around 10 mm, the cross-sectional areas of this geometry is around 64% of the TM surface area, making this a feasible device for surgical placement. If drug delivery studies show a transfer rate higher than the aforementioned 0.1%, the device size and the volume of IL contained could be decreased.

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Example 2

[00397] Described herein is EarFlow, a drug delivery device placed on the eardrum to elute antibiotic- and steroid-loaded ionic liquids into the middle ear space to treat acute otitis media.

[00398] The current otitis media market is underserved and there is a need for non-invasive, effective drug delivery approach. Oral administration requires Multiday, multidose per day – making adherence and compliance challenging [2], has ineffective delivery and low bioavailability of drugs in the middle ear space [23], has systemic side effects due to high dosage [24], and may lead to antibiotic resistance [23]. Ear drops have limited permeability across the eardrum [3] and are only indicated when used with tympanotomy tubes [24]. Tympanostomy tubes feature invasive placement leaving a perforation in the eardrum, requires general anesthesia, is ineffective, has surgical complications [25], and is conducted in hospital or ambulatory surgery center (ASC)—more costly due to facility overhead [30]. Intratympanic injections are invasive and have surgical complications [23,27].

[00399] Drug delivery to the middle and inner ear has faced various challenges. Common treatment methods of middle and inner ear ailments often have systemic side effects, are invasive and cause surgical complications, or are ineffective in delivering drugs to the middle ear space. To achieve localized drug delivery, it would be ideal to place drugs on the tympanic membrane (TM), or eardrum, via the external auditory canal (EAC) to allow non-invasive drug delivery into the middle ear space. However, the TM is a near-impermeable membrane to most drugs.

[00400] Described herein is a hydrogel drug delivery device to be placed on the TM to sustain drug delivery across the TM. The hydrogel is composed of customizable hydrogel monomers and crosslinkers. The hydrogel is polymerized and then lyophilized to create a porous structure to hold high volumes of drug-loaded carrier solutions, including ionic liquids, which have been shown to be a superior drug delivery carrier than traditional saline solutions. The carrier solution and drugs, as well as their volumes and concentrations, can be tuned to adapt to different diseases or individual needs. The hydrogel can also be created in various geometries and sizes.

[00401] The hydrogel device will be placed on the TM via the EAC to readily elute drugs into the middle ear space. During treatment period, the device can be reloaded or replenished with drug solutions. After the treatment period is complete, the device can be removed from the TM via the EAC. This procedure can be achieved in an office setting without the need for general anaesthesia, indicating practical feasibility of device placement and removal.

[00402] Ionic liquids delivery more dexamethasone compared to saline, across intact ex vivo eardrums. Fig. 37.

[00403] Earflow permits rapid customization. The hydrogel starting materials can be customized to create gels with different mechanical and drug eluting properties. The hydrogel can also be loaded with different drug carriers and therapeutics to treat the desired indications.

[00404] Earflow achieves non-invasive and sustained drug delivery. EarFlow is shown to deliver drugs successfully across an intact membrane over a sustained period. As it is placed on the eardrum to elute drugs, it is noninvasive.

[00405] EarFlow has low cytotoxicity. EarFlow has low cytotoxicity to the two types of cells (human dermal fibroblasts and epidermal keratinocytes) on the eardrum.

[00406] EarFlow has low impact on hearing. Based on in silico modelling results, it is anticipated that the placement of EarFlow on the TM will have a low impact on the patient's hearing.

[00407] EarFlow delivers drugs locally and should not cause systemic side effects. Given that EarFlow delivers drugs locally into the middle ear space, it is anticipated that it will reduce systemic side effects associated with current oral antibiotic delivery.

[00408] EarFlow is surgically convenient to use. EarFlow can be easily and quickly placed on the TM in an office setting without the need for general anaesthesia. In vitro cell adhesion assays shows

that EarFlow remains bioinert, preventing any tissue or scar formation around the device and allowing it to be easily removed after the treatment period.

[00409] EarFlow can be administered non-invasively. There are no systemic side effects or development of antibiotic resistance. Procedures (like tympanostomy tube placement) conducted in hospitals and ambulatory surgery centers are more costly to patients and their parents due to higher copayments or coinsurance amounts. These procedures may also be subject to higher deductibles compared to services provided in a physician practice [30]. Ultimately EarFlow can improve compliance and save parents time and costs in the long term.

[00410] EarFlow is safer than tympanostomy tube insertion: 1. a reduction in the occurrence of a known serious adverse event—EarFlow would reduce surgical complications associated with tympanostomy tube placement 2. a reduction in the occurrence of a known device failure mode—tympanostomy tubes may prematurely fall out or are ineffective in draining fluids; EarFlow bypasses the need of tympanostomy tubes.

[00411] EarFlow hydrogel can hold tunable ionic liquids (ILs) and drugs. In vitro diffusion studies show improved delivery compared to control saline solutions. Device is easy to place on the eardrum through the ear canal, eliminating the need for surgical procedures and general anesthesia. Superior to existing eardrops: utilizes both the superior membrane permeability of ILs and the sustained delivery ability of hydrogels.

[00412] Fig. 38 depicts the input design factors and their influence on outcome.

[00413] Potential therapeutics for delivery by EarFlow include steroids, antibiotics, small molecules, viral vectors / gene therapy, growth factors, proteins. Ionic liquid-hydrogel platform may be used to load other therapeutics for treating other ailments. EarFlow can reduce incidence of systemic side effects and antibiotic resistance; reduce medical spending; improve patient experience. EarFlow is a non-invasive platform to achieve localized, sustained drug delivery to treat otitis media.

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[00415] *Example 3*

[00416] Fig. 44 depicts a graph of EarFlow mediated drug delivery across intact tissues.

Dexamethaone delivery amounts are shown for the time periods and tissues (porcine skin) indicated.

[00417] The compositions described herein are contemplated for use in drug delivery through the eye and mucosal membranes; drug delivery through the skin; drug delivery through the gastrointestinal tissue/tract or genitourinary tract; for treatment of: otitis externa (swimmer’s ear), sudden sensorineural hearing loss, age and noise related hearing loss, infection, cancer, and trauma related hearing loss; and drug delivery via subcutaneous implant of hydrogel-drug depot in cavities of interest.

What is claimed herein is:

1. A composition comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid.
2. The composition of claim 1, wherein the at least one loading liquid comprises at least one ionic liquid.
3. A kit or combination comprising at least one hydrogel scaffold and at least one of: at least one drug and at least one loading liquid.
4. The kit or combination of claim 3, wherein the at least one loading liquid comprises at least one ionic liquid.
5. The composition, kit, or combination of any one of the preceding claims, wherein the at least one ionic liquid has a cationic component and an anionic component; and

wherein the cationic component is selected from the group consisting of benzyl pyridinium, benzyl dimethyl dodecyl ammonium, a choline cation, phosphonium, tetraalkylphosphonium, and benzethonium; and

wherein the anionic component is selected from the group consisting of bistriflimide, a geranate anion, oleate, 2-octenoic acid, hexanoate, dodecyldimethyl ammonia propane sulfonate, N-Lauryl sarconsinate, and geraniolate.
6. The composition, kit, or combination of any one of the preceding claims, wherein the at least one ionic liquid comprises choline and geranic acid (CAGE), choline and hexenoic acid (CAHA), or choline and 2-octenoic acid.
7. The composition, kit, or combination of any one of the preceding claims, wherein the at least one ionic liquid comprises choline and geranic acid (CAGE) or choline and hexenoic acid (CAHA).
8. The composition, kit, or combination of any one of the preceding claims, comprising at least 30 μL of the at least one ionic liquid.
9. The composition, kit, or combination of any one of the preceding claims, comprising at least 40 μL of ionic liquid.

10. The composition, kit, or combination of any one of the preceding claims, comprising at least 200 μ L of ionic liquid.
11. The composition, kit, or combination of any one of the preceding claims, comprising at least 1000 μ L of ionic liquid.
12. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs are selected from: antibiotics; steroids; growth factors; biologics; viral vectors; polypeptides; nucleic acid molecules; polysaccharides; anesthetics; and external, middle or inner ear disease therapeutics.
13. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs comprise at least one antibiotic.
14. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs comprise at least one steroid.
15. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs comprise at least one antibiotic and steroid.
16. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs are selected from dexamethasone and ciprofloxacin.
17. The composition, kit, or combination of any one of the preceding claims, wherein the one or more drugs comprise dexamethasone and ciprofloxacin.
18. The composition, kit, or combination of any one of the preceding claims, comprising at least 1 mg/mL of dexamethasone.
19. The composition, kit, or combination of any one of the preceding claims, comprising 100-400 mg/mL of dexamethasone.
20. The composition, kit, or combination of any one of the preceding claims, comprising 200 mg/mL of dexamethasone.
21. The composition, kit, or combination of any one of the preceding claims, comprising at least 1 mg/mL of ciprofloxacin.
22. The composition, kit, or combination of any one of the preceding claims, comprising 100-500 mg/mL of ciprofloxacin.

23. The composition, kit, or combination of any one of the preceding claims, comprising 250 mg/mL of ciprofloxacin.
24. The composition, kit, or combination of any one of the preceding claims, comprising 200 mg/mL of ciprofloxacin.
25. The composition, kit, or combination of any one of the preceding claims, wherein the hydrogel scaffold comprises, consists of, or consists essentially of one or more of: at least one polyacrylate, at least one polymethacrylate, at least one protein, at least one polysaccharide, poly(glycerol sebacate) (PGS), and poly-(d,l-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA triblock polymer.
26. The composition, kit, or combination of claim 25, wherein the at least one polyacrylate and/or at least one polymethacrylate comprises, consists of, or consists essentially of one or more of: poly(2-hydroxyethyl methacrylate) (pHEMA), poly(acrylic acid) (PAA), poly(methyl methacrylate) (PMMA), hydroxyethyl acrylate (HEA), poly(2-hydroxyethyl acrylate) (pHEA), polyacrylamide (PAM), poly(acrylonitrile-co-acrylic acid) (PANCAA), gelatin methacrylate (GelMA), hyaluronic acid methacrylate (MeHA); poly(epoxy methacrylate) (pEMA), poly(acryl amide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylamido glycolic acid), poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), poly(ethyl acrylate) (PEA), and poly(ethyl methacrylate) (PEMA).
27. The composition, kit, or combination of claim 25, wherein the at least one protein comprises, consists of, or consists essentially of one or more of: collagen, gelatin, and silk fibroin.
28. The composition, kit, or combination of claim 25, wherein the at least one polysaccharide comprises, consists of, or consists essentially of one or more of: hyaluronic acid, glycosaminoglycans, agarose, alginate, and chitosan.
29. The composition, kit, or combination of any one of the preceding claims, wherein the hydrogel scaffold comprises, consists of, or consists essentially of poly(2-hydroxyethyl methacrylate) (pHEMA).
30. The composition, kit, or combination of any one of the preceding claims, further comprising at least one hydrogel crosslinker.
31. The composition, kit, or combination of claim 30, wherein the at least one hydrogel crosslinker comprises at least one of: ethylene glycol dimethacrylate (EGDMA), N,N'-methylenebisacrylamide (MBAA-crosslinker), ammonium persulfate (APS-initiator), tetramethylethylenediamine (TEMEDA-catalyst), and Fe³⁺ (ionic cross linker).

32. The composition, kit, or combination of any one of the preceding claims, wherein the hydrogel scaffold does not comprise a crosslinker.
33. The composition, kit, or combination of any one of the preceding claims, further comprising a radical initiator or photoinitiator.
34. The composition, kit, or combination of claim 33, wherein the radical initiator or photoinitiator is azobisisobutyronitrile (AIBN).
35. The composition, kit, or combination of any one of the preceding claims, wherein the hydrogel scaffold is not formulated with water.
36. The composition, kit, or combination of any one of the preceding claims, wherein the composition has a size no greater than 12 mm by 12 mm by 12 mm.
37. The composition, kit, or combination of any one of the preceding claims, wherein the composition has a size no greater than 8 mm by 8 mm by 8 mm.
38. The composition, kit, or combination of any one of the preceding claims, wherein the composition has a size of at least 8 mm by 8 mm by 7.5 mm.
39. The composition, kit, or combination of any one of the preceding claims, wherein the composition has a size of at least 5.4 mm by 5.4 mm by 2.7 mm.
40. A method of drug delivery, the method comprising administering, to a patient in need of a drug, the composition of any of the preceding claims, wherein the composition comprises the drug.
41. The method of claim 40, wherein the composition is administered by placing the composition in contact with the ear, the outer ear, ear canal, tympanic membrane, oval window, round window membrane, eustachian tube, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.
42. A method of treating an ear condition or disease, the method comprising administering to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient the composition of any of the preceding claims.
43. The method of any one of the preceding claims, wherein the administration comprises circumferential application or contact with the skin of the ear canal.
44. The method of any one of the preceding claims, wherein the administration comprises contact with the auricular concha and pinna.

45. The method of any one of the preceding claims, wherein the ear condition or disease is selected from the group consisting of:

otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss.

46. The method of any one of the preceding claims, wherein the ear condition or disease is otitis media.

47. The method of any one of the preceding claims, wherein the composition is administered by placing the composition in contact with the tympanic membrane, oval window, round window membrane, eustachian tube or ear canal.

48. The method of any one of claims 42-47, wherein the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, oval window or the round window membrane.

49. The method of any one of claims 42-47, wherein the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the round window, and/or the oval window.

50. The method of any one of the preceding claims, wherein the ear condition or disease is otitis externa.

51. The method of any one of claims 42-47, wherein the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.

52. The method of any one of the preceding claims, wherein the composition is left in the ear for at least 2 days.

53. The method of any one of the preceding claims, wherein the composition is left in the ear for at least 3 days.

54. The method of any one of the preceding claims, wherein the composition is left in the ear for at least 5 days.

55. The method of any one of the preceding claims, wherein the composition is left in the ear for at least 7 days.
56. The method of any one of the preceding claims, wherein the composition is left in the ear for no more than 10 days.
57. The method of any one of the preceding claims, wherein the composition is left in the ear for no more than 28 days.
58. The method of any one of the preceding claims, wherein the composition is left in the ear for no more than 12 months.
59. The method of any one of the preceding claims, wherein the composition is physically removed from the ear after at least 7 days.
60. The method of any one of the preceding claims, wherein the composition is physically removed from the ear after at least 10 days.
61. The method of any one of the preceding claims, wherein the composition is physically removed from the ear within 10 days.
62. The method of any one of the preceding claims, wherein the composition is physically removed from the ear within 28 days.
63. The method of any one of the preceding claims, wherein the composition is physically removed from the ear within 12 months.
64. The composition of any one of claims 1-40, for use in a method of drug delivery.
65. The composition of claim 64, wherein the method comprises administering the by placing the composition in contact with the ear, the outer ear, ear canal, tympanic membrane, oval window, round window membrane, eustachian tube, the skin, the eye, the gastrointestinal tissue/tract, the genitourinary tract, a mucosal membrane (e.g., mouth or nose), or a subcutaneous tissue or cavity.
66. The composition of any one of claims 1-40, for use in a method of treating an ear condition or disease, the method comprising administering the composition to the outer ear, ear canal, tympanic membrane, oval window, round window membrane, or eustachian tube of a patient.
67. The composition of any one of claims 64-66, wherein the administration comprises circumferential application or contact with the skin of the ear canal.

68. The composition of any one of claims 64-67, wherein the administration comprises contact with the auricular concha and pinna.
69. The composition of any one of claims 64-68, wherein the ear condition or disease is selected from the group consisting of:
- otitis media; otitis externa; sensorineural hearing loss; conductive hearing loss; age and noise related hearing loss; infection; inflammation; tympanic membrane perforation; genetic forms of hearing loss; Meniere's disease; vestibular hypofunction; labyrinthitis; vestibular neuritis; vertigo; chronic eczematous otitis externa (itchy ear canal); chronic stenosing otitis externa; myringitis; mucosalization of the ear drum, ear canal or mastoid bowl; cancer; and trauma related hearing loss.
70. The composition of any one of claims 64-69, wherein the ear condition or disease is otitis media.
71. The composition of any one of claims 64-70, wherein the composition is administered by placing the composition in contact with the tympanic membrane, oval window, round window membrane, eustachian tube or ear canal.
72. The composition of any one of claims 69-71, wherein the ear condition or disease is otitis media, sudden sensorineural hearing loss, or age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, oval window or the round window membrane.
73. The composition of any one of claims 69-71, wherein the ear condition or disease is age and noise related hearing loss and the composition is administered by placing the composition in contact with the tympanic membrane, the round window, and/or the oval window.
74. The composition of any one of claims 69-71, wherein the ear condition or disease is otitis externa.
75. The composition of any one of claims 69-71, wherein the ear condition or disease is otitis externa and the composition is administered by placing the composition in contact with the ear canal epithelium.
76. The composition of any one of claims 64-75, wherein the composition is left in the ear for at least 2 days.
77. The composition of any one of claims 64-76, wherein the composition is left in the ear for at least 3 days.

78. The composition of any one of claims 64-77, wherein the composition is left in the ear for at least 5 days.
79. The composition of any one of claims 64-78, wherein the composition is left in the ear for at least 7 days.
80. The composition of any one of claims 64-79, wherein the composition is left in the ear for no more than 10 days.
81. The composition of any one of claims 64-80, wherein the composition is left in the ear for no more than 28 days.
82. The composition of any one of claims 64-81, wherein the composition is left in the ear for no more than 12 months.
83. The composition of any one of claims 64-82, wherein the composition is physically removed from the ear after at least 7 days.
84. The composition of any one of claims 64-83, wherein the composition is physically removed from the ear after at least 10 days.
85. The composition of any one of claims 64-84, wherein the composition is physically removed from the ear within 10 days.
86. The composition of any one of claims 64-85, wherein the composition is physically removed from the ear within 28 days.
87. The composition of any one of claims 64-86, wherein the composition is physically removed from the ear within 12 months.

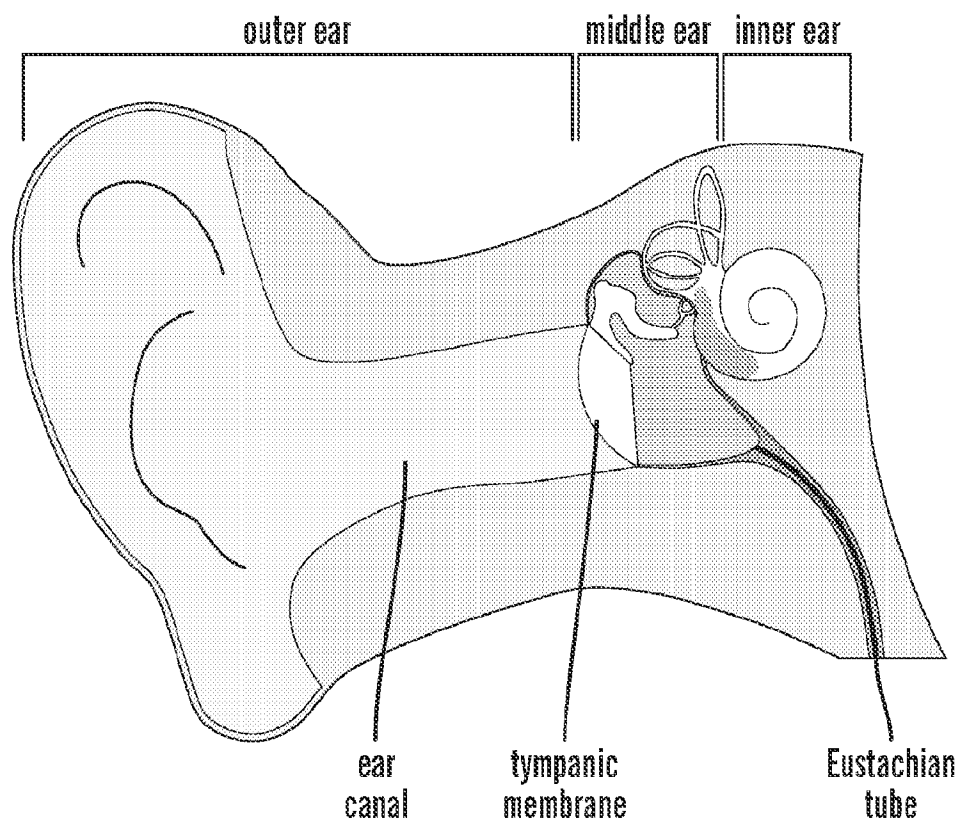


FIG. 1

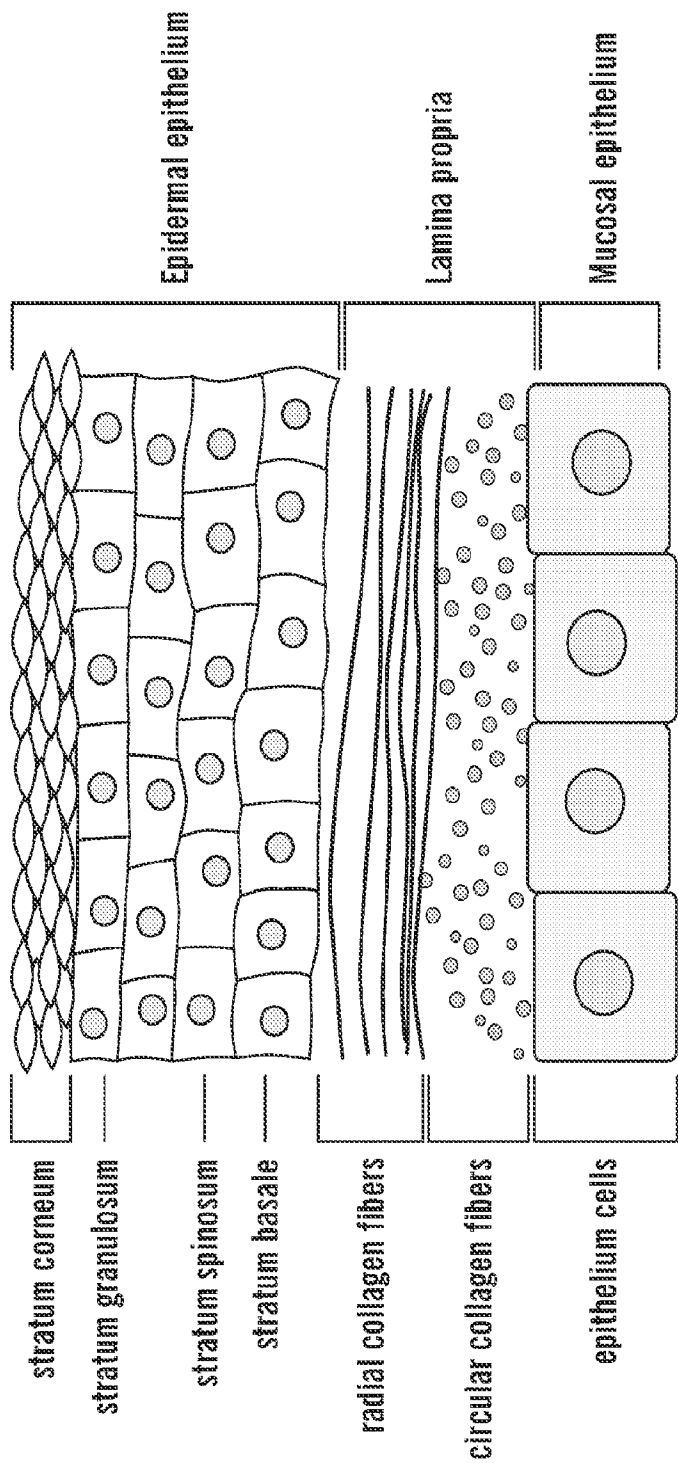


FIG. 2

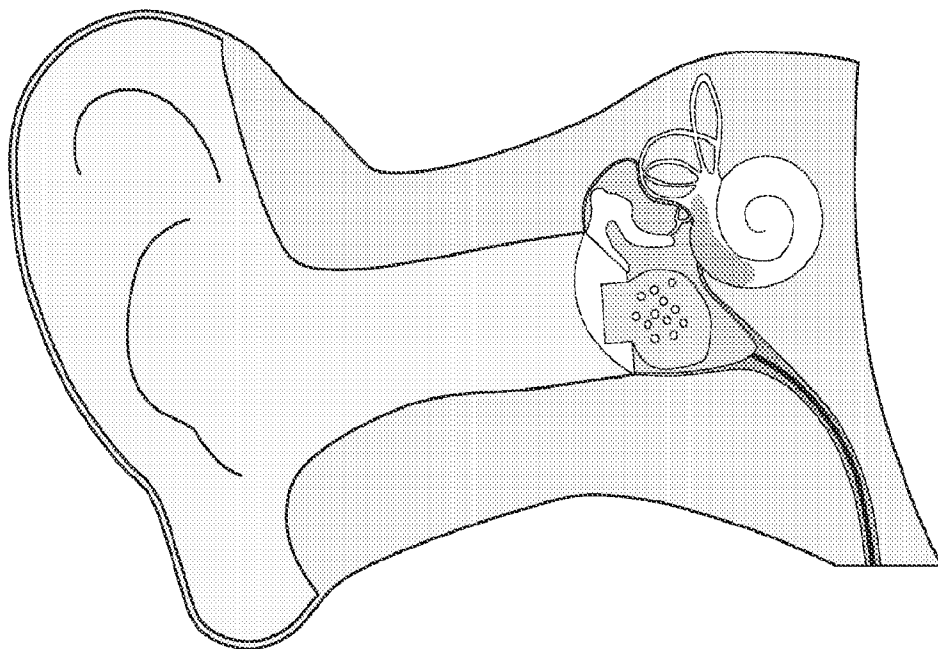


FIG. 3

	Efficacy	Invasiveness	Cytotoxicity	Impact on Hearing	Local vs. Systemic Effects	Convenience
Oral administration of antibiotics	Low	Low	Low	Low	Systemic	Inconvenient; multi-day/multi-dose treatment
Antibiotic otic drops (ear drops)	Low	Low	Low	Low	Local	Convenient
Tympanostomy tubes	Low	High	Low	High	Local	Inconvenient; requires general anesthesia
Iontophoresis coupled with tympanostomy tubes	High	Low	Low	High	Local	Inconvenient; requires bulky external device
Intratympanic injection	High	High	Low	High	Local	Inconvenient; requires general anesthesia
Hydrogel drug delivery	High	Low	High	High	Local	Convenient

FIG. 4

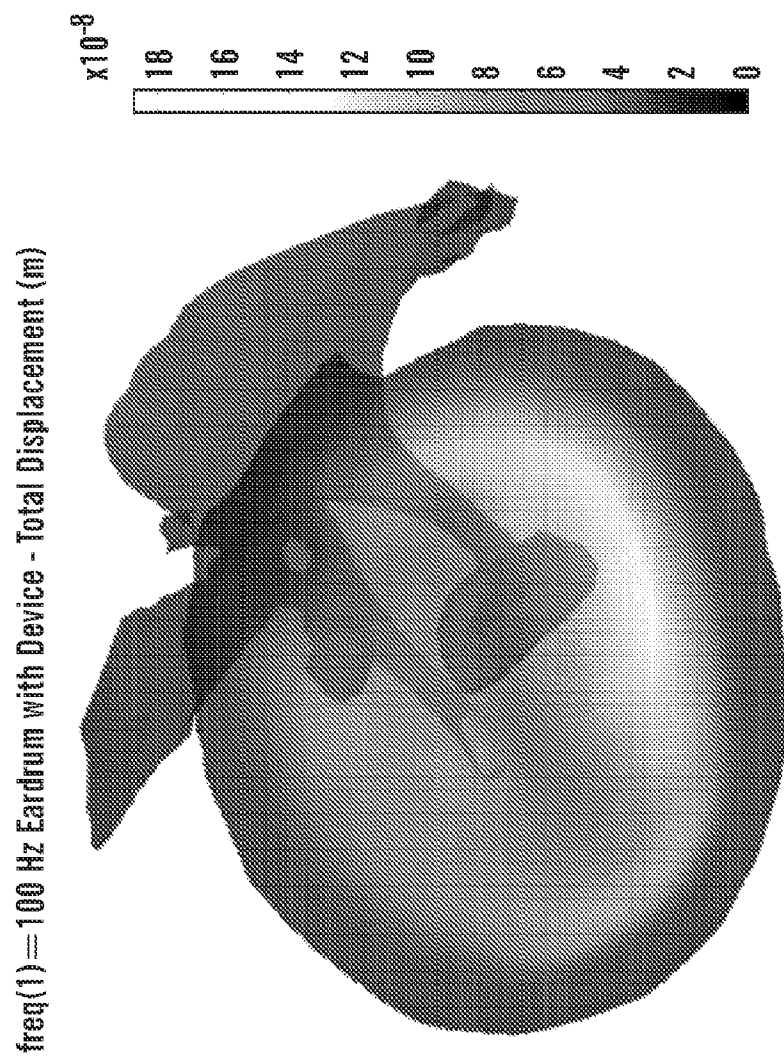


FIG. 5

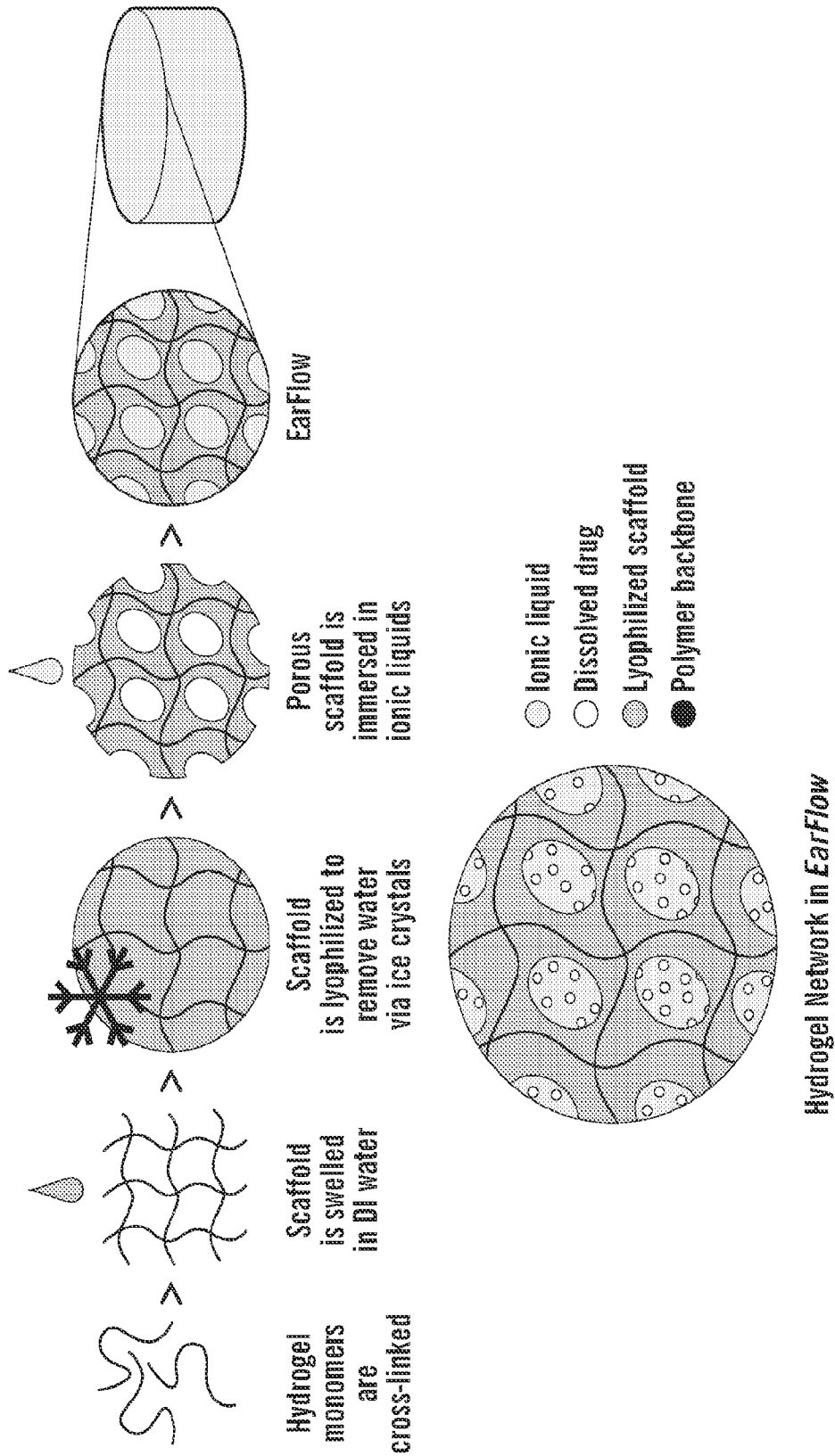


FIG. 6

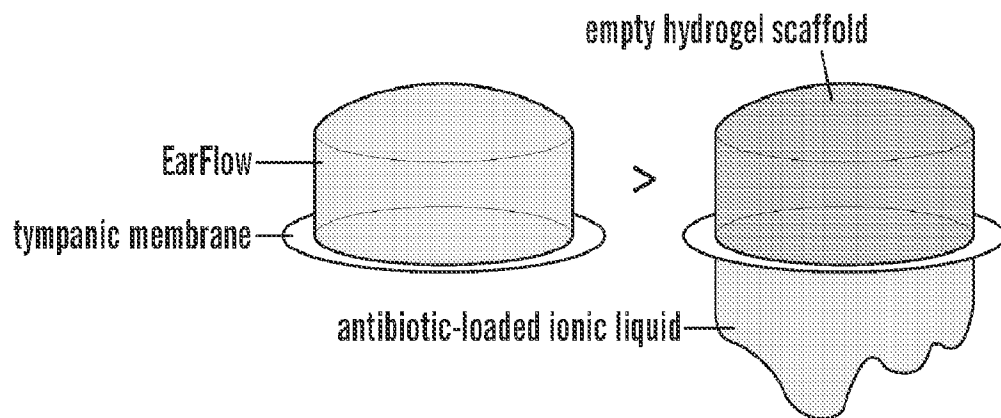


FIG. 7

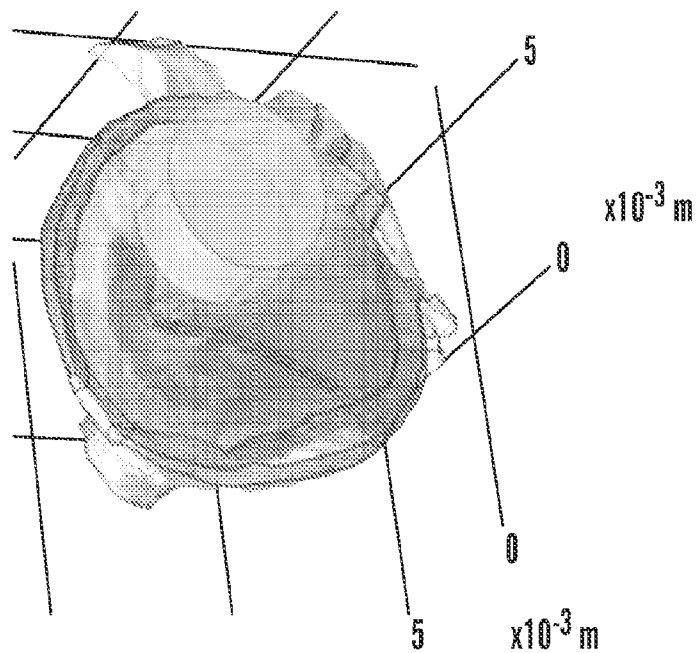
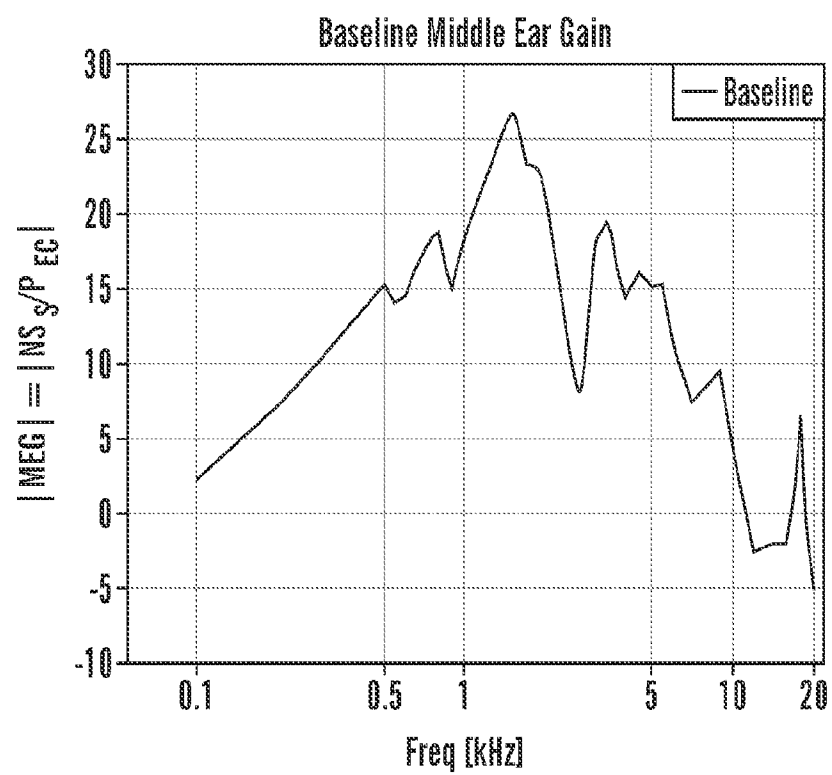


FIG. 8A

***FIG. 8B***

Specification	Target Range	Reasoning	Measurement
Device Manufacturing			
Device radius	≤ 4 mm	The human TM has a diameter of 8-10 mm; <i>EarFlow</i> should occupy less than 70% of the TM surface to minimize its effects on hearing	Select biopsy punch of ≤ 8 mm diameter
Porosity of scaffold	$> 80\%$	This porosity would allow the minimum size scaffold (a 2 mm radius and 2 mm height cylinder) to hold enough volume of ILs that contain one course of antibiotic and steroid treatment	Swelling measurement of hydrogels
Drug Delivery and Release			
Loading concentration of drugs in IL (dosage)	≥ 250 mg/mL ciprofloxacin	Based on calculations in consideration of the minimal inhibitory concentration of ciprofloxacin against <i>H. influenza</i> and <i>S. pneumoniae</i> , and a treatment period of 7 days, 250 mg/mL was the necessary concentration of ciprofloxacin to clear OM	Weigh the amount of drug loaded in IL
Total volume of IL held in device	> 40 μ L	40 μ L was the anticipated volume necessary for delivering one course of antibiotics before reloading the device	Loading with pipettes and measurement of swelling ratios of lyophilized hydrogels in IL

FIG. 9A

Specification	Target Range	Reasoning	Measurement
Sustained delivery timeframe	>7 days	Antibiotics courses are typically of 7 days to prevent antibiotic resistance and recurring infection	Measure <i>in vitro</i> drug release in a diffusion chamber system via UV spectrophotometry
Device degradation rate	No degradation in 7 days	The degradation of hydrogel scaffolds may significantly affect their physical properties (including the drug release profile). The device should not show any sign of degradation within 7 days (the length of which the device would be placed on the TM)	Manufacture the device with non-hydrolysable hydrogel monomer, pHEMA
Cytotoxicity			
Cytotoxicity of ionic liquids	>90% cell viability	The IL should be biocompatible when in contact with live cells	<i>In vitro</i> cytotoxicity assays with fibroblasts and keratinocytes
Cytotoxicity of hydrogel device	>90% cell viability	Treatment with <i>EarFlow</i> must not destroy the trilaminar structure of the TM; the trilaminar structure of the TM protects the middle ear space from external stimuli or bacteria. The physical structure of TM is important for proper sound conduction	<i>In vitro</i> cytotoxicity assays with fibroblasts and keratinocytes
Clinical Considerations			
Hearing changes	<15 dB decrease	Existing devices worsen hearing by at least 15 dB, which is considered a significant change in hearing, based on consultation with ENT surgeons	Validate <i>in silico</i> with COMSOL model and potentially in animal studies

FIG. 9B

Specification	Target Range	Reasoning	Measurement
Easy to place into and remove from the EAC	Device placement in under 10 minutes	Allows ENT surgeons to place the device easily without anesthesia procedures; improves patient compliance. This value was also based on literature review of existing in-office procedures	Consult ENT surgeons and test feasibility of placement in EAC and test the device placement in an anatomically accurate model of the human EAC
Device adhesion	Device must be able to adhere to the TM	Adhesion to the TM allows the device to effectively sustain drug elution without dislodging during normal human motion	Validate that the device can adhere to porcine tissues without dislodging
Cell adhesion	$\leq 10\%$ of cell attachment (i.e. bioinert)	To ensure that the device does not integrate into the TM and can be easily removed from the TM after treatment, the device must not allow for more than 10% of cell adhesion	Cell adhesion studies with human keratinocytes

FIG. 9C

Specification	Reasoning	Verification
The complete TM in the model is between 80 -100 μm thick	This is the physiological accurate range of the human TM thickness; using an anatomically accurate representation of the human middle ear maximizes the translatability of <i>in silico</i> results of <i>EarFlow</i> placement, allowing stronger prediction of the effects in a human middle ear	The Puria group's FE model was used, which is based on a micro-CT scan and thus anatomically accurate
The TM has a diameter of around 10 mm	This is the physiological accurate range of the human TM size; using an anatomically accurate representation of the human middle ear maximizes the translatability of <i>in silico</i> results of <i>EarFlow</i> placement, allowing stronger prediction of the effects in a human middle ear	The Puria group's FE model was used, which is based on a micro-CT scan and thus anatomically accurate

FIG. 10A

Specification	Reasoning	Verification
The TM is conically shaped	The conical shape of the TM allows recapitulating of a key geometry of the actual human TM and allows accurate modeling of the TM	The Puria group's FE model has a conically shaped TM, as produced from the micro-CT scan
The TM must have a Young's modulus of between 10 - 300 MPa	The Young's modulus of the TM has been previously reported as between 10 - 300 MPa	The Puria group's finite element model has a Young's modulus of $0.75\text{e}8 - 1.4\text{e}8$ Pa, depending on the direction of measurement
Model must be acoustically and mechanically coupled	This allows the data output from the model with <i>EarFlow</i> placement to be an accurate representation of the changes in both the acoustics (hearing) and mechanics (motion) of the TM	The Puria group's FE model is acoustically and mechanically coupled
Model is amenable to changes in device placement, geometry, density, and size	This allows the analysis of how changes in parameters – including device location, radius, size, density, and geometry – affects hearing	The FE model was verified to allow these variations in parameters
Model outputs data representative of sound conduction	This allows the analysis of the impact of <i>EarFlow</i> on the TM on sound conduction	The FE model outputted data that can be used to calculate MEG, a proxy for sound conduction

FIG. 10B

Specification	Reasoning	Verification
The complete TM in the model is between 80 - 100 μm thick	This is the physiologically accurate range of the human TM thickness; using an anatomically accurate representation of the human middle ear maximizes the translatability of <i>in silico</i> results of <i>EarFlow</i> placement, allowing for better prediction of the effects in a human middle ear	The FE model was built based on this literature-reported value of the TM thickness
The epidermal epithelium layer of the TM should be around 20 μm for a 80 μm thick TM	This value was obtained from measurements based on a histologically stained image of the TM (Figure A.1)	The FE model was built based on these proportions
The radial collagen fiber layer and circular collagen fiber layer should be around 30 μm and 10 μm for a 80 μm thick TM, respectively	This value was obtained from measurements based on a histologically stained image of the TM (Figure A.1)	The FE model was built based on these proportions
The mucosal epithelium layer should be around 20 - 25 μm for a 80 μm thick TM	This value was obtained from measurements based on a histologically stained image of the TM (Figure A.1)	The FE model was built based on these proportions
The model should allow the diffusion coefficient of the transported species to be varied between $1\text{e-}5 - 1\text{e-}11 \text{ m}^2/\text{s}$	This is a relevant range of diffusion coefficient values, as determined by prior literature review	The model was run with values within this range and underwent troubleshooting if the model does not run successfully
The model should output diffusion data	To allow the analysis of drug diffusion across the TM, the model should output diffusion data	Able to generate data on the diffused molecule concentration

FIG. 11

Solution	HEMA	EGDMA	MilliQ water
1A	100%	0%	0%
1B	95%	5%	0%
2A	100%	0%	20%
2B	95%	5%	20%

FIG. 12

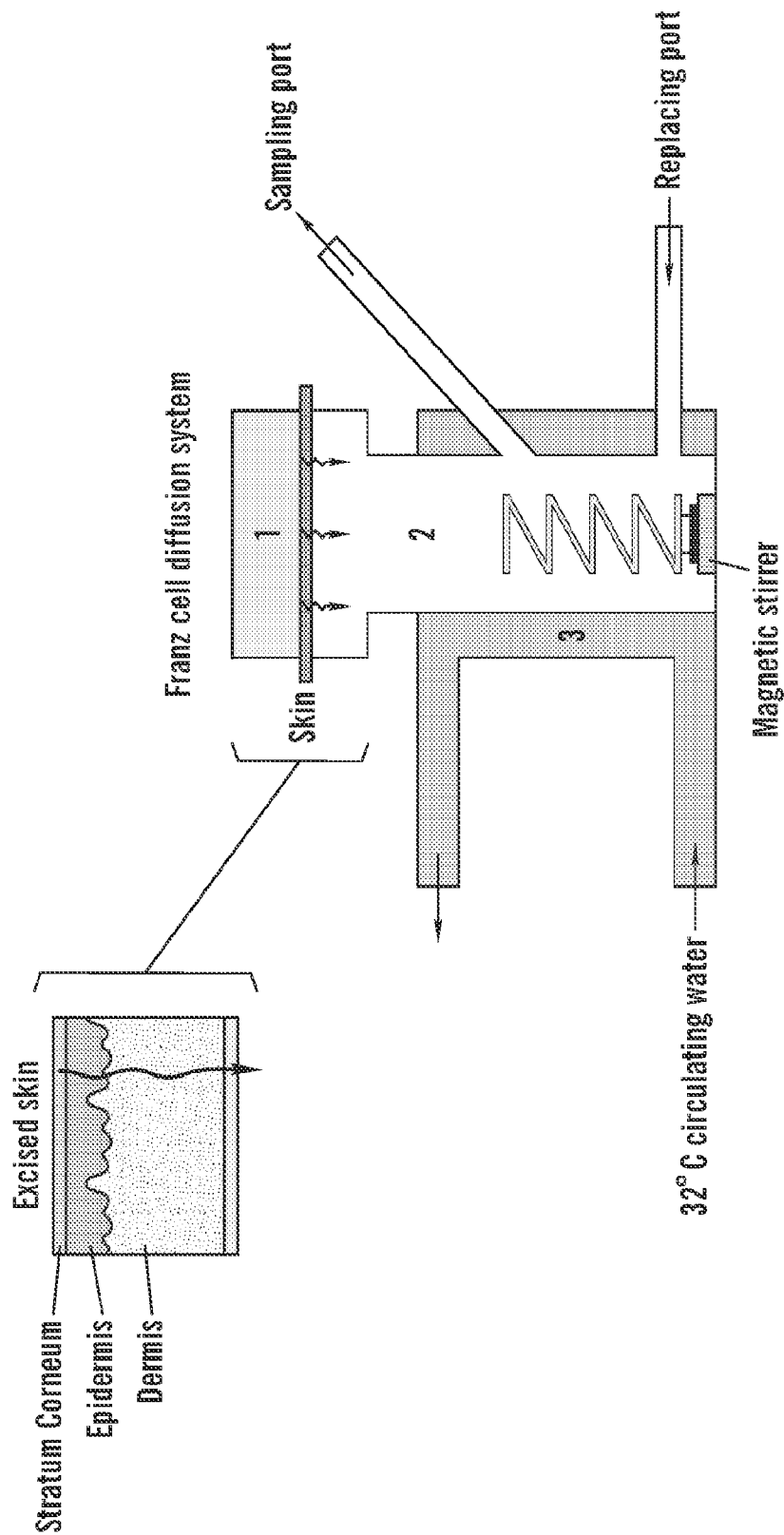


FIG. 13

Parameter	Range of values to test
Device radius	2, 2.25, 2.50, 2.75 mm
Device geometry	Cylinder, hemisphere, square
Device location on the TM	Anterior-inferior, anterior-superior, posterior-inferior, posterior-superior
Device density	1000, 1200, 1400, 1600, 1800, 2000 $\frac{kg}{m^3}$

FIG. 14

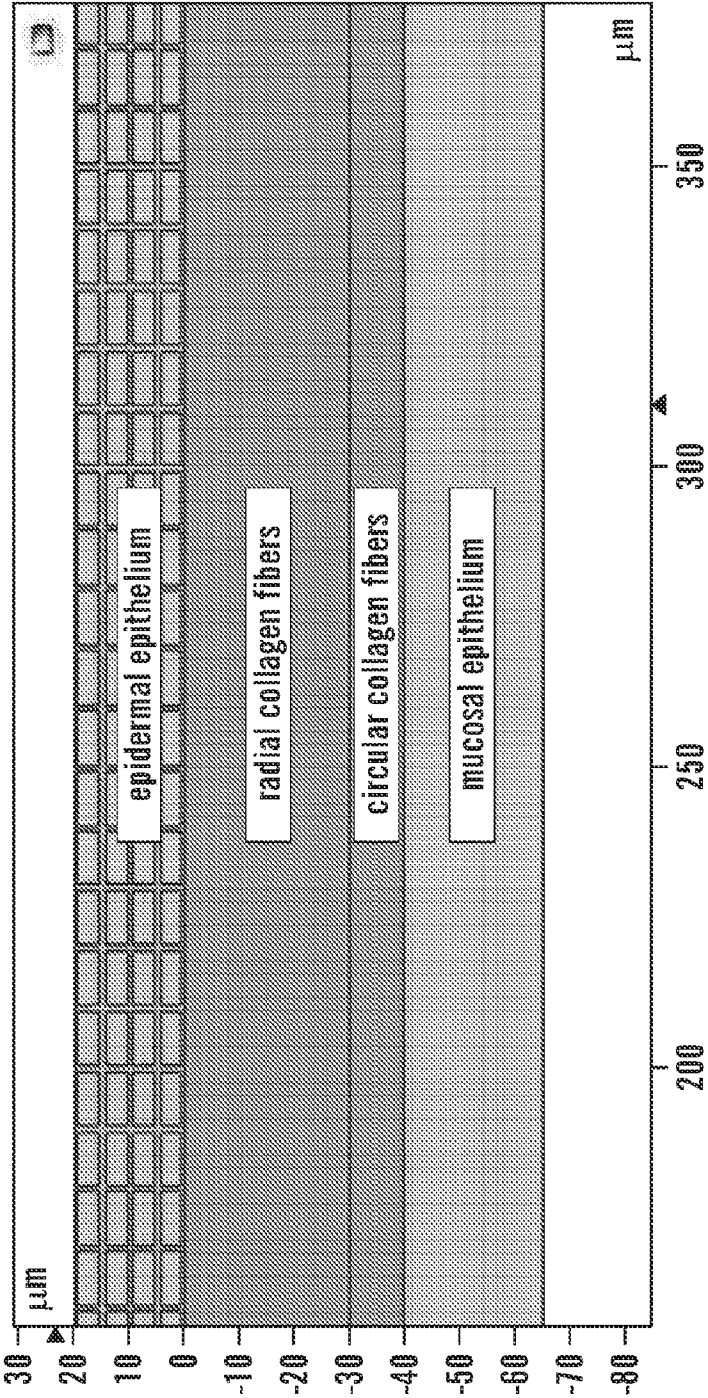


FIG. 15

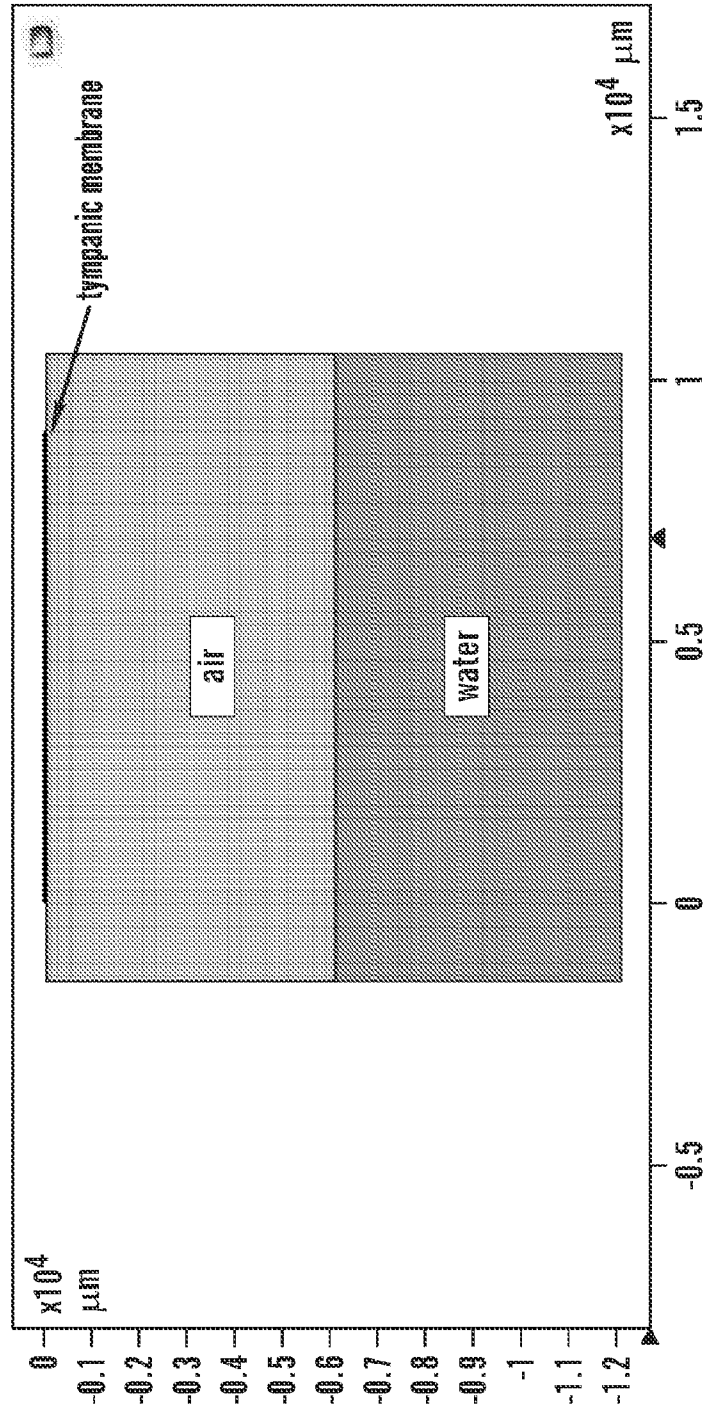


FIG. 16

Parameter	Value
Average velocity at the inlet	0.0001 m/s
Initial concentration of ciprofloxacin in middle ear	0 mol/m ³
Initial concentration of ciprofloxacin at inlet	100 mg/mL or 301.8 mol/m ²
Permeability of keratinocytes/cells (K_c)	10 ⁻¹⁷ m ²
Permeability of lipids (K_l)	10 ⁻¹⁷ m ²
Diffusion coefficient in keratinocytes/cells (D_c)	10 ⁻¹⁰ cm ² /s = 10 ⁻¹⁴ m ² /s
Diffusion coefficient in lipids (D_l)	10 ⁻¹⁹ cm ² /s = 10 ⁻¹³ m ² /s
TM thickness	85 μ m
TM 2D length	9000 μ m
Presence of liquid in middle ear space chamber	50%

FIG. 17

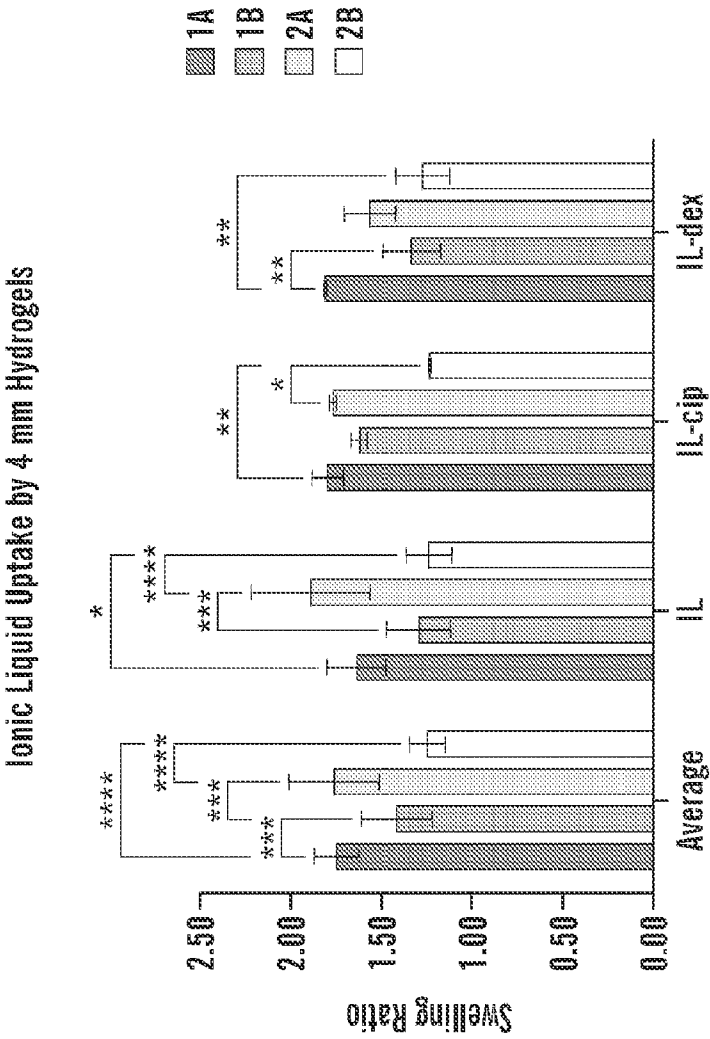


FIG. 18

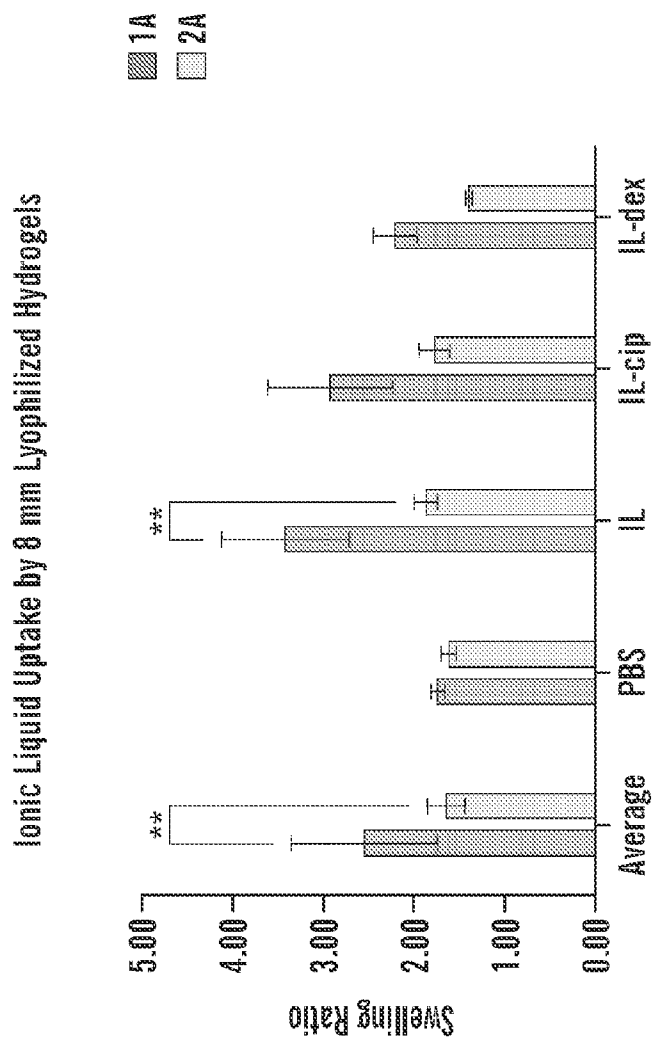


FIG. 19

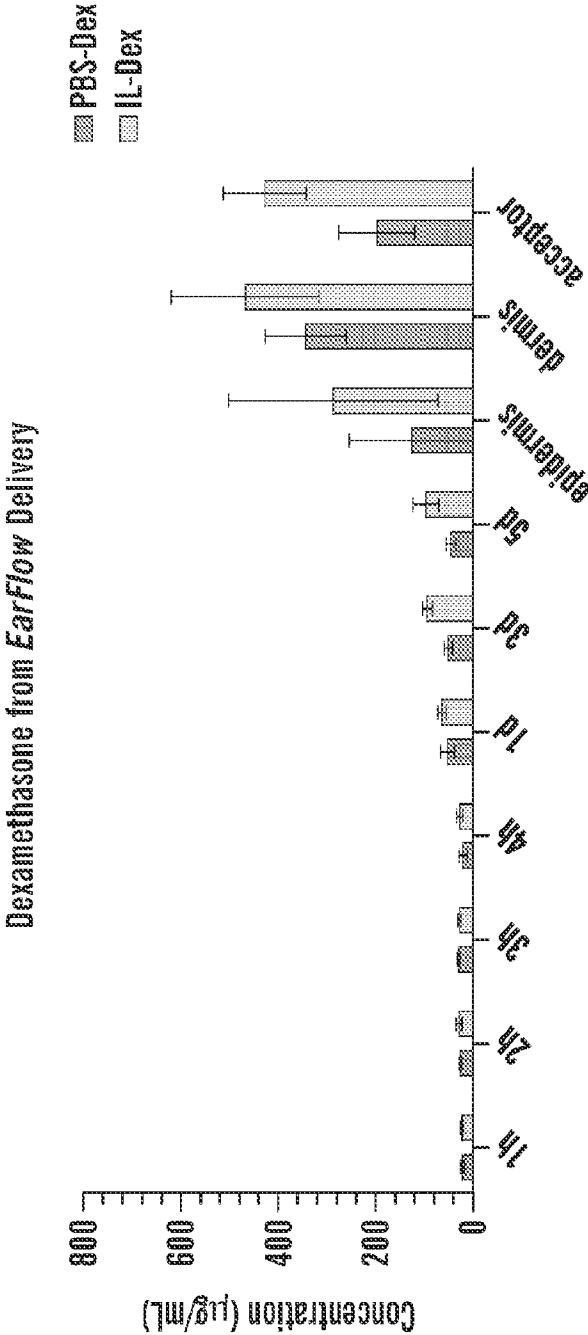


FIG. 20

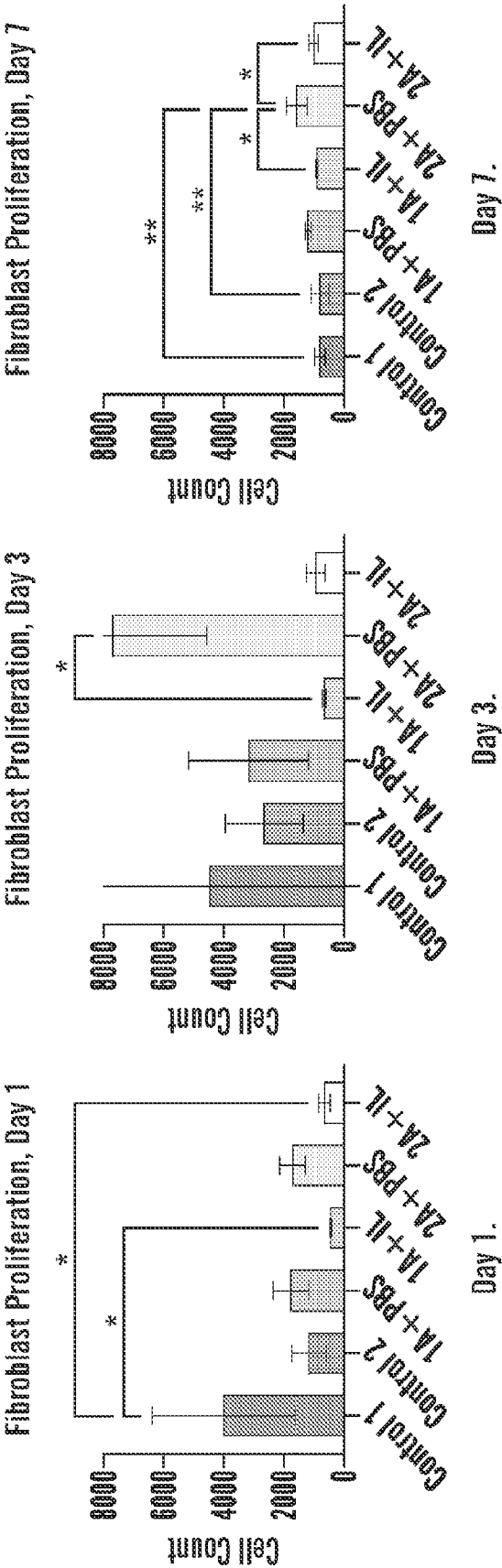


FIG. 21A

FIG. 21B

FIG. 21C

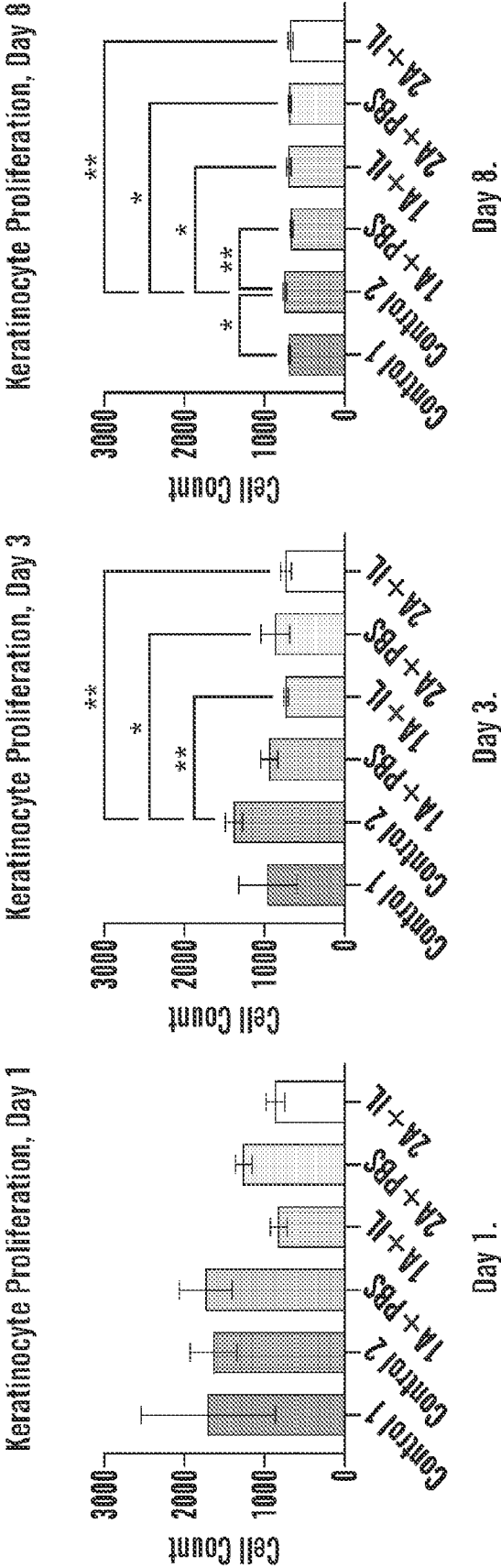


FIG. 22A

FIG. 22B

FIG. 22C

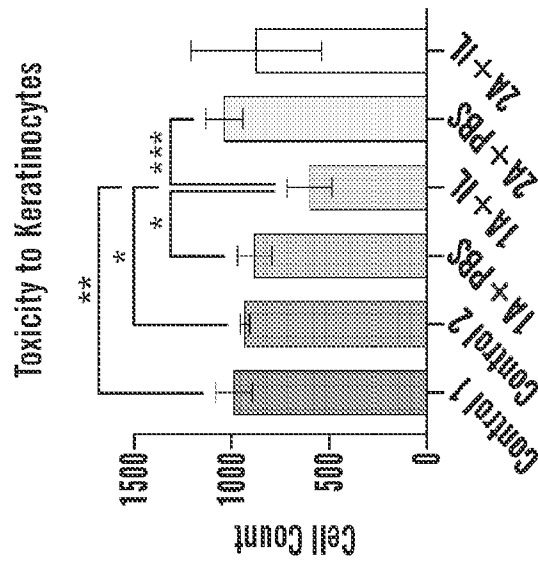


FIG. 23B

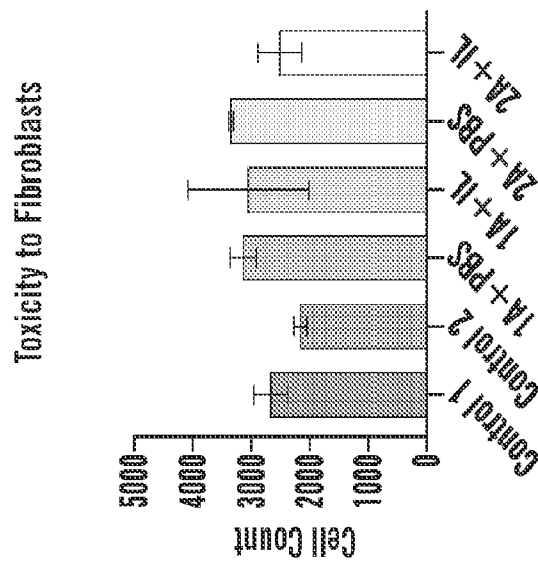


FIG. 23A

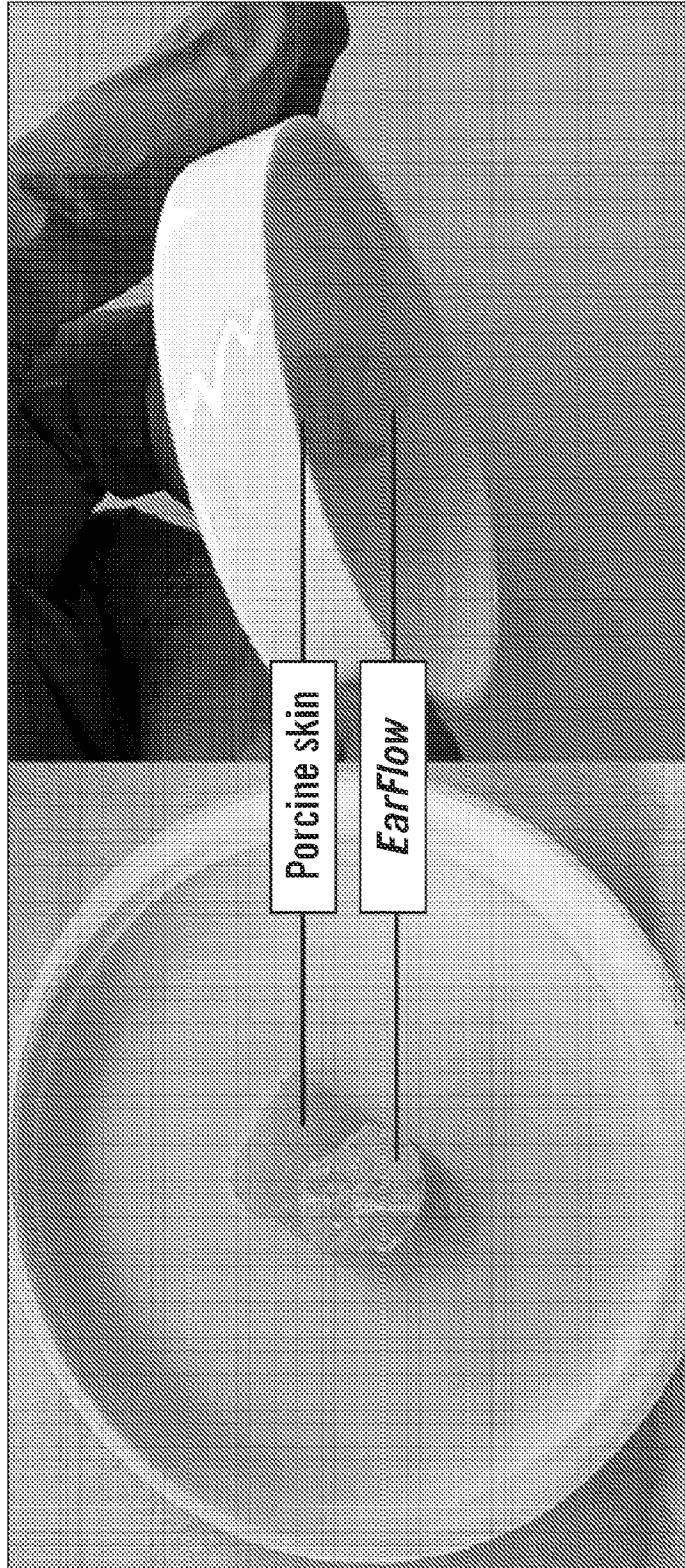
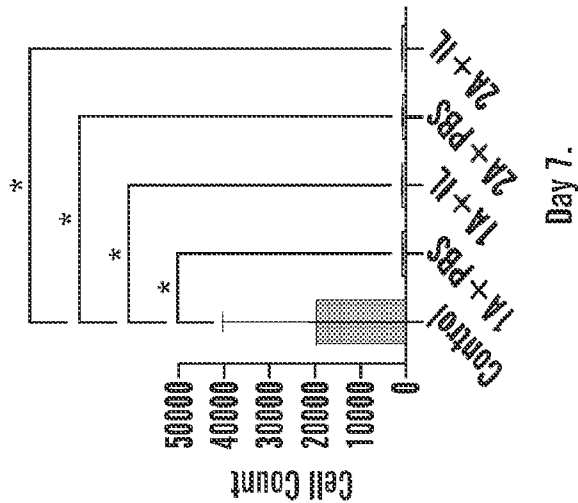
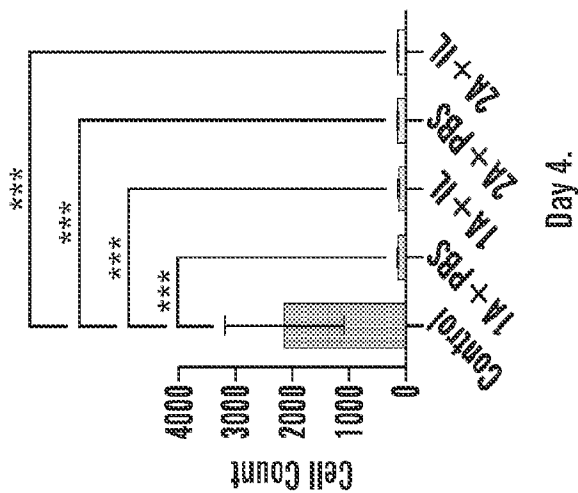


FIG. 24

Keratinocyte Attachment, Day 7



Keratinocyte Attachment, Day 4



Keratinocyte Attachment, Day 1

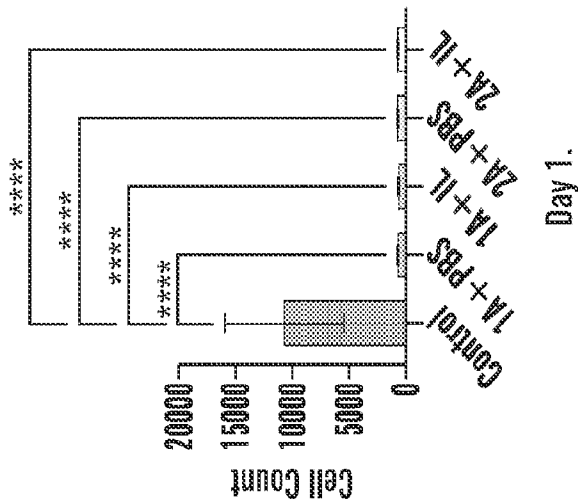
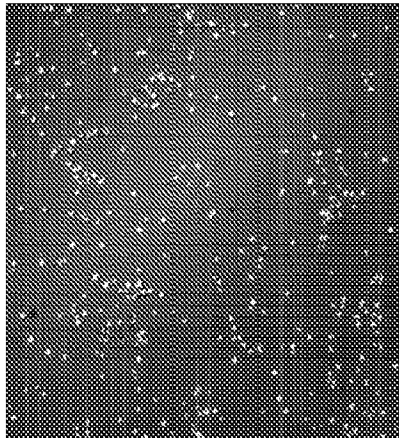


FIG. 25C

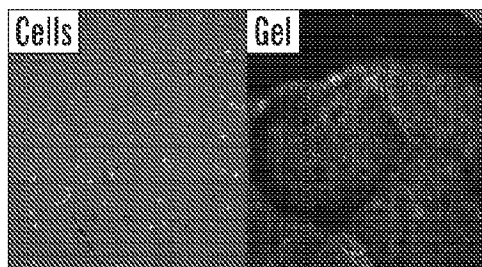
FIG. 25B

FIG. 25A



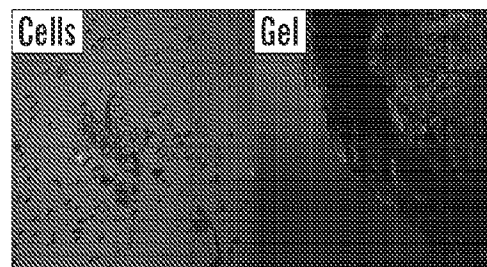
Cells cultured without hydrogel.

FIG. 26A



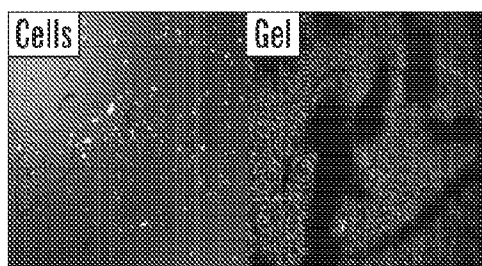
Cells cultured with 1A hydrogel
swelled with saline.

FIG. 26B



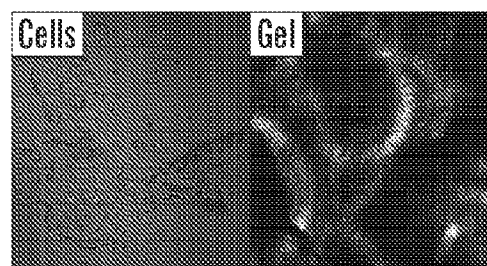
Cells cultured with 1A hydrogel
swelled with ionic liquid.

FIG. 26C



Cells cultured with 2A hydrogel
swelled with saline.

FIG. 26D



Cells cultured with 2A hydrogel
swelled with ionic liquid.

FIG. 26E

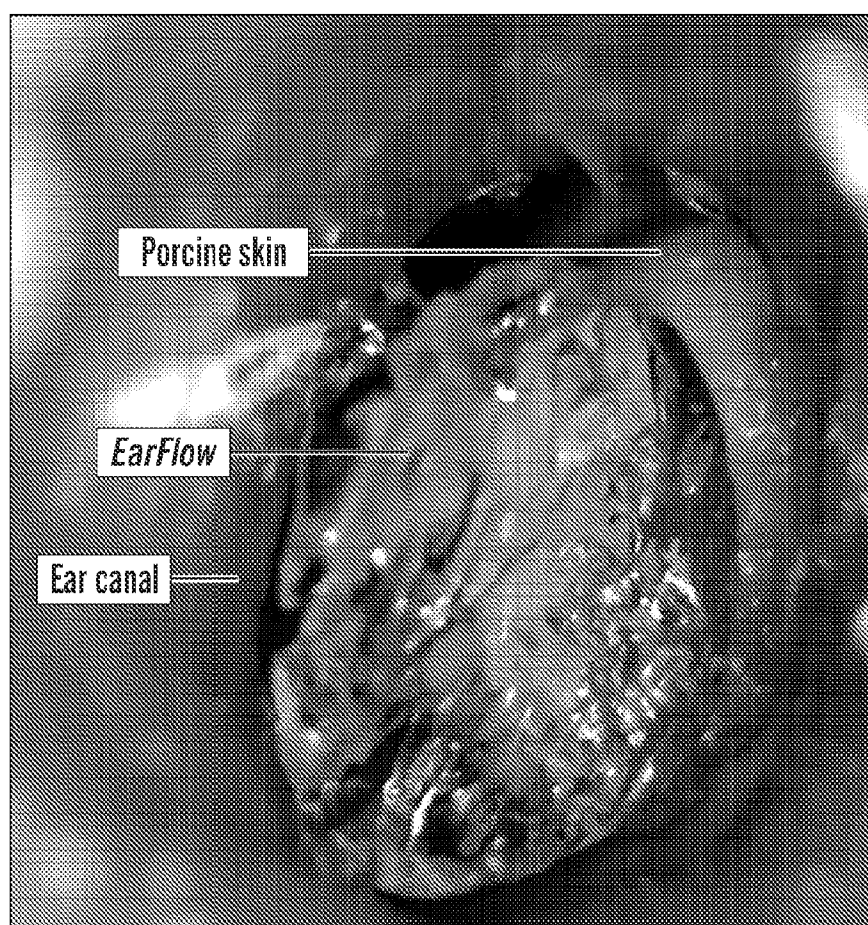


FIG. 27

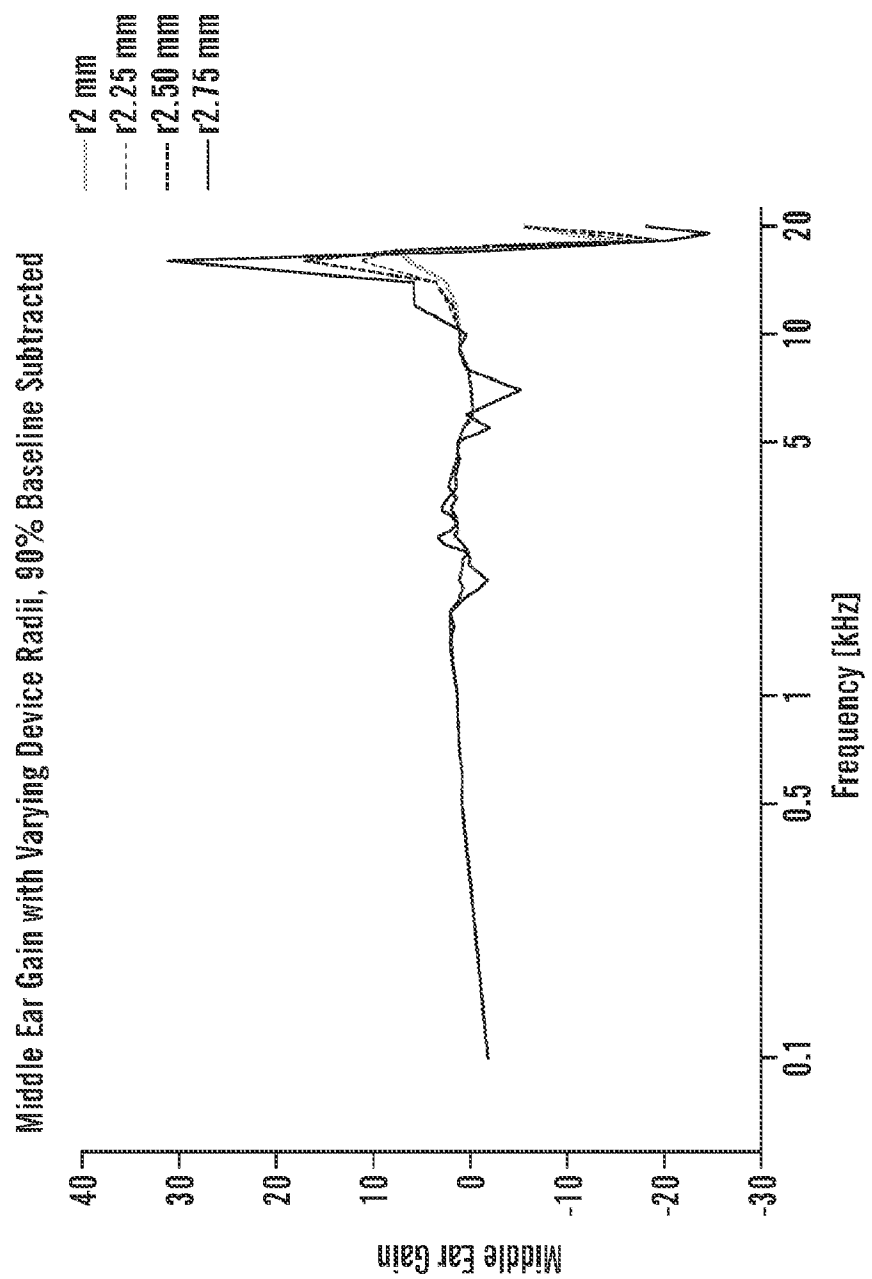


FIG. 28A

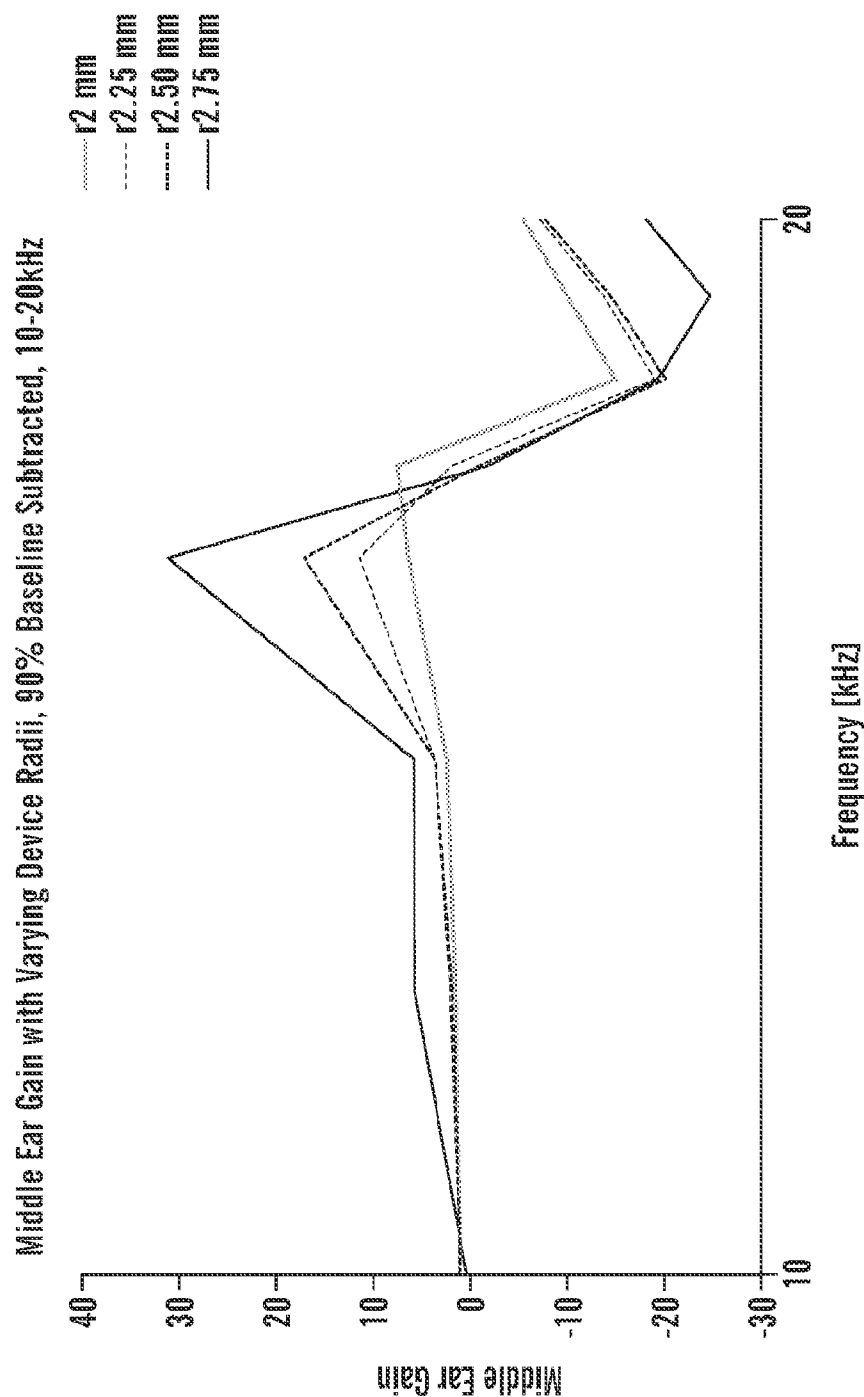


FIG. 28B

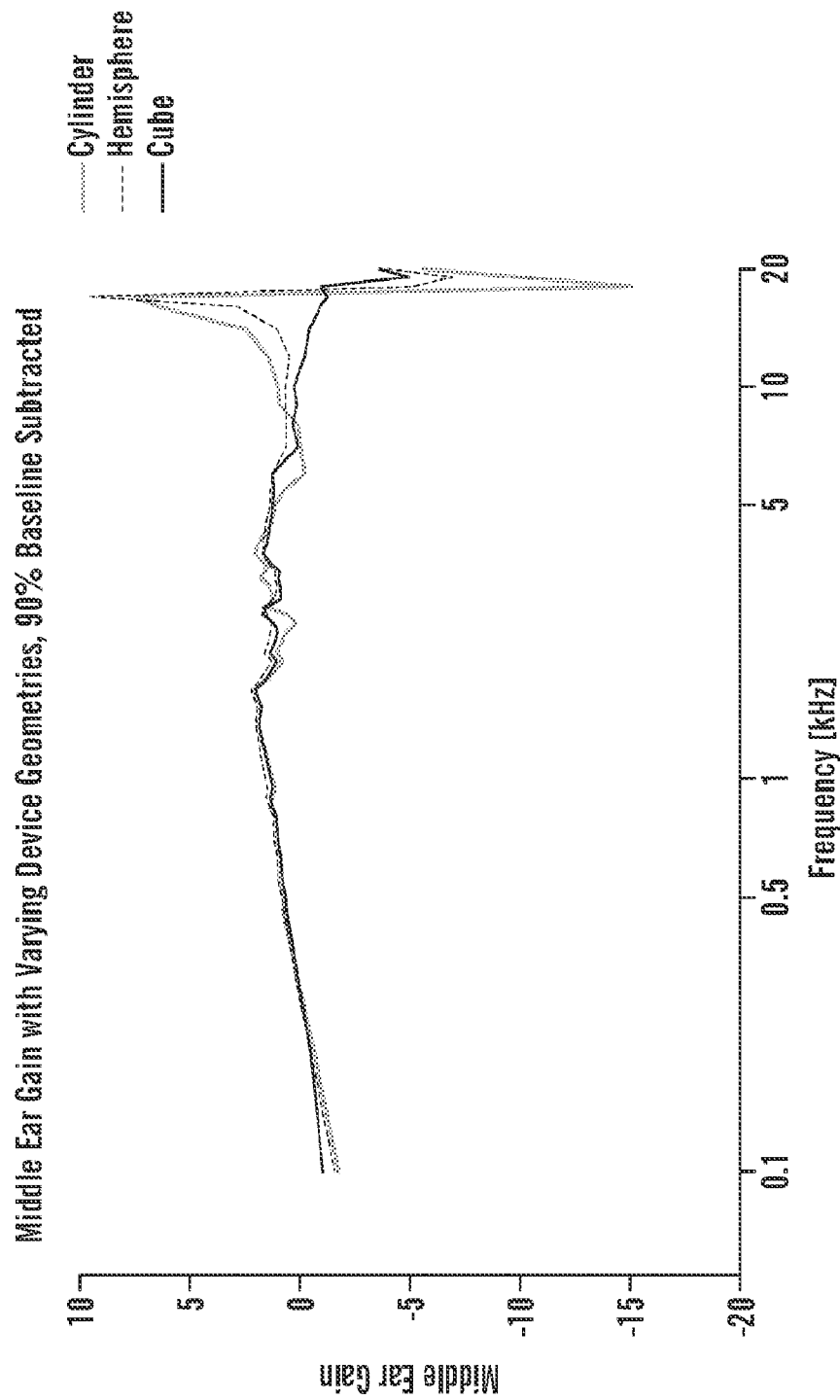


FIG. 29A

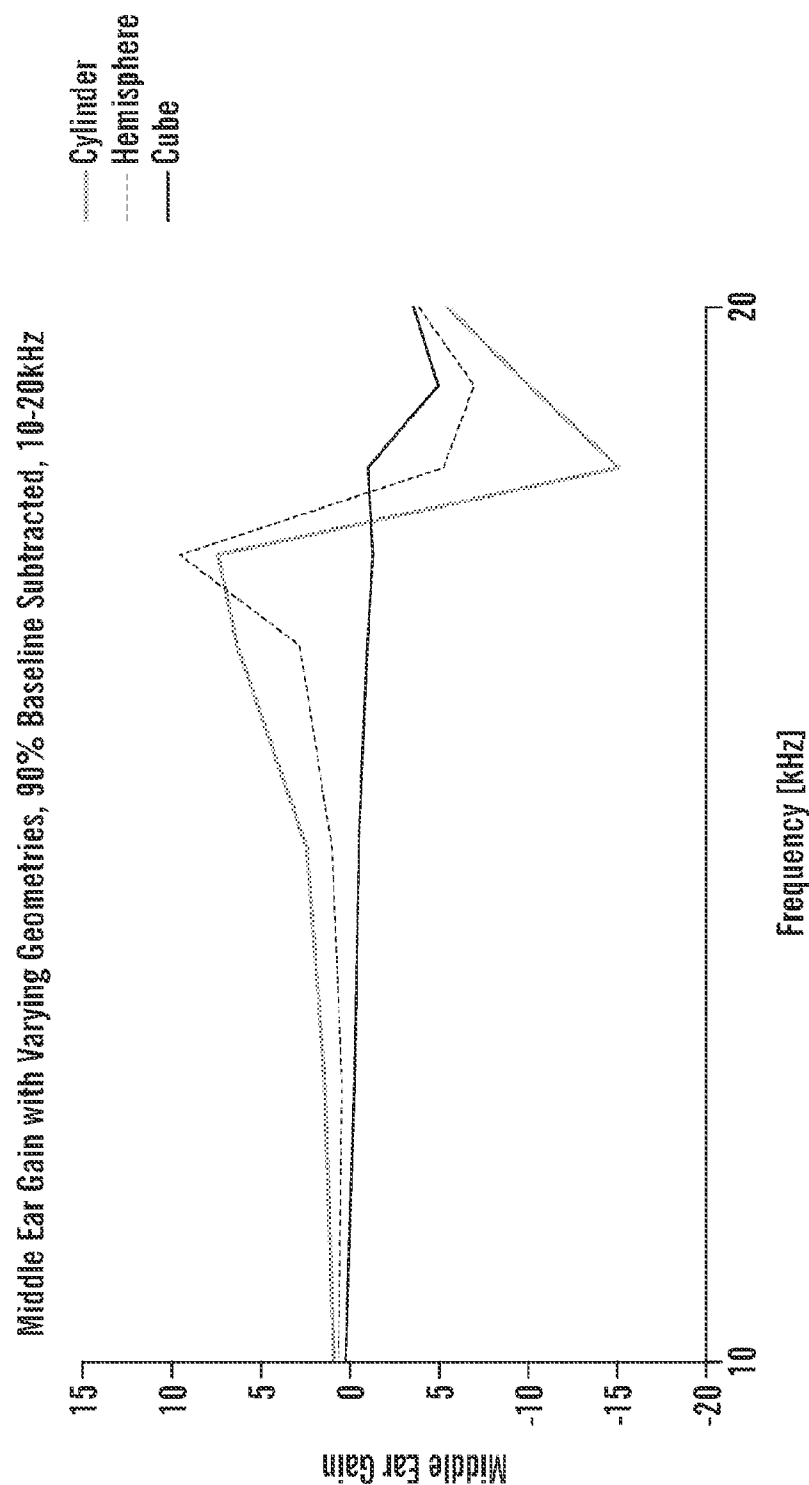


FIG. 29B

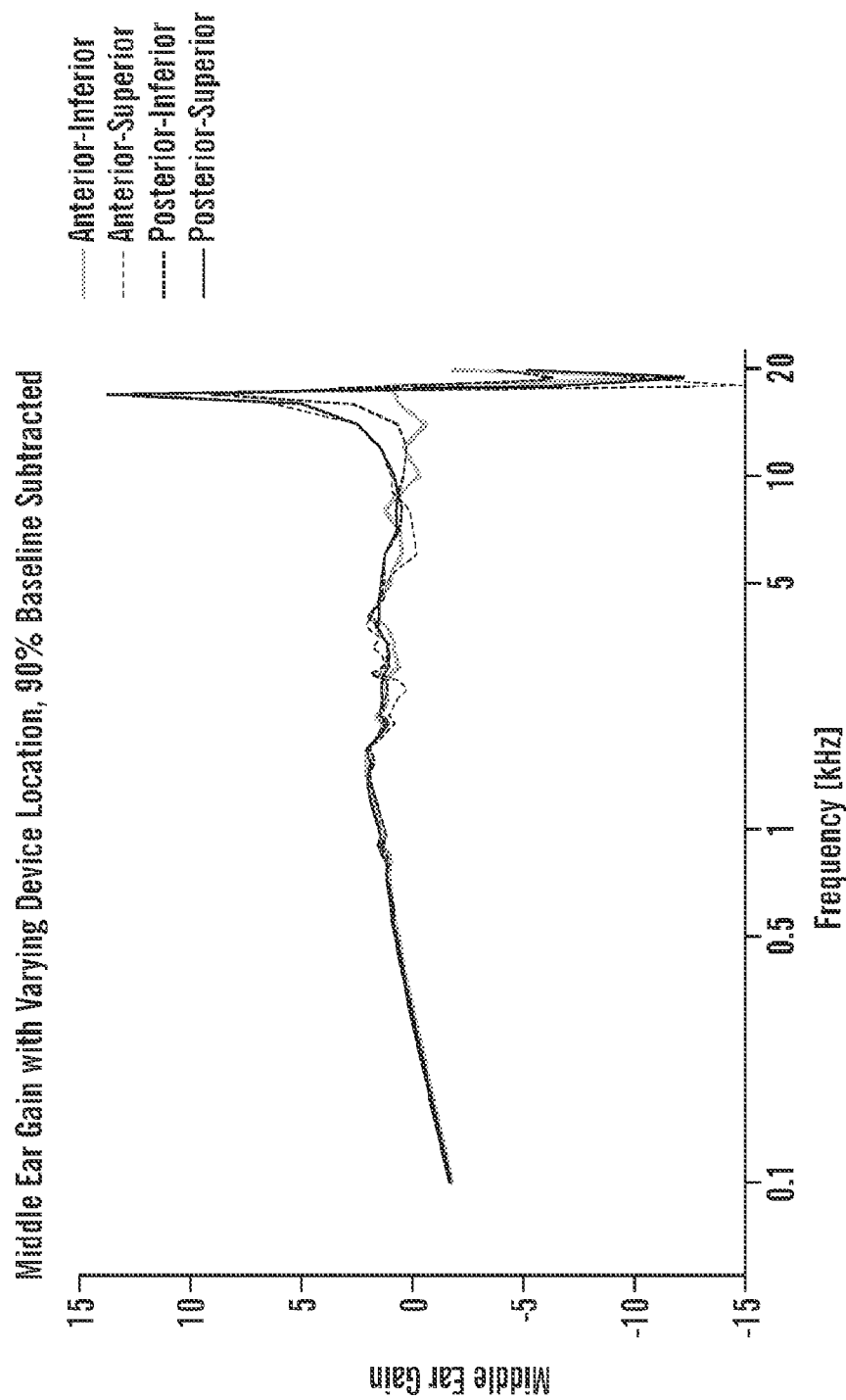


FIG. 30A

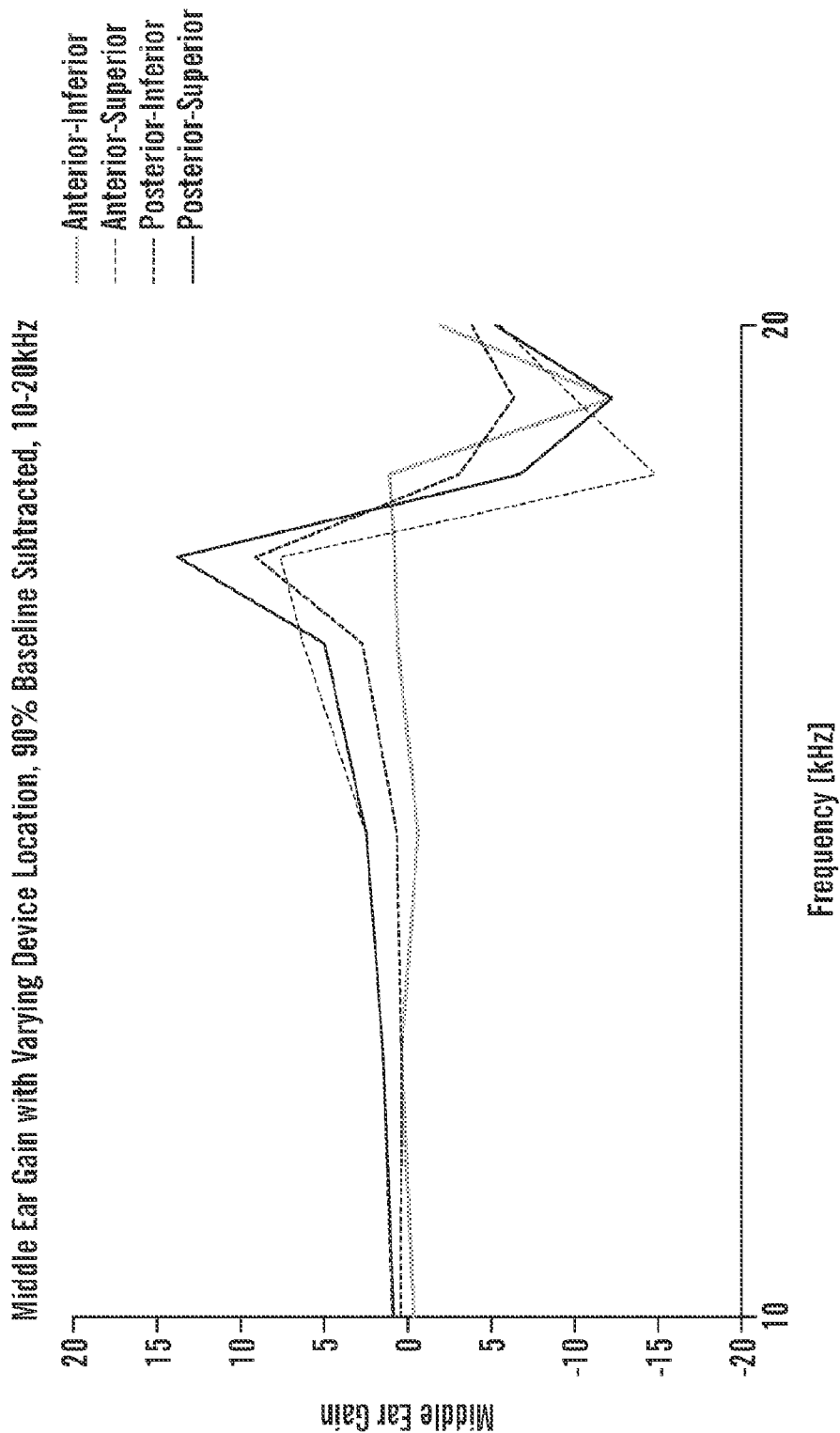


FIG. 30B

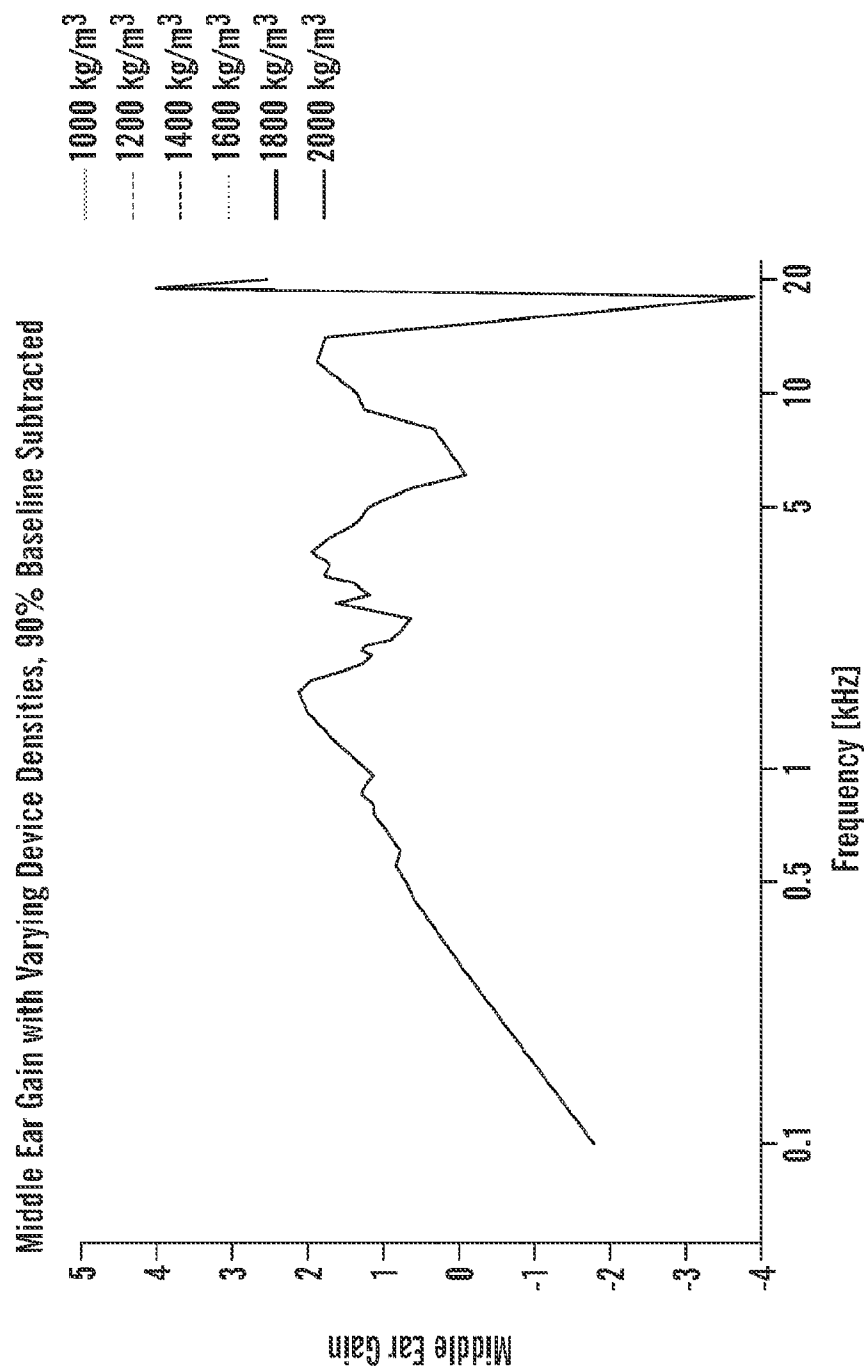
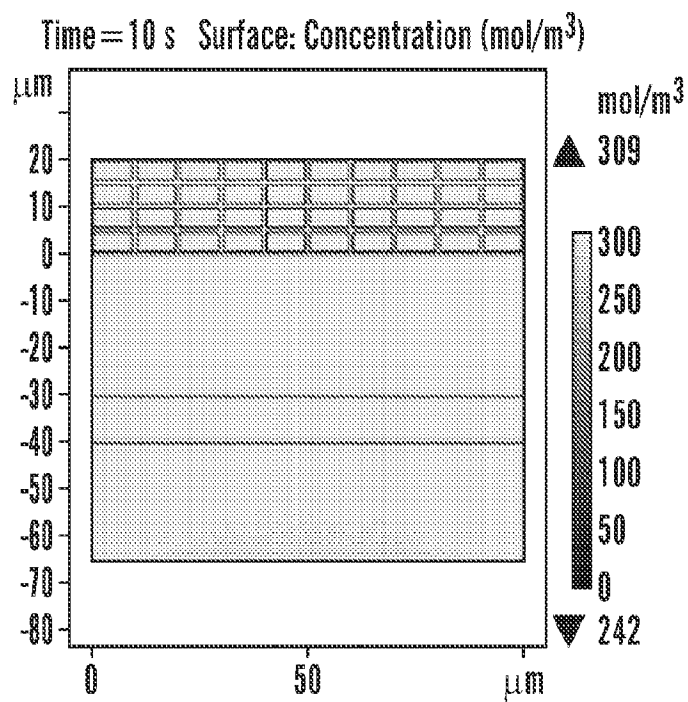
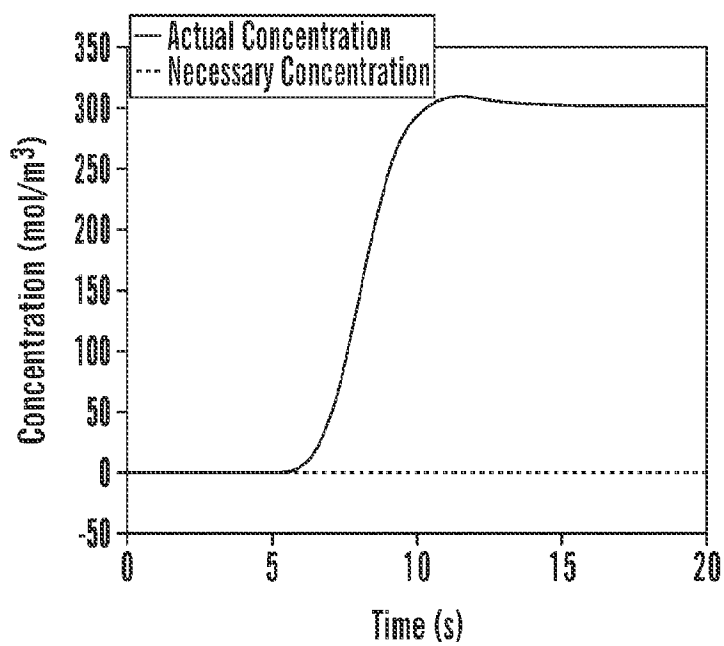


FIG. 31

**FIG. 32A****FIG. 32B**

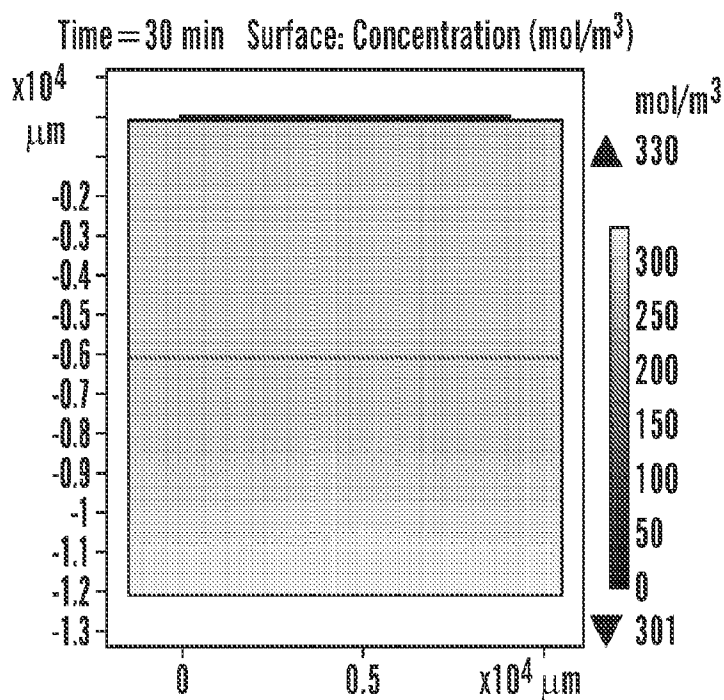


FIG. 33A

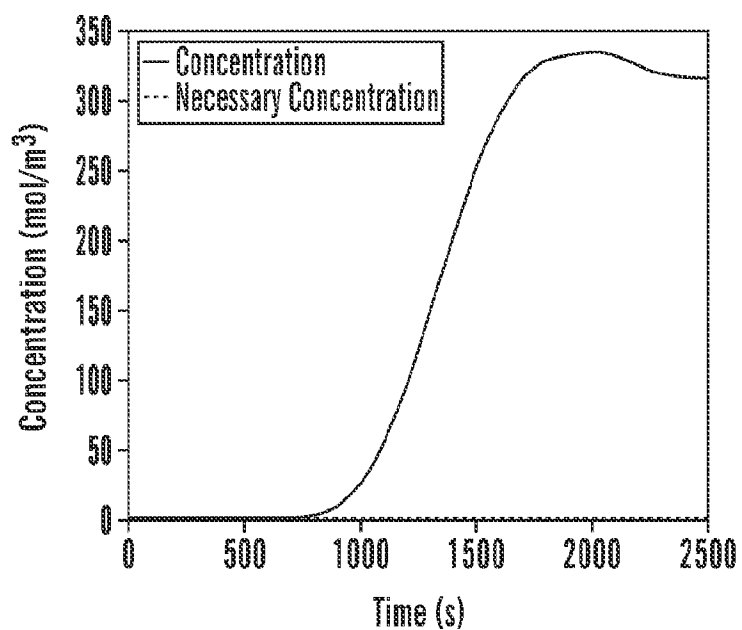


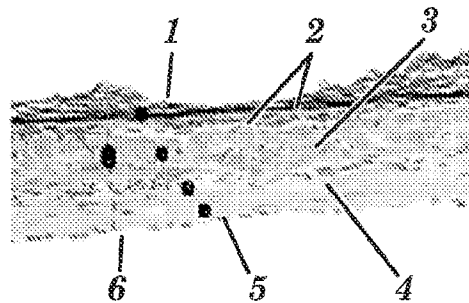
FIG. 33B

Specification	Target Range	Measured Value
Device Manufacturing		
Device radius	≤ 4 mm	Achieved – device radius can be varied from 2 mm to 4 mm
Porosity of scaffold	$> 80\%$	Not evaluated, though results from hydrogel-IL uptake studies suggested that the porosity is sufficient for holding the desired volume of IL
Drug Delivery and Release		
Specification	Target Range	Measured Value
Loading concentration of drugs in IL (dosage)	≥ 250 mg/mL ciprofloxacin	Achieved – IL can load ciprofloxacin at a concentration of 250 mg/mL (note that 200 mg/mL was used in diffusion studies)
Total volume of IL held in device	> 40 μ L	Achieved – 8 mm diameter device held > 40 μ L of IL
Sustained delivery timeframe	> 7 days	Achieved – detectable concentration of dexamethasone in the acceptor chamber throughout the 7-day treatment period, as well as in the epidermis and dermis layers of the porcine skin
Device degradation rate	No degradation in 7 days	Achieved – device was built with pHEMA hydrogel monomer, which is not hydrolyzable or enzymatically degradable
Cytotoxicity		
Cytotoxicity of ionic liquids	$> 90\%$ cell viability	Achieved – $> 90\%$ cell viability for fibroblasts; some toxicity to keratinocytes with 2A+IL combination; <i>in vivo</i> studies necessary
Cytotoxicity of hydrogel device	$> 90\%$ cell viability	Achieved – $> 90\%$ cell viability for both fibroblasts and keratinocytes
Clinical Considerations		
Hearing changes	< 15 dB decrease	Not evaluated <i>in vivo</i> , but COMSOL modelling suggested that this is feasible

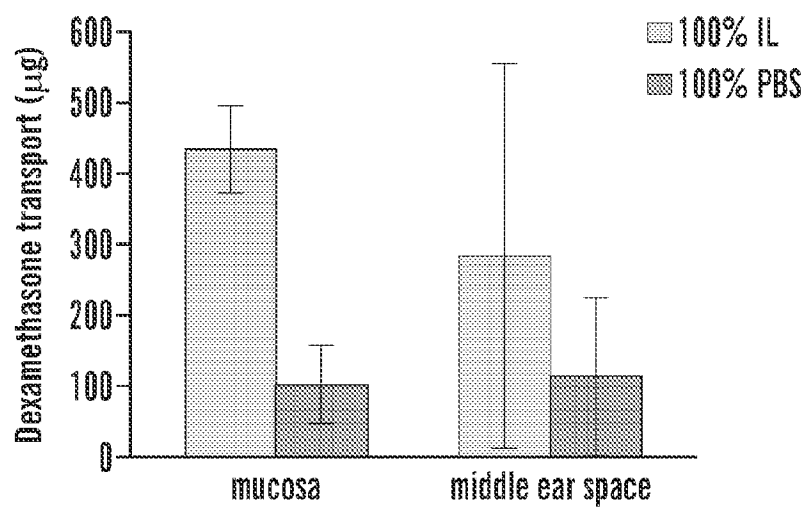
FIG. 34A

Specification	Target Range	Measured Value
Easy to place into and remove from the EAC	Device placement in under 10 minutes	Achieved – placement in <1 minute
Device adhesion	Device must be able to adhere to the TM	Achieved – <i>EarFlow</i> adhered to <i>ex vivo</i> porcine skin without dislodging from the tissue
Cell adhesion	$\leq 10\%$ of cell attachment (i.e. maintain biologically inert)	Achieved – experimental groups on average had around $4.1 \pm 0.27\%$ of cells of the control group at Day 7

FIG. 34B

**FIG. 35**

	Label	Length
2	Epidermal epithelium	19.026
3	Radial collagen fibers	30.265
4	Circular collagen fibers	9.618
5	Mucosal epithelium	21.095
	Total TM	80.004

FIG. 36**FIG. 37**

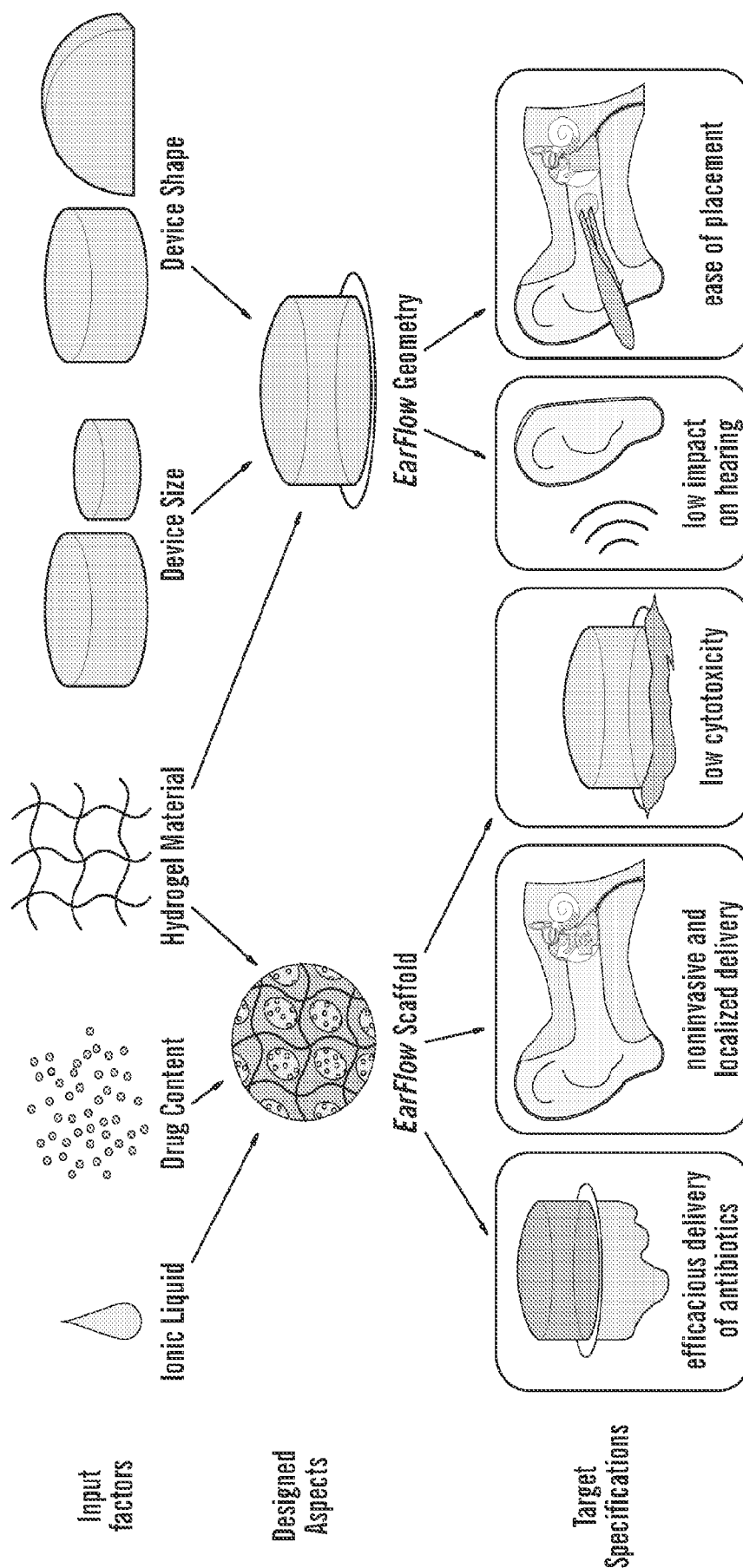


FIG. 38

Specification	Target Range	Reasoning	Measurement
Drug Delivery and Release			
Loading consideration of drugs in IL (dosage)	> 100 mg/mL ciprofloxacin	Based on calculations in consideration of the minimal inhibitory concentration (MIC) of ciprofloxacin against pathogens	Weight the amount of drug loaded in IL
Total volume of IL held in device	> 40 μ L	40 μ L is the anticipated volume necessary for delivering one course of antibiotics	Percent swelling of lyophilized hydrogels in IL
Sustained delivery timeframe	> 7 days	Antibiotics courses are typically 7 days to prevent recurring infections	Measure <i>ex vivo</i> drug release via UV spectrophotometry
Device degradation	No degradation in 7 days	Degradation of the scaffold may affect its drug release profile	Use non-hydrolysable monomer for synthesis
Cell Toxicity			
Cytotoxicity of the device	> 80% cell viability	Treatment with <i>EarFlow</i> must not destroy the trilaminar structure of the TM	Cytotoxicity studies with keratinocytes and fibroblasts
Clinical Considerations			
Easy to place into and remove from the ear canal	Device placement in under 10 minutes	Allows ENT surgeons to place the device easily without anesthesia procedures	Consult ENT surgeons and test feasibility of placement in EAC
Device adhesion to the eardrum	Physically adheres to the eardrum without falling off	Ensures that the device will continue eluting drugs over 7 days	Validate that the device can adhere to <i>ex vivo</i> tissues without falling
Cell adhesion	< 10% of cell attachment	To ensure biocompatibility and prevent integration with TM	Cell adhesion studies with human keratinocytes
Hearing changes	No more than 15 dB decrease	Existing devices worsens hearing by at least 15 dB – a significant change in hearing	Validate <i>in vivo</i> with COMSOL model and potentially in animal studies

FIG. 39

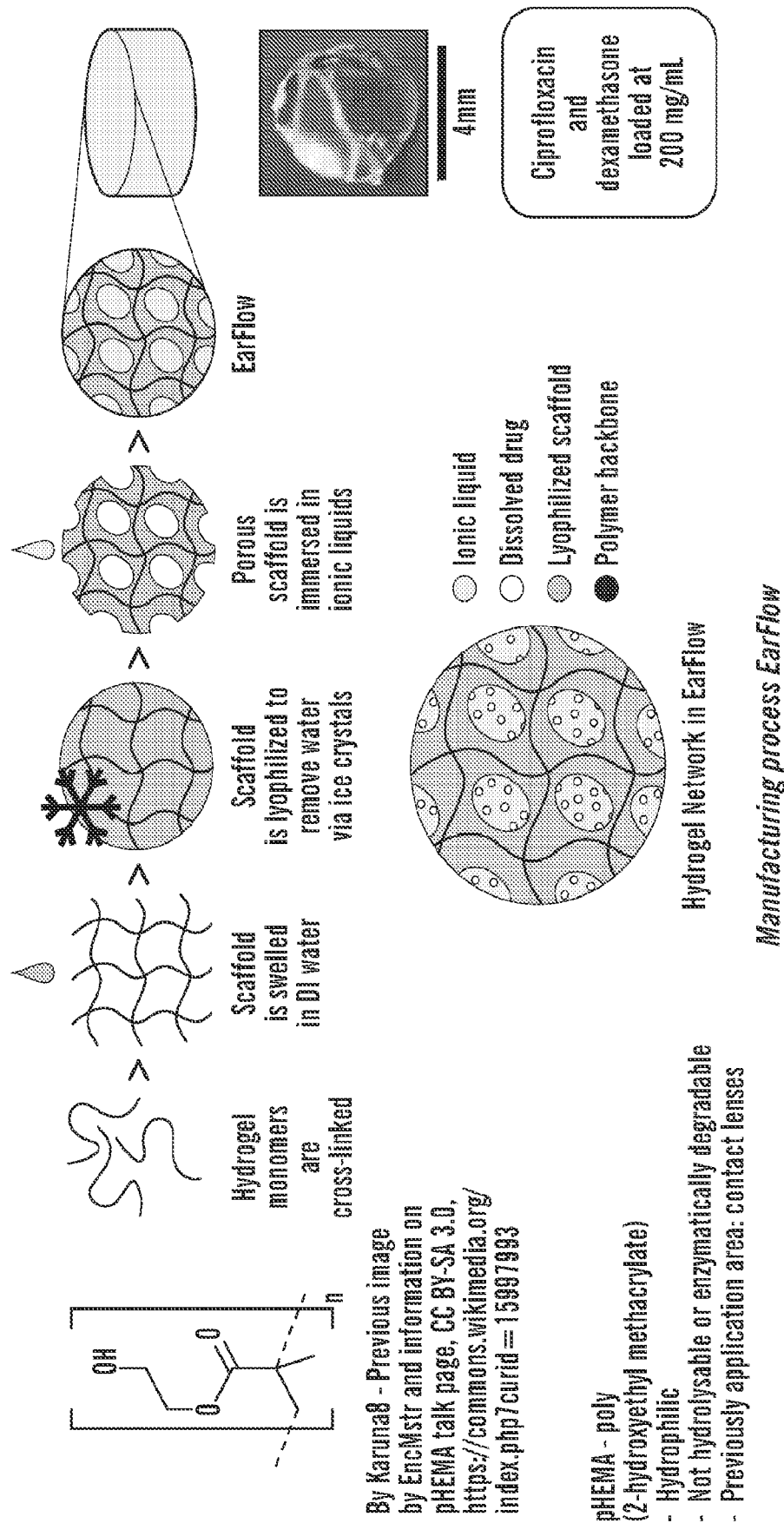


FIG. 40

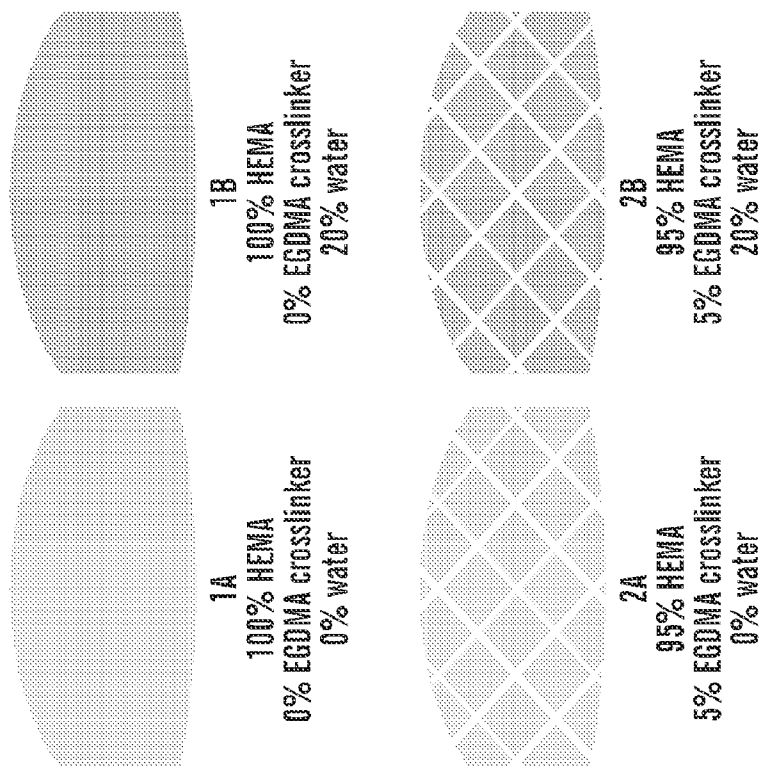
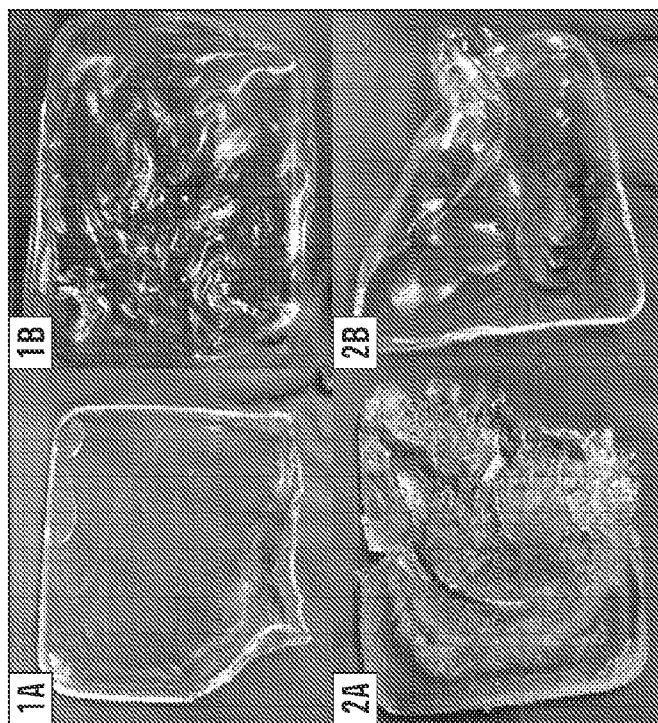


FIG. 41

Specification		Target Range	Measurement
Drug Delivery and Release			
✓ Loading concentration of drugs in IL (dosage)	> 100 mg/mL ciprofloxacin	200 mg/mL ciprofloxacin and dexamethasone	
✓ Total volume of IL held in device	> 40 μ L	1A: 81.5 ± 46.7 μ L, 2A: 38.0 ± 14.7 μ L	
✓ Sustained delivery timeframe	> 7 days	Achieved - detectable [dexamethasone]	
✓ Device degradation	No degradation in 7 days	Achieved by fabricating with PHEMA	
Cell Toxicity			
✓ Cytotoxicity of the device	> 90% cell viability	> 90% fibroblast survival, some toxicity to keratinocytes in 1A+IL combination	
Clinical Considerations			
✓ Easy to place into and remove from the ear canal	Device placement in under 10 minutes	Placement in < 1 minute	
✓ Device adhesion to the eardrum	Adheres to the eardrum without falling off	Adheres to porcine skin without falling	
✓ Cell adhesion	< 10% of cell attachment	Experimental groups on average have $4.1 \pm 0.27\%$ of cells of the control group at Day 7	
Hearing changes	No more than 15 dB decrease	Potentially feasible based on <i>in silico</i> COMSOL modelling results; further validation with <i>in vivo</i> animal studies	

FIG. 42

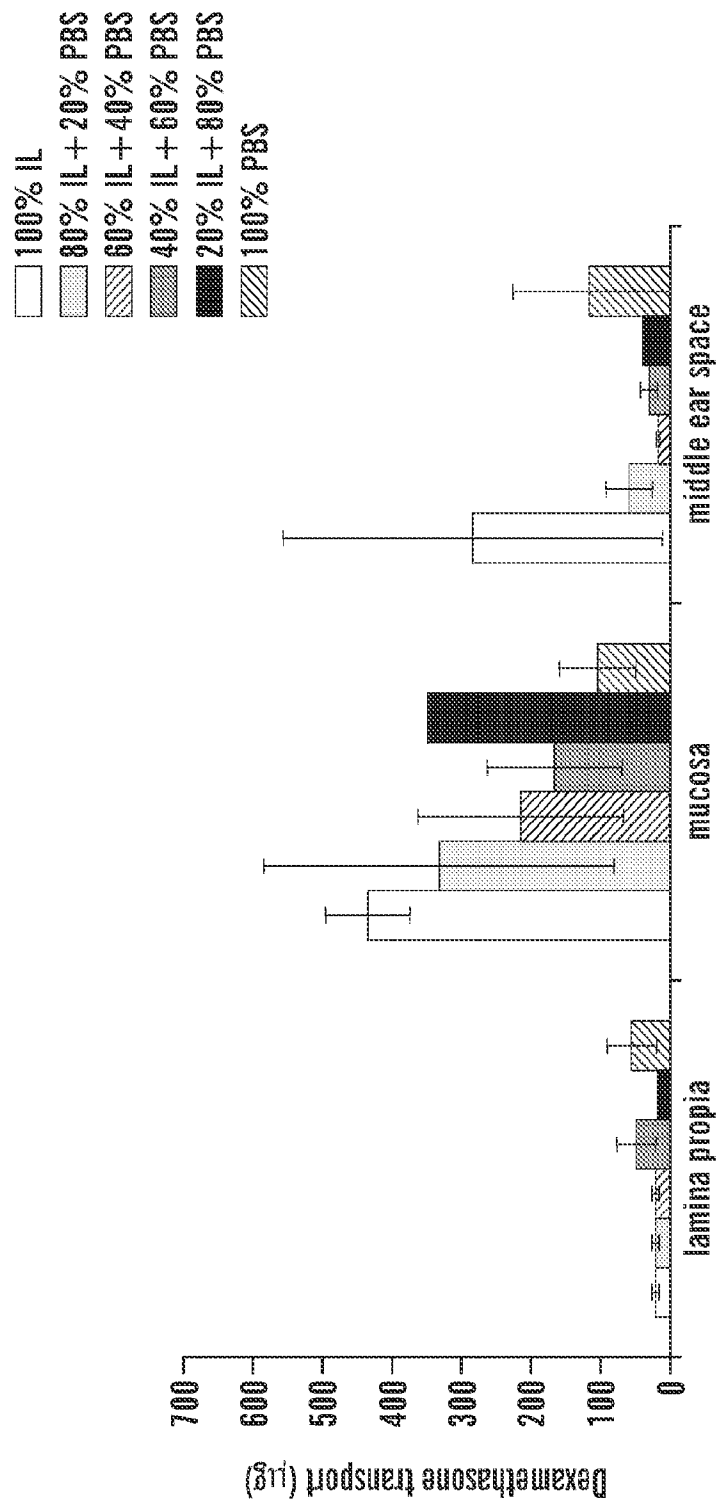


FIG. 43

Dexamethasone from EarFlow Delivery

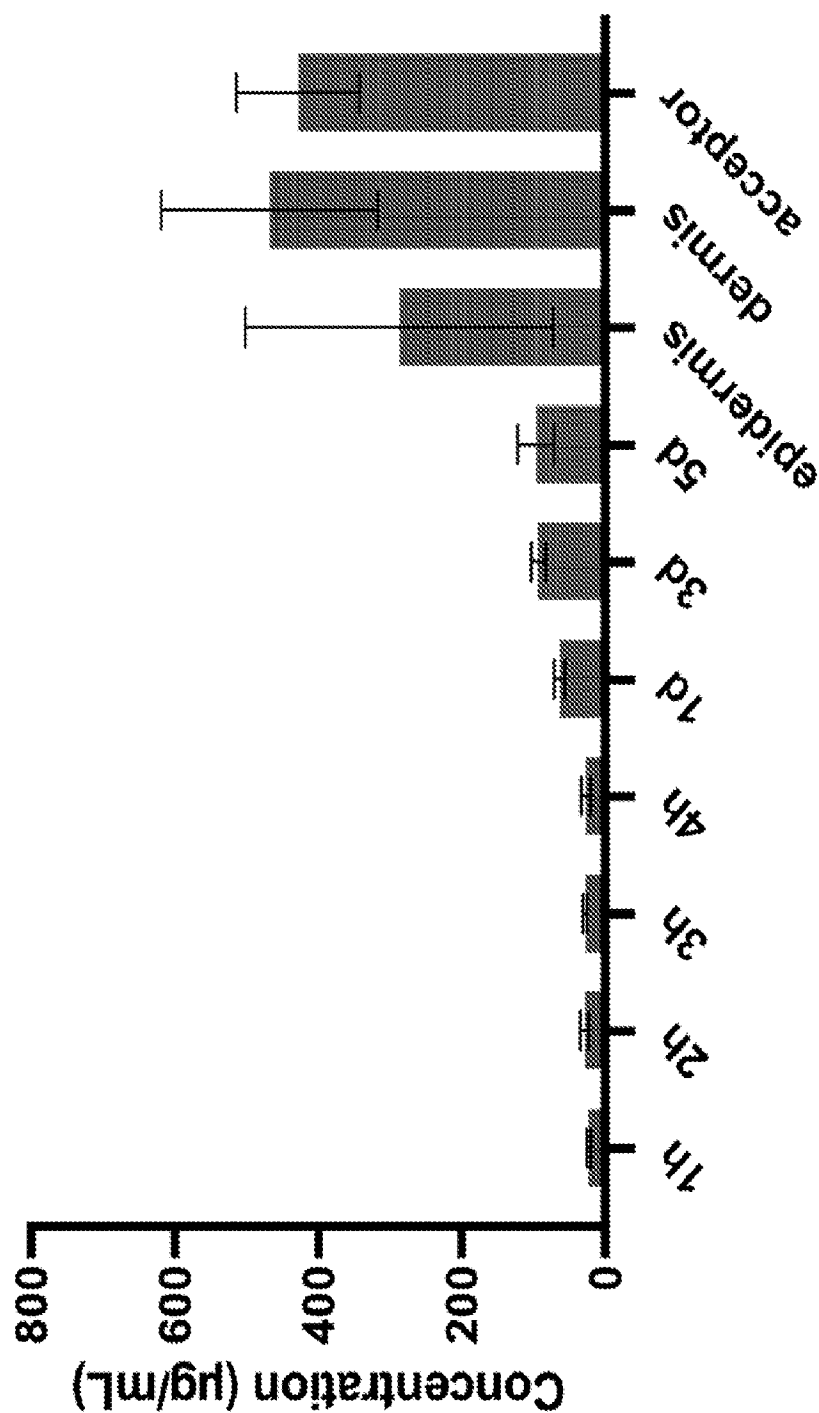


Fig. 44

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/031522

A. CLASSIFICATION OF SUBJECT MATTER

INV. **A61K31/573** **A61L27/14** **A61L27/18** **A61L27/20** **A61L27/22**
A61L27/24 **A61L27/52** **A61L27/54**

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/228903 A1 (KOHANE DANIEL S [US] ET AL) 16 August 2018 (2018-08-16)	1-5, 8-87
Y	paragraphs [0006] - [0012], [0036] - [0050], [0198], [0279], [0301], [0302], [0325] - [0344] paragraphs [0477] - [0550] claims 1-131	6, 7
Y	WO 2021/102084 A1 (HARVARD COLLEGE [US]) 27 May 2021 (2021-05-27) paragraphs [0013], [0097] - [0099], [0139], [0145], [0236] claims 1-67	6, 7
A	WO 2015/066647 A2 (UNIV CALIFORNIA [US]; LOS ALAMOS NAT SECURITY LLC [US]) 7 May 2015 (2015-05-07) cited in the application claims 1-20	1-87

☐

Further documents are listed in the continuation of Box C.

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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

6 September 2022

Date of mailing of the international search report

14/09/2022

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Authorized officer

Burtan, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/031522

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