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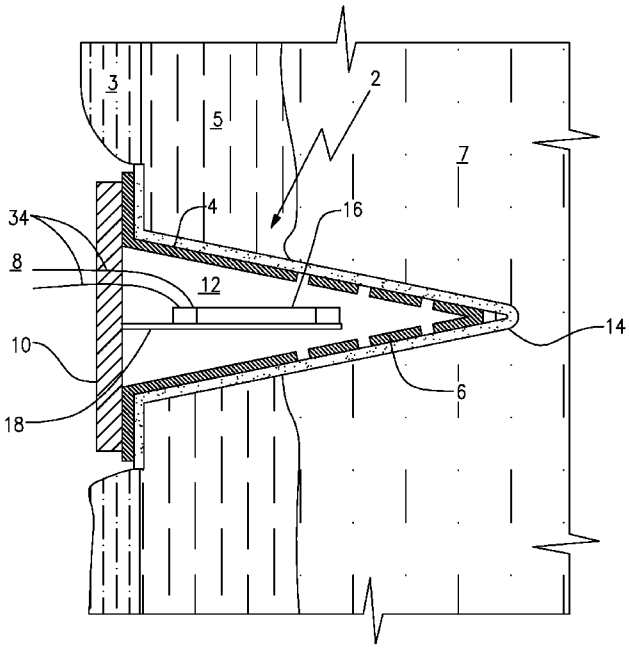

FIG.1

(57) Abstract: A microtensiometer is provided that includes a substrate layer having an enclosed reservoir formed therein. A porous membrane is disposed on a surface of the substrate layer. The membrane includes a liquid side fluidly coupled to the reservoir and a vapor side fluidly coupled to a vapor interface. The porous membrane includes a plurality of through holes fluidly coupling the liquid reservoir to the vapor interface, and a nanoporous filler material disposed within the plurality of through holes. The filler material includes a plurality of open pores having a maximum diameter in the range of 0.2 to 200 nanometers. In one embodiment, the microtensiometer includes a molecular membrane disposed adjacent to the vapor side of the porous membrane. In one example, the molecular membrane is a highly crystalline polytetrafluoroethylene polymer having a microstructure characterized by nodes interconnected by fibrils. In one application, the microtensiometer may be used to measure the average water potential within a network of plant or tree xylem. In another application, the microtensiometer may be useful in real-time determination of the water potential in soil.

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MICROTENSIOMETER

Cross Reference to Related Application

[0001] Reference is made to and this application claims priority from and the benefit of U.S. Provisional Application Serial No. 61/170,223, filed April 17, 2009, entitled "MICROFLUIDIC XYLEM PROBE", which application is incorporated herein in its entirety by reference.

Field of the Invention

[0002] This invention relates generally to microelectromechanical sensors, and more specifically, to a microfluidic microtensiometer for measuring chemical potential at negative pressures or in sub-saturated phases.

Background of the Invention

[0003] Plants form a complex interface between the soil and the atmosphere, two spatially and temporally variable systems. In this role, plants mediate and respond to – in their growth potential, yield, and seed maturation – the mass and energy fluxes that are defined by these boundaries. Conventional agricultural and ecological assessments of the physical and chemical state of this coupled system depend on measurements of low temporal and spatial resolution in the soil and the atmosphere. Thus, the instantaneous state of the plants must be extrapolated from this indirect data, and, conversely, the role of the plants in defining their environment must be estimated via models. Furthermore, the behavior of individual plants or sub-populations within a heterogeneous environment must be deconvolved from global measurements.

[0004] Water potential (ψ_w) is a form of chemical potential of water (μ) that is the most widely measured plant water status parameter and is a useful indicator to predict plant and fruit growth, yield, and fruit composition and quality. One advantage of using water potential to quantify plant water status is that at equilibrium, both liquid and vapor (gas) phases have identical values of water potential. "Water potential" refers to the chemical potential of sap water (μ_w) relative to that of pure water (μ_0) at the same temperature and atmospheric pressure, and is synonymous with "plant water status" or the "chemical potential" of the sap within the xylem. The relationship between water potential (ψ_w) as used

by the plant scientific community and chemical potential (μ) as used by the general scientific community may be expressed as:

$$\psi_W = \frac{\mu_W - \mu_o}{\overline{V_W}} \quad (1)$$

where $\overline{V_W}$ is the partial molal volume of water. For purposes of discussion herein, the term "chemical potential" will be used with the understanding that the term could also mean water potential.

[0005] Plant water relations are governed by chemical (water) potential and its gradients, not by water content. There have been many studies of the effects of water stress on plant productivity and product quality though these have not always led to predictive tools. The main problem is that in large woody plants such as trees, or grapevine variations in water status are very dynamic, both daily and seasonally, as plant water stress responds to both soil moisture and atmospheric evaporative demand. However, the mechanisms are not yet fully understood. Part of the reason for this is that there are presently no effective tools available to easily quantify and monitor continuously plant water status. Ideal measures must be direct measurement of chemical potential, continuous to capture the dynamics, and inexpensive enough to allow spatial resolution.

[0006] Probing and determining the plant water status, or state of sap water, in xylem (the water-transporting vascular system) presents fundamental challenges. For example, during active transpiration, the xylem conduits of plants maintain an extreme physical environment. Under common physiological conditions the sap water is at large negative pressures, *e.g.*, $P_{xylem} < -10$ atmospheres (atm.), due to extreme capillary pull generated in the leaves as evaporation occurs. The liquid is thermodynamically metastable with respect to its vapor. This metastability has been a major obstacle to the development of synthetic interfaces with xylem.

[0007] There is currently no known effective way to monitor the state of water sap continuously in the field. Soil moisture, an indirect measure, can be monitored with several methods and can be useful. However, due to the strong role of aerial evaporative demand and variable root distributions, indirect soil moisture measurement is often inadequate. Other methods have been tested in an effort to determine plant water status. For example, pressure chamber methods provide accurate measurement and allow for a direct estimate of chemical potential within the plant, but are manual, slow and do not provide continuous data.

Electronic stem dendrometers provide continuous monitoring of the shrinking and swelling of the plant stem, but the relation to chemical potential is not consistent. Another method utilizes remote sensing of canopy temperature by IR thermometry or multispectral scanning, but these methods are technically complex to implement, expensive, indirect and normally only done very occasionally. Measurement of $^{13}C/^{12}C$ isotope ratios of carbon in plant tissues can be a useful integrator of water stress at over extended periods, but not for monitoring real-time. A "stem psychrometer" has been used in research to directly monitor plant water status in the stem of a plant. A stem psychrometer has a small chamber with a thermocouple junction that is cooled to reach the dewpoint of the enclosed air. The stem version has one side of the chamber open and it is sealed against the xylem of a plant to make a tight chamber. With extremely accurate measurement and control of temperature, the stem psychrometer can estimate the chemical potential at a point in the tissue. These have been used successfully by a few scientists but they are very difficult to use, are often unstable and give many artifacts. The Agricultural Electronics Corp. has manufactured the Phytogram (covered by U.S. Patent No. 6,870,376), that is a metal wire embedded inside the plant tissue. The manufacturer claims to estimate the tissue hydration or water content by measuring protons in the spaces between cells. This method, however, is related to tissue water content and only indirectly to tissue chemical potential.

Summary of the Invention

[0008] Disclosed is a microfluidic probe including a probe body comprising a platform and a semi-permeable tip, and a microtensiometer coupled to the platform. The microtensiometer includes a substrate layer having an enclosed reservoir formed therein, and a porous membrane disposed on a surface of the substrate layer. The membrane includes a liquid side fluidly coupled to the reservoir and a vapor side fluidly coupled to the vapor interface. The membrane includes a plurality of pores having a maximum diameter in the range of 0.2 to 200 nanometers. The microfluidic probe further includes a pressure sensor coupled to the reservoir for measuring changes to a pressure of a liquid disposed within the reservoir.

[0009] In one aspect of the invention, the porous membrane includes a plurality of through holes fluidly coupling the liquid reservoir to the vapor interface. A nanoporous filler material is disposed within the plurality of through holes. The filler material includes a plurality of open pores having a maximum diameter in the range of 1 to 100 nanometers.

[0010] In one example, the filler material is a sol-gel comprising a plurality of open pores having a maximum diameter in the range of 1 to 10 nanometers.

[0011] In another aspect of the invention, the sensor is a diaphragm-based pressure sensor comprising piezo-resistive strain gauges.

[0012] In another aspect of the invention, a microtensiometer is provided that includes a substrate layer having an enclosed reservoir formed therein. A porous membrane is disposed on a surface of the substrate layer. The membrane includes a liquid side fluidly coupled to the reservoir and a vapor side fluidly coupled to a vapor interface. The porous membrane includes a plurality of through holes fluidly coupling the liquid reservoir to the vapor interface, and a nanoporous filler material disposed within the plurality of through holes. The filler material includes a plurality of open pores having a maximum diameter in the range of 0.2 to 200 nanometers.

[0013] In another aspect of the invention, the microtensiometer further includes a molecular membrane disposed adjacent to the vapor side of the porous membrane.

[0014] In one example, the molecular membrane is a hydrophobic highly crystalline polytetrafluoroethylene polymer having a microstructure characterized by nodes interconnected by fibrils.

[0015] In another aspect of the invention, a method for measuring chemical potential in plant xylem is provided. The method includes the steps of providing a plant or tree having xylem conduits for transporting sap water, placing a microtensiometer adjacent to a plurality of intact xylem conduits, equilibrating the average chemical potential of the sap water with a liquid disposed in the microtensiometer, sensing a change in the chemical potential of the sap water with a pressure sensor coupled to the microtensiometer, transmitting a voltage response from the pressure sensor to a transponder coupled to conductive leads of the pressure sensor, and transmitting an output from the transponder to a receiver.

[0016] In another aspect of the invention, the microfluidic tensiometer comprises a substrate layer having an enclosed liquid reservoir formed therein and a porous membrane disposed on a surface of the substrate layer. The membrane includes a plurality of pores fluidly coupling the liquid reservoir to a vapor interface of the sap water. The pressure sensor is coupled to the liquid reservoir for measuring changes to pressure in the liquid reservoir.

[0017] In another aspect of the invention, the step of equilibrating includes exposing the sap water to a semi-permeable material that permits sap water vapor transfer and prohibits sap water liquid transfer, exposing the liquid in the reservoir to the porous membrane such

that the membrane permits vapor transfer and prohibits liquid transfer, and allowing the sap water vapor to reach equilibrium with the reservoir vapor.

Brief Description of the Drawings

[0018] The features described herein can be better understood with reference to the drawings described below. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the drawings, like numerals are used to indicate like parts throughout the various views.

[0019] FIGS. 1 and 1A are schematic diagrams of a microfluidic probe in accordance with two embodiments of the invention;

[0020] FIG. 2 is a schematic diagram of a microtensiometer from FIG. 1;

[0021] FIGS. 3A and 3B are schematic diagrams of a top view of the microtensiometer from FIG. 2;

[0022] FIG. 4 is a schematic diagram of one embodiment of a porous membrane from FIG. 2;

[0023] FIG. 5 is a schematic diagram of an alternate embodiment of the porous membrane from FIG. 2;

[0024] FIG. 6 is a schematic diagram of an alternate embodiment of the porous membrane from FIG. 2;

[0025] FIG. 7 is a perspective view of a water management system in accordance with one embodiment of the invention; and

[0026] FIG. 8 is a perspective view of one embodiment of the water management system of FIG. 7.

Detailed Description of the Invention

[0027] Microtensiometry is a required method for sensing the negative sap chemical potential *in situ* in the plant due to its range of measurement, accuracy, compatibility with continuous operation, and the ease with which the signal can be transduced electronically. Tensiometry allows chemical potential to be measured via the change in pressure within an enclosed volume of liquid water. Briefly, the enclosed liquid is allowed to equilibrate with the water vapor in an external environment across a porous wick. For sub-saturated vapors (*e.g.*, relative humidity < 100%) the pressure in the enclosed liquid drops below atmospheric and reaches negative values, referred to as tension.

[0028] Monitoring water status in vines presents at least two significant drawbacks in currently-available tensiometers currently used in soils. One is the large form factor - existing designs are too large for direct embedding in the plant. The other is insufficient range. The maximum tensions measurable with current tensiometers are approximately -1 atm., far short of the physiologically important range in vines and many other plants (-20 atm.). This limitation on negative pressure measurement is set by the material used as the tensiometer wick. As the pressure within the enclosure drops, it overtakes the capillary forces within the wick and pulls vapor into the cavity, causing the device to dry out and become inoperable. This limitation also arises from the large internal volume of liquid within a conventional tensiometer (*e.g.*, 10 milliliters). This large volume is highly susceptible to cavitation (boiling) of the internal liquid under tension due to homogeneous nucleation, presence of impurities, and mechanical shocks.

[0029] In an effort to overcome these and other noted problems, the inventors have devised a microtensiometer with a millimeter-scale form factor capable of measuring negative pressures down to -100 atm.

[0030] Referring to FIG. 1, a microfluidic probe 2 according to the present invention is illustrated after insertion through a plant's bark 3 and phloem 5 into the network of plant xylem vessels 7. The microfluidic probe 2 includes a substantially solid probe body 4 with a semi-permeable tip 6 that is placed in the tissue adjacent to intact xylem conduits 7, such as xylem conduits in a grape vine or a tree, such that the chemical potential of water in the conduit can equilibrate through the intervening tissue. In one example, a grapevine trunk may include between 250 and 500 xylem vessels or conduits, and the microfluidic probe 2 may measure the average chemical potential in 10 to 100 of the nearby xylem conduits 7. The semi-permeable tip 6 may be cone-shaped to ease insertion into a plant, and an opposing end 8 of the probe body 4 may include a cover 10 forming a hermetic seal with the body. The semi-permeable tip 6 functions as a fluid barrier to prevent the liquid sap water from crossing over, but allows vapor transfer. In this manner, an interior portion of the probe body 4 defines a vapor interface 12 in equilibrium with the sap water within the xylem 7.

[0031] In one embodiment, the semi-permeable tip 6 may include a coating 14 thereon with specific reactivity to proteins for biocompatibility with plant xylem 7 and fusion with plant tissue. Major concerns regarding biocompatibility include fouling of external and internal surfaces of the probe 2 and rejection by the host plant. With the coating 14, the plant xylem 7 is less likely to reject the microfluidic probe 2 and may continue to grow around it. In one example, the coating 14 is a hydrogel formulation.

[0032] The microfluidic probe 2 includes a microtensiometer 16 secured to the interior portion of the probe body 4. In the disclosed embodiment, the microtensiometer 16 is glued to a platform 18 that is secured to the cover 10.

[0033] Referring to FIG. 1A, in another embodiment the microfluidic probe 2 may be inserted into a slit in the plant bark 3, rather than creating a hole for a cone-shaped body. The probe body 4 includes the platform 18 and cover 10, with the microtensiometer 16 being secured to the platform 18. The semi-permeable tip 6 may comprise a hydrophobic highly crystalline polytetrafluoroethylene polymer which has a microstructure characterized by nodes interconnected by fibrils. In one example, the polytetrafluoroethylene polymer is a GORE-TEX® membrane available from W. L. Gore & Associates, Inc. The GORE-TEX® membrane may be secured to the surface of the microtensiometer 16 by an adhesive backing, for example. In this embodiment, the pores in the GORE-TEX® membrane are approximately 0.02 micrometers to 10 micrometers in size, which is about 20,000 times smaller than a water droplet, but 700 times larger than a water vapor molecule. The hydrophobic GORE-TEX® membrane will therefore establish the water vapor interface 12 at the surface of the microtensiometer 16. One advantage to this embodiment over the cone-shaped probe body is that the vapor interface 12 is close-coupled to the microtensiometer 16, so the response time of the probe 2 may be significantly improved. Another advantage to this embodiment is that the small pore size and hydrophobicity will allow the membrane to exclude the passage of liquid water and aqueous solutions out to pressures of approximately 1 bar.

[0034] Turning now to FIG. 2 for a magnified view, the microtensiometer 16 includes a substrate layer 20 that serves as a backbone structure for the sensor elements. The substrate layer 20 may be fabricated from a silicon wafer, for example, due to its wide acceptance and compatibility with micro-fabrication techniques, such as on-substrate integration of sensing elements. Other substrate materials are contemplated without departing from the scope of the invention, such as other semiconductor materials, metals, oxides, or ceramics. However, alternate materials may not optimize the requirements for the overall design. The microtensiometer 16 further includes an enclosed liquid reservoir 22. In one example, the reservoir 22 is configured to hold approximately 10 microliters of water. In one possible construction, the reservoir 22 is isotropic wet etched into a glass plate 24, and the top of the plate 24 (with the etched feature) is secured to the bottom of the substrate layer 20 by anodic bonding, thereby forming a hermetic seal to enclose the reservoir 22.

[0035] The microtensiometer 16 further includes a molecularly porous membrane 26 disposed on a surface of the substrate layer 20. One side of the porous membrane 26 is

adjacent the liquid reservoir 22, and the other side of the membrane is exposed to the vapor interface 12. Once inserted into the plant xylem 7, the vapor interface 12 of the microfluidic probe 2 reaches equilibrium with the sap water, and the liquid reservoir 22 reaches equilibrium with the vapor interface 12. This vapor mediated coupling to the sap water may be utilized to directly detect changes in the sap chemical potential of the xylem 7. That is, the chemical potential of the sap water may be measured via the hydrostatic pressure in the pure liquid within the tensiometer once equilibrium is achieved between the two liquids. At equilibrium, the chemical potentials of the liquid in the xylem (μ_w^{xylem}) and in the tensiometer (μ_w^{tensio}) are approximately equal, giving:

$$\mu_w^{xylem} = \mu_w^{tensio} = \mu_0(T) + \bar{v}(P_{tensio} - P_{atm}) \qquad (2)$$

where $\bar{v}$ and μ_0 are the molar volume and reference chemical potential of the pure liquid (known values). This is an approximate expression in which the liquid has been assumed to be inextensible such that $\bar{v}$(P) equals $\bar{v}$(ambient). This approximation is good within 1% out to 100 bars of tension.

[0036] In the microtensiometer 16 of the present invention, the pressure, $P_{tension}$ will be generated in the discrete volume of water coupled via the molecularly porous membrane 26 to the vapor interface 12 in equilibrium with the local sap water. The sap water in the xylem moves along gradients of chemical potential, from higher (less negative) potentials to lower potentials. The liquid water disposed in the reservoir 22 is near pure, consequently it is near zero chemical potential. The chemical potential of a plant tissue, or other material like soil for example, will be more negative because any solutes in the solution or interactions with small particles, *e.g.*, soil, will reduce the chemical potential to negative values. Thus, there will be a gradient from the reservoir 22 in the microtensiometer 16 to the xylem 7. The microtensiometer 16 adjacent to the xylem 7 equilibrates via a vapor phase, and liquid will be pulled from the microtensiometer to the xylem 7 tissue (or soil, for example). As some vapor moves moisture from the reservoir 22, the remaining liquid will develop tension, which is then sensed by a pressure transducer, discussed in detail below. This negative pressure reduces the chemical potential in the reservoir 22 until the chemical potential of the microtensiometer 16 equals the chemical potential of the xylem 7 at equilibrium. The greater the initial difference, the more the xylem 7 will draw from the microtensiometer 16 and the greater the tension (*e.g.*, negative pressure) in the reservoir 22.

[0037] The difference of hydrostatic pressure $\Delta P_{tensio} = P_{tensio} - P_{atm}$ in the liquid reservoir 22 may be transduced into a voltage with an appropriate sensor 28. Referring to FIGS. 2, 3A, and 3B, in one embodiment the sensor 28 is a diaphragm-based pressure sensor using piezo-resistive strain gauges 30 formed as thin films of poly-silicon. A diaphragm 32 may be positioned above the liquid reservoir 22, formed in the silicon substrate layer 20 using conventional fabrication techniques. Conductive leads 34 extend from the sensor 28 through the cover 10 (FIG. 1) and terminate at a data logging apparatus (not shown). Although not illustrated in FIG. 2, a support structure such as honeycomb may be necessary below the membrane 26 and the diaphragm 32 to support the thin members when large negative pressures are achieved in the reservoir 22.

[0038] Local temperature changes may affect the molar volume of the liquid in the reservoir 22, the standard chemical potential, or the response of the diaphragm 32. Accordingly, the probe 2 may further include temperature compensation circuitry. In one embodiment, the temperature compensation circuitry is integrated directly into the sensor 28. In another embodiment, the temperature compensation circuitry may comprise a stand-alone temperature probe located proximate to the microtensiometer 16, such as on the platform 18. The temperature compensation circuitry may be formed by microfabrication techniques known in the art. In one example, a platinum resistance probe is constructed with four leads to allow for a "four-probe" measurement of resistance. In another example, a thin-film thermocouple may be formed by layering two materials such as gold and nickel (or gold and aluminum), each layer having thickness of approximately 300 nm.

[0039] The microtensiometer disclosed herein may comprise a microelectromechanical system (MEMS) capable of being fabricated on a silicon chip, for example. Referring to FIG. 3A, a plurality of microtensiometers 16 may be fabricated on a single silicon chip 36. In fact, the silicon chip 36 may include thousands of microtensiometers 16.

[0040] The molecularly porous membrane 26 must have the capacity to withstand large capillary stresses in the liquid to accommodate the large negative pressures present in the sap water in the xylem 7. The relationship between the membrane maximum pore diameter $d_p^{\max}$ and the maximum capillary pressure $\Delta P_{cap}^{\max}$ may be expressed according to the Young-Laplace equation:

$$\Delta P_{cap}^{\max} = P_{vap} - P_{liq} = \frac{4\gamma\cos\theta_r}{d_p^{\max}} \quad (3)$$

where P_{vap} and P_{liq} are the pressures of the vapor above the pore and of the liquid in the pore, respectively, γ[N/m] is the surface tension, and θ_r is the receding contact angle in the pore (a wetting characteristic). Solving for $d_p^{\max}$,

$$d_p^{\max} = \frac{4\gamma\cos\theta_r}{P_{vap} - P_{liq}} \quad (4)$$

[0041] As can be seen with reference to Equation 3, the maximum pore diameter must be very small for the liquid to withstand the large pressure differential without vapor breakthrough. The inventors have recognized that the pore sizes in the porous membrane 26 required to hold the large negative pressures may be an order of magnitude smaller than existing structures in the art. Turning now to FIG. 4, an enlarged cross section of one possible construction of the porous membrane 26 illustrates that in one embodiment the pore structure is comprised of the interstitial voids formed in the lattice structure of the substrate layer 20. Stated another way, the pores 38 occupy the region situated in-between the atoms that corresponds to the maximum diameter sphere which can fit in the free space bounded by the neighboring atoms. The mean diameter of the interstitial voids may be calculated or determined experimentally using known techniques. The interstitial voids may be formed in the crystalline structure or the amorphous structure of silicon, for example. In the example of single crystalline silicon, the interstitial voids provide a fluid path that, although somewhat tortuous, will fluidly couple a liquid in the reservoir 22 and the vapor interface 12. In this embodiment, the pores 38 (interstitial voids) have a mean diameter in the range of 20 to 200 nanometers.

[0042] The inventors have recognized that the interstitial voids by themselves may withstand sufficient negative pressure for some applications, but to achieve very large negative pressures smaller diameter pores are required. Alternately, the maximum pore size of the naturally-occurring interstitial voids may not provide sufficient repeatability.

[0043] To that end, FIG. 5 illustrates another embodiment of a porous membrane 126. The membrane 126 includes a plurality of through holes 140 fluidly coupling a liquid reservoir 122 to a vapor interface 112. In the illustrated embodiment, the through holes 140 have a diameter in the range of 2 nanometers to 20 micrometers, and extend straight through

the substrate layer 120. The through holes 140 may be formed in the silicon substrate layer 120 by electrochemically etching the silicon substrate layer through a lithographically patterned mask, chemical etching, or high temperature annealing, for example. One example fabrication method includes etching the through holes 140 from the liquid-side of the substrate layer 120, which corresponds to the bottom or underside of the layer shown in FIG. 2. As shown, the etch is performed through a portion (approximately half) of the substrate layer thickness. Then, material is removed from the opposing side of the substrate layer 120 until break-thru occurs with the through holes 140. The resulting porous membrane 126 may have a thickness in the range of 100 to 500 micrometers.

[0044] A nanoporous filler material 142 may be disposed within the plurality of through holes 140. The filler material 142 includes a plurality of molecular-scale open pores 138 fluidly coupling the liquid reservoir 122 to the vapor interface 112. The molecular-scale pores 138 may be sized to provide a pre-determined pressure differential across the porous membrane 126, in accordance with Equation 3 above. As used herein, the term "open pore" means an open passageway from the vapor-side to the liquid-side of the substrate. The open passageway may be straight-through, tortuous, or branched. In one embodiment, the filler material 142 comprises a molecular gel. As used herein, a molecular gel is a cross-linked system comprising an amorphous mixture of an interconnected phase and a solvent. The three-dimensional cross-linked network within the solvent provides a molecular-scale pathway through the structure of the gel, herein referred to as the open pores. The diameter of the pores 138 in the molecular gels range from 1 to 100 nanometers. The molecular gel may include both organic forms and inorganic forms. In one example, an organic form is a hydrogel.

[0045] In another example, an inorganic form is a sol-gel. One example of a sol-gel that is particularly well-adapted for use in the present invention is an amorphous silica sol-gel comprising a tetraethoxysilane precursor and having a pore size in the range of 1 to 2 nanometers. With reference to the equations above, this filler material 142 may provide negative pressures in the liquid reservoir 122 of less than -100 atmospheres (-10 megapascals). The sol-gel may be formed via spin-coating the precursor solutions onto the etched through holes 140. Alternately, the composite comprising porous silicon and silica sol-gel may be formed in the through holes 140 by drop-casting the pre-gel solution onto the porous matrix. The reagents will wick into the through holes 140 prior to thermal curing in ethanol.

[0046] In other examples, the filler material 142 may comprise nanoporous materials such as zeolytes, ceramics, and porous oxides such as alumina and silica. The size of the pores 138 in these examples may range from 0.2 nanometers (for zeolytes) to 200 nanometers (for porous silicon). In one example, the filler material 142 is porous silicon having a mean pore diameter of approximately 20 nanometers. The corresponding negative pressure in the liquid conduit 16 may be less than -0.1 atmospheres (-0.01 megapascals), and in some examples, may be less than -100 atmospheres (-10 megapascals).

[0047] Turning to FIG. 6 of the drawings, yet another embodiment of a porous membrane 226 is shown wherein a molecular membrane 244 is disposed adjacent to a filler material 242 to add an extra measure of robustness. In one example, the molecular membrane 244 is a wicking hydrogel membrane disposed on the vapor-side of the porous membrane 226. The inventors have determined that the hydrogel membrane 244, being a molecular-scale mixture of polymer and water, is able to mediate the generation of negative pressures through an osmosis-like mechanism and provides excellent wicking capability. In another example, the molecular membrane 244 comprises a solution of acrylate monomer (or oligomers), a cross-linker, an initiator, and an acrylo-silane binder. The hydrogel solution may be spin cast onto the external surface of the sol-gel filled, porous silicon, then cured.

[0048] The molecular membrane 244 may be utilized to ease manufacturing and reduce costs. In one example, the porous membrane 226 may have relaxed tolerances on pore diameter, thereby making it cheaper to produce. Since the pressure differential from the vapor interface 212 to the reservoir 222 is dependent upon the largest pore diameter (Eq. 4), the pore size in the porous membrane 226 may be relaxed without adversely affecting the maximum achievable pressure differential, as long as the pore diameter in the molecular membrane 244 remains tightly controlled. In this manner, a hierarchically structured or graduated membrane may be constructed within the scope of the invention.

[0049] In another embodiment, the molecular membrane 244 comprises a non-wicking (*e.g.*, hydrophobic) highly crystalline polytetrafluoroethylene polymer which has a microstructure characterized by nodes interconnected by fibrils. In one example, the polytetrafluoroethylene polymer is a GORE-TEX® membrane available from W. L. Gore & Associates, Inc. In this embodiment, the pores in the GORE-TEX® membrane are approximately 0.02 micrometers to 10 micrometers in size, which is about 20,000 times smaller than a water droplet, but 700 times larger than a water vapor molecule. The GORE-TEX® membrane will therefore establish a water vapor interface at the surface of the porous membrane 226.

[0050] The disclosed microfluidic probe may benefit agricultural crop producers, such as wine grape growers, because the probe allows dynamic data gathering to predict the effect of water stress on the overall vineyard or on portions thereof. In a broader sense, data gathered may also lead to proper regulation of irrigation to impose an intended water deficit to achieve the desired fruit style with higher reliability. Turning now to FIGS. 7 and 8, a water management system 346 includes a plurality of microfluidic probes 302 to provide precision ecology and agriculture. In one example, a tract of land may include a forest portion 348, an orchard portion 350, and a vineyard portion 352, each having variable soil conditions such as chemical potential and nutrient concentrations, μ_w^{soil} and $\{c_w^{soil}\}$, respectively. The portions of the tract 348, 350, 352 further include variable atmospheric conditions such as temperature, T_{atm}, vapor pressure, p_w, solar radiance, R_s, and wind speed V. The plurality of microfluidic probes 302 may allow for monitoring and control of water (μ_w^{plant}) and nutrient ($\{c_{nut}^{plant}\}$) status directly in plants using a wireless interface 354. The probe data may allow for closure of mass and energy balances, (J_w [moles/(m^{2}s)]) and (q [W/m^2]), respectively.

[0051] FIG. 8 illustrates the microfluidic probe 302 with a wireless transponder 356 bound to the trunk of a woody plant. In one embodiment, the probes 302 make multiple insertions to the xylem 307 conduits of the plant to assess the health of the plant and provide nutrients according to a pre-determined schedule. A first insertion point includes an analytical xylem tap 358*a* for sensing chemical potential μ_w^{plant}. A second insertion point includes an analytical xylem tap 358*b* for sensing nutrient concentration $\{c_{nut}^{plant}\}$. Data gathered from the water dynamics and nutrient content may be broadcast from the wireless transponder 356 to a receiver 360 at a control point where data is gathered from all the other probes 302. The interfaces may allow for the transmission and remote logging of data from sensors as well as those from conventional thermocouples and air hygrometers deployed on the plant. The data may be compared to historical or desired properties, for example, and corrective action may be undertaken to alter the chemical potential or nutrient content in any number of plants.

[0052] In one example, the current state of chemical potential is determined to be higher than desired, meaning the plant may be experiencing undue stress from lack of moisture. A feedback action may be initiated responsive to the data reading, such as actuating an irrigation system. The action may continue until current readings indicate the chemical potential is within acceptable limits. In one example, the water (or nutrient) is added via a feedback-controlled supply tap 362 for direct delivery to the xylem 307.

[0053] The disclosed microfluidic probe has particular potential for use in measuring the chemical potential in grapevines. Water status not only determines growth in grapevines, but there is also recent evidence that water status is a critical component of *terroir*, or the special characteristics that geography, climate and culture bestows upon wines from specific locations (e.g. Burgundy, France). The effect of water stress on fruit and wine quality is a high priority on national wine industry surveys, reflecting the industry's understanding of this relationship. But the invention is not so limited. The inventors contemplate the microfluidic probe may be used to measure chemical potential in virtually any type of tree, including any variety of fruit tree or forest trees. The microfluidic probe may further be adapted to measure chemical potential in annual plants and the like, but the probe body and/or coating on the semi-permeable tip may require small modifications due to the soft tissue on the plants.

[0054] An additional use of the microfluidic probe may be to measure the chemical potential in soil replacing the current large soil tensiometers to provide a much greater and more useful range of chemical potentials. In one application of the invention, real-time measurements of soil water potential can be made. Current methods assess soil water status by measuring soil water content or soil water potential. Soil water content is measured using feel and appearance, the gravimetric technique, or using instruments such as neutron probes, or time-domain or frequency-domain reflectometers. However, these techniques are prone to errors and have limitations. For example, neutron probes have a radioactive source and therefore requires a licensed professional to operate the instrument while reflectometers, although very accurate, tend to be expensive. Soil water content is better for calculating soil water budgets and water use of plants, but does not give a direct measure of plant stress. The relationship between soil water content and soil water potential is non-linear, but due to the heterogenous nature of soils, the mathematical description of the relationship varies for each type of soil and cannot be easily predicted. Additionally, as soil changes with depth and roots occur across this variation, predicting plant water potential from soil water content is exceedingly difficult. Consequently, soil water content is rarely used to estimate soil water potential. Very general empirical relationships between soil water content and plant performance are used (for example, with a given soil and plant a 50% depletion of the plant-available soil water may found from grower experience to relate to initial losses in yield). Soil water tension is measured by instruments such as porous (typically ceramic or gypsum) blocks and tensiometers. Porous blocks can be problematic in alkaline soils (they break down) and erroneous in saline soils due to high electrical conductivity of the soil solution. Current soil tensiometers have a practical operating range of only 0 to 75 centibars (0 to

0.075 megapascals), which may be adequate for coarse-textured soils but inadequate for fine-textured soils such as silt loams and clay loams. They are useful for irrigation scheduling to avoid any stress, but are not suited for measuring even moderately dry soil. They also require refilling of the water reservoir on a regular basis (depending on soil dryness).

[0055] Owing to its very small form factor, the disclosed microtensiometer may have useful applications in environments other than plant xylem or soils. In one example, the microtensiometer may be utilized in food service industries as a means for measuring the water chemical potential in foods. In another example, the microtensiometer may be used to measure the water chemical potential in industrial or chemical processes. In yet another example, the microtensiometer may be useful to measure the water chemical potential in materials processing.

[0056] One advantage of the disclosed microfluidic xylem probe is that the large negative water pressures (less than -10 atm.) present in the xylem may be directly measured to aid in determining the water content and ultimately the health of the plant, vine, or tree.

[0057] Another advantage of the disclosed microfluidic xylem probe is that they are compatible with long-term deployment directly within the primary vascular system of plants (xylem) and could therefore revolutionize the management of high value crops and the refinement of ecological and climatological data and models.

[0058] Yet another advantage of the disclosed microfluidic xylem probe is that real-time data may be obtained on a continuous basis, allowing scientists to correlate soil and climate models with actual responses in the chemical potential of the plant.

[0059] A general advantage of tensiometry is the potential for high accuracy at very high relative humidity (ΔP_{tensio} = 1 bar at RH = 99.99%). A particular advantage of the disclosed MEMS tensiometer is the potential to extend the functional range of tensiometry to ~85% RH (for water). Conventional tensiometers fail for RH <~ 99% RH. Improvements in performance due to membrane design and small internal volume.

[0060] While the present invention has been described with reference to a number of specific embodiments, it will be understood that the true spirit and scope of the invention should be determined only with respect to claims that can be supported by the present specification. Further, while in numerous cases herein wherein systems and apparatuses and methods are described as having a certain number of elements it will be understood that such systems, apparatuses and methods can be practiced with fewer than the mentioned certain number of elements. Also, while a number of particular embodiments have been described, it

will be understood that features and aspects that have been described with reference to each particular embodiment can be used with each remaining particularly described embodiment. For example, although the liquid in the reservoir has been described as pure water, other liquids are contemplated within the scope of the invention, such as alcohols or organic solvents.

We Claim:

1. A microfluidic probe comprising:

a probe body comprising a platform and a semi-permeable tip;

a microtensiometer coupled to the platform, the microtensiometer comprising:

a substrate layer having an enclosed reservoir formed therein;

a porous membrane disposed on a surface of the substrate layer, the membrane comprising a liquid side fluidly coupled to the reservoir and a vapor side fluidly coupled to a vapor interface, the membrane comprising a plurality of pores extending from the vapor interface to the reservoir, the pores having a maximum diameter in the range of 0.2 to 200 nanometers; and

a pressure sensor coupled to the reservoir for measuring changes to a pressure of a liquid disposed within the reservoir.

2. The microfluidic probe of claim 1, wherein the probe has a millimeter-scale form factor.

3. The microfluidic probe of claim 1, wherein the semi-permeable tip comprises a cone-shaped body, an interior portion of the cone-shaped body defining the vapor interface.

4. The microfluidic probe of claim 1, wherein the semi-permeable tip comprises a hydrophobic highly crystalline polytetrafluoroethylene polymer having a microstructure characterized by nodes interconnected by fibrils.

5. The microfluidic probe of claim 1, further comprising a transponder coupled to the pressure sensor for transmitting pressure data to a receiver.

6. The microfluidic probe of claim 5, wherein the transponder is a wireless transponder.

7. The microfluidic probe of claim 1, wherein the plurality of pores are interstitial voids formed in the lattice structure of the substrate layer.

8. The microfluidic probe of claim 7, wherein the mean diameter of the interstitial voids are in the range of 20 to 200 nanometers.

9. The microfluidic probe of claim 1, wherein the porous membrane comprises a plurality of through holes fluidly coupling the liquid reservoir to the vapor interface and a nanoporous filler material disposed within the plurality of through holes, the filler material comprising a plurality of open pores having a maximum diameter in the range of 1 to 100 nanometers.

10. The microfluidic probe of claim 9, wherein the through holes have a diameter in the range of 1 to 10 micrometers.

11. The microfluidic probe of claim 9, wherein the filler material comprises a molecular gel.

12. The microfluidic probe of claim 11, wherein the molecular gel is a sol-gel.

13. The microfluidic probe of claim 12, wherein the sol-gel comprises a plurality of open pores having a maximum diameter in the range of 1 to 10 nanometers.

14. The microfluidic probe of claim 11, wherein the molecular gel is a hydrogel.

15. The microfluidic probe of claim 1, wherein the sensor is a pressure sensor.

16. The microfluidic probe of claim 15, wherein the pressure sensor is a diaphragm-based pressure sensor comprising piezo-resistive strain gauges.

17. The microfluidic probe of claim 16, wherein the strain gauges are formed as thin films of poly-silicon.

18. The microfluidic probe of claim 1, wherein the probe body further comprises a cover, the pressure sensor having conductive leads attached thereto for transmitting a voltage responsive to the changes in pressure of the liquid in the reservoir, the conductive leads extending from the pressure sensor through the cover.

19. The microfluidic probe of claim 1, wherein the semi-permeable tip comprises a coating thereon with specific reactivity to proteins for biocompatibility with plant xylem.

20. The microfluidic probe of claim 19, wherein the coating is a hydrogel formulation.

21. A microtensiometer, comprising:

a substrate layer having an enclosed reservoir formed therein;

a porous membrane disposed on a surface of the substrate layer, the membrane comprising a liquid side fluidly coupled to the reservoir and a vapor side fluidly coupled to a vapor interface, the porous membrane comprising a plurality of through holes fluidly coupling the liquid reservoir to the vapor interface and a nanoporous filler material disposed within the plurality of through holes, the filler material comprising a plurality of open pores having a maximum diameter in the range of 0.2 to 200 nanometers; and

a pressure sensor coupled to the reservoir for measuring changes to a pressure of a liquid disposed within the reservoir.

22. The microtensiometer of claim 21, wherein the through holes have a diameter in the range of 1 to 10 micrometers.

23. The microtensiometer of claim 21, wherein the filler material comprises a molecular gel.

24. The microtensiometer of claim 23, wherein the molecular gel is a sol-gel.

25. The microtensiometer of claim 24, wherein the sol-gel comprises a plurality of open pores having a maximum diameter in the range of 1 to 10 nanometers.

26. The microtensiometer of claim 23, wherein the molecular gel is a hydrogel.

27. The microtensiometer of claim 21, wherein the pressure sensor is a diaphragm-based pressure sensor comprising piezo-resistive strain gauges.

28. The microtensiometer of claim 27, wherein the strain gauges are formed as thin films of poly-silicon.

29. The microtensiometer of claim 21, further comprising a molecular membrane disposed adjacent to the vapor side of the porous membrane.

30. The microtensiometer of claim 29, wherein the molecular membrane is a wicking hydrogel membrane.

31. The microtensiometer of claim 29, wherein the molecular membrane is a hydrophobic, highly crystalline polytetrafluoroethylene polymer which has a microstructure characterized by nodes interconnected by fibrils.

32. A method for measuring chemical potential in plant xylem, the method comprising the steps of:

providing a plant or tree having xylem conduits for transporting sap water;

placing a microtensiometer adjacent to a plurality of intact xylem conduits;

equilibrating the average chemical potential of the sap water in the plurality of intact xylem conduits with a liquid disposed in the microtensiometer;

sensing a change in the average chemical potential of the sap water with a pressure sensor coupled to the microtensiometer;

transmitting a voltage response from the pressure sensor to a transponder coupled to conductive leads of the pressure sensor; and

transmitting an output from the transponder to a receiver.

33. The method of claim 32, wherein the microfluidic tensiometer comprises:

a substrate layer having an enclosed liquid reservoir formed therein;

a porous membrane disposed on a surface of the substrate layer, the membrane comprising a plurality of pores fluidly coupling the liquid reservoir to a vapor interface of the sap water; and

wherein the pressure sensor is coupled to the liquid reservoir for measuring changes to pressure in the liquid reservoir.

34. The method of claim 33, wherein the step of equilibrating comprises:

exposing the sap water to a semi-permeable material that permits sap water vapor transfer and prohibits sap water liquid transfer;

exposing the liquid in the reservoir to the porous membrane such that the membrane permits vapor transfer and prohibits liquid transfer; and

allowing the sap water vapor to reach equilibrium with the reservoir vapor.

35. The method of claim 33, wherein the pores have a maximum diameter in the range of 0.2 to 200 nanometers.

36. The method of claim 32, wherein the transponder is wireless.

37. The method of claim 32, wherein the step of placing a microtensiometer adjacent to a plurality of intact xylem conduits comprises inserting a plurality of microtensiometers at different locations within one plant.

38. The method of claim 32, wherein the step of placing a microtensiometer adjacent to a plurality of intact xylem conduits comprises inserting a plurality of microtensiometers into a plurality of plants, the receiver adapted to receive the output from each transponder coupled to the plurality of microtensiometers.

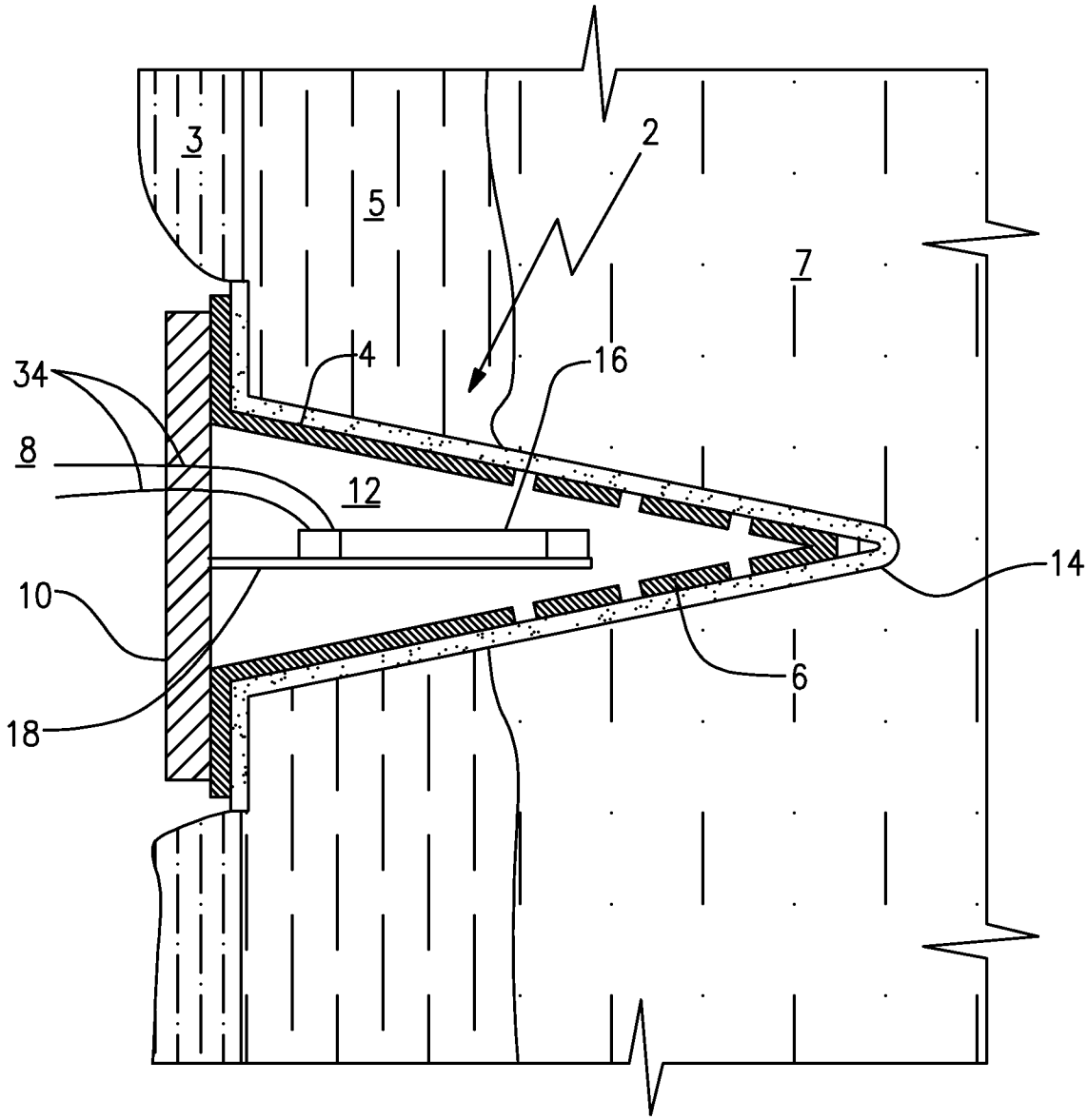


FIG.1

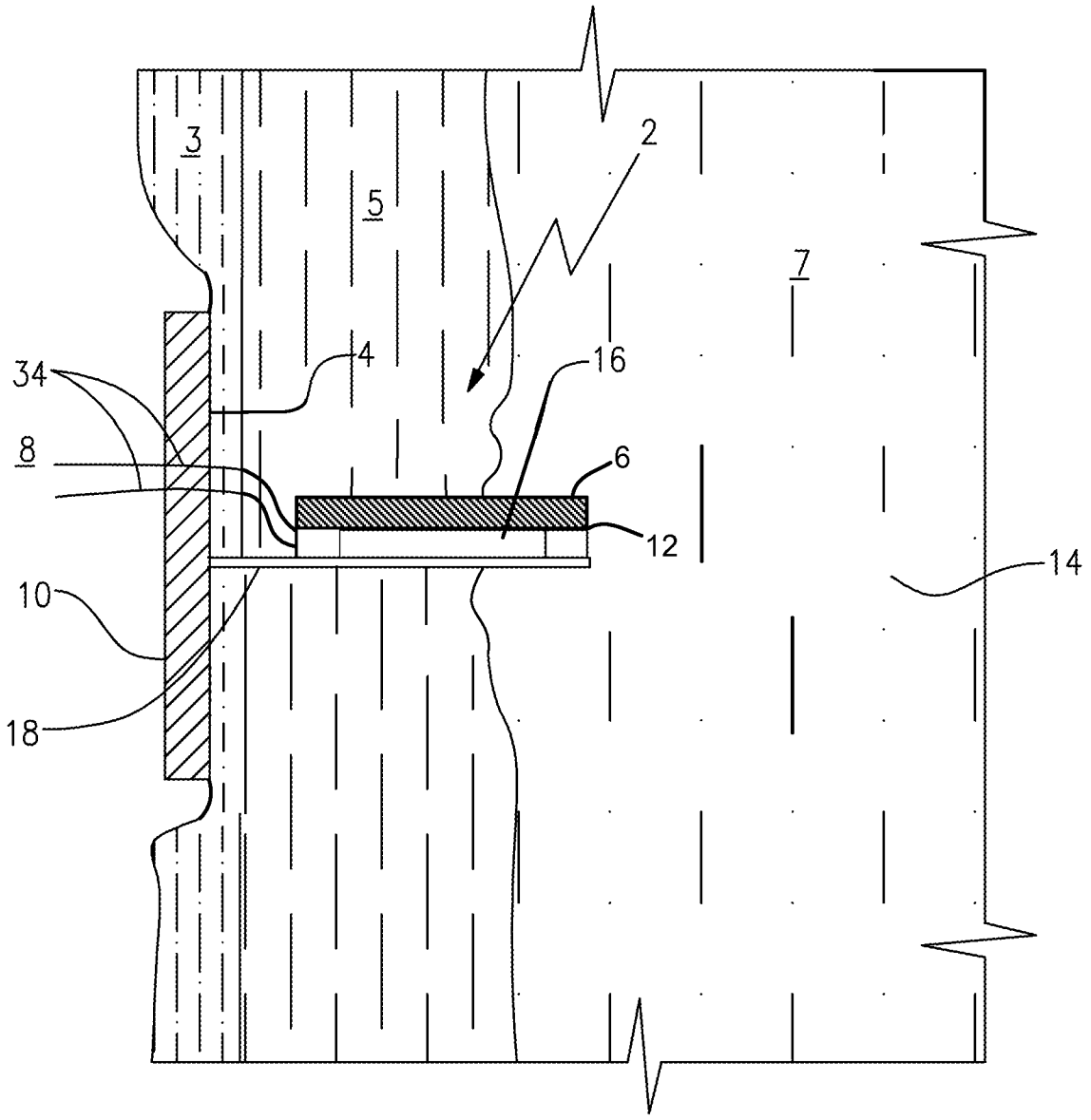


FIG.1A

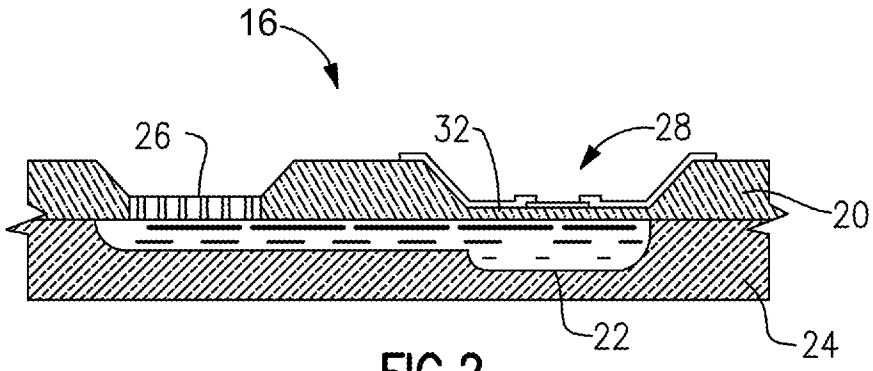


FIG.2

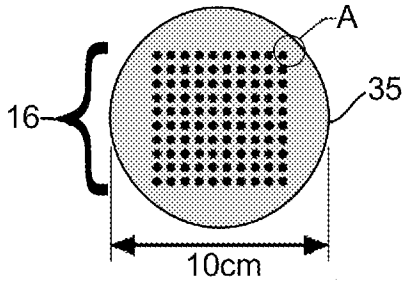


FIG.3A

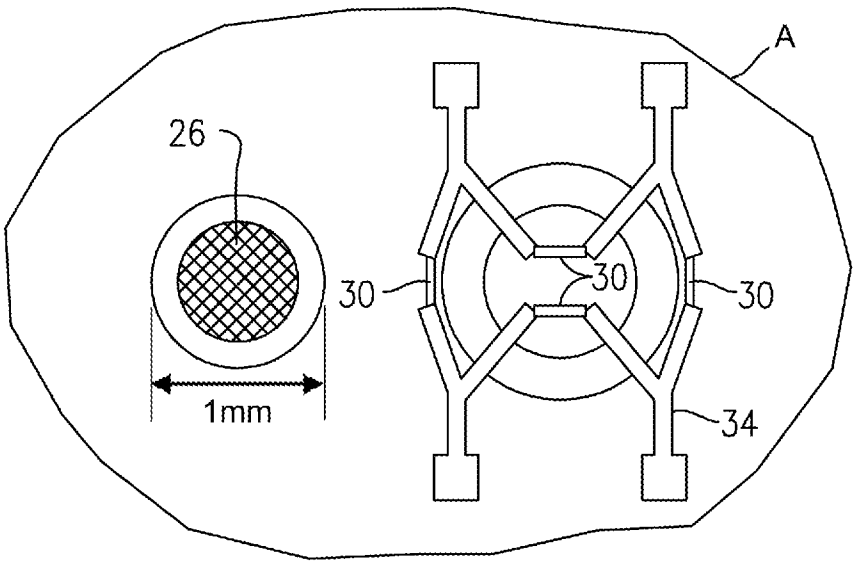


FIG.3B

FIG.4

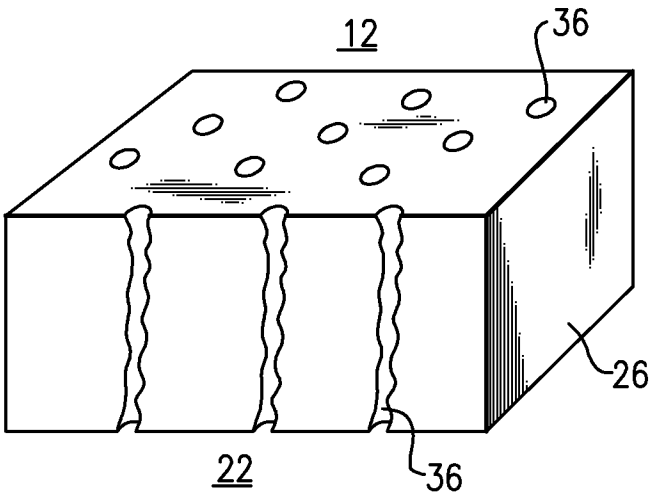
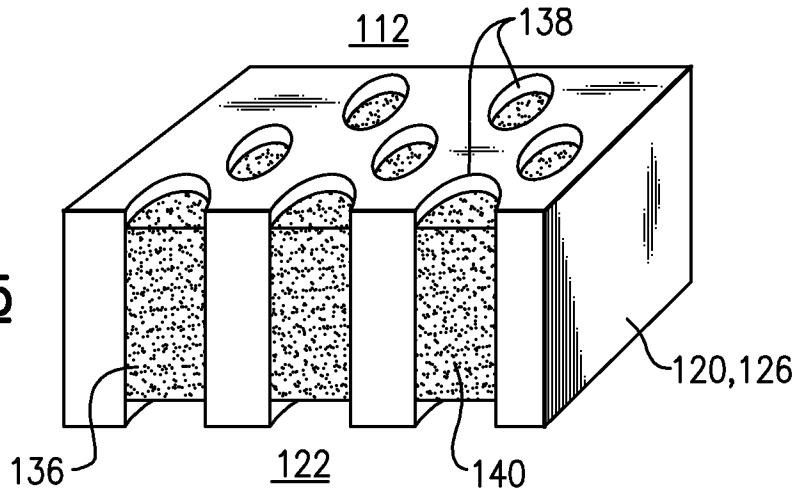


FIG.5



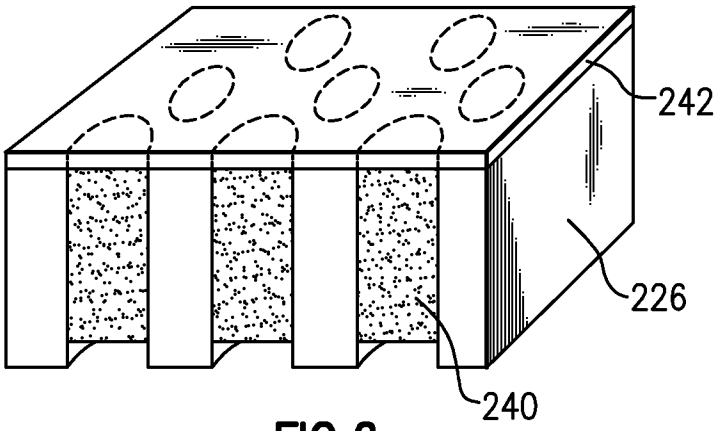


FIG.6

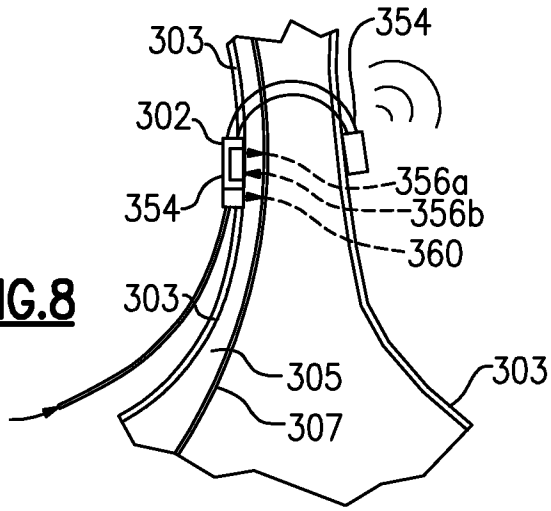


FIG.8

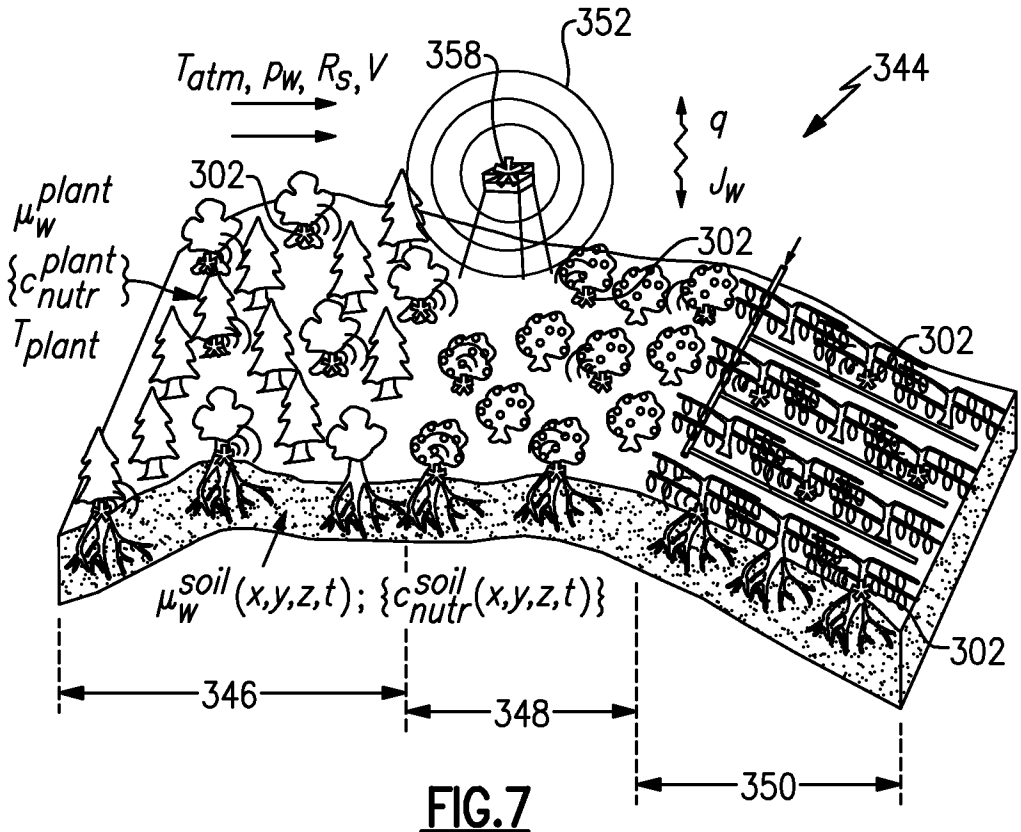


FIG.7