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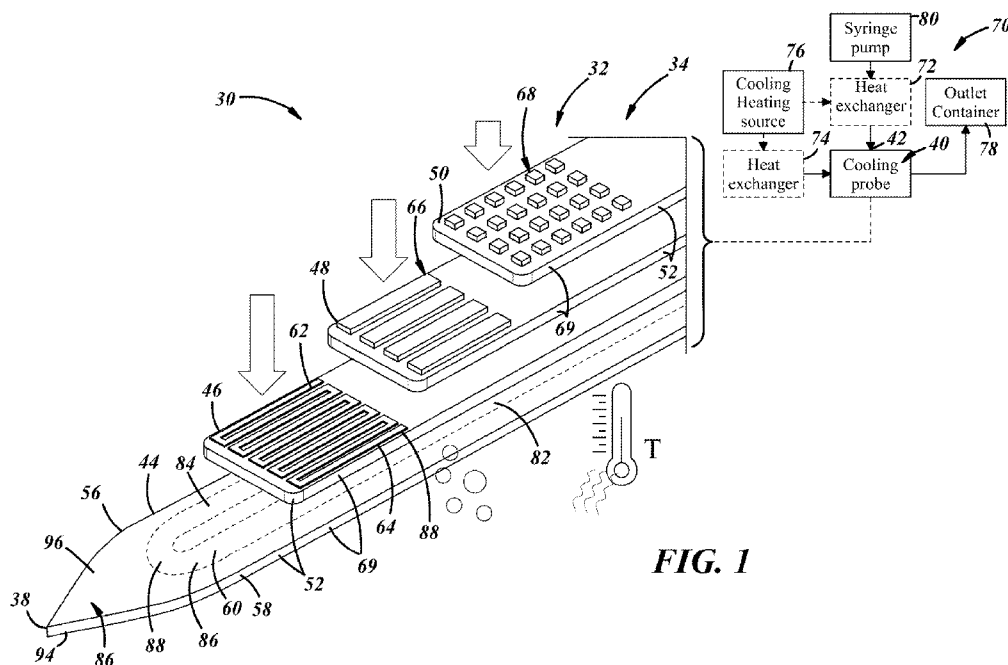


FIG. 1

(57) Abstract: A neural probe assembly and implantable neural probe include a fluid-based temperature control system that helps controls the temperature of neural tissue. In one implementation, the neural probe includes a probe shank extending from a tip end portion to a backend portion, and a channel extending from the backend portion at least partially down the probe shank toward the tip end portion. The channel is fully sealed along the probe shank toward the tip end portion, and the channel is configured to sealingly house a fluid. A head stage adapter may be employed to house one or more heat exchangers for modulating the temperature of the fluid in the channel.

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NEURAL PROBE ASSEMBLIES, METHODS OF USE AND MANUFACTUREFIELD

[0001] This disclosure relates generally to probe assemblies, and more particularly, to temperature controlled neural probe assemblies and methods of use and manufacture.

BACKGROUND

[0002] Brain temperature control is a potential treatment for reducing neural activity, with its working principle possibly related to decreasing neural activity. Although some clinical trials are ongoing, the mechanism by which temperature changes affect neural activity remains unclear. It has been found that deep hypothermia (20°C for 30 minutes) can reduce EEG power and seizure activity, without causing widespread breakdown of the blood-brain barrier (BBB) or significant macrophage infiltration in areas of neuronal injury, compared to the control group. However, whole-body cooling can be impractical and ineffective. For research purposes, intracranial cooling offers greater spatial selectivity and faster temperature reduction (10–30 times faster than traditional systemic hypothermia) with minimal invasiveness. This approach could be a more practical and effective way to study the effects of temperature changes on neural activity. Controlling the probe temperature may be beneficial in clinical settings, such as epilepsy and stroke treatment, to cite a few examples. Cooling the probe may be desirable in some instances, but heating may be helpful in situations as well, such as for research purposes to determine the impact of increased temperatures on brain tissue.

[0003] Researchers in the past have used Peltier devices, also known as thermoelectric coolers (TECs) or Peltier modules, to achieve precise focal brain temperature control. These solid-state heat pumps can transfer heat from one side to the other based on the direction of the applied electrical current. Peltier devices are popular in cooling and temperature control applications, such as electronic components, scientific instruments, and small-scale refrigeration systems, due to their compact size, absence of moving parts, and precise temperature control by adjusting input voltage. However, Peltier devices have some limitations, such as relatively low efficiency compared to traditional cooling methods, a limited temperature differential between the hot and cold sides, and decreased efficiency as the temperature of the hot side increases. This can further restrict their effectiveness in high-temperature applications. In conjunction with Peltier devices, researchers have attached silver wires (e.g., 260 µm diameter) or gold pins (e.g., 330 µm

diameter). Heat conduction is the primary mode of heat transfer, necessitating high thermal conductivity materials, typically metals, for efficient heat transfer. This constraint means that the probe can only be based on metal. Due to their low efficiency, these probes are larger than standard neural probes, and their high Young's modulus could potentially cause damage to brain tissue.

[0004] Cooling probes can be considered a new category of neural probes. Neural probes are devices designed to record, stimulate, or modulate the electrical activity of neurons, enabling researchers and clinicians to study brain function and develop targeted therapies. When compared to other advanced probes, the focus is on miniaturization and increased channel count. Researchers are striving to create smaller, more compact probes capable of accommodating a greater number of recording and stimulation channels. This would enable more precise and targeted interactions with neuronal populations while minimizing tissue damage during implantation. Another trend is the application of flexible and biocompatible materials. Incorporating biocompatible and flexible materials, such as polymers, in neural probe design can enhance the device's long-term stability and biocompatibility. This can help reduce the foreign body response and improve signal quality. It may also be possible to harness the heat distribution characteristics of flexible probe materials to improve temperature control outcomes.

SUMMARY

[0005] In accordance with one embodiment, there is provided a neural probe comprising a probe shank extending from a tip end portion to a backend portion, and a channel extending from the backend portion at least partially down the probe shank toward the tip end portion. The channel is fully sealed along the probe shank toward the tip end portion, and the channel is configured to sealingly house a fluid.

[0006] In some embodiments, the channel has a U-shaped longitudinal extent along the probe shank. An inlet branch and an outlet branch of the U-shaped longitudinal extent join at a pointed tip end at the tip end portion. The channel can be embedded in a flexible body of the probe shank.

[0007] In some embodiments, there is an external temperature-controlled source configured to change a temperature of the fluid, and a head stage adapter located between the implantable neural probe and the external temperature-controlled source. The head stage adapter is

configured to allow for flow of the fluid from the external temperature-controlled source to the channel. A heat exchanger can be at least partially housed in the head stage adapter.

[0008] In some embodiments, there is an insulative sheath at least partially surrounding the probe shank. The insulative sheath can leave the tip end portion exposed and wrap around a full radial extent of the probe shank. The insulative sheath has one or more curvilinear contours and a plurality of fluid bubbles.

[0009] In accordance with one embodiment, there is provided a neural probe assembly comprising an implantable neural probe, an external temperature-controlled source configured to change a temperature of a fluid, and a head stage adapter located between the implantable neural probe and the external temperature-controlled source. The head stage adapter is configured to allow for flow of the fluid from the external temperature-controlled source to the implantable neural probe.

[0010] In some embodiments, a heat exchanger is at least partially housed in the head stage adapter, along with a second heat exchanger that is bonded to the first heat exchanger via a heat sink layer.

[0011] In some embodiments, the head stage adapter comprises an inlet/outlet segment, an interface segment, and a connection port configured to couple the interface segment with a back end of the implantable neural probe. The interface segment can include an O-ring, and the connection port can be a rigid plate body.

[0012] In some embodiments, the implantable neural probe comprises a probe shank extending from a tip end portion to a backend portion, and a channel extending from the backend portion at least partially down the probe shank toward the tip end portion. The channel is fully sealed along the probe shank toward the tip end portion, and the channel is configured to sealingly house the fluid.

[0013] In accordance with one embodiment, there is provided a method of controlling a temperature of an implanted neural probe assembly, comprising the step of administering a fluid to a closed channel on a neural probe of the neural probe assembly. The flow rate of the fluid can be 0-1 mL/min, inclusive, or 40-200 μ L/min, inclusive, or advantageously 100-160 μ L/min, inclusive. In some embodiments, the method also includes steps of receiving feedback from a temperature sensor on the neural probe, and adjusting a temperature of the fluid depending on the feedback from the temperature sensor.

[0014] In accordance with one embodiment, there is provided a method of manufacturing a neural probe assembly comprising the steps of molding a channel into a channel layer, the channel having a U-shaped cross-section and a U-shaped longitudinal extent, and laminating the channel layer to a backing layer to form a probe shank. In some embodiments, the method also includes the steps of forming a plurality of fluid bubbles into an insulative sheath layer, and folding the insulative sheath layer at least partially around the probe shank.

[0015] Various aspects, embodiments, examples, features, and alternatives set forth in the preceding paragraphs, in the claims, and/or in the following description and drawings may be taken independently or in any combination thereof. For example, features disclosed in connection with one embodiment are applicable to all embodiments in the absence of incompatibility of features.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Preferred example embodiments will hereinafter be described in conjunction with the appended drawings, wherein like designations denote like elements, and wherein:

FIG. 1 is a partial, exploded view of a neural probe and neural probe assembly, according to one embodiment, with a backend system schematically illustrated;

FIG. 2 shows a tip end portion of the neural probe of FIG. 1;

FIG. 3 is a cross-section view of the neural probe of FIGS. 1 and 2, also showing a delivery shuttle;

FIG. 4 is a simulation model from COMSOL, with a comparison of two probe types (panel A) and the border of the heat distribution map (panels B, C, and D);

FIG. 5 shows a tip end portion and cross-section of the tip end portion of a neural probe in accordance with one embodiment;

FIG. 6 is a schematic drawing of a neural probe according to one embodiment;

FIG. 7 schematically represents a process flow manufacturing method of the neural probe of FIG. 6;

FIG. 8 is a side, schematic view of a neural probe assembly in accordance with an embodiment;

FIG. 9 schematically shows a neural probe assembly in accordance with an embodiment;

FIG. 10 shows one example embodiment of a head stage adapter that can be used with a neural probe assembly;

FIG. 11 shows another example embodiment of a head stage adapter;
FIG. 12 shows another example embodiment of a head stage adapter;
FIG. 13 is a side view of the head stage adapter of FIG. 12;
FIG. 14 is a top, perspective view of the head stage adapter of FIGS. 12 and 13;
FIG. 15A is a view of the heat exchanger from the head stage adapter of FIGS. 12-14;
FIG. 15B is a view of the heat exchanger of 15A with a heat sink layer;
FIG. 16 schematically represents an implanted neural probe assembly;
FIG. 17 shows the heat absorption rates for different dimensions compared to flow rate;
FIG. 18 shows flow rate versus temperature drop for different inlet fluid temperatures;
FIG. 19 shows flow rate versus temperature drop for different probe dimensions;
FIG. 20A shows flow rate versus temperature drop for different fluids;
FIG. 20B shows flow rate versus heat absorbed for different fluids;
FIG. 21 shows temperature sensor resistance change correlated with temperature change;
FIG. 22 shows flow rate versus temperature drop, comparing in-vitro testing results with simulation results;
FIG. 23 shows a heat loss plot, illustrating temperature change versus tubing length;
FIG. 24 shows brain temperature changes for different flow rates;
FIG. 25 shows temperature change versus flow rate under different heat exchanger settings;
FIG. 26 shows power fluctuations during cooling treatment within the 0-40 Hz spectrum;
FIG. 27 shows the head stage adapter of FIGS. 12-14, in an angled configuration;
FIG. 28 is a schematic diagram showing one embodiment of a heat exchanger operation scheme;
FIG. 29 is a graph showing *in vivo* test results, showing the feasibility of the wide temperature control range of greater than $\pm 10^{\circ}$;
FIG. 30 shows an embodiment of a neural probe having an insulative sheath, with an inset showing the insulative sheath and another inset showing the cross-section;
FIG. 31 shows the insulative sheath of the embodiment of FIG. 30; and
FIG. 32 shows simulation results of temperature profile along the shank without and with differently structured insulative layers.

DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

- [0017] Temperature can play a vital role in physiological processes, with meticulous control of brain temperature surfacing as an advantageous determinant in modulating neural activity. Current Peltier-based cooling modules grapple with problems like irregular heat distribution and substantial brain damage due to their rigid and sizeable structure. In response to these challenges, a flexible microfluidic channel-based temperature control probe is described herein. In the illustrated embodiments, the neural probe has cross-sectional dimensions of 150 μm x 45 μm , although other sizes are certainly possible. While microfluidic channels exist that facilitate drug dosing or the like, the present disclosure relates to closed channels that facilitate distribution of a cooling fluid along the tip end of the implantable probe. The designs described herein merge cutting-edge neural interface technology with an external cooling source, thereby imparting the ability to achieve exceptional cooling performance.
- [0018] Compared with other Peltier-based cooling probes, the neural probe assemblies described herein showed a six-fold decrease in brain tissue damage by reducing the probe size, a 46-fold reduction in the count of polyimide insulation tubes, and Young's modulus that is 20,000 times smaller. The use of flexible materials such as PDMS and polyimide can also improve heat distribution, which can enhance the cooling effect at the tip end of the cooling probe.
- [0019] In one embodiment, a temperature reduction of 14 degrees Celsius in the rat hippocampus and a 10-degree Celsius reduction in the nucleus accumbens was achieved with the neural probes described herein, and sustained for a continuous period of 30 minutes. Paired with a focal temperature sensor and a 64-channel flexible neural recording probe in one embodiment, the electrophysiological data reveal varying responses in amplitude and firing rate across different cell types in the hippocampus.
- [0020] To reduce the size and enhance the flexibility of cooling probes, evaluating their heat transfer mechanism can be helpful. Heat transfer occurs through three methods: convection, conduction, and radiation. Convection can be classified into two types: natural convection and forced convection. Forced convection occurs when a fluid (such as air or water) is compelled to flow over a surface by an external force like a pump or fan. This fluid flow can improve the heat transfer rate between the surface and the fluid, compared to natural convection driven solely by temperature differences. Microfluidic channels can be employed to utilize heat

convection for cooling brain regions. These small-scale channels, typically measuring in micrometers, are used to manipulate fluids at a microscale. Commonly employed in microfluidics research, these channels help control the flow and behavior of fluids in various applications, including medical diagnostics, drug delivery, chemical analysis, and DNA sequencing. Flexible microfluidics is an emerging field impacting numerous research areas, such as chemistry, electronics, biology, and medicine. Various flexible and stretchable materials have been utilized in flexible microfluidics, allowing for robust fluid-structure interactions. By incorporating flexible microchannels into cooling probe designs, as described herein, it is possible to create more compact and adaptable devices for brain temperature control. While microfluidic channels are generally known, they are typically for drug delivery and do not incorporate a closed or sealed channel structure to accommodate fluid delivery and flow for temperature control purposes.

[0021] In at least some embodiments, sealed U-shaped microfluidic channel-based neural probes leverage low Young's modulus materials and forced heat convection. This design has the potential to reduce the temperature of the targeted brain region by at least 5 °C, presenting a promising method for focal brain temperature control. The potential for reduced brain damage may allow for recording spike or broader electrophysiological data and a wider range of temperature control. By altering the targeted brain temperature and the duration of the cooling treatment, as well as employing a mix of neural recording devices and temperature sensors, both brain temperature and electrophysiological information can be consistently tracked. This real-time feedback allows for the measurement of correlation between localized brain temperature fluctuations and seizure events, facilitating the refinement of the cooling approach.

[0022] Existing cooling probes for rodent animal research suffer from inflexible and bulky designs due to material and heat transfer limitations. The large size of these probes can lead to significant brain damage, making it difficult to acquire neural spike signals. To tackle these challenges, a flexible microfluidic channel-based temperature control probe is described herein that integrates advanced neural interface technology with an external cooling source, resulting in improved cooling performance.

[0023] Advanced neural probes focus on minimizing probe size and using more flexible materials, which can significantly decrease brain tissue damage and inflammation. However, as described herein, current cooling probes typically rely on Peltier devices as cooling sources and attach a

metal wire for focal brain cooling, resulting in large, rigid wires limited by heat transfer methods. To overcome this limitation, sealed microfluidic channels and materials including PDMS and polyimide with a low Young's modulus can be used. Utilizing forced convection with high flow rate fluids can also boost cooling efficiency, resulting in a smaller cross-sectional dimension of $180\text{ }\mu\text{m} \times 45\text{ }\mu\text{m}$ in one implementation.

[0024] Cooling effects can impact focal brain temperature control range, cooling efficiency, and cooling distribution. As described, current cooling probes use Peltier devices attached to metal to conduct heat, leading to uneven heat distribution due to the material's high thermal conductivity. As a result, most heat is concentrated at the probe's back end, and the probe tip might only have a limited cooling effect area. Using flexible materials like polyimide and PDMS, both with low thermal conductivity, can offer more even heat distribution and a larger cooling effect area compared to metal. Peltier devices, as solid-state devices, can absorb heat from the cold junction, but their efficiency is typically low, from 6.88% to 14.32%. The microfluidic channel probes described herein can leverage an external cooling source with continuous water flow, and can achieve greater heat transfer efficiency. In some embodiments, a targeted cooling effect of $5\text{ }^{\circ}\text{C}$ is desirable. To ensure accuracy, high-precision temperature sensors can be integrated into the probe.

[0025] FIG. 1 shows a neural probe assembly 30 having a neural probe 32 and a probe shank 34 that extends from a tip end portion 36 at a tip end 38 to a backend portion 40 at a back end 42 (the back end is only schematically shown in FIG. 1 but more visible in other embodiments/figures such as FIG. 6, FIG. 9, and FIG. 28). In some embodiments, the neural probe assembly 30 has a single neural probe 32 and probe shank 34, as illustrated, and in other embodiments, the neural probe assembly 30 has a plurality of neural probes 32, with one or more having the cooling structure described herein, or a neural probe 32 may have a plurality of probe shanks 34 that emanate from a single backend portion 40, to cite a few examples. The assembly 30 comprises at least one intracortical neural probe 32 configured to be inserted into neural tissue (e.g., the neural probe 32 is for brain tissue, neural tissue, or other tissue in which electrical recording and/or stimulation could be beneficial), although it is possible for the assemblies, probes, and methods to be used in alternate implementations.

[0026] FIG. 1 shows an example of a probe shank 34 having a plurality of probe sub-units 44, 46, 48, 50. Each probe sub-unit 44, 46, 48, 50 is in essence its own separate probe, with the sub-

units then being assembled together. While a multi-layer implementation is advantageous, it is possible for the probe shank 34 to only be a single functional layer or sub-unit, with a channel layer integrated therewith or otherwise attached thereto, depending on the desired implementation. Each of the probe sub-units 44, 46, 48, 50 has a flexible body 52 that serves as the main probe structure and shape. The flexible body is advantageously one or more polymer thin film layers, or more particularly, patterned PDMS and/or polyimide thin film layers (e.g., PI-2610). The low Young's modulus and high yield strain material properties of polymer thin films allow them to provide better heat distribution and a larger cooling effect area. The probe sub-units 44, 46, 48, 50 can be assembled together to create the probe shank 34 having a pointed tip end 38 configured to be inserted into neural tissue, the back end 42 configured to connect to one or more PCBs 54 (possibly via a flexible cable, see e.g., FIG. 9). The probe shank 34 also has a first longitudinal side 56 and a second longitudinal side 58. The first and second longitudinal sides 56, 58 are the longest sides of the probe shank 34 and extend from the tip end 38 to the back end 42.

[0027] In the embodiment illustrated in FIGS. 1-3, the probe sub-unit 44 has a channel 60, which is used to control the temperature of the probe shank 34 and the tip end portion 36, as detailed further below. The probe sub-unit 46 has a thermal resistor 62 that is used as the temperature sensor 64. The probe sub-unit 48 has a plurality of chemical sensing electrodes 66, and the probe sub-unit 50 has a plurality of recording/stimulating electrodes 68 (recording/stimulating electrodes may include recording sites, stimulation sites, or a combination of both recording and stimulation sites). Each probe sub-unit 44, 46, 48, 50 has its own functionality, and are operable on their own as a distinct probe. Advantageously, the probe sub-units 44, 46, 48, 50 are laminated together to align each of the first and second longitudinal sides 68, 70 and create a multi-layer probe shank 34. The narrow sidewall 69 of each of the probe sub-units 44, 46, 48, 50 can help to maintain a low profile for the probe shank 34. However, as addressed above, it is possible in some implementations to only use one probe sub-unit as the probe shank. Other functional probe sub-units may also be added depending on the desired functionality of the probe assembly 30, or some probe sub-units may be multi-functional. Additionally, various traces, interconnections, etc. may be included that are not particularly illustrated in the figures.

[0028] The neural probe 32 of the neural probe assembly 30 is coupled to a backend system 70 which is schematically illustrated in FIG. 1. The backend system 70 helps facilitate the cooling

effects by strategically introducing a cooling fluid to the channel 60 in the probe shank 34. The backend system 70, as detailed further below, may include one or more heat exchangers 72, 74, a cooling/heating source 76, an outlet container 78, and a pump 80. The arrangement and components of the backend system 70 may vary from what is particularly illustrated, and generally, the backend system 70 is configured to cooperate with the channel 60 to enhance temperature control precision as compared with other cooling probes.

[0029] The microfluidic channel 60 is a fully sealed and embedded fluid passageway to accommodate temperature-controlled fluid from the external heating/cooling source 76. As opposed to drug delivery devices or the like, the channel 60 is not configured to allow fluid escape into the surrounding tissue upon implantation. In this embodiment, the channel 60 is fully sealed along the probe shank 34 toward the tip end portion 36 in order to sealingly house a fluid from the external heating/cooling source 76. It is possible, however, for the neural probe assembly 30 to be equipped with a drug delivery microchannel in addition to the channel 60. In some embodiments, if the neural probe assembly 30 comprises a plurality of neural probes 32, one or more of the neural probes 32 may be equipped with one or more microfluidic cooling channels 60, and then one or more of those channels 60 may be used to selectively administer cooling fluid as needed during operation of the probe assembly.

[0030] In the embodiments illustrated in FIGS. 1-3, the channel 60 has a U-shaped longitudinal extent 82 with an inlet branch 84 and an outlet branch 86. The inlet branch 84 and the outlet branch 86 meet at a curved junction 88 at the pointed tip end 38 of the tip end portion 36. The U-shaped longitudinal extent 82 may also have more of an angled-V shaped junction or a rectangular shaped junction, or could include other features such as sinusoidal curves, projections out from the inlet branch 84 and/or the outlet branch 86. The configuration of each of the inlet branch 84 and/or the outlet branch 86 may also vary from what is particularly illustrated. For example, including a widened area or curved portion may help more selectively change the temperature at a desired area, as the flow rate would change depending on the channel 60 configuration. It is advantageous, however, to have the U-shaped longitudinal extent 82 as it helps promote a more consistent and controllable fluid flow rate within the channel 60, which can help with precision temperature control. Additionally, routing the channel 60 all the way to the pointed tip end 38 allows for improved cooling at the tip portion 36, which is usually closer to the functional components of the probe 32 which may impact temperature (e.g., microLEDs for

optoelectrode applications). The sharp pointed tip end 38 is designed to simplify implantation into the brain and provide a larger bonding area to prevent channel 60 leakage.

[0031] The channel 60 also has a U-shaped cross-section, as shown in FIG. 3. Like the curved junction 88, the channel base 92 can be rounded or curved, or have a V-shape or rectangular-shape as illustrated. In the illustrated embodiment, the rectangular U-shaped cross section provides for a channel that is about twice as deep as it is wide, but other sizes, shapes, configurations, etc. for the channel 60 are certainly possible. The dimensions of the microfluidic channel 60 should be sufficiently sized to provide a suitable flow rate for cooling purposes. In this embodiment, the width of the channel 60 is designed to range from 20 μm to 60 μm , and the total width of the probe shank 34 is 100 μm to 180 μm . In an advantageous embodiment, as illustrated the width, of the channel 60 is consistent along the entirety of the probe shank 34, which can help with fluid flow. However, it is possible for portions of the width to change (e.g., small reservoirs or the like), but the fluid dynamic impact should be considered in order to achieve sufficient temperature change.

[0032] FIG. 3 shows that the channel 60 may be comprised of a channel layer 94 that is attached to a backing layer 96, which in this embodiment, together make up the probe sub-unit 44 of the neural probe 32. In other embodiments, it is possible to have alternate arrangements, such as a channel that is fully embedded within a single material layer, or the channel being an added tube-like component on the surface of another, wider probe shank, or another operable embodiment. One or more other probe sub-units 46, 48, 50 can be bonded to the back of the backing layer 96, and then, as shown in FIG. 3, a glass shuttle 98 may be used to help implantation. In this embodiment, a sharpened glass pipette with a tip diameter of approximately 30 μm was used for the shuttle 98. This shuttle structure may be beneficial with the flexible probes 32 detailed herein.

[0033] Advantageously, the channel layer 94 and the backing layer 96, as well as the other main probe sub-units 46, 48, 50 are made of a flexible polymer-based material to form the flexible body 52 of the neural probe 32. A flexible material to make the flexible body 52 may include materials having a Young's modulus of less than 10 GPa, compared with more rigid silicon probes that have shanks with a Young's modulus of about 140 GPa. In one particular embodiment, which can be both easier to manufacture and improve thermal distribution capabilities, the channel layer 94 is made from PDMS and the backing layer 96 is made from polyimide.

[0034] Silicon has traditionally been the primary material used in neural probe device fabrication due to its ease of processing, handling, and packaging. However, flexible materials such as SU-8, parylene-C, polyimide (PI), and PDMS may allow for improved long-term monitoring applications. When selecting a material for microfluidic probes, thermal conductivity should be considered, as it may be undesirable to have the fluid heat up too much before reaching the targeted brain tissue. As a result, materials with low thermal conductivity are preferred. Parylene is a preferred material due to its low thermal conductivity. However, the weak bonding strength between parylene layers may limit the operating flow rate, with the highest flow rate achieved in experimental tests being 10 $\mu\text{L}/\text{min}$. Thus, parylene may be a better material for larger dimension channel 60 implementations. Polyimide films are commonly used in harsh environments, such as automotive, aerospace, and microelectronic applications, due to their excellent mechanical performance, high thermal stability, and good electrical properties. However, it may be a bit more difficult to create a conformal surface when the channel thickness is too large (e.g., more than 10 μm). PDMS may be beneficial to use in microfluidic device fabrication due to its ease of fabrication, physical properties, and cost-effectiveness. PDMS channels can be made with a thickness of 40 μm or more, compared to polyimide channels with a thickness of 10 μm or less. However, the low Young's modulus of PDMS could make it unsuitable for integrated circuits, which may present challenges for integrating temperature sensors 100 at a later stage.

[0035] Therefore, a probe 32 combining PDMS and PI is proposed, which can facilitate future integration of temperature sensors 100 while also allowing for the fabrication of microfluidic channels 60 with a larger space. Material selection also affects the heat distribution of the cooling probe 32. Heat distribution of the cooling probe 32 can help with understanding the cooling effect along the longitudinal extent of the probe shank 34. In FIG. 4, the left heat map shows a silver-based probe, while the right is a PDMS/PI-based probe tip end portion 36. The two models are based on microfluidic channel flow at 150 $\mu\text{L}/\text{min}$ and only differ in material. Since the target is generally to control the temperature of the brain tissue around the probe tip end 38, the temperature distribution was measured within a range of 0, 100, 200, and 500 μm in diameter, with the probe tip 38 as the center. The PDMS-based probe 32 achieved temperature changes of 11.1°C, 8.3°C, 5°C, and 2.8°C, while the silver-based probe only achieved temperature changes of 6°C, 4.7°C, 1.9°C, and 0.2°C, respectively. The silver-based cooling probe requires a higher flow rate, and colder fluid from the inlet to achieve a similar cooling effect to that of the flexible

material-based probe 32. Panels B, C, and D of FIG. 4 show the border of the heat distribution map. An 8 mm x 8 mm x 10 mm brain tissue model was built in COMSOL, with the border set at a constant 37 °C.

[0036] FIG. 5 shows integration of a temperature sensor 100 onto the probe shank 34. The temperature sensor 100 is similar in structure to the temperature sensor 64 shown in FIG. 1. In this embodiment, however, the resistor of the temperature sensor 100 was made by patterning a single 100-nm thick and 1- μ m wide Ti/Au/Ti trace into a meander pattern on a PCB. The thermal resistor had a footprint of 200 μ m by 56 μ m. To improve the sensitivity of the thermal resistor of the temperature sensors, Wheatstone bridge circuits can be implemented. In this embodiment, polyethylene glycol (PEG) was coated on the temperature sensor 100 and the backing layer 96 to facilitate attachment. In some implementations, feedback from one or more of the temperature sensors 64, 100 may be used to adjust a temperature of the fluid administered to the channel 60, depending on the feedback from the temperature sensor(s).

[0037] FIG. 6 shows an example neural probe 32 having a channel 60, and FIG. 7 shows example manufacturing steps taken at the cross-section A-A' in FIG. 6. For the channel 60 and its outline shape, two SU-8 layers 102 were spin-coated on a Si wafer 104 with thicknesses of 30 μ m and 60 μ m. After the lithography process and Silane treatment for four hours, a 45 μ m thick 10:1 (weight ratio, w/w prepolymer to curing agent) PDMS (Sylgard 184, DOW CORNING) channel layer 94 was spin-coated at 1800 rpm for 60 s. The PDMS layer 94 on the SU-8 mold was cured at 80°C for four hours, and a silane treatment was carried out for 30 mins. Next, 30 grams of PDMS were poured on top of the PDMS channel surface. The poured PDMS was used as a buffer layer 106 to facilitate the transfer process of the PDMS channel layer 94. The PDMS/PDMS surface energy is stronger than the PDMS channel layer 94 and SU-8 mold 102 surface.

[0038] For the PI film backing layer 96, chrome (20 nm), gold (200 nm), and another chrome layer (100 nm) (not shown) were deposited on an Si wafer 108 for the subsequent probe 32 release. Then, a 5 μ m PI backing layer 96 was spin-coated at 3000 rpm for 60 s. A 20 nm titanium layer was sputtered after the polyimide curing process as an adhesion layer to enhance the bonding strength between the SiO₂ and PI layer. The SiO₂ layer was deposited chemically using an O₂ plasma (Plasma-Term 790 Series) with 250 nm by using 3.0 sccm SiH₄ flow, 250 sccm N₂O flow, 150 sccm He flow, 150 W stage power, and 700 mTorr chamber pressure for 5 min. Accordingly, in this embodiment, the adhesion layer 110 between the channel layer 94 and the backing layer

96 is an SiO₂/Ti layer between the PDMS and PI layers. The bonding step between PI/PDMS was pre-treated with O₂ plasma, followed by baking at 120°C for three hours. The PDMS buffer layer 106 was then peeled off, leaving the PDMS thin-film channel layer 94 bonded with the SiO₂-Ti-PI substrate or backing layer 96. The RIE process was subsequently employed to etch the outline of the probe 32, thin the PDMS layer 94, and etch the SiO₂-Ti-PI substrate 96 for 50 mins using 50 sccm SF₆ and 3 sccm O₂ by Plasma-Term 790. Finally, the sample was soaked in the Cr etchant for four hours and then carefully packaged for use.

[0039] FIGS. 8-16 illustrate various portions and components of the backend portion 40 of the neural probe 32 and the backend system 70 of the neural probe assembly 30. FIG. 8 shows the neural probe assembly 30 corresponding to the probe 32 illustrated in FIG. 6 with the temperature sensor 100. As shown, the PCB 54, having at least a portion of the temperature sensor 100, is located toward the backend portion 40. Glass slides 112, 114 may be used to enhance rigidity toward the backend portion 40, particularly at the inlet/outlet connectors 116, 118. The PCB 54 is coupled to the glass slide 114 in this embodiment.

[0040] A stable and sealed backend assembly 70 is advantageous, especially for chronic experiments. Chronic experiments are preferred in neuroscience research to study the long-term effects of treatments or conditions on the brain and behavior. Unlike acute experiments, chronic experiments involve long-term manipulations for several weeks or months, followed by analysis of resulting changes in the brain and behavior, enabling researchers to study gradual changes in synaptic connectivity or neuroplasticity. To facilitate broader applications for the cooling probe 32, a head stage 120 for chronic experiments can be used, along with an external pumping system 80. The pump 80 may be a syringe pump as illustrated, and it may be possible to have an inlet pump and/or an outlet pump (e.g., force fluid in and vacuum fluid out, respectively). The cooling probe sub-unit 44 can be integrated with other functional probes, including neural recording probes 50 and temperature sensor(s) 64, 100, in a chronic freely moving animal setup. The cross-sectional dimensions of the probe shank 34 can be reduced to 180 μm x 45 μm to help minimize brain damage while preserving sufficient cooling efficacy. The probe 32 is intended to have a longevity of 1.5 months, or more, consistent with other neural probes and potential applications.

[0041] FIGS. 9 and 12-16 show an embodiment of the head stage adapter 120 and its subcomponents that may be used with the backend system 70. FIGS. 10 and 11 show alternate embodiments for a head stage adapter 120', 120'' respectively, that can be used with a backend

system 70 for a neural probe assembly 30. The head stage adapter 120' shown in FIG. 10 comprises a steel tube connector 122. The steel tube connector 122 provides a simple solution for connecting PDMS-based microfluidic devices. However, it may be fragile and unable to withstand too much pressure, so it may be less desirable for use in a freely moving animal setup. To address this issue, the adapter 120" shown in FIG. 11 may be used, in which a primary O-ring seal 124 sealing backend design is illustrated. The microfluidic fitting 126 (IDEX N-333) can provide a good seal to connect the tubing with the external pump 80, which can withstand over 1000 PSI. The O-ring 124 is pressed by four screws tightened evenly to avoid leakage.

[0042] With reference to FIGS. 9 and 12-14, the head stage adapter 120 includes an inlet/outlet segment 128, an interface segment 130, and a connection port 132 configured to couple the interface segment to the back end 42 of the implantable neural probe 32. In this embodiment, the inlet/outlet segment 128 includes an inlet 134 and an outlet 136 that communicate and are operably connected with the inlet branch 84 and outlet branch 86 of the channel 60, respectively. The inlet 134 receives fluid 138 from an external temperature-controlled source 76, and fluid flow may be impacted by the pump 80. Fluid 138 then passes through the interface segment 130, through the connection port 132, into the inlet branch 84 of the channel 60, around the curved junction 88, through the outlet branch 86, back through the connection port 132, the interface segment 130, to the outlet 136 and back to the source 76 or another operable reservoir or drain. The inlet/outlet segment 128 may include a flexible housing 139 in which the inlet 134 and the outlet 136 are integrally molded, or the inlet 134 and outlet 136 may be separate tubes or the like to facilitate delivery of fluid 138.

[0043] In the illustrated embodiments, the interface segment 130 includes a sealing structure such as the O-rings, 140, 142. Other sealing configurations are possible, such as a single sealing layer made of a polymer material or the like with openings to facilitate the fluid flow to the inlet 134 and the outlet 136. The interface segment 130 provides a sealing interface between the inlet/outlet segment 128 and the connection port 132. The connection port 132 in the illustrated embodiment comprises customized rigid plate bodies 144, 146, which in this embodiment, form a glass slide 148, although other operable rigid materials may be employed. In some implementations, uneven tightening force can block the microfluidic channel 60, so the rigid plate bodies 144, 146 can be used as the connection port 132 or transit between the PDMS cooling probe back end 42 and the

inlet O-ring 140 and outlet O-ring 142 that are used. To minimize the head stage adapter 120 size, the number of fasteners or screws can be reduced to two.

[0044] Returning to FIG. 1, which shows the schematic representation of the backend system 70, one or more heat exchangers 72, 74 may be employed to help improve thermal management. Heat exchanger 72 was implanted to cool down the tubing of the syringe pump 80 and microfluidic inlet 134. The tubing used may be pressure and thermal resistant, with an outer diameter of 1/16 inch and an inner diameter of 1/32 inch in one particular embodiment. However, with a low flow rate (e.g., less than 200 $\mu\text{L}/\text{min}$), the heat loss could significantly impact the inner fluid 138 temperature, especially if the tubing is longer between the heat exchanger 72 and the inlet 134. Accordingly, it may be beneficial to use a heat exchanger 74, in addition to or as an alternative to the heat exchanger 72.

[0045] FIGS. 14, 15A, and 15B show an example embodiment of the heat exchanger 74. The heat exchanger 74 can be attached at the back end 42 of the probe 32. As shown in FIGS. 14 and 15A, the heat exchanger 74 includes a milli-fluidics channel passageway 150 that can accommodate or couple to the channel 60. The heat exchanger 74 in this embodiment is a 3D printed plate with the channel passageway 150, which may be sealed using a heat sink layer 152, as shown in FIG. 15B (the view of FIG. 15A shows the heat sink layer 152 removed). In this embodiment, the heat sink layer 152 is a copper tape 154, although other materials and configurations for creating and/or sealing the passageway 150 are certainly possible. The heat sink layer 152 advantageously serves to seal the channel passageway 150 and improve heat transfer, even at higher flow rates. In one example, an external pump, in addition to or as an alternative to the syringe pump 80, can be used to circulate the channel passageway 150 at 1L/min, which is about 5000 times higher than a preferred microfluidic flow rate. In some embodiments, an extra external pump may be associated with the external fluid source 76.

[0046] FIG. 16 shows an example chronic set up where the head stage adapter 120 is located on a carrier 156. The carrier 156 may be used to strategically position the head stage adapter 120 and neural probe 32 with respect to the neural tissue of a user 158. A freely moving rat with the head stage 120 setup was demonstrated, with the cooling probe 32 functioning at week three of the experiment. An adhesive (e.g., dental cement) may be used to embed the main probe's backend structure and carrier 156 and bond it with the skull. In experiments, the head stage adapter 120 remained undamaged and in place even when the users 158 (e.g., rats or another species) try to

scratch their heads, even after two months. Additionally, the bottom cap design at the inlet/outlet segment 128 can be used to help protect against scratches from the rats' forelimbs. To evaluate the cooling impact across different brain areas, in-vivo implantation of numerous temperature sensors 100 can be employed, strategically placing the sensors at diverse distances from the cooling probe 32. FIG. 27 illustrates how the head stage adapter 120 and carrier 156 can be angled (see e.g., angle θ) to enhance insertion capabilities.

[0047] To validate and develop the neural probe assembly 30, the heat absorption rate of the channel 60 was compared to a Peltier device implementation. Additionally, the change in brain tissue temperature at the probe's tip 38 was analyzed. To compare the heat absorption power, the Peltier device's performance can be assessed, including the curves of Q_c (the amount of heat absorbed at the cold junction in watts (W) versus the current under different conditions). The optimal condition is 0.2 W maximum in this particular embodiment. As for the microfluidic channel-based cooling probe 32, formula 1 can be utilized to calculate the heat energy (Q) transferred, as follows:

$$Q = mc \Delta T \quad (\text{Formula 1})$$

[0048] In this equation, Q represents the heat energy transferred (measured in Joules, J), m is the mass of the object or substance being heated or cooled (measured in kilograms, kg), c is the specific heat capacity of the object or substance (measured in J/kg·°C), which is a measure of its ability to store heat, and ΔT is the change in temperature of the inlet and outlet of the microfluidic channel 60 (measured in °C). This value can be further calculated by combining heat conduction and convection and using Newton's law of cooling to derive an equation for determining the temperature of the cooling probe tip surface T(t), as shown in formula 2.

$$T(t) = T(env) + (T(0) - T(env)) * e^{\frac{-t}{\tau}} \quad (\text{Formula 2})$$

[0049] The equation presented involves several parameters, including T(env), the initial temperature of the brain region, T(0), the initial temperature of the fluid at the channel inlet, t, the total time taken for a unit of liquid to flow from the inlet to the cooling point, and τ , the time constant. To calculate τ and t, formulas 3 and 4 can be used:

$$\tau = m * c * R \quad (\text{Formula 3})$$

$$t = \frac{L}{v} \quad (\text{Formula 4})$$

[0050] Here, m and c refer to the mass and heat capacitance of the fluid in the channel, respectively. R represents the thermal resistance of the probe 32, while L denotes the probe length, set at 5 mm and 7 mm for different depths of the brain region. V refers to the fluid flow velocity. To determine R , we used formula 5:

$$R = \frac{1}{h \cdot A} + \frac{L}{A \cdot K} \quad (\text{Formula 5})$$

[0051] K represents the thermal conductivity of the fluid, A represents the area of the surface in contact with the fluid, and h represents the heat transfer coefficient. To find the value of h , the team used formula 6:

$$Nu = \frac{h \cdot D}{K} \quad (\text{Formula 6})$$

[0052] Nu represents the Nusselt number, which is 3.66 in this case, and D represents the characteristic length parameter, which is the diameter of the pipe or cylinder with a unit of meters. The initial temperature of the fluid at the channel inlet 134, $T(0)$, was set to 15°C, while the environment temperature was set to 37°C. In summary, utilizing the parameters, it was possible to simulate the correlation between the probe tip 38 temperature changes and the flow rate. As discussed herein, the microfluidics channel-based probe 32 includes a sealed microfluidic channel 60 within the probe shank 34, which uses an external cooling source 76 and pump 80 to cool the brain. In contrast, a Peltier-based cooling probe uses a metallic or silver wire attached to a Peltier pad, and the other end of the silver wire is inserted into the brain tissue to achieve cooling. For a more detailed simulation of the cooling probe 32 heat transfer in brain tissue, we employed COMSOL to develop a model that provided improved results (see e.g., FIG. 4).

[0053] FIG. 17 illustrates the heat absorption rate of the cooling probe 32 with different dimensions and flow rates. As mentioned, a preferred heat absorption rate (Q_c) is 0.2 W. Peltier device efficiencies typically range from 10% to 50%, so it can be beneficial to compare against the maximum efficiency. A probe 32 with a 60 μm width can achieve a higher absorption rate if the flow rate is over 106 $\mu\text{L}/\text{min}$, which falls within the cooling probe flow rate range (0-200 $\mu\text{L}/\text{min}$). In one implementation, the flow rate of the fluid is 40-200 $\mu\text{L}/\text{min}$, and in a more preferred embodiment, is 100-160 $\mu\text{L}/\text{min}$. These ranges, given the size of the channel 60, can help to achieve a sufficient cooling effect. Three main parameters may be manipulated to obtain desirable dimensions for the probe 32 and cooling efficiency: the inlet fluid temperature, probe channel 60 dimensions, and fluid 138 material.

[0054] In FIG. 18, colder water resulted in a better temperature difference between the inlet fluid temperature and the brain temperature at the tip 38 of the probe 32. In FIG. 19, the dimensions of the probe 32 were not changed as much as the inlet temperature changed. FIG. 20A compares the flow rate versus the temperature drop for different fluids 138, namely water and FC-72 coolant, which are two potential fluids for the assembly 30. Other operable fluids may be used as well. The Y-axis (ΔT) represents the temperature difference between the inlet fluid temperature and the fluid temperature at the tip 38 of the probe 32. The simulation results indicated that the coolant could maintain a large temperature difference at the tip 38 of the probe 32, but due to its low thermal conductivity, the heat absorption rate was significantly lower than water, as demonstrated in FIG. 20B. It may be beneficial to select a fluid 138 that has low viscosity, low freezing point, biocompatibility, appropriate thermal conductivity, chemical stability, and no large particles to avoid blocking the channel 60. Based on these criteria, water can be advantageously used for the fluid 138.

[0055] FIG. 21 shows data from characterization of four temperature probes 32, showing the temperature sensor 100 resistance change correlated with the temperature change. During bench-top characterization, the resistance was measured using a 4-point measurement with a source meter, and it was found to be $15.35 \pm 0.45 \text{ k}\Omega$ at 37°C . The responsivity of the sensor 100 was within a range of 32 to 42°C was $11.761 \pm 1.403 \text{ }\Omega/\text{K}$, with a linear correlation coefficient of 0.996 . The thermal coefficient of resistance was $907 \pm 96 \text{ ppm/K}$. By performing the 4-point measurement with a pulse of $100 \text{ }\mu\text{A}$ current using the source meter, the minimum resolution was below 0.01 K with an accuracy of 0.1 K . It may also be beneficial to ascertain the practical temperature sensing resolution with in vivo validation.

[0056] To conduct the initial testing of temperature change in the cooling probe 32, the first step was to stack the temperature sensor 100 with the cooling probe. The back ends 42 of both probe subunits were the PCB 54 and the inlet/outlet end of the PDMS microfluidic channel 60, respectively, and their positions are shown for example, in FIG. 8. It can be helpful to align the temperature sensor tip with the cooling probe tip 38. For the external setup of the cooling probe 32, the syringe pump 80 can be used to drive the fluid 138 into the microfluidic channel 60, and the flow rate ranged from 0 - $200 \text{ }\mu\text{L/min}$. The outlet water flowed into a container 78 from the outlet of the probe 32.

- [0057] FIG. 22 shows a comparison between simulated results and in-vitro testing. Two cooling probes 32 of different dimensions were tested, with the cross-section of the microfluidic channel 60 being 60 μm by 30 μm and 60 μm by 20 μm . While the temperature change was less pronounced in vitro as compared to the simulation, desirable effects were shown, particularly at flow rates of 100 $\mu\text{L}/\text{min}$ and up.
- [0058] FIG. 23 shows a comparison of temperature change versus tubing length. The tubing is preferably pressure and thermal resistant, with an outer diameter of 1/16 inch and an inner diameter of 1/32 inch in one embodiment. However, due to the low flow rate ($<200 \mu\text{L}/\text{min}$), the heat loss impacted the inner fluid temperature, especially if the tubing was longer between the heat exchanger 74 and the inlet.
- [0059] Owing at least partially to the heat exchanger 74, the cooling effect can be significantly improved from 5 $^{\circ}\text{C}$ to a maximum of 14 $^{\circ}\text{C}$ in one example embodiment. This expanded cooling range suggests a broader scope of potential applications for the setup. FIG. 24 illustrates the temperature variation in the mouse hippocampus brain region, with the heat exchanger 74 operational throughout the experiment. The activation and deactivation of the syringe pump 80 altered the temperature, causing it to quickly return to baseline when the pump was halted. FIG. 25 conveys the relationship between temperature change and microfluidic channel 60 flow rate. When the heat exchanger 74 is off and the syringe pump 80 introduces room temperature water into the microfluidic channel 60, the maximum temperature change is approximately - 5 $^{\circ}\text{C}$. In contrast, the heat exchanger 74 with 10 $^{\circ}\text{C}$ water can achieve a -14 $^{\circ}\text{C}$ temperature change, while 55 $^{\circ}\text{C}$ water can increase brain temperature by approximately 8 $^{\circ}\text{C}$.
- [0060] Normalized LFP data demonstrates power fluctuations during cooling treatment within the 0-40 Hz spectrum, as depicted in FIG. 26, with a 30-second window scale. The amplitude of 0-20 HZ frequency band is significantly decreased during the cooling treatment. Local field potentials (LFPs) are low-frequency electrical signals recorded in the brain that represent the summed synaptic activity of a group of neurons in a local area. LFPs provide valuable information about the functional organization of neuronal circuits and are often used to study brain function and dysfunction. The LFP spectrum refers to the distribution of signal power across different frequency bands (e.g., delta, theta, alpha, beta, and gamma).
- [0061] Brain temperature can influence the biophysical properties of neuronal membranes, such as ion channel kinetics and membrane capacitance. These properties directly impact the generation

and propagation of electrical signals, including LFPs. When the brain temperature changes, it can alter the relative contributions of different ion channels and affect the overall shape of the LFP spectrum. Brain temperature can affect the LFP spectrum due to several factors, including synaptic transmission, metabolism and blood flow, and neural network dynamics, to cite a few examples. Changes in brain temperature can affect the release of neurotransmitters and the sensitivity of postsynaptic receptors. This, in turn, can influence synaptic transmission and the balance of excitation and inhibition in neuronal networks. As LFPs represent the aggregate synaptic activity of local neuronal populations, alterations in synaptic transmission due to temperature changes can affect the LFP spectrum. Brain temperature is also closely linked to brain metabolism and blood flow. Changes in temperature can impact the availability of energy substrates (e.g., glucose, oxygen) and alter the overall metabolic rate of the brain. These changes may have secondary effects on neuronal activity and LFPs, as the brain's energy demands can influence the balance of excitatory and inhibitory processes. Neuronal networks exhibit distinct patterns of activity and synchronization depending on the temperature. As the brain temperature changes, the dynamics of these networks can shift, leading to changes in the LFP spectrum. For example, cooling the brain has been shown to slow down the frequency of oscillations in some cases, while heating may increase the frequency or lead to more desynchronized activity.

[0062] Although an LFP spectrum can provide complementary information about brain function, spike signals are considered the "gold standard" for neural activity analysis due to their role as the primary means of neuron communication, specificity to individual neurons, high temporal resolution, direct causal relationship with behavior, ease of quantification, and compatibility with computational modeling. The probe assemblies 30 described herein can help create a comprehensive analysis of electrophysiological data correlated with temperature changes, particularly if spike data is sought.

[0063] FIG. 28 shows a schematic representation of the probe assembly 30 with the first heat exchanger 72 (stage 1) and second heat exchanger 74 (stage 2). Stage 1 on its own can induce a modest temperature change, typically a reduction of approximately 5°C, due to the low flow rate of the water (maximum 200 µL/min) within the microfluidic channel 60. However, due to heat loss in the microchannels, even when ice-cold water is introduced at the start, only room-temperature water reaches the tip end 38 of the channel 60. To address this limitation and achieve a broader range of temperature modulation ($\geq \pm 10^\circ\text{C}$), the second stage 74 incorporates a

secondary water loop with a higher flow rate (2 L/min) integrated into a custom 3D-printed heat exchanger, attached to the back end 40 along the back side of the stage 1 heat exchanger 72. To maximize the efficiency of heat transfer, copper tape and thermal conductive paste are together used in this embodiment as the heat sink layer 152 applied between the two stages 72, 74. This two-stage approach allows precise control of the temperature differential between the tip 38 of the microfluidic probe 32 and the surrounding tissue by adjusting the water temperature in the heat exchanger 74 (stage 2, typically ranging from 2°C to 50°C) and the flow rate in the microfluidic channel 60 (stage 1, heat exchanger 72). FIG. 29 shows the validation *in vivo* test results.

[0064] As discussed herein, for the flexible and planar microfluidic platform, polyimide (PI-2610, HD Microsystems) and PDMS (Sylgard-184) were selected as substrate materials in the illustrated embodiment. Polyimide offers high biostability and durability, ideal for neural applications, while PDMS is recognized for its versatility and biocompatibility in various biochip and microfluidic applications. The cooling/heating module of the backend assembly 70 is designed with cross-sectional dimensions of $<120\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$ and a length of 5 mm at the implantable end. Variations of these dimensions can be used to accommodate different potential applications. The internal microfluidic channels 60 are U-shaped, with dimensions of 20 μm in height and 30 μm in width. The fabrication process involves bonding a PDMS microfluidic channel layer onto a thin polyimide capping layer. Due to the pressure drop in the long, narrow microfluidic channel 60, the bonding between these materials must withstand internal pressures of up to 500 KPa, achieved through the integration of a Ti/SiO₂ adhesion layer to ensure robust bonding between the polyimide and PDMS layers.

[0065] At the back end 40 of the microfluidic module, the channel 60 includes two openings—a pair of inlet and outlet ports 134, 136 for water circulation. These are bonded to a custom-made circular glass slide for structural support. Room-temperature water is pumped from a micro syringe into the microfluidic channel 60 through a custom 3D-printed port system 116, 118, completing stage 1 of the process.

[0066] FIGS. 30-32 relate to embodiments of the assembly 30 in which the probe shank 32 is at least partially covered with an insulative sheath 160. The insulative sheath 160 has a plurality of fluid bubbles 162 and is made of a flexible material so as to allow for the formation of curvilinear contours 164. The fluid bubbles 162 may include one or more circular or more spherically shaped

structures and/or more of a cylindrical or alternatively shaped pillar, to cite a few examples. In one embodiment, the insulative sheath 160 surrounds an entire radial extent of the probe shank 32, while leaving the tip end portion 36 exposed. In some embodiments, there may be multiple insulative sheath layers, or just the one layer as illustrated. Employing the sheath 160 can enable precise focal temperature modulation at targeted regions while preventing unintended thermal effects on surrounding superficial brain structures. Maintaining accurate thermal control can be helpful for avoiding erroneous experimental outcomes that can arise from uncontrolled heat transfer to non-targeted areas.

[0067] In the illustrated embodiment, the insulative sheath 160 comprises a micro-air bubble wrap shield, along with alternative air layer configurations such as air pillars, to provide effective thermal insulation along the probe shank. These fluid bubbles 162 act as barriers to heat conduction, utilizing air's inherently low thermal conductivity ($0.022 \text{ W/m}\cdot\text{K}$) in comparison to materials typically used in such devices, such as polyimide ($0.4 \text{ W/m}\cdot\text{K}$) and PDMS ($0.27 \text{ W/m}\cdot\text{K}$). By employing discrete air-filled structures 162, such as bubbles/pillars, instead of a continuous air layer, the insulation 160 remains compliant yet robust during implantation, reducing the risks of compression or air displacement and ensuring consistent thermal protection (see FIG. 31).

[0068] The insulative sheath layer 160 can be strategically wrapped around the shank 32 of the probe assembly 32, leaving the tip end portion 36 exposed to allow for localized heat transfer and electrophysiological recordings in the target brain region. According to simulations, insulation layers 160 with a thickness greater than $20 \mu\text{m}$ provide effective thermal isolation along the shank 32, resulting in negligible temperature variation ($\pm 0.5^\circ\text{C}$) in the insulated areas, while facilitating a substantial temperature modulation ($\geq 10^\circ\text{C}$) at the exposed tip end portion 36 (see FIG. 32).

Initial validation has demonstrated that both the micro-air bubble and air pillar layers can be successfully fabricated using a SU-8 mold and soft lithography techniques. A PDMS air bubble/pillar layer was formed and, following a saline surface treatment, was easily peeled off then bonded on a polyimide layer to achieve the micro-air bubble structure of the insulative sheath layer 160, after the shape etching, it can be released and applied to the probe shank 32.

[0069] It is to be understood that the foregoing description is of one or more preferred exemplary embodiments of the invention. The invention is not limited to the particular embodiment(s) disclosed herein, but rather is defined solely by the claims below. Furthermore, the statements

contained in the foregoing description relate to particular embodiments and are not to be construed as limitations on the scope of the invention or on the definition of terms used in the claims, except where a term or phrase is expressly defined above. Various other embodiments and various changes and modifications to the disclosed embodiment(s) will become apparent to those skilled in the art. All such other embodiments, changes, and modifications are intended to come within the scope of the appended claims.

[0070] As used in this specification and claims, the terms “for example,” “e.g.,” “for instance,” and “such as,” and the verbs “comprising,” “having,” “including,” and their other verb forms, when used in conjunction with a listing of one or more components or other items, are each to be construed as open-ended, meaning that the listing is not to be considered as excluding other, additional components or items. Other terms are to be construed using their broadest reasonable meaning unless they are used in a context that requires a different interpretation. In addition, the term “and/or” is to be construed as an inclusive OR. Therefore, for example, the phrase “A, B, and/or C” is to be interpreted as covering all the following: “A”; “B”; “C”; “A and B”; “A and C”; “B and C”; and “A, B, and C.”

CLAIMS

1. A neural probe, comprising:
 - a probe shank extending from a tip end portion to a backend portion; and
 - a channel extending from the backend portion at least partially down the probe shank toward the tip end portion, wherein the channel is fully sealed along the probe shank toward the tip end portion, and wherein the channel is configured to sealingly house a fluid.
2. The neural probe of claim 1, wherein the channel has a U-shaped longitudinal extent along the probe shank.
3. The neural probe of claim 2, wherein an inlet branch and an outlet branch of the U-shaped longitudinal extent join at a pointed tip end at the tip end portion.
4. The neural probe of any one of claims 1 to 3, wherein the channel is embedded in a flexible body of the probe shank.
5. The neural probe of any one of the preceding claims, further comprising an external temperature-controlled source configured to change a temperature of the fluid, and a head stage adapter located between the implantable neural probe and the external temperature-controlled source, wherein the head stage adapter is configured to allow for flow of the fluid from the external temperature-controlled source to the channel.
6. The neural probe of claim 5, wherein a heat exchanger is at least partially housed in the head stage adapter.
7. The neural probe of any of the preceding claims, further comprising an insulative sheath at least partially surrounding the probe shank.
8. The neural probe of claim 7, wherein the insulative sheath leaves the tip end portion exposed and wraps around a full radial extent of the probe shank.
9. The neural probe of claim 7 or claim 8, wherein the insulative sheath has one or more curvilinear contours and a plurality of fluid bubbles.

10. The neural probe of any of the preceding claims, wherein the probe shank is made from polyimide, SU-8, parylene, or PDMS.

11. A neural probe assembly, comprising:
an implantable neural probe;
an external temperature-controlled source configured to change a temperature of a fluid;
and
a head stage adapter located between the implantable neural probe and the external temperature-controlled source, wherein the head stage adapter is configured to allow for flow of the fluid from the external temperature-controlled source to the implantable neural probe.

12. The neural probe assembly of claim 11, wherein a heat exchanger is at least partially housed in the head stage adapter.

13. The neural probe assembly of claim 12, further comprising a second heat exchanger, wherein the heat exchanger and the second heat exchanger are bonded via a heat sink layer.

14. The neural probe assembly of any one of claims 11 to 13, wherein the head stage adapter comprises an inlet/outlet segment, an interface segment, and a connection port configured to couple the interface segment with a back end of the implantable neural probe.

15. The neural probe assembly of claim 14, wherein the interface segment includes an O-ring.

16. The neural probe assembly of claim 14 or 15, wherein the connection port is a rigid plate body.

17. The neural probe assembly of any one of claims 11 to 16, wherein the implantable neural probe comprises a probe shank extending from a tip end portion to a backend portion, and a channel extending from the backend portion at least partially down the probe shank toward the tip end portion, wherein the channel is fully sealed along the probe shank toward the tip end portion, and wherein the channel is configured to sealingly house the fluid.

18. A method of controlling a temperature of an implanted neural probe assembly, comprising the step of:

administering a fluid to a closed channel on a neural probe of the neural probe assembly.

19. The method of claim 18, wherein a flow rate of the fluid is 0-1 mL/min, inclusive.

20. The method of claim 19, wherein a flow rate of the fluid is 100-160 μ L/min, inclusive.

21. The method of any one of claims 18 to 20, further comprising the steps of receiving feedback from a temperature sensor on the neural probe and adjusting a temperature of the fluid depending on the feedback from the temperature sensor.

22. A method of manufacturing a neural probe assembly, comprising the steps of:

molding a channel into a channel layer, the channel having a U-shaped cross-section and a U-shaped longitudinal extent; and

laminating the channel layer to a backing layer to form a probe shank.

23. The method of claim 22, further comprising the steps of forming a plurality of fluid bubbles into an insulative sheath layer, and folding the insulative sheath layer at least partially around the probe shank.

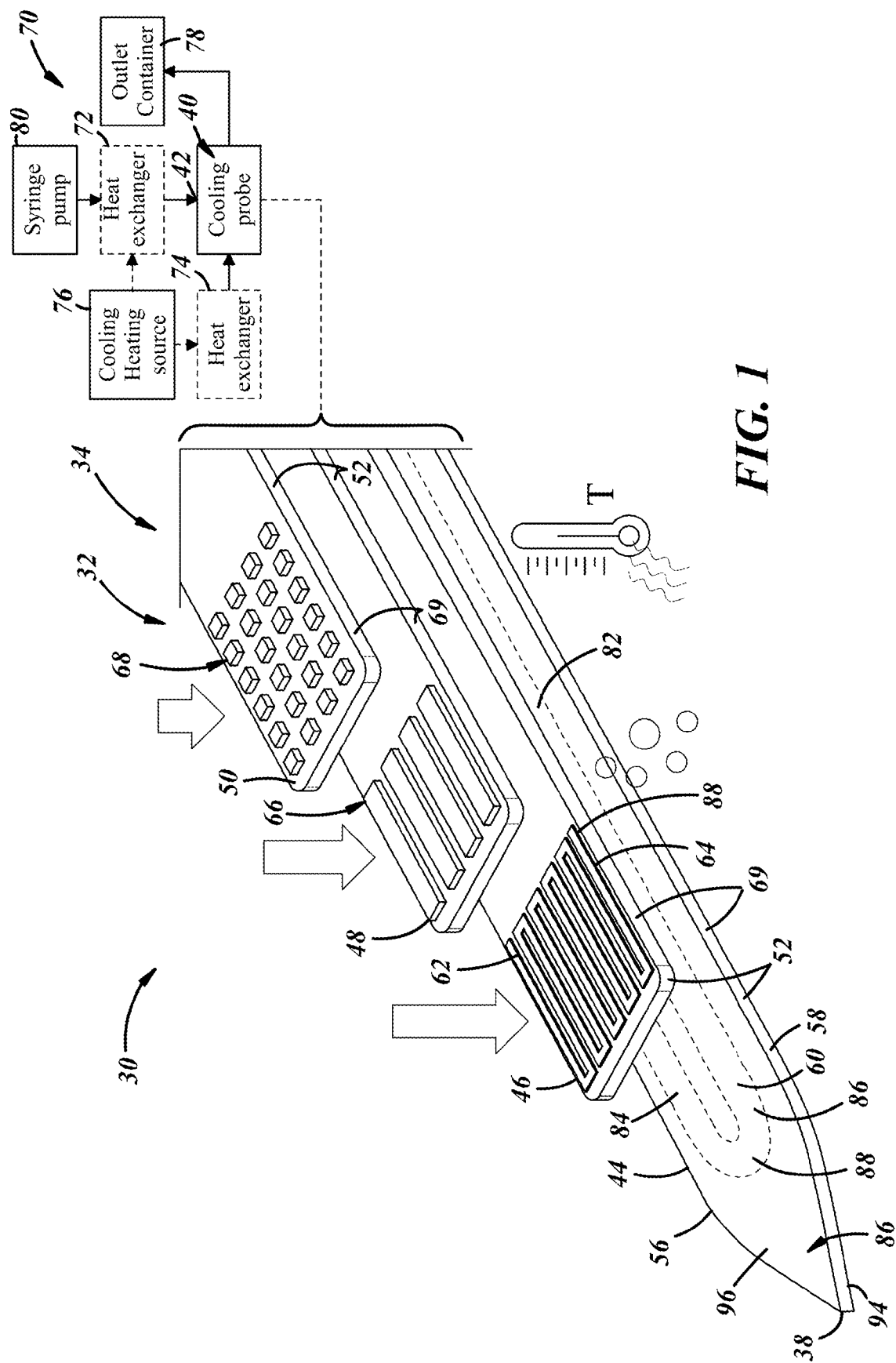
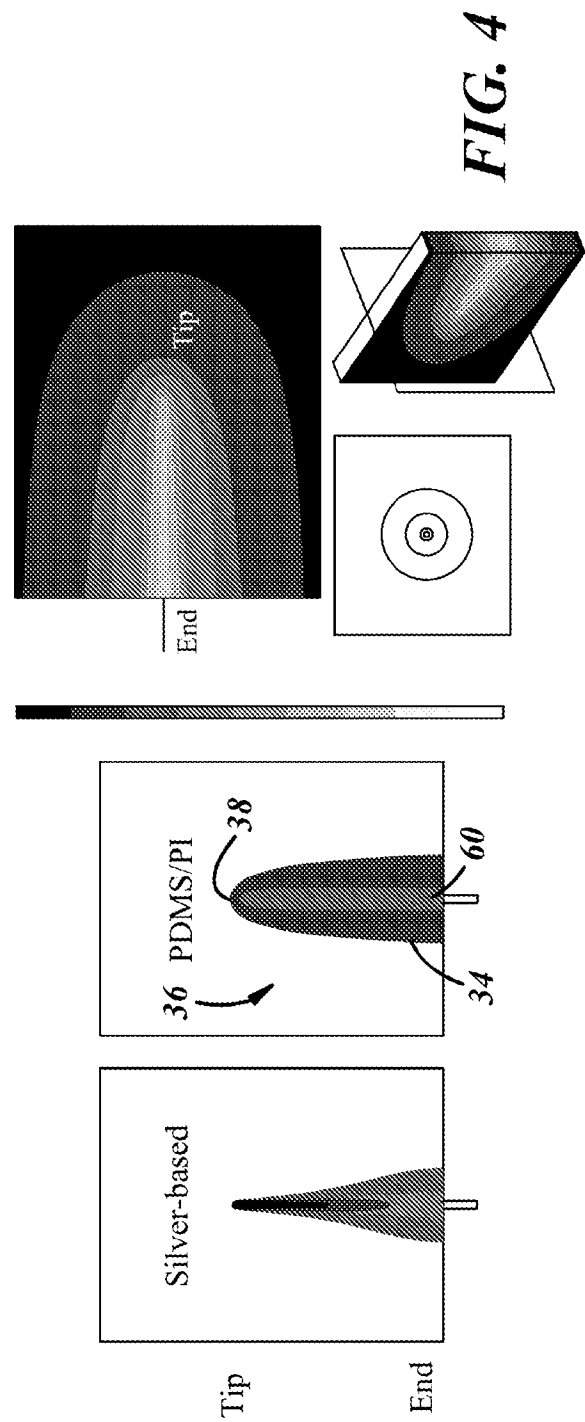
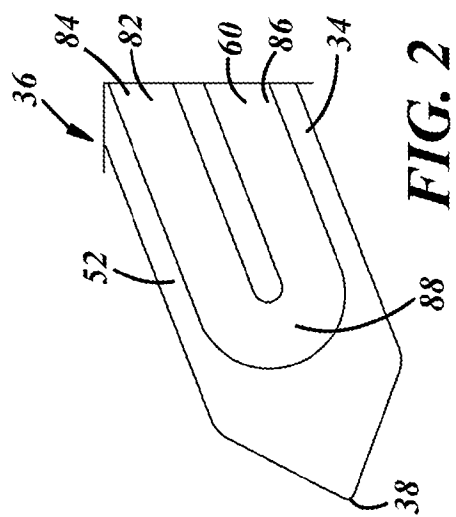
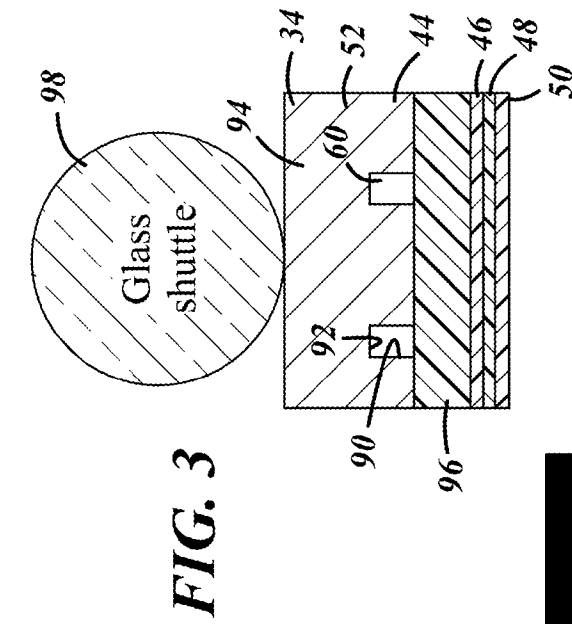
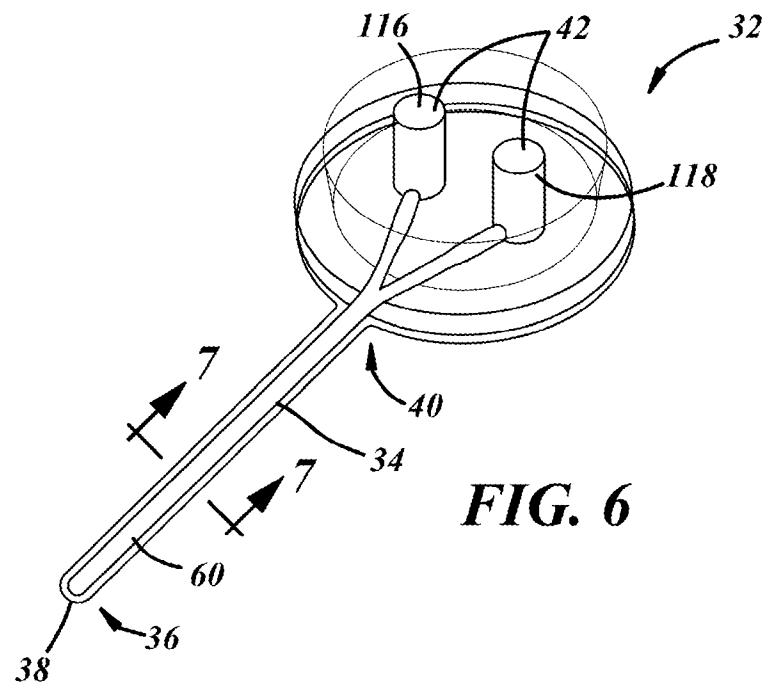
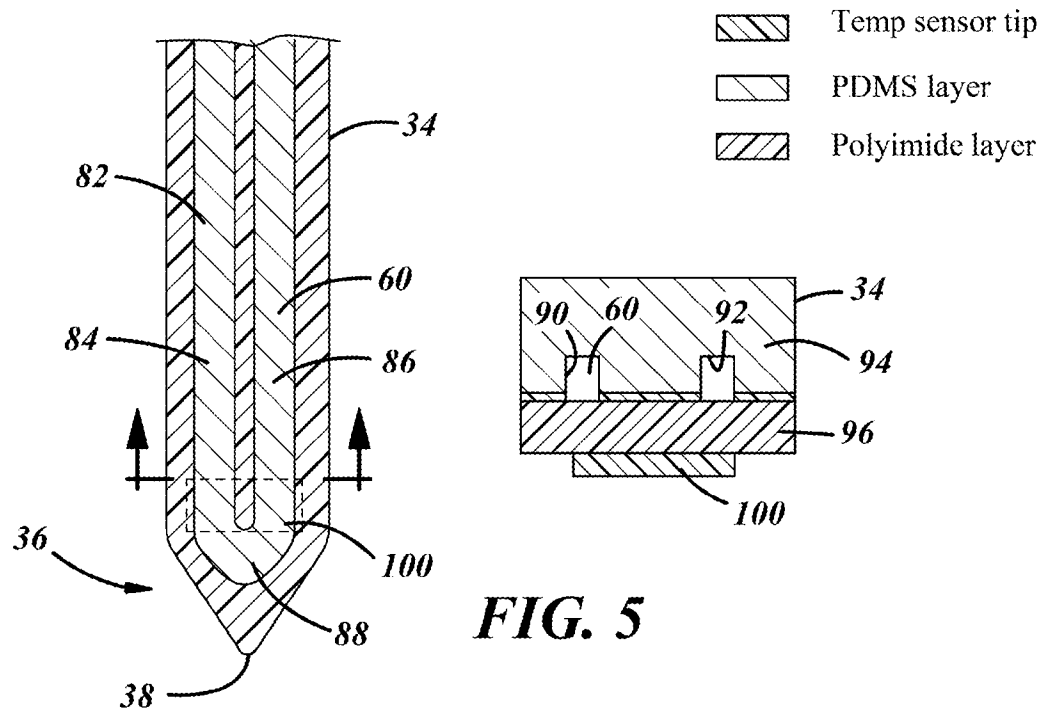
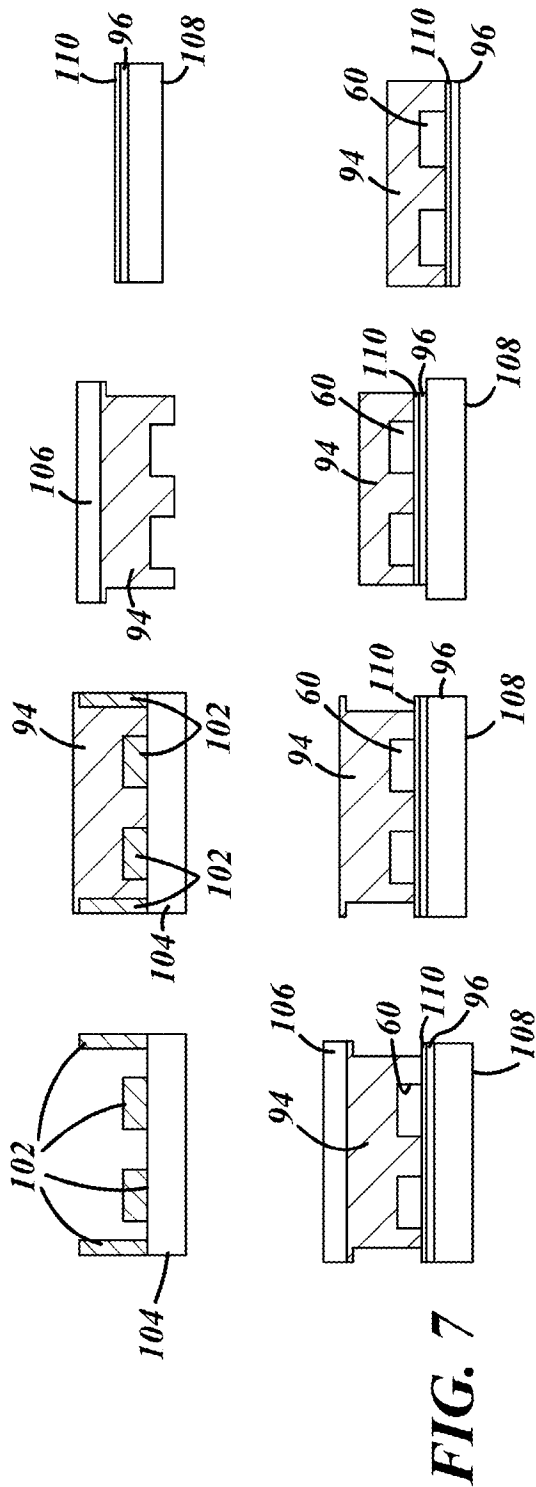


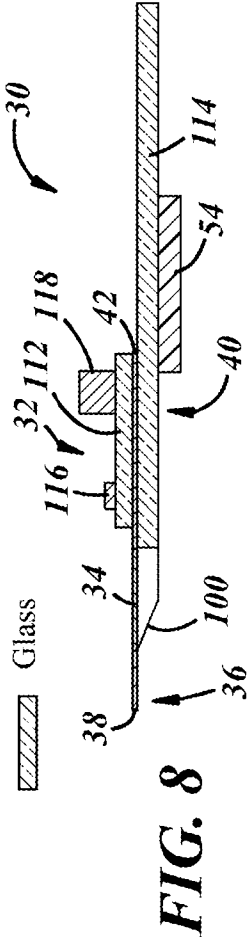
FIG. 1







- Inlet/outlet connector
- Cooling probe
- Temp probe/recording probe
- PCB board
- Glass



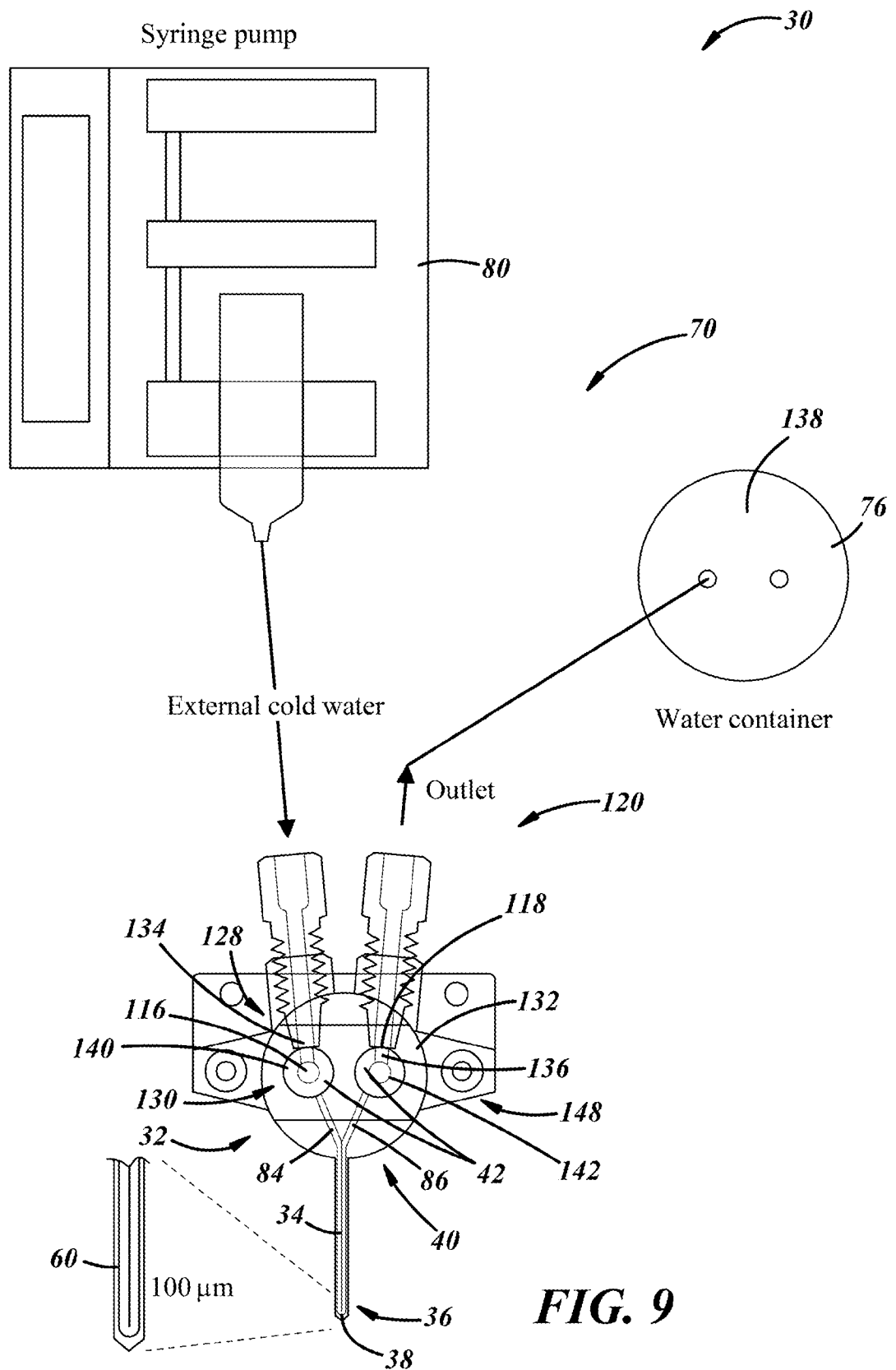


FIG. 9

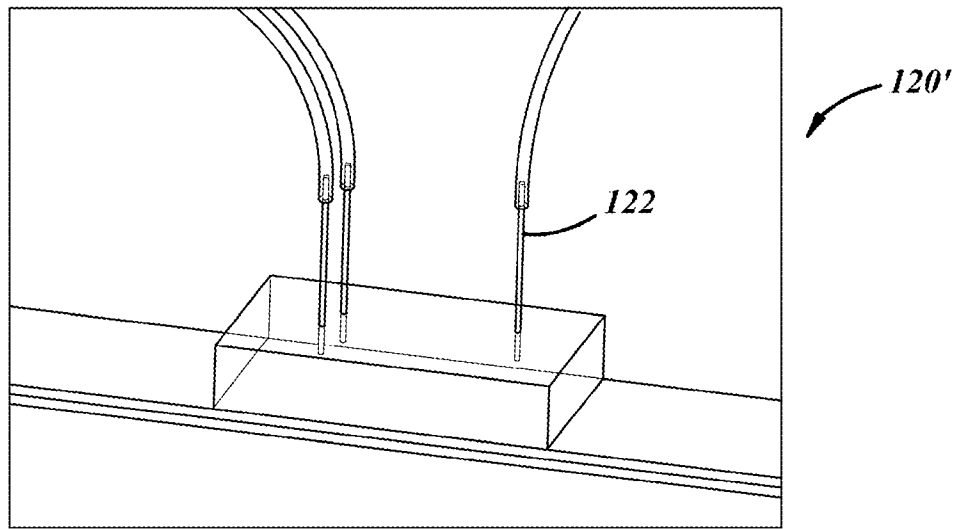


FIG. 10

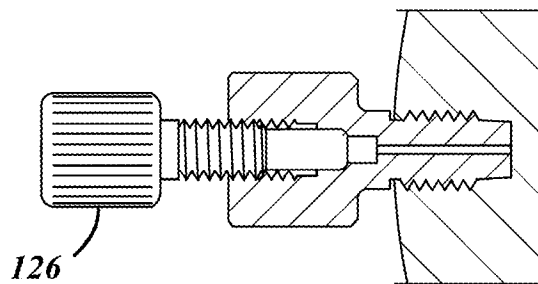
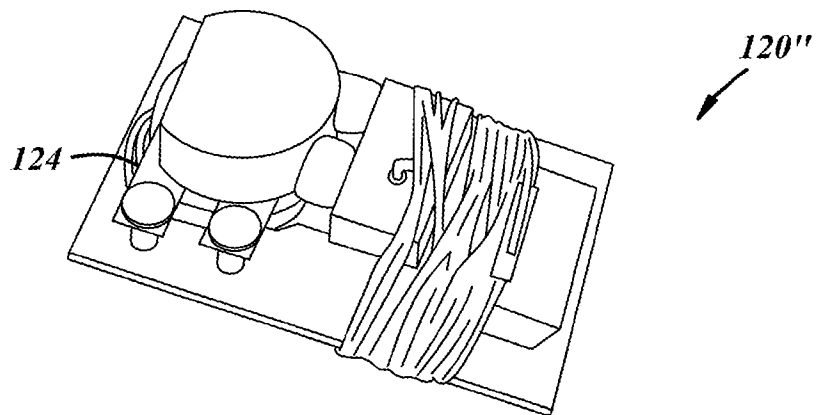
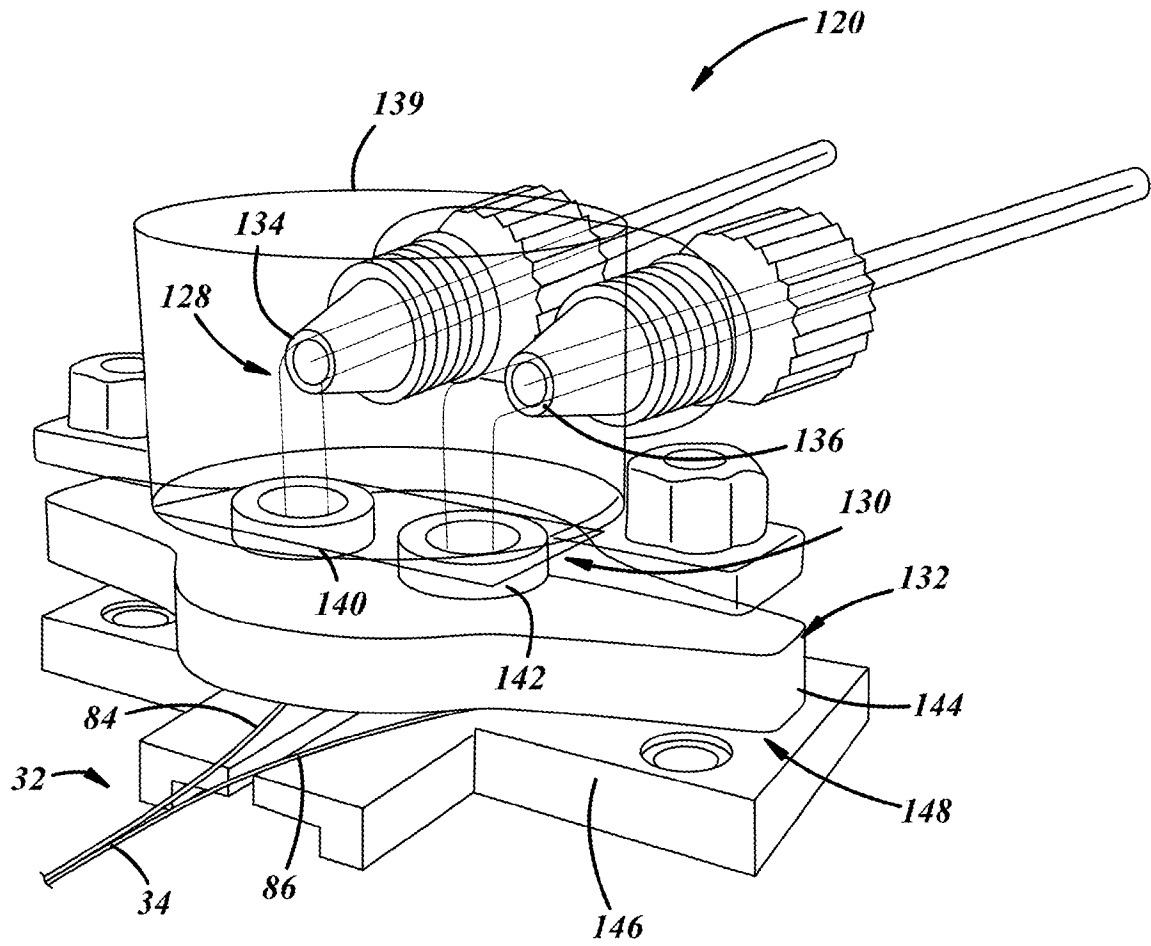
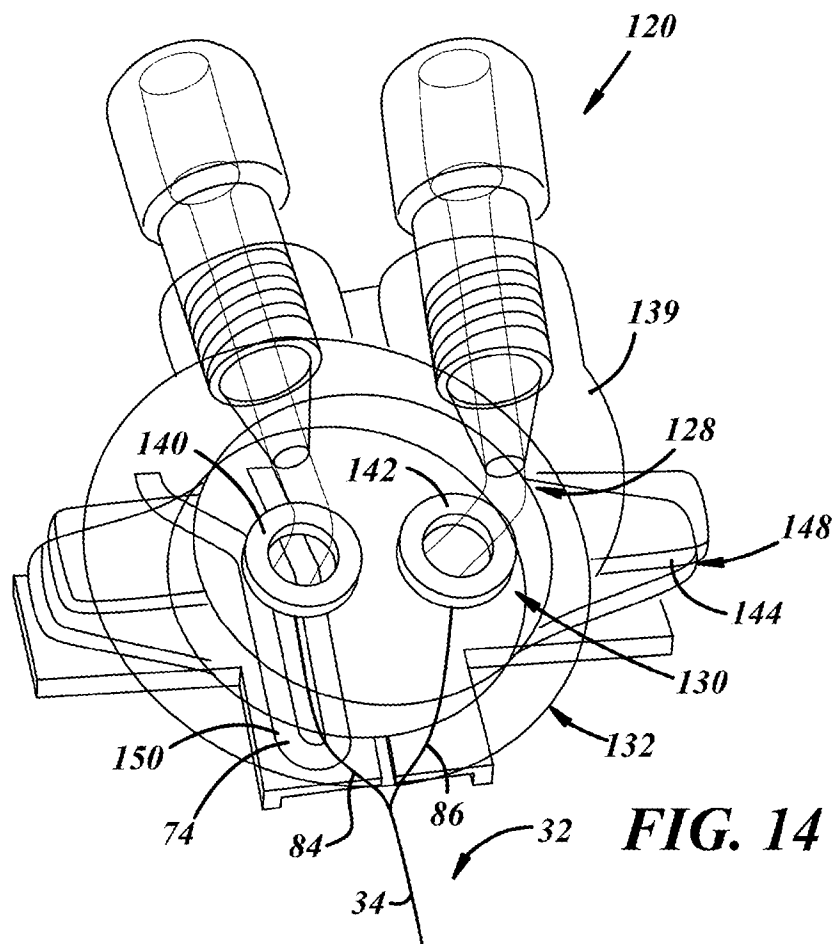
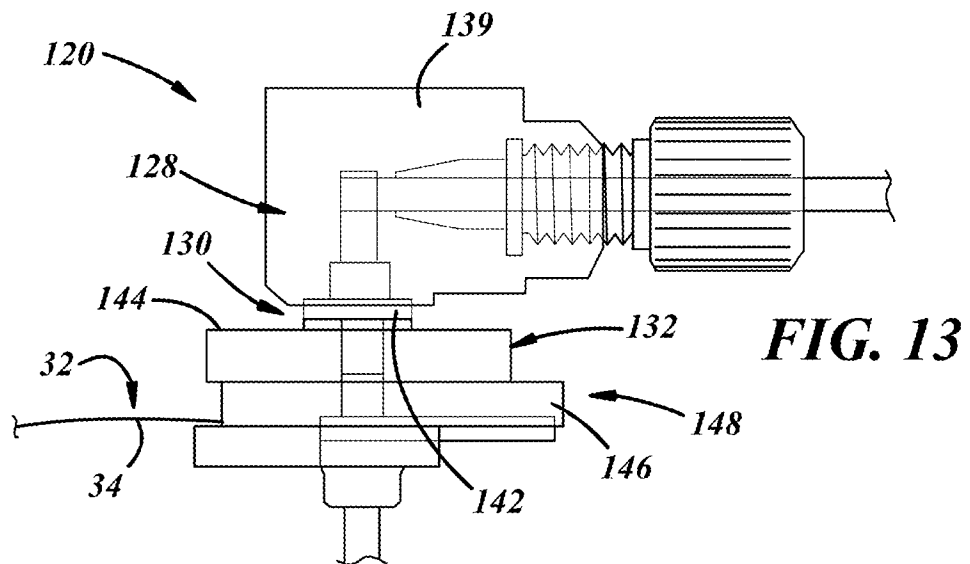


FIG. 11

**FIG. 12**



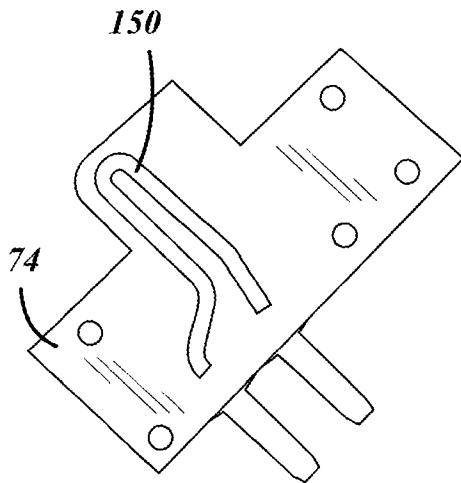


FIG. 15A

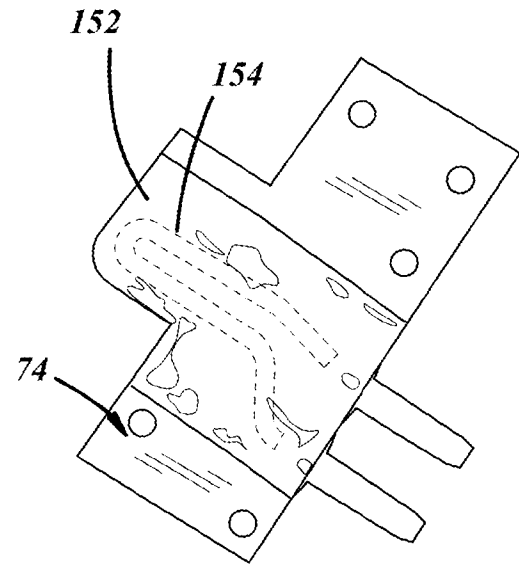


FIG. 15B

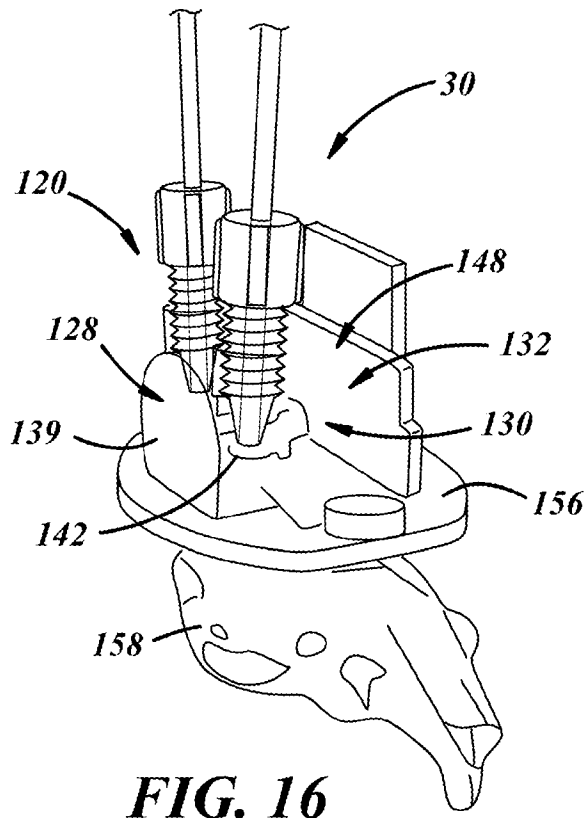


FIG. 16

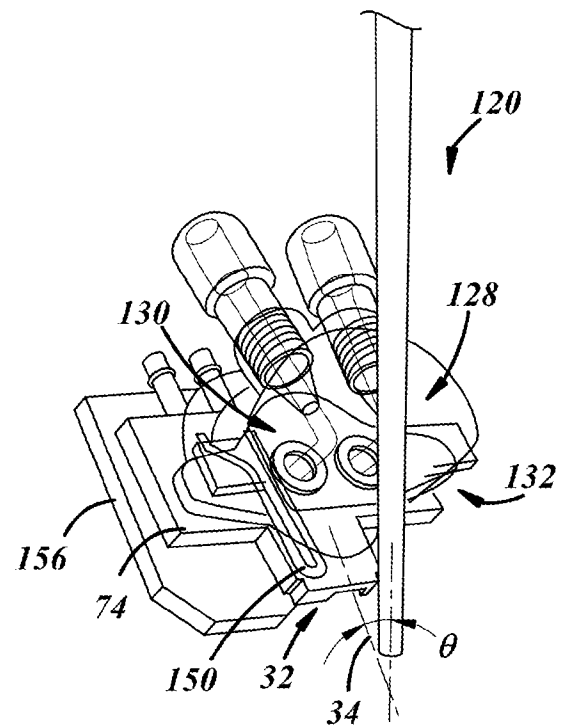


FIG. 27

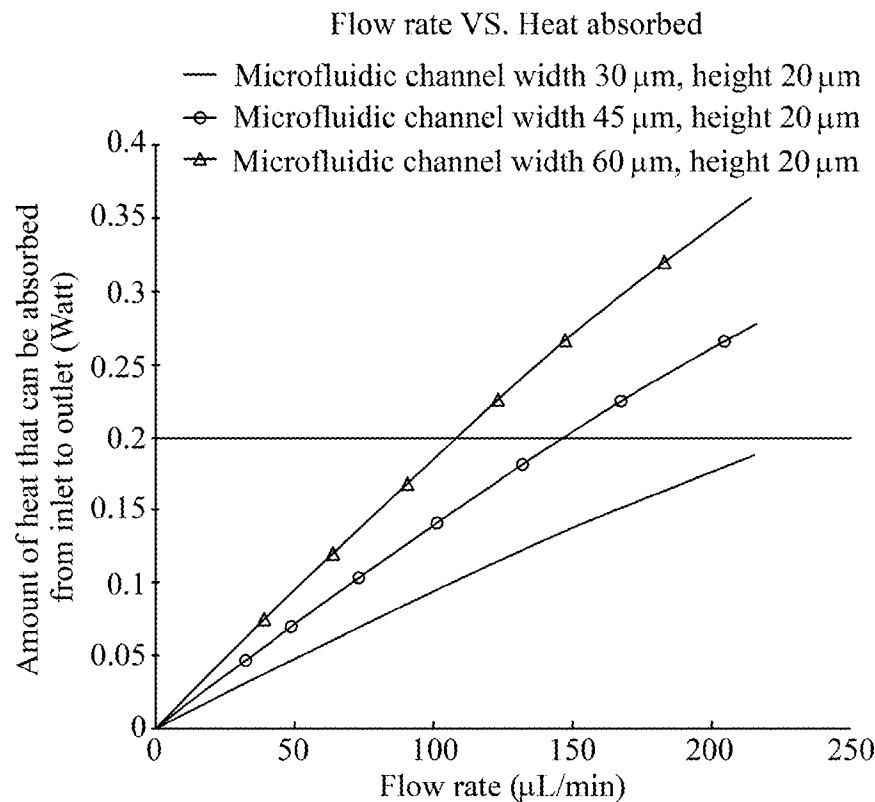


FIG. 17

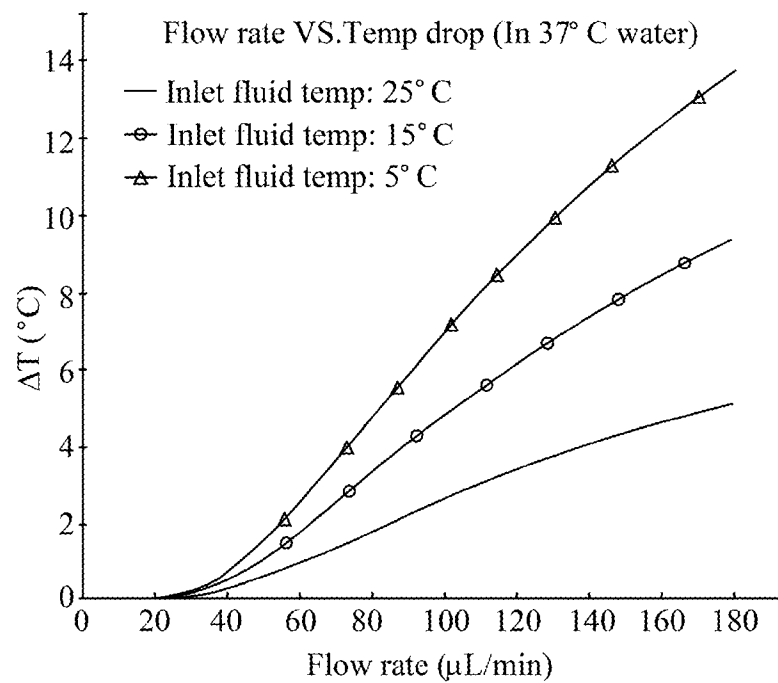


FIG. 18

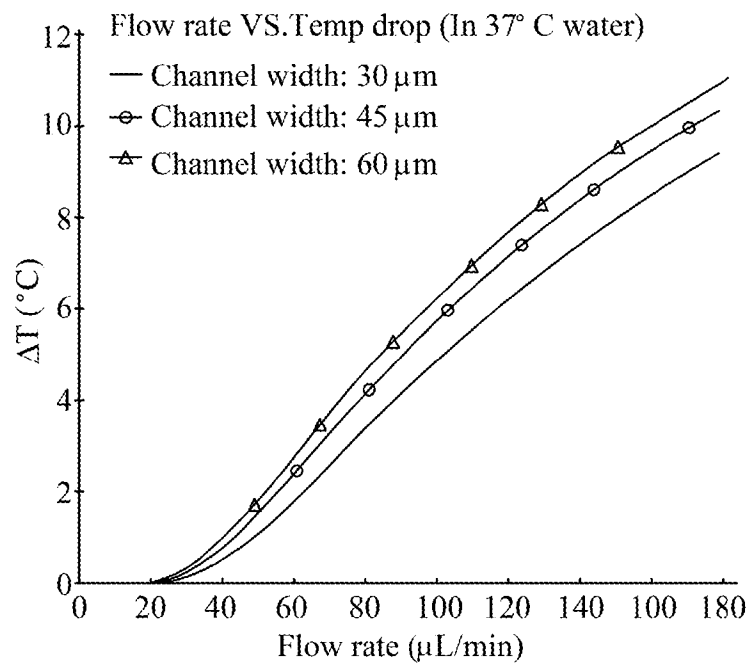


FIG. 19

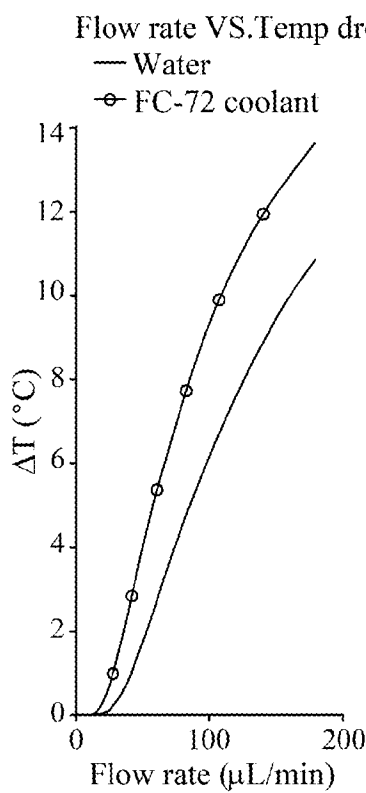


FIG. 20A

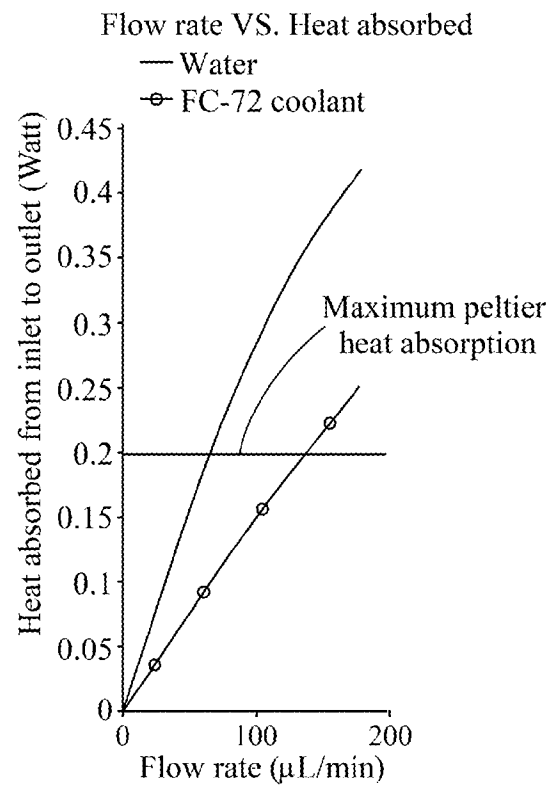


FIG. 20B

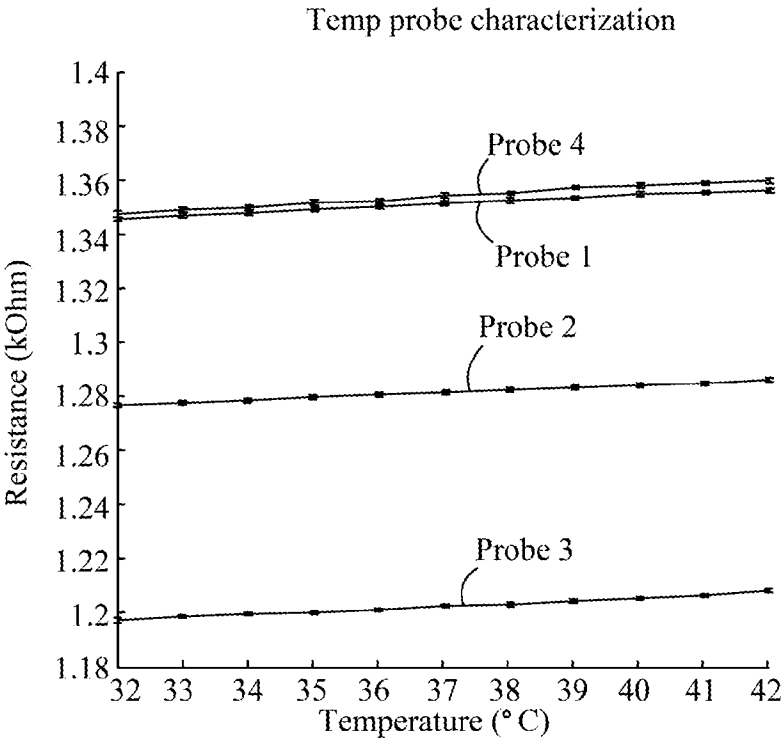


FIG. 21

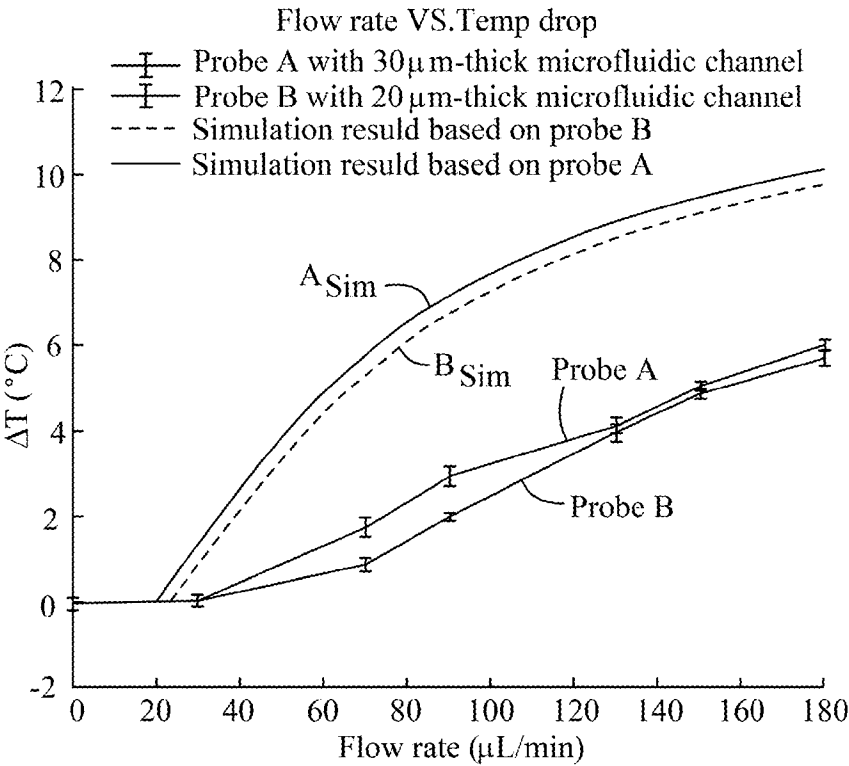


FIG. 22

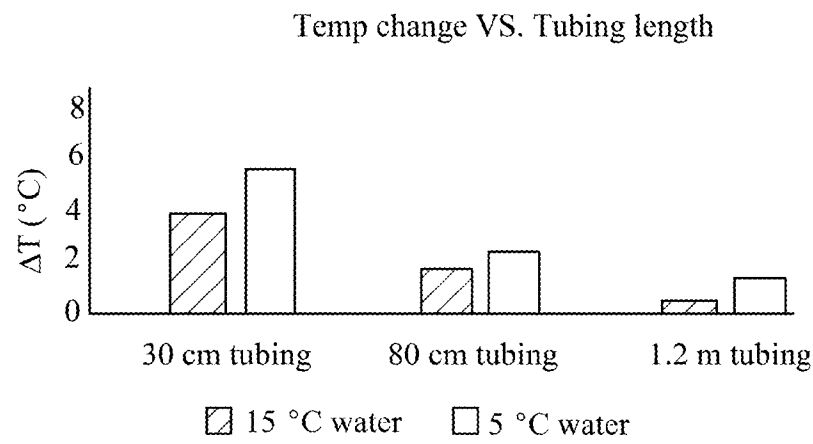
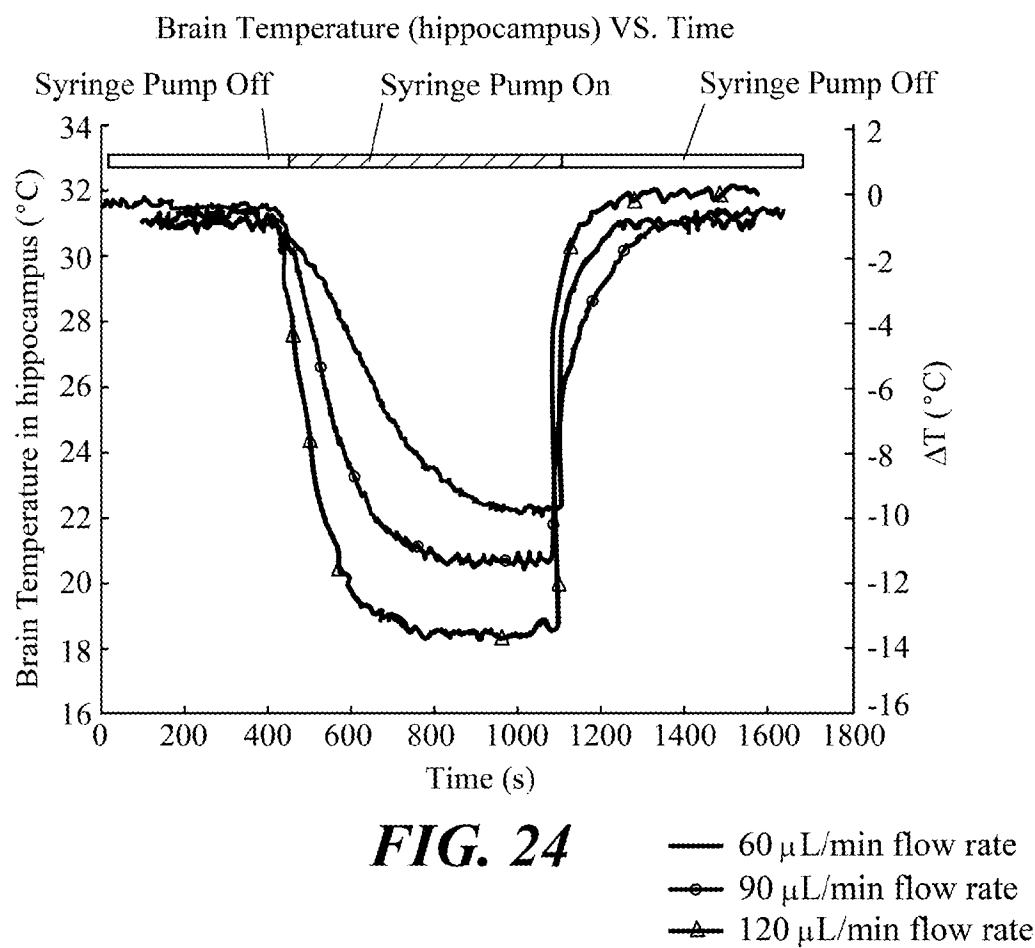


FIG. 23



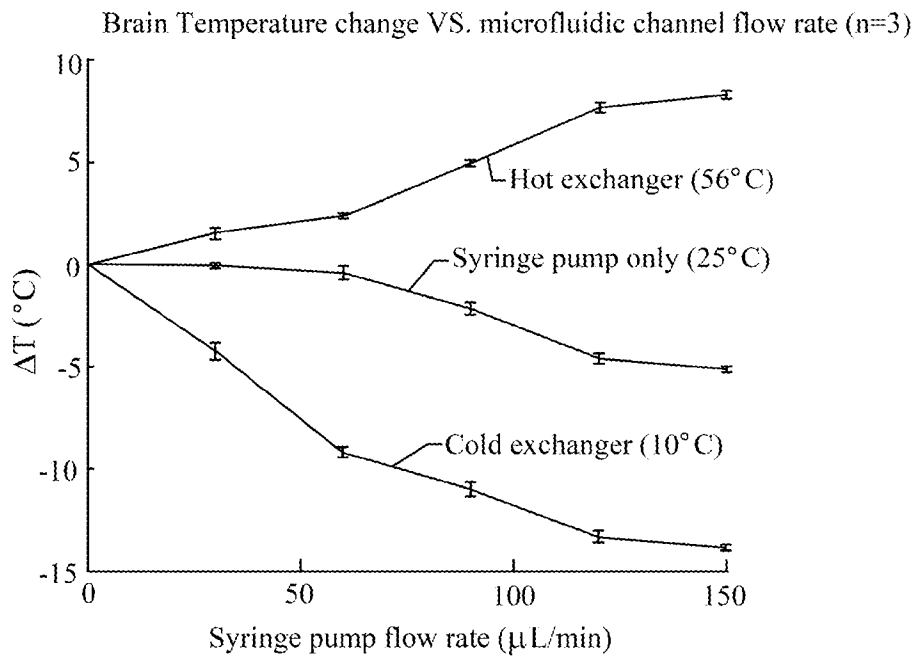


FIG. 25

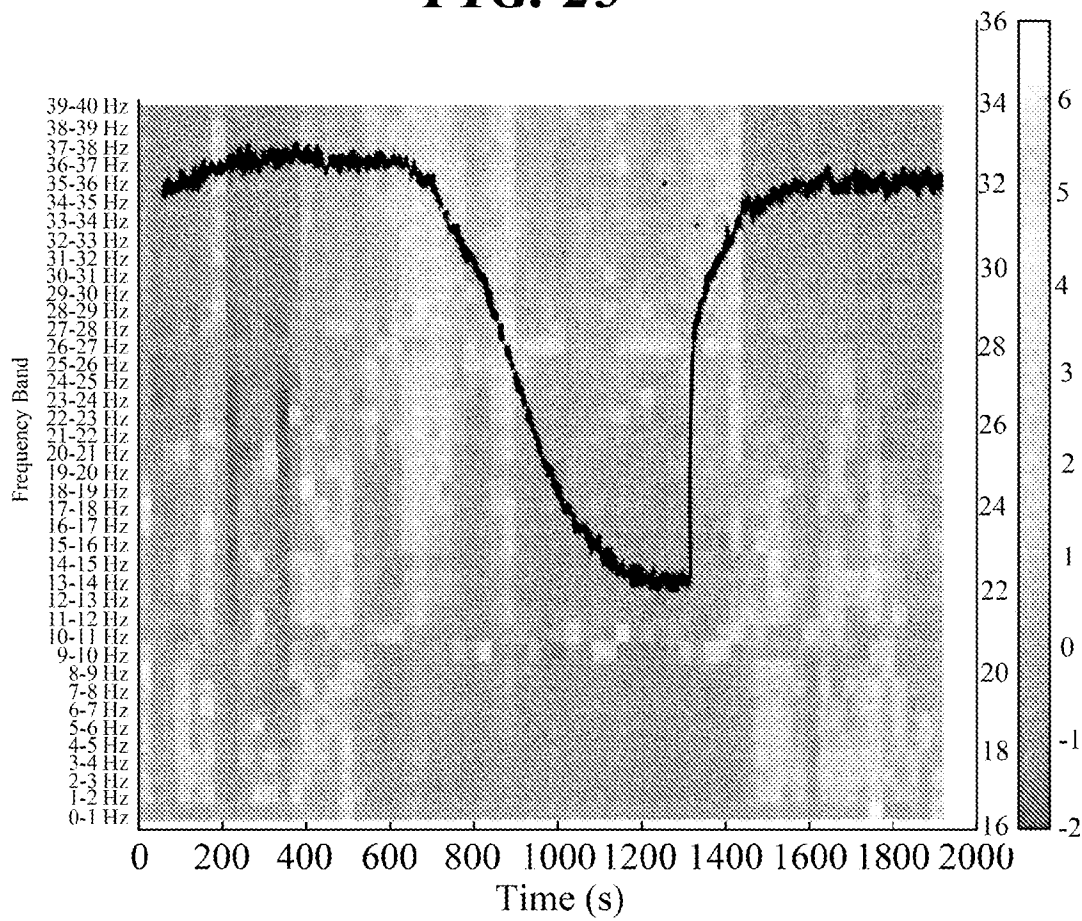
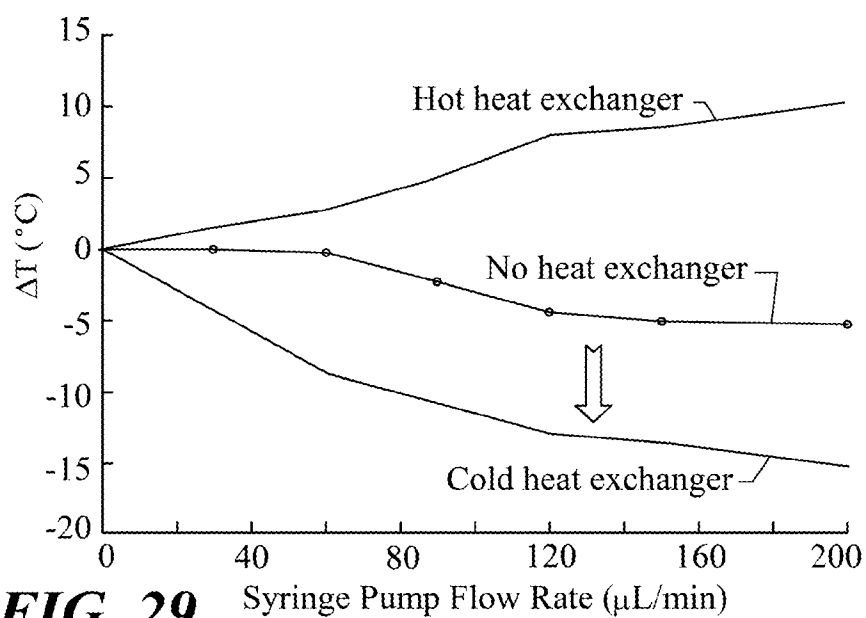
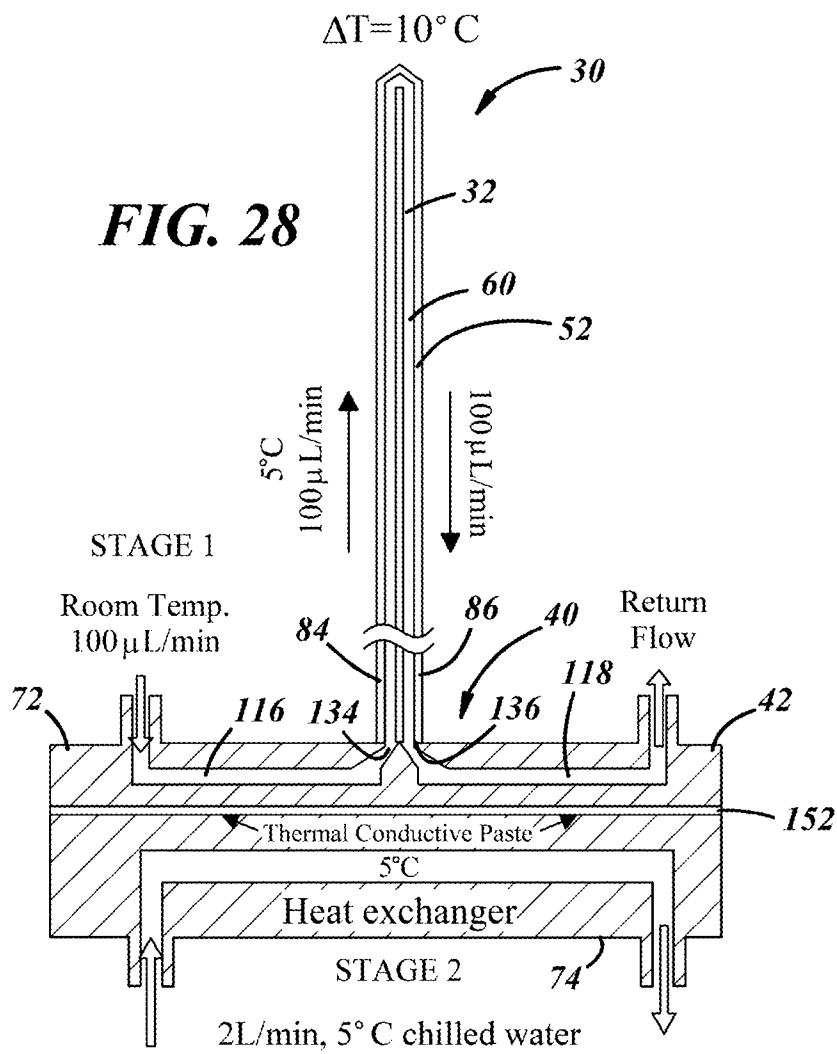
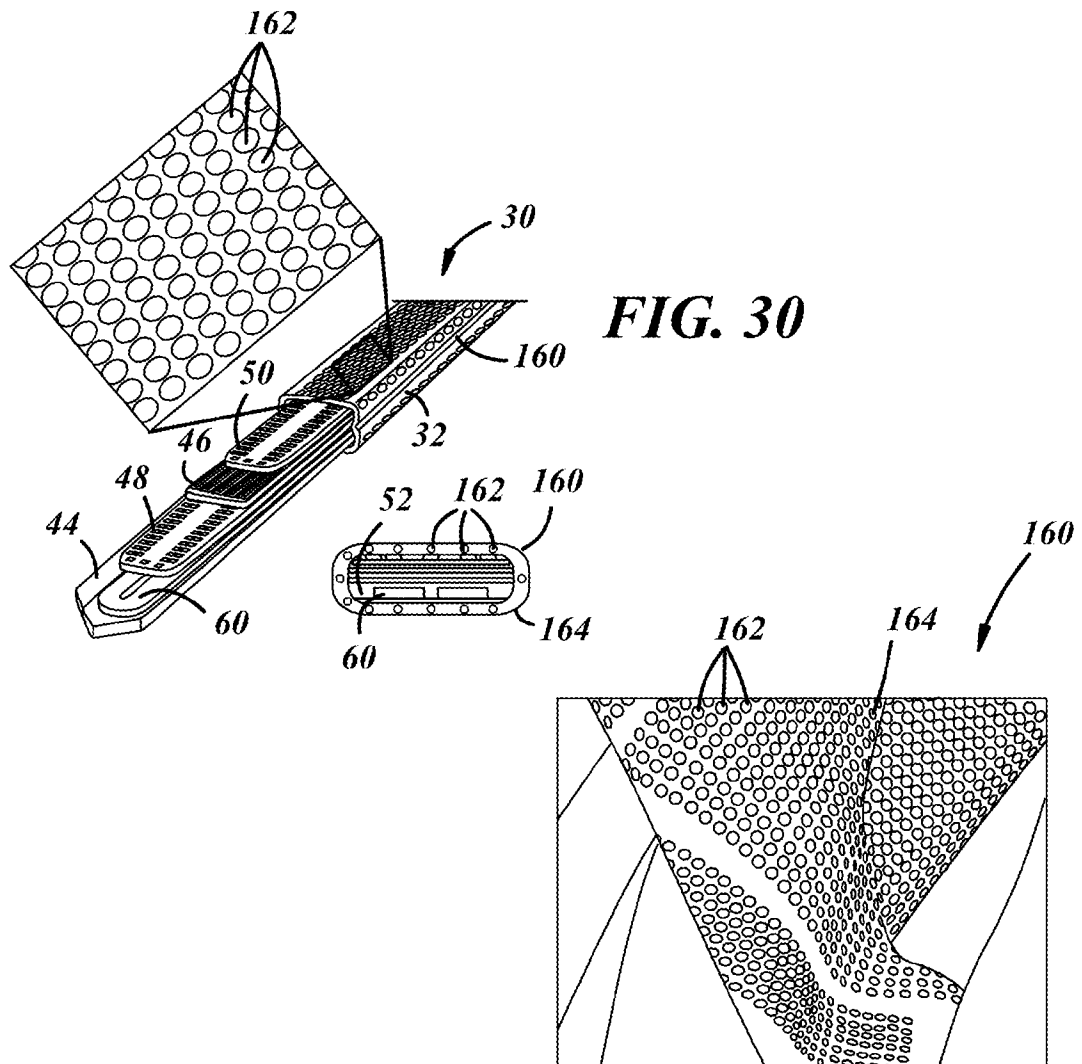
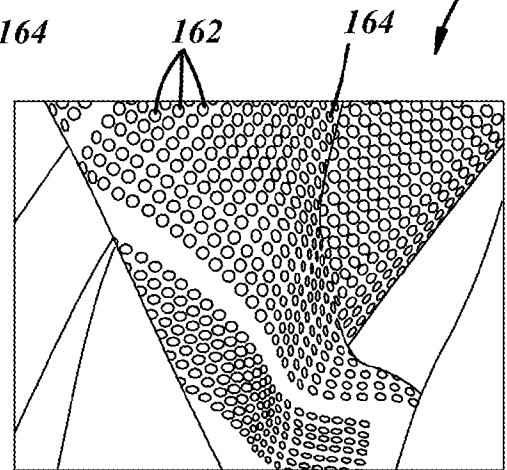
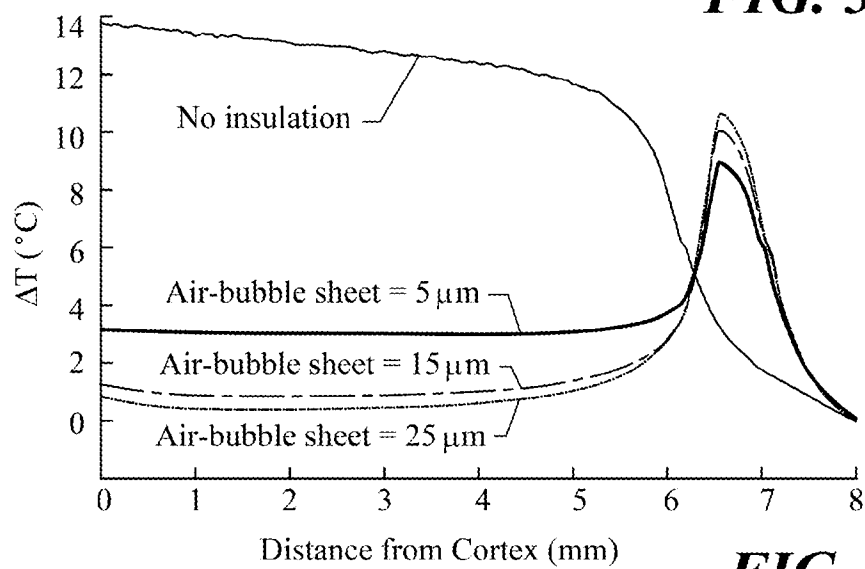


FIG. 26



**FIG. 30****FIG. 31****FIG. 32**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/055305

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61B 18/02** (2024.01); **A61B 5/25** (2024.01); **A61B 18/04** (2024.01); **A61B 5/00** (2024.01); **A61B 18/00** (2024.01)
CPC: **A61B18/02**; **A61B5/25**; **A61B5/40**; **A61B5/6868**; **A61B18/04**; **A61B2018/00005**; **A61B2018/00011**; **A61B2018/00434**;
A61B2018/00446; **A61B2018/0262**; **A61B2018/046**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/0093512 A1 (NEURONANO AB) 26 March 2020 (26.03.2020) figure 4; paras [0001], [0094], [0096]	1-3
A	GB 1123632 A (AEROJET GENERAL CORP) 14 August 1968 (14.08.1968) Entire Document	1-4
A	US 2018/0028119 A1 (DIAGNOSTIC BIOCHIPS, INC.) 01 February 2018 (01.02.2018) Entire Document	1-4
X	"Control of neural probe shank flexibility by fluidic pressure in embedded microchannel using PDMS/PI hybrid substrate" to Rezaei et al [July 24, 2019]; Retrieved from the Internet: https://pmc.ncbi.nlm.nih.gov/articles/PMC6655783/ ; Retrieved on February 26, 2024 page 2, paras [3], [5]; page 3, para [1]; page 5, paras [1], [2], [5]; figures 1, 2a, 2c, 2d	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"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

26 February 2025 (26.02.2025)

Date of mailing of the international search report

06 March 2025 (06.03.2025)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/055305

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **5-10, 16-17**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fee must be paid.

Group I: Claims 1-4 are directed toward a neural probe.

Group II: Claims 11-15 are directed toward a neural probe assembly.

Group III: Claims 18-21 are directed toward a method of controlling a temperature of an implanted neural probe assembly.

Group IV: Claims 22-23 are directed toward a method of manufacturing a neural probe assembly.

The special technical features of Group I include at least a probe shank extending from a tip end portion to a backend portion; and a channel extending from the backend portion at least partially down the probe shank toward the tip end portion, wherein the channel is fully sealed along the probe shank toward the tip end portion, and wherein the channel is configured to sealingly house a fluid.

The special technical features of Group II include at least an external temperature-controlled source configured to change a temperature of a fluid; and a head stage adapter located between the implantable neural probe and the external temperature-controlled source, wherein the head stage adapter is configured to allow for flow of the fluid from the external temperature-controlled source to the implantable neural probe.

The special technical features of Group III include at least administering a fluid to a closed channel on a neural probe of the neural probe assembly.

The special technical features of Group IV include at least molding a channel into a channel layer, the channel having a U-shaped cross-section and a U-shaped longitudinal extent; and laminating the channel layer to a backing layer to form a probe shank.

The common technical features shared by Groups I-IV are a neural probe, comprising: a probe shank and a channel.

However, these common features are previously disclosed by US 2018/0028119 A1 to DIAGNOSTIC BIOCHIPS, INC. (hereinafter "DIAGNOSTIC"). DIAGNOSTIC discloses a neural probe, comprising: a probe shank and a channel (neural probe containing shank and area (channel) for interconnects; [0035]).

Since the common technical features are previously disclosed by the DIAGNOSTIC reference, these common features are not special and so Groups I-IV lack unity.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/055305

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-4**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.