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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

[Continued on next page]

(54) Title: SIGNAL TRANSMITTER AND METHODS FOR TRANSMITTING SIGNALS FROM ANIMALS

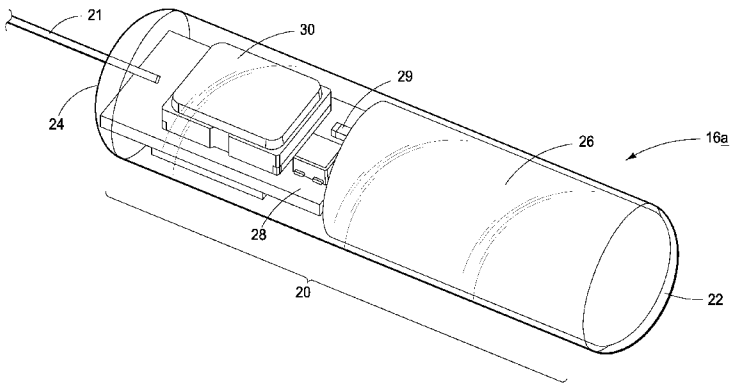


FIG. 2

(57) Abstract: Injectable transmitters are provided that can include a body with the body housing a power source and an oscillator, the injectable transmitter also including an antenna extending from the body, the body and antenna being of sufficient size to be injected through a 9 gauge needle. Radio frequency transmitters are provided that can include a body extending from a nose to a tail with the body housing a power source and RF signal generator components. The power source of the transmitter can define at least a portion of the nose of the body. The transmitters can have an antenna extending from the tail. Methods for attaching a radio frequency (RF) transmitter to an animal are provided. The methods can include providing an RF transmitter and providing an injection device having a needle of gauge of 9 or smaller; providing the RF transmitter into the injection device; and providing the RF transmitter through the 9 gauge or smaller needle and into the animal.

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Signal Transmitter and Methods for Transmitting Signals from Animals

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/267,797 which was filed on December 15, 2015, and U.S. Patent Application Serial No. 15/087,936 which was filed March 31, 2016, the entirety of which is incorporated by reference herein.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under Contract DE-AC0576RL01830 awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

TECHNICAL FIELD

The present disclosure relates to signal transmitters and methods for transmitting signals from animals. In particular embodiments, the transmitters can be injectable and/or provide radio frequency signals.

BACKGROUND

Transmitters have revolutionized biologists' understanding of both terrestrial and aquatic animal movements since they were first attached to animals about 50 years ago. Accurate information on fish movement, for example, is needed to understand the impacts of hydroelectric dams on fish migration and survival so that mitigation techniques can be applied to recover endangered populations (or to prevent endangerment in the first place). However, biologists are limited by the relatively large size of transmitters because of the potential to negatively impact and bias animal behavior. For example, the American Ornithologists' Union suggested that the transmitter weight should not exceed 5% of the body weight of birds. American

Society of Mammologists recommend transmitter weight should be less than 5% of the bats' body weight. Miniature radio-frequency (RF) transmitters used for tracking small aquatic, airborne, or terrestrial animals/objects that may be injected are provided herein.

SUMMARY OF THE DISCLOSURE

Injectable transmitters are provided that can include a body with the body housing a power source and an oscillator, the injectable transmitter also including an antenna extending from the body, the body and antenna being of sufficient size to be injected through a 9 gauge needle.

Radio frequency transmitters are provided that can include a body extending from a nose to a tail with the body housing a power source and RF signal generator components. The power source of the transmitter can define at least a portion of the nose of the body. The transmitters can have an antenna extending from the tail.

Methods for attaching a radio frequency (RF) transmitter to an animal are provided. The methods can include providing an RF transmitter and providing an injection device having a needle of gauge of 9 or smaller; providing the RF transmitter into the injection device; and providing the RF transmitter through the 9 gauge or smaller needle and into the animal.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a depiction of implantation of a transmitter according to an embodiment of the disclosure.

Figure 2 is one perspective view of a transmitter according to an embodiment of the disclosure.

Figure 3 is another perspective view of the transmitter of Figure 2 according to embodiment of the disclosure.

Figure 4 is one perspective view of a transmitter according to another embodiment of the disclosure.

Figure 5 is a depiction of a transmitter of the prior art and a transmitter of the present disclosure.

Figure 6 is a block diagram depicting components of a transmitter according to an embodiment of the disclosure.

Figure 7 is a block diagram depicting component of a transmitter according to another embodiment of the disclosure.

Figure 8 is one perspective view of a transmitter according to an embodiment of the disclosure.

Figure 9 is a depiction of a component of a transmitter according to an embodiment of the disclosure.

Figure 10 is a depiction of another component of a transmitter according to an embodiment of the disclosure.

Figure 11 is a part list of components of a transmitter according to an embodiment of the disclosure.

Figures 12A and 12B are circuit diagrams of component configurations of a transmitter without a crystal and with a crystal according to two embodiments of the disclosure.

Figures 13-19 are graphical representations of animal wound data acquired when implanting transmitters of the present disclosure according to methods of the present disclosure.

DESCRIPTION

To enhance the ability to study the survival of small fishes through hydroelectric dams, a smaller and more powerful radio-frequency (RF) transmitter, which can be injected into fish using a 9-gauge needle, is provided. Designs of two transmitters were developed: one that transmits coded signals and one that transmits

un-coded signals. To accommodate different transmitter life requirements, each design can be configured to provide a high or low signal strength.

The coded transmitter is 2.95 mm diameter and 11.85 mm long, and weighs merely 160 mg. Depending on the ping rate (or PRI, pulse rate interval), the coded low-signal-strength transmitter has a projected service life of 11 days@2s, 27 days@5s and 52 days@10s. By way of comparison, the smallest commercially available radio frequency transmitter for animals (NTQ-1, Lotek Wireless Inc., Newmarket, ON, Canada) has 6-58% lower operating lifetimes, despite being somewhat larger than the disclosed designs. The coded high-signal-strength transmitter has a comparable service life to the Lotek NTQ-1.

The un-coded transmitter is 2.95 mm diameter and 11.22 mm long, and weighs 152 mg. It provides even longer service life than the coded transmitter. Its low-signal-strength variant can last 15 days@2s, 37 days@5s and 69 days@10s.

Definitive determination of a preferred surgical implantation method was complicated due to several factors in the bio-effects evaluation. However, at the end of the study (day 14), the Incision-Cath method had the lowest percentage of open wounds (4.2%), the smallest wound size (1.14 mm^2), and the lowest percentage of transmitter loss (10%), and is likely the best method for transmitter implantation based on this pilot evaluation. Although the injection method had the fastest implantation time, and may allow cost-savings for telemetry studies with large sample sizes of tagged fish, the percentage of dropped transmitters (47.5%) was the highest of any treatment.

Referring to Fig. 1, a method for attaching a radio frequency transmitter 16 through a needle 14 into an animal 12 is depicted. Transmitter 16 is as described herein and needle 14 has a gauge of 9

or less. As depicted, transmitter 16 can be injected into animal 12. This can be a subcutaneous injection if desired or more or less invasive injection. Animal 12 can be any ambulatory being including but not limited to fish for example.

The transmitter can have a cylindrical body with a diameter of 2.95 mm or less to be compatible with a 9-gauge needle, for example. The transmitter can be encapsulated in an epoxy resin for rigidity and the transmitter body can be coated with a 25-μm layer of Parylene-C to provide waterproofness and/or bio-compatibility.

Referring next to Fig. 2, transmitter 16a is shown according to an embodiment of the disclosure. According to this embodiment, transmitter 16a includes a body 20 and an antenna 21. Body 20 can extend between a nose 22 and a tail 24. Preferably, injection of the transmitter proceeds nose first, for example. Transmitter 16a can include a power source 26 that can define at least a portion of the nose. This power source can be a battery such as that battery disclosed in U. S. Patent Application Serial No. 14/014,035 filed August 29, 2013, the entirety of which is incorporated by reference herein. Transmitter 16a can include RF signal generating components such as voltage regulator 28, capacitor 29 and oscillator 30.

Referring next to Fig. 3, according to another view of transmitter 16a, additional components are shown in operable alignment. For example, transmitter 16a can include LED 31 (blue), microcontroller 35, capacitor 32, resistor 33, and another capacitor 34.

Other component configurations of the transmitters are contemplated. For example, transmitter 16b is shown in Fig. 4. Transmitter 16b can include LED 31 mounted next to oscillator 30, for example.

Referring next to Fig. 5, a transmitter of the present disclosure is pictured (bottom) side by side with the commercially available radio frequency transmitter referenced above (Lotek NTQ-1, top).

The transmitters can include three components: an antenna component for transmitting RF signals, a circuit board component containing the controlling circuitry, and a cylindrical micro-battery component that powers the transmitter. The antenna can be a Teflon PFA (perfluoroalkoxy)-coated stainless steel wire that has a diameter of 38.1 μm. The length of the antenna can be varied to achieve the desired resonance frequency of the transmitter (e.g., 17.8 cm = ~164 MHz). To meet the transmitter life goal, the transmitter can use a Li/CF_x micro-battery consisting of lithium metal anode and carbon fluoride cathode, which has a capacity of 6 mAh and is 6 mm long. Compared to the traditional silver oxide button-cell batteries, which are commonly used in small commercial radio transmitters, the Li/CF_x batteries have the advantages of high energy and power density as well as high average operating voltage (3.1-3.4 volts), long shelf life and a wide operating temperature range.

To fabricate the prototype transmitter, the micro-battery can be first attached to the circuit board using a silver-filled epoxy such as the 8331 (MG Chemicals, Ontario, Canada). The antenna can be soldered to the circuit board. For encapsulation, this RF transmitter assembly can be coated with an insulating epoxy such as the EPO-TEK 301 epoxy (Epoxy Technology Inc., Billerica, MA) using a flexible mold, which can be made from flexible materials (e.g. silicone rubber). The mold can have cavities that define the shape of the transmitter. After the RF transmitter assembly is placed into the cavity, the insulating epoxy can be injected into the bottom of the cavity using a syringe with a small needle until the epoxy fills up the cavity. The mold may be left to stand overnight for the epoxy to cure. After removal from the mold, the transmitter may be gently sanded with sand paper and polished using a rotary polishing tool to eliminate any sharp edges or burrs. Finally, the RF transmitter can be coated with 25-μm thick Parylene-C to become waterproof and/or bio-compatible.

Two different designs (Option 1 and Option 2 hereinafter) of the injectable RF transmitter are provided as example implementations, but other implementations are contemplated. Option 1 transmits un-coded RF signals at a set ping rate, whereas Option 2 transmits coded RF signals.

A first embodiment (i.e. Option 1) can be 11.22 millimeters in length and weighs less than 152 milligrams.

A second embodiment (i.e. Option 2) can have an extra crystal to control the timing of RF signal so that it can generate coded RF signals. The second embodiment can be about 0.63 mm longer and 8 mg heavier than the first embodiment.

A block diagram of the first embodiment (i.e. Option 1) transmitter is shown in Figure 6. This design includes a microcontroller, a resistor–capacitor (RC) network, a light emitting diode (LED), a low-dropout voltage (LDO) regulator and a programmable oscillator.

The microcontroller controls various circuits and functions within the injectable RF transmitter. It executes embedded firmware (source code) that defines its operation, which includes turning the programmable oscillator on and off to control the transmitted signal.

The RC circuit isolates the microcontroller from the voltage drop of the battery and keeps it from entering brownout state.

The LED provides an optical link for programming through a personal computer. This component is not used in the typical manner: rather than generating light when a voltage is applied across its terminals, the LED generates a voltage across its terminals when exposed to ultraviolet light. A configuration apparatus (not shown) may utilize a USB-to-TTL converter circuit and a second LED to convert serial commands from a personal computer to a coded series of “on” and “off” pulses of light, which then may be converted back

into electrical signals by the first LED on the transmitter. This first LED is then coupled to one of the pins on the microcontroller. The above mechanism provides a small yet effective way to activate the microcontroller and specify operating parameters such as the PRI.

The LDO regulator outputs a fixed voltage at 1.8 V to power the programmable oscillator, because the typical input voltage of the programmable oscillator ranges from 1.6 V to 2.2 V.

The programmable oscillator generates a symmetric square wave signal. The Fourier series of a square wave is

$$\text{square}(t) = \frac{4}{\pi}\sum_{n=1}^{\infty}\frac{\sin(nt)}{n} = \frac{4}{\pi}\left(\frac{\sin(t)}{1} + \frac{\sin(3t)}{3} + \frac{\sin(5t)}{5} + \cdots\right)$$

A square wave only contains odd harmonics and the amplitude decreases in inverse portion to harmonic order n. For this application, the FCC frequency range can be 164~168 MHz. The programmable oscillator can be programed to 54.667~56.000 MHz for high-strength signals to generate a sine wave at 164~168 MHz using the 3rd harmonic. This option consumes a current of 2.7 mA. The programmable oscillator can alternatively be programed to 32.8~33.6 MHz for low-strength signals using the 5th harmonic. This option consumes a current of 2.1 mA. The duration of the un-coded RF signal can be set to about 16 ms using the internal clock of the microcontroller.

A block diagram of the second embodiment (i.e. Option 2) transmitter is shown in Figure 7. This design includes a microcontroller that uses an external clock signal from an added quartz crystal to accurately control the timing of the oscillator. This mechanism allows the transmitter to generate coded RF signals. The microcontroller can also use the crystal to calibrate its internal clock.

To allow multiple transmitters broadcasting on the same frequency, a pattern of pulses unique to each individual transmitter can be used.

Referring next to Fig. 8, a depiction of the second embodiment (i.e. Option 2) transmitter 16c that includes crystal 80 and additional capacitors 81 and 82 is shown. Referring to Fig. 9, a more detailed view of the oscillator 30, voltage regulator 28 and capacitor 29 are shown associated with a circuit board of the transmitter. Referring to Fig. 10, additional components of the same circuit board, but opposing side can include microcontroller 35, capacitor 32, resistor 33, LED 31, and capacitor 29.

Referring next to Fig. 11 a general electronic component parts list is provided that can be arranged in accordance with the circuit diagram of Figs. 12A and 12B. Fig. 12A is representative of the first embodiment (i.e. Option 1) transmitter and Fig. 12B is representative of the second embodiment (i.e. Option 2) transmitter.

Signal output can be compared between the two prototype transmitters and the smallest commercially available transmitter. The test can be performed outdoors to minimize the effects of electromagnetic noise and other signals. The test transmitters can be placed about 10-cm apart and parallel to each other and the signal receiver (Sigma Eight Orion receiver with an omnidirectional whip antenna) was located about 6 m away. The receiver antenna was arranged perpendicular to the transmitter antennas. All transmitters and receiver/antenna remained in the same place during the test.

Hatchery-reared spring Chinook salmon (*Oncorhynchus tshawytscha*) were used for all bio-effects experiments. After hatching, the juveniles were held in cold water until July 2015, when they were slowly acclimated to a water temperature of 12°C to allow them to grow to an appropriate size for juvenile salmonid radio transmitter implantation (i.e., >95 mm). For 2 months prior to tagging and for the duration of this study, fish remained at 12°C (±2°C). Food was

restricted for 24 hours prior to and 24 hours following implantation. On the day of implantation, fish ranged from 103 - 153 mm (mean = 129.9 mm) in fork length (FL) and weighed 10.9 - 36.8 g (mean = 23.6g). All tools were disinfected (by ultraviolet light for surgical blade) or sterilized (by autoclave for stainless steel needles and catheters) prior to use. The 9-gauge needles were new on the day of tagging. After first use with the injection treatment, they were autoclaved and still very sharp for the 9ga-needle with catheter treatment.

Since implantation time was an important variable in this study, rather than randomize the treatment order throughout the tagging day, each treatment was completed in a block. Using this design, the tagger was able to standardize the technique of implantation and to become efficient with the process. Thus, theoretically, this tagging order produced the fastest implantation times for each of the three treatments with the radio transmitter tested. In addition, the efficiency is representative of the process in which fish would be tagged in a field study where only one technique is used.

"Dummy" transmitters of the second embodiment described above that were equal in dimensions and mass to the functioning transmitter (i.e., the larger prototype suitable for coded transmitters) were used for the bioeffects study. It had dimensions of 2.95 mm x 2.95 mm x 11.85 mm and contained within its volume an 8.4 mm long passive integrated transponder (PIT) tag (HPT8, Biomark). This PIT tag permitted unique identification of the dummy transmitter if it were shed during the post-tagging holding period. Currently in a radio telemetry study in the Willamette River basin, PIT tags also are being implanted alongside radio transmitters in smolts for PIT tag detection downstream of Foster Dam. Therefore, for this laboratory study, a 12.5 mm PIT tag (HPT12, Biomark) was implanted along with the dummy radio transmitter. Unfortunately, the presence of 2 PIT tags in a single fish makes neither tag detectable due to their proximity. However, every tag dropped could be immediately identifiable. At the

completion of this study, fish were again identifiable during necropsy by scanning each tag individually.

On September 15, 2015, fish were netted from a 4-ft diameter circular rearing tank and placed in a 20-L bucket filled with aerated river water. One at a time, fish were placed in an anesthetic bucket with a dosage of 80 mg/L Tricaine Methanesulfonate for ~3 min or after a complete loss of equilibrium. Fish were then immediately weighed, measured, and tag codes assigned. The tagger then implanted the anesthetized fish using one of three treatment methods: surgical incision with catheter (aka Incision-Cath), 9-gauge needle injection (aka Injection), and 9-gauge needle with catheter (aka 9GA-Cath). For both of the catheter treatments, fish were placed on a wet, grooved, surgery pad (coated with PolyAqua, a water conditioner) for stabilization and their gills were irrigated with fresh water through rubber tubing from a gravity-fed tank. For the injection treatment, fish were stabilized in the tagger's hand. Detailed descriptions of the treatments are explained below.

Method 1: Surgical incision with catheter (Incision-Cath) - With the fish facing ventral side up, a surgical blade was used to make ~3-mm incision on the linea alba (mid-ventral line). The incision was made ~5 mm anterior of the pelvic girdle. To place the antenna through the body wall, a 19-gauge stainless steel needle shielded with a 16-gauge stainless steel catheter was carefully guided through the body cavity posterior to the pelvic girdle. Then, the 19-ga needle was unshielded to make a hole through the body wall. The needle remained in the fish while the catheter was pulled back out through the incision. The end of the transmitter antenna was then threaded through the tubing of the needle. Both the needle and antenna were pulled posteriorly until the needle was out of the fish and the antenna was threaded through the body wall. Next, the PIT tag was inserted into the peritoneal cavity. Lastly, the transmitter body was guided into the peritoneal cavity as the antenna was gently guided posteriorly.

Unlike most field radio tagging studies, a suture was not used to close the incision. Recent laboratory studies using an injectable acoustic transmitter of similar size showed 100% long-term transmitter retention with a ~3 mm incision in salmon as small as 80 mm in length (unpublished data).

Method 2: 9-gauge needle injection (Injection) - To prepare the tags for injection, first the transmitter was loaded into the 9-ga needle by inserting the rounded transmitter end through the hub end of the needle. If the dummy transmitter body was slightly too wide to be inserted through the hub, then the slower loading method was used. The slower method consisted of threading a short section of the transmitter antenna through the 16-ga catheter and guiding it through the pointed end of the needle. The antenna could not be threaded directly through the needle and hub (without the aid of the catheter) because the end of the antenna would catch on an edge inside the needle and the antenna could become kinked. After loading the transmitter into the needle, the needle hub was screwed onto an implanter (Biomark MK10 implanter). Modifications to the implanter included removal of the spring and notching the tip of the implanter to permit the antenna wire to hang outside the implanter body. Next, the PIT tag was loaded into the pointed end of the needle and this completed the preparation phase of tagging. The fish was then held ventral side up with its head facing away from the tagger. Using a bevel-down technique similar to that described by Cook et al. (2014), the needle was used to puncture the body wall ~3-5 mm anterior of the pelvic girdle and ~5 mm off the linea alba, on the left side of the fish. The needle was inserted to a shallow depth to create a hole just large enough for the transmitter. With a plunge of the implanter, the PIT tag, followed by the dummy transmitter, were injected anteriorly into the peritoneal cavity. Pressure was applied to the wound with the left thumb to ensure the transmitter was retained while the needle was drawn out of the fish and the antenna moved forward through the implanter and needle.

Method 3: 9-gauge needle with catheter (9GA-Cath) - Before the fish was placed on the surgery pad, the 19-ga needle, shielded with the 16-ga catheter, was inserted into the hub end of the 9-ga needle (the implanter was not used for this technique). With the fish facing ventral side up on the pad, the 9-ga needle was used to make a small opening (~3 mm; equal to the width of the transmitter) near the distal end of the right pectoral fin ~3-5mm from the linea alba. Then, the shielded 19-ga needle was carefully guided through the body cavity posterior to the pelvic girdle. The 19-ga needle was unshielded to make a hole through the body wall. The needle remained in the fish while the catheter and 9-ga needle were pulled away from the fish. The end of the transmitter antenna was then threaded through the tubing of the needle. Both the needle and antenna were pulled posteriorly until the needle was out of the fish and the antenna was threaded through the body wall. Next, the PIT tag was inserted into the peritoneal cavity. Lastly, the transmitter body was guided into the peritoneal cavity as the antenna was gently guided posteriorly.

After tagging, images were taken of the implantation wounds: two wounds each for fish implanted by methods 1 or 3, and one wound each for fish implanted by method 2. Fish were placed ventral-side up on a pad and they were supplied with water through rubber tubing throughout the imaging process. They were returned quickly to a recovery bucket supplied with aerated river water. Once ~10 fish were recovered, they were transferred to the holding tank where they resided for the duration of the study.

At 14 days post-surgery, fish were euthanized in 250 mg/L MS-222. Images were taken of the wounds as the external fish assessment was completed. Wounds were examined for openness, redness/inflammation, and ulceration. It was also noted whether the antenna was still present outside of the fish. The fish was then necropsied to determine radio transmitter and PIT tag retention and to identify the fish (by scanning each tag). Evaluation of tag

encapsulation and/or adhesions was also completed at this time. Measurements of the wound area were made post-hoc.

All analyses were done with JMP version 7 (The SAS Institute, Cary, NC) at an alpha = 0.05. Assumptions of equal variances and normality were verified prior to parametric statistical procedures.

The signal strengths of both high and low-signal-strength variants of the second embodiment prototype were tested to compare with that of the Lotek NTQ-2. Both of the prototype transmitters were found to be consistently about 10 dB stronger (-76 and -77 dBm, respectively for the high and low-signal-strength designs) than the Lotek NTQ-2's (-88 dBm).

Transmitters of the present disclosure also have similar or better service life compared to Lotek NTQ-1 and NTQ-2. The energy consumption of the transmitters was calculated by

$$E_{trans} = V * I * T$$

V is the battery voltage in volts; we choose an average value of 2.5 Volts in the service life calculations. *I* is the average current consumed during transmission and *T* is the duration of each transmission.

For transmitters of the first embodiment, the duration is 16 ms for each RF signal transmission. For low signal strength, current I is 2.1 mA and the energy consumption is 84 µJ. For high signal strength, current I is 2.7 mA and the energy consumption is 105 µJ.

For transmitters of the second embodiment, the total pulse duration is about 22 ms for each RF signal transmission. For low signal strength, current I is 2.1mA, the energy consumption is 115.5 µJ. For high signal strength, current I is 2.7 mA, the energy consumption is 148.5 µJ.

The service life calculation in Table 1 was based on the battery capacity 6mAh, constant static current 0.5 uA that flows through the transmitter circuit, PRI.

The total energy of battery in transmitters can be calculated by

$$E_{battery} = V * C * 3600/1000$$

V is the battery voltage in volts, C is the battery capacity in mAh, the number 3600 is used to convert hours to seconds and 1000 is used to convert mAh to Ah.

The total energy of battery can also be calculated by

$$E_{battery} = E_{total_trans} + E_s$$

E_{total_trans} is the total energy consumed by transmissions throughout the service life of the transmitter and E_s is the energy consumed by the static current that constantly flows through the transmitter circuit.

Therefore, the total energy of battery can be expressed as:

$$E_{battery} = E_{trans} * n + V * I_s * T = E_{trans} * \frac{T}{PRI} + V * I_s * T = \left(\frac{E_{trans}}{PRI} + V * I_s\right) * T$$

E_{trans} is energy consumption of each transmission, n is total number of transmission throughout the service life. I_s is constant static current 0.5 μA that flows through the transmitter circuit. PRI is the pulse rate interval (ping rate) and T is the service life in seconds.

Because the total number of transmission n can be calculated by

$$n = \frac{T}{PRI}$$

The service life in days can be calculated as

$$T = \frac{E_{battery}}{\frac{E_{trans}}{PRI} + V * I_s} = \frac{V * C * 3600/1000}{\frac{E_{trans}}{PRI} + V * I_s} /3600/24 = \frac{V * C/1000/24}{\frac{E_{trans}}{PRI} + V * I_s}$$

It is worth noting that the actual service life of the transmitter is usually slightly longer than the projected values based on this equation, because the battery voltage gradually decreases at a slow rate as it discharges, which consequently causes the E_{trans} decreases over time.

Table 1 provides the size, weight, and calculated service life comparisons between the Lotek transmitters and the example transmitters.

Table 1: The comparison of Lotek, example transmitters

Transmitter	Size	Weight	Calculated Life (days)			
	w x h x l (mm)	(air) (mg)	2s PRI	5s PRI	10s PRI	60s PRI
Lotek						
NTQ-1	5*3*10	260	10	21	33	59
NTQ-2	5*3*10	310	16	33	52	94
1st Embodiment						
Low signal strength	2.95*11.22	152	15	37	69	245
High signal strength	2.95*11.22	152	12	30	56	217
2nd Embodiment						
Low signal strength	2.95*11.85	160	11	27	52	206
High signal strength	2.95*11.85	160	9	21	41	176

The actual service life of the first embodiment transmitter was tested at a 3s PRI (Table 2). The test results are consistent with the projected value obtained using the equation described above.

Table 2: The prototype RF transmitter life testing results

1st Embodiment (3s PRI) Transmitter	Calculated Life (days)	Measured Life (days)
Low-signal-strength Transmitter (test sample 1)	23	30
Low-signal-strength Transmitter (test sample 2)	23	24

No mortalities occurred throughout the 14-day duration of the experiment; however, surgery time and surgery maladies differed between the three surgical techniques/treatments. Implantation time significantly differed between the 3 treatment types ($P<0.0001$) with the Injection treatment having the fastest time (mean = 12 s, SE = 1.3 s) and the 9GA-Cath and Incision-Cath treatments having times of 48 s (SE = 1.3) and 45 s (SE = 1.3), respectively (Figure 13). Preparation time for the Injection treatment was also measured for 15 surgeries, and when combined with surgery time, resulted in an average total surgery time of 29 seconds (SE = 3.14).

Figure 13 depicts Implantation time (seconds) of the 3 surgical implantation treatments. The horizontal line in the green diamond represents the mean, upper and lower bounds of the diamond represent 25th and 75th percentiles, and blue horizontal lines indicates 5th and 95th percentiles. Means comparisons are also shown in the right panel using the Tukey-Kramer HSD test.

The percentage of dropped tags and dropped antennas differed significantly between treatments (tags, $P=0.0002$; antennas, $P=0.001$). Injection treatment fish lost the most tags (47.5%) whereas 9GA-Cath (15%) and Incision-Cath (10%) lost fewer tags (Figure 14). Injection fish also lost the most antennas (65%) compared to either

9GA-Cath (27.5%) or Incision-Cath (40%). The difference in percentages between lost antennas and tags was due to the antennas falling off of the tag bodies and the tag bodies remaining inside the fish.

Figure 14 depicts percentages of fish that retained (dotted "Yes") or lost (cross-hatched, "No") their RF transmitters through the final day of the study (Day 14).

Wound area at Day 0 (day of tagging) for the Injection-Incision site (i.e., hole made that tag body was inserted through) was significantly different between treatments ($P<0.0001$; Figure 15). The Injection treatment wound (mean = 2.04 mm^2, SE = 0.07) and 9GA-Cath wound (mean = 1.84 mm^2, SE = 0.07) were relatively large and statistically similar whereas the Incision-Cath wound was significantly smaller (mean = 0.63 mm^2; Tukey HSD). Catheter site wound area did not differ between the 9GA-Cath and Incision-Cath treatments ($P=0.0885$; Figure 16). Specifically comparing the wound area of the antenna-exit holes for each treatment (i.e., same as injection site for Injection treatment), the Injection treatment was significantly larger than either the 9GA-Cath or Incision-Cath wound made by the catheter, which likely played a role in whether fish retained or lost their transmitters.

Figure 15 depicts a comparison of the wound area of the injection-incision site at day 0. The horizontal line in the green diamond represents the mean, upper and lower bounds of the diamond represent 25^{th} and 75^{th} percentiles, and blue horizontal lines indicates 5^{th} and 95^{th} percentiles.

Figure 16 depicts a comparison of the wound area of the catheter (Cath) site at day 0. The horizontal line in the green diamond represents the mean, upper and lower bounds of the diamond

represent 25th and 75th percentiles, and blue horizontal lines indicates 5th and 95th percentiles.

Wound area on Day 14 (end of study) was significantly different among treatment groups (P<0.0001; Figure 17) with Injection fish having the largest wound area (5.77 mm^2), 9GA-Cath having an intermediate wound area (3.17 mm^2), and Incision-Cath having the smallest wound area (1.14 mm^2). However, it's important to note that fish that lost their tags or antennas were not included in these results because of inherent bias that losing a tag/antenna would have had on wound healing; thus, this analysis (and Figure) only include fish that retained their transmitter and antenna. Similar to Day 0, the wound area of the catheter site did not differ between 9GA-Cath and Incision-Cath treatments (P=0.5592, Figure 18).

Figure 17 depicts a comparison of the wound area of the injection-incision (Inj-Inc) site at day 14 (end of study) for each of the 3 surgical treatments. The horizontal line in the green diamond represents the mean, upper and lower bounds of the diamond represent 25th and 75th percentiles, and blue horizontal lines indicates 5th and 95th percentiles. Tukey-Kramer HSD comparisons are included for reference.

Figure 18 depicts a comparison of the wound area of the catheter (Cath) site at day 14. The horizontal line in the green diamond represents the mean, upper and lower bounds of the diamond represent 25th and 75th percentiles, and blue horizontal lines indicates 5th and 95th percentiles.

Predicting dropped transmitters. Using a stepwise multivariate modeling procedure, tag loss was significantly related to the treatment method (P<0.0001) as well as fish fork length (P=0.0168). However, the predictability of the final model was relatively weak (r^2 = 0.1837) with treatment contributing most to the model's prediction power (r^2 =

0.1391). Original predictor variables included treatment, fish fork length, inj-inc wound area at day 0, and catheter wound area at day 0.

Of the variables measured on the day 14 necropsy, only wound openness was significantly different among treatments (P<0.0001; Figure). All Injection treatment fish (N=13) had open injection wounds (i.e., unhealed with visible opening to internal organs) on day 14 whereas 24.1% (7 of 29) of the 9GA-Cath treatment and 4.2% (1 of 24) of the Incision-Cath fish had open injection wounds.

No significant differences were found between catheter wound openness of the two catheter treatments (P=0.8916), redness/inflammation (P=0.3916), tag encapsulation (P=0.4931), or tag adhesion (P=0.2548; Table 3). No ulcerations were present on any necropsied fish.

Figure 19 depicts percentage of fish, by surgical treatment, with open Incision-Injection (Inc-Inj) wounds (dotted, “Yes”) or closed wounds (cross-hatched, “No”) during day 14 necropsies.

Table 3. Percentages of necropsy maladies (variable) by surgical treatment groups and wound locations.

	Treatment/Wound					
Variable	Injection	9GA-Cath/tag	9GA-Cath/cath	Inc-Cath/tag	Inc-Cath/cath	P
Catheter wound open	NA	NA	3.5% (1 of 29)	NA	4.2% (1 of 24)	0.8916
Redness/inflammation present	15.4% (2 of 13)	3.5% (1 of 29)	10.3% (3 of 29)	4.2% (1 of 24)	8.3% (2 of 240	0.3916
Tag encapsulation	30.8% (4 of 13)	51.7% (15 of 29)	NA	37.5% (9 of 24)	NA	0.4931
Tag adhesion	7.7% (1 of 13)	0% (0 of 29)	NA	4.2% (1 of 24)	NA	0.2548

Definitive determination of a preferred surgical implantation method was complicated due to several factors in the bio-effects evaluation. However, at the end of the study (day 14), the Incision-Cath method had the lowest percentage of open wounds (4.2%), the smallest wound size (1.14 mm2), and the lowest percentage of tag loss (10%), and is likely the best method for transmitter implantation based on this pilot evaluation. Although the injection method had the fastest implantation time, and may allow cost-savings for telemetry

studies with large sample sizes of tagged fish, the percentage of dropped tags (47.5%) was the highest of any treatment.

Tag loss and antenna loss for all implantation treatments was likely affected and biased by the kinking and tangling of the antenna material. At least 10 transmitter antennas were found to be tangled with each other, either within fish, or after tags had fallen out of fish. Further, the Injection, 9GA-Cath, and Incision-Cath treatments lost 17.5%, 12.5%, and 30% of their antennas, respectively, while tag bodies remained inside study fish. However, because fish from all treatments were located within the same tank during the 14 day observation period, we assume that all treatments had equal probability of having their antenna tangled and despite this, there were differences in tag loss with the Injection technique having greatest tag loss (47.5%) and the 9GA-Cath (15%) and Incision-Cath (10%) methods having lower loss. Thus, we presume that tag loss was related more to the size of the open wound – the Injection technique had the largest wounds on both day 0 (2.04 mm2) and day 14 (5.77 mm2) – rather than due to the tangling of antennas alone.

Surgical implantation time is approximately the time that a fish is “out of water” during the surgical procedure; either on a surgical pad or held in the tagger’s hand. The out of water time for the Injection treatment was faster than the 2 catheter techniques and would likely have beneficial effects with respect to fish health and survival. However, researchers should be cautious when relating the time savings of the Injection technique to budget/cost savings because the additional preparation time required to load the tags in the implanter needle ranged from 5–50 s (i.e. total load + implant time = 13–59 s; mean = 29 seconds, SE = 3.14). Otherwise, if the tagger did not load the needle themselves, budgets would likely require an extra person tasked with loading tags for the entire tagging day. Alternatively, small modifications could be made to the transmitter or

9-gauge needle/implanter to keep loading times to ~5 s, thereby potentially improving the cost-savings estimates.

The pilot laboratory evaluation using the prototype injectable radio transmitter has provided an array of ideas on methods to improve and refine the transmitter design and implantation techniques. Qualitative improvements to the transmitter design based on the laboratory evaluation include creating a smoother antenna coating, using a different antenna material, and standardizing the tag body size. A smoother antenna coating would have likely reduced the implantation times of the Incision-Cath and 9GA-Cath techniques due to jagged "barbs" of the coating that snagged on the surgery materials as the antenna was threaded through the 19-gauge needle. Tagging times were much faster (i.e., ~ 30 s) using uncoated transmitters on dead fish during our pre-experiment "practice" tagging. A smoother antenna coating or a different antenna material would also likely minimize the tangling of antennas in holding tanks following implantation, which is an important factor to consider for future field studies that necessitate holding fish in close proximity in small buckets. Standardizing the tag body size would also likely reduce the implantation times of the Injection technique. With a more consistent body size, the transmitter could be consistently loaded from the hub end, which resulted in tagging load times of about 5 s.

Improvements to surgical tools could also be beneficial for improving the tagging technique and reducing surgery time for the Injection and 9GA-Cath techniques studied in this pilot evaluation. Modifications to the tubing used for the 9-gauge needle in the Injection technique could further reduce tag-loading time. Additionally, modification of the notched implanter used in the Injection technique could reduce implantation time by reducing antenna snags in the implanter during transmitter injection. The 9-gauge needle used in the 9GA-Cath treatment was also cumbersome and designing a

combination needle-implanter may improve tag retention, reduce wound size, and reduce implantation time.

CLAIMS

1. An injectable transmitter comprising:

a body, the body housing a power source and an oscillator; and

an antenna extending from the body, the body and tail being of sufficient size to be injected through a 9 gauge needle.

2. The injectable transmitter of claim 1 further comprising:

an LED;

RC network;

microcontroller; and

low dropout regulator.

3. The injectable transmitter of claim 1 further comprising:

an LED;

RC network;

microcontroller;

low dropout regulator; and

crystal.

4. The injectable transmitter of claim 1 wherein the body further comprises a waterproof housing.

5. The injectable transmitter of claim 1 wherein the body is substantially capsule in shape.

6. The injectable transmitter of claim 1 wherein the body is less than 12 mm in length.

7. The injectable transmitter of claim 1 wherein the body has a diameter of less than 3 mm in one cross section.

8. A radio frequency transmitter comprising:

a body extending from a nose to a tail, the body housing a power source and RF signal generator components, the power source defining at least a portion of the nose of the body; and

an antenna extending from the tail.

9. The radio frequency transmitter of claim 8 wherein the power source is a battery.

10. The radio frequency transmitter of claim 8 wherein the power source is a cylindrical micro-battery

11. The radio frequency transmitter of claim 10 wherein the battery comprises a Li/CF_x micro-battery comprising a lithium metal anode and a carbon fluoride cathode.

12. The radio frequency transmitter of claim 10 wherein the battery has a capacity of 6 mAh and is less than 6 mm long.

13. The radio frequency transmitter of claim 8 wherein the antenna comprises Teflon PFA (perfluoroalkoxy)-coated stainless steel wire

14. The radio frequency transmitter of claim 8 wherein the antenna defines a diameter of less than 38.1 μm and a length of less than 17.8 cm.

15. A method for attaching a radio frequency (RF) transmitter to an animal, the method comprising:

providing an RF transmitter and providing an injection device having a needle of gauge of 9 or smaller;

providing the RF transmitter into the injection device; and

providing the RF transmitter through the 9 gauge or smaller needle and into the animal.

16. The method of claim 15 wherein the animal is a fish.

17. The method of claim 16 wherein the animal is a salmon.

18. The method of claim 15 wherein the RF transmitter includes an antenna.

19. The method of claim 15 further comprising tracking the animal using an RF signal generated by the RF transmitter.

20. The method of claim 15 further comprising providing a signal from the RF transmitter for at least 24 days.

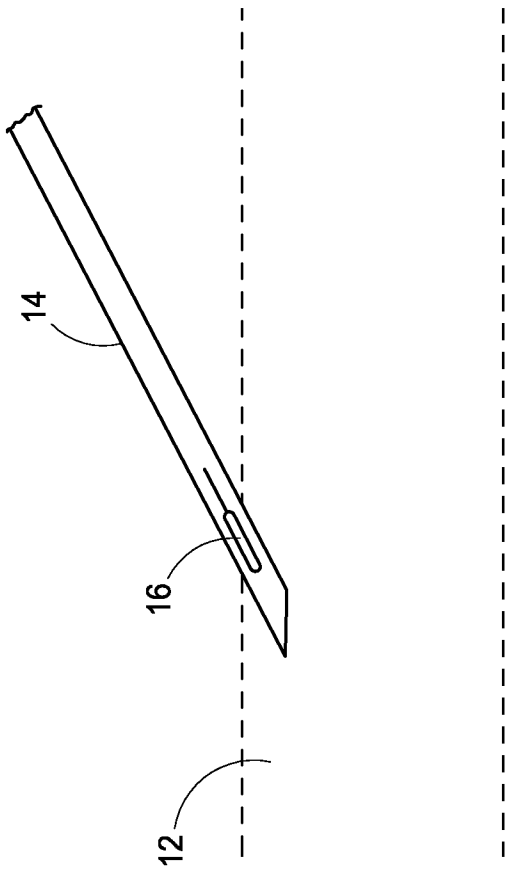


FIG. 1

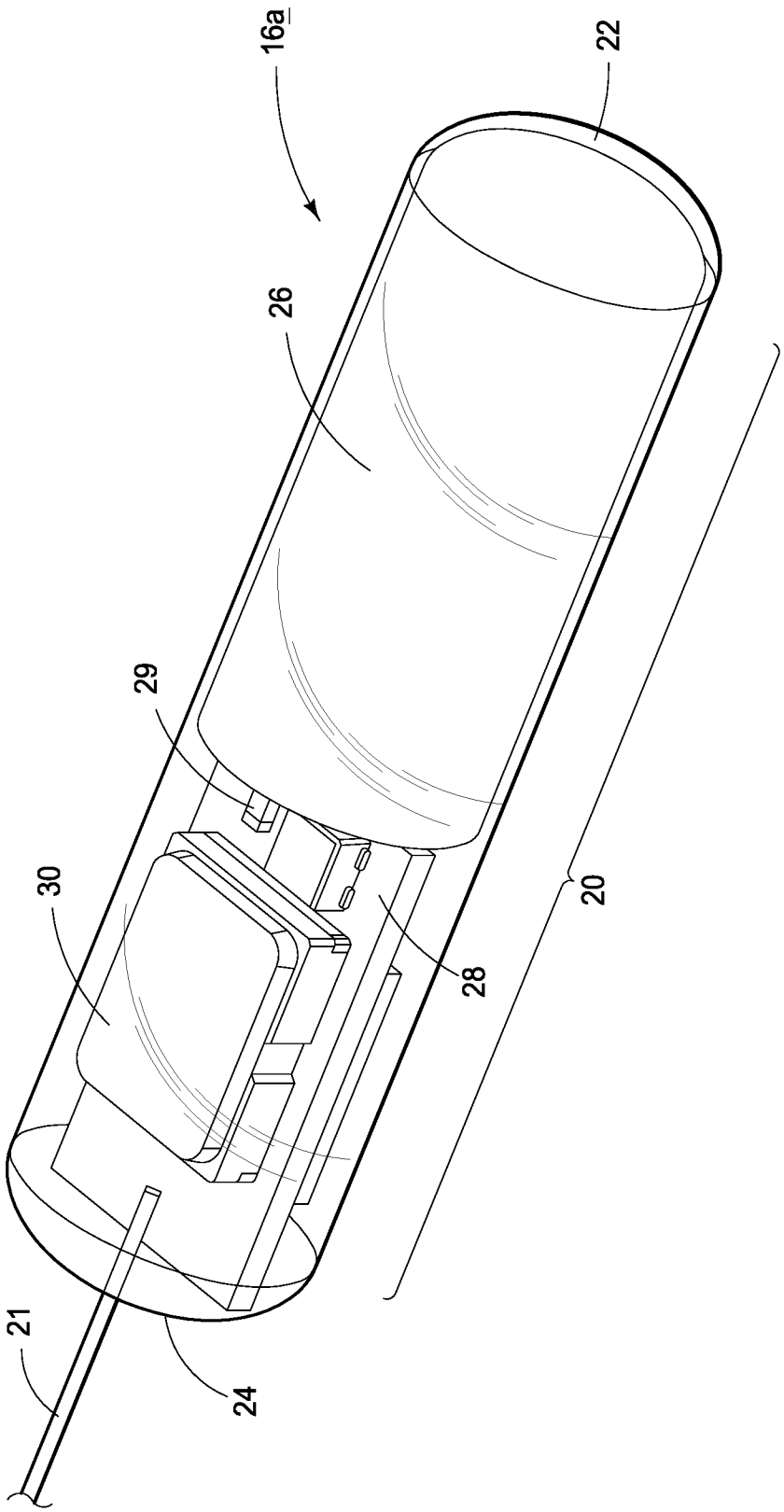


FIG. 2

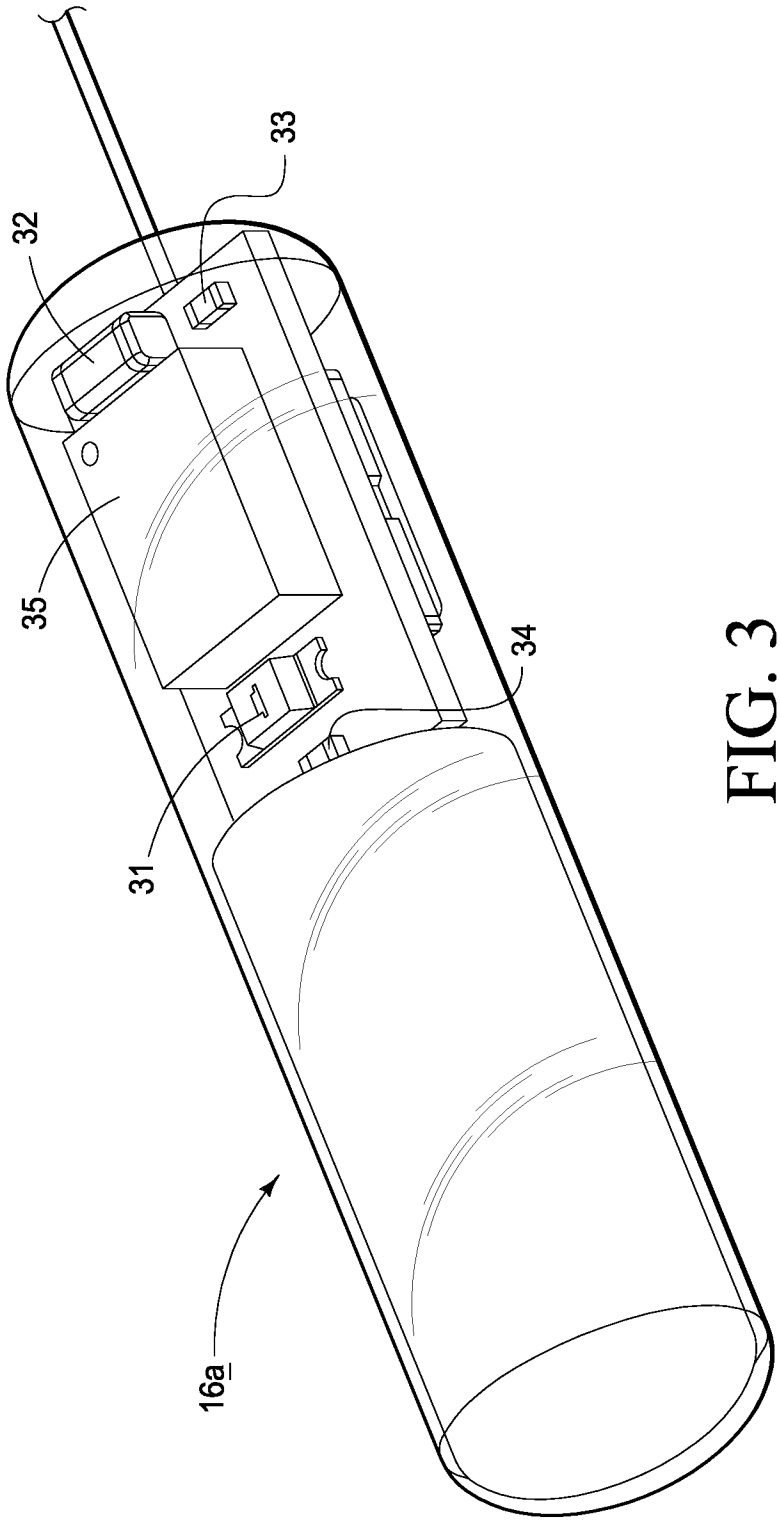


FIG. 3

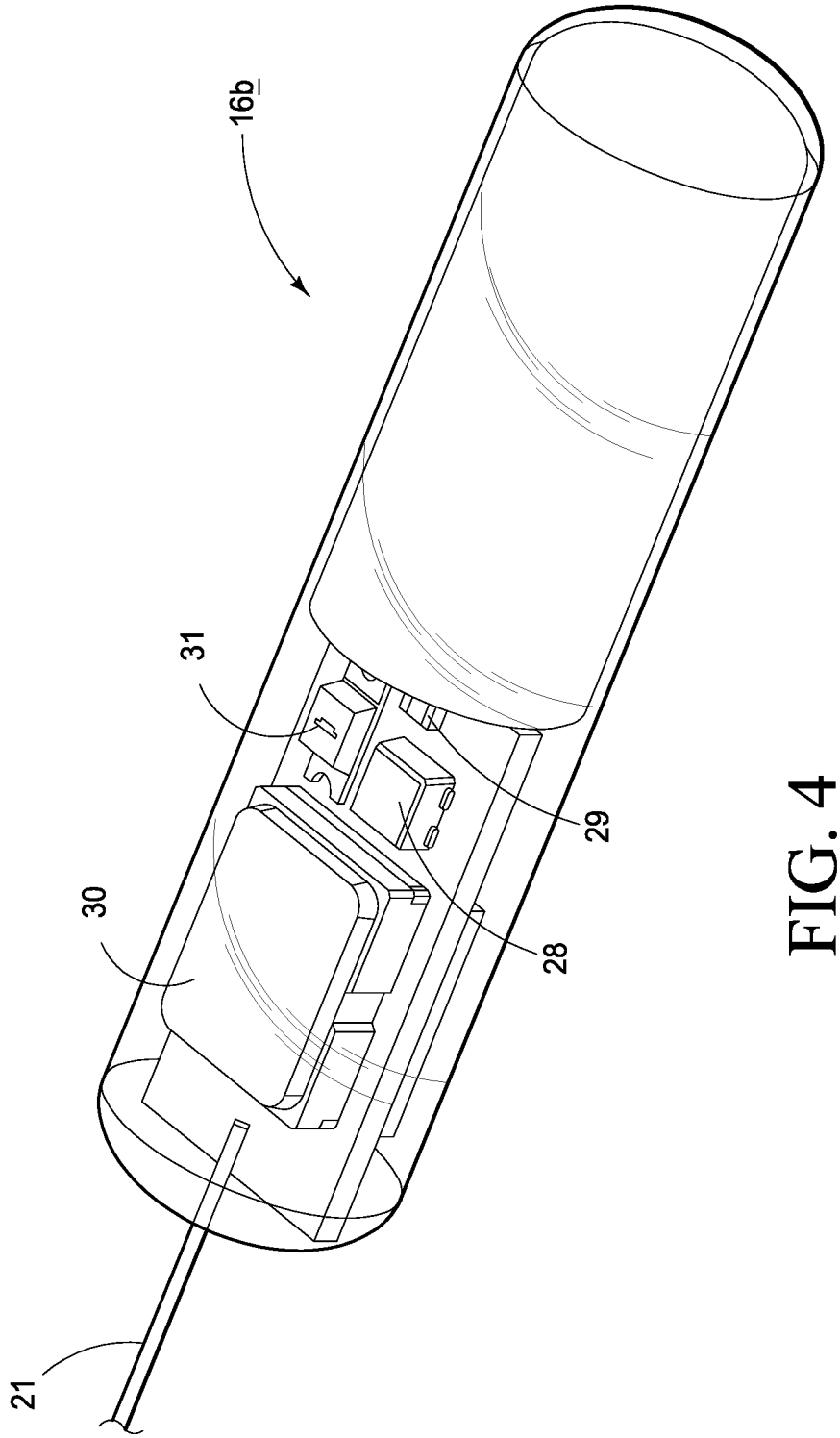


FIG. 4

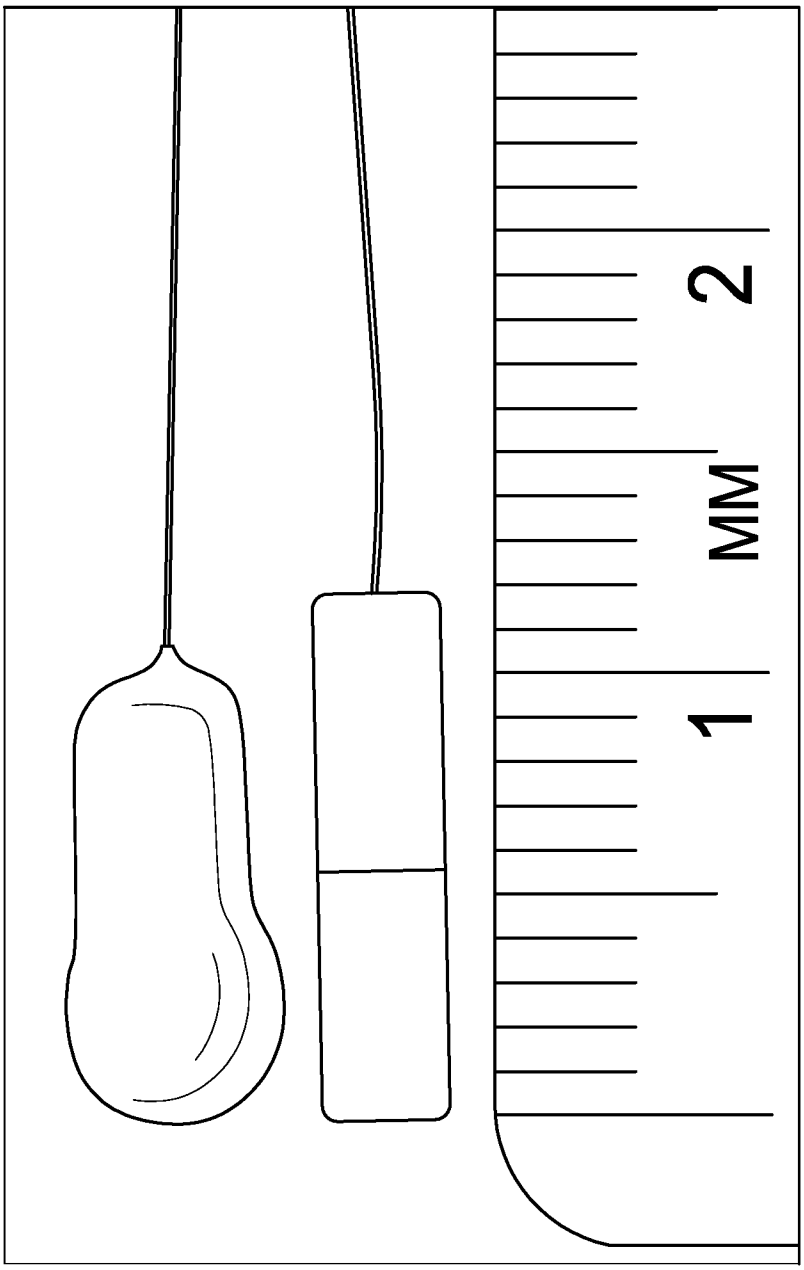


FIG. 5

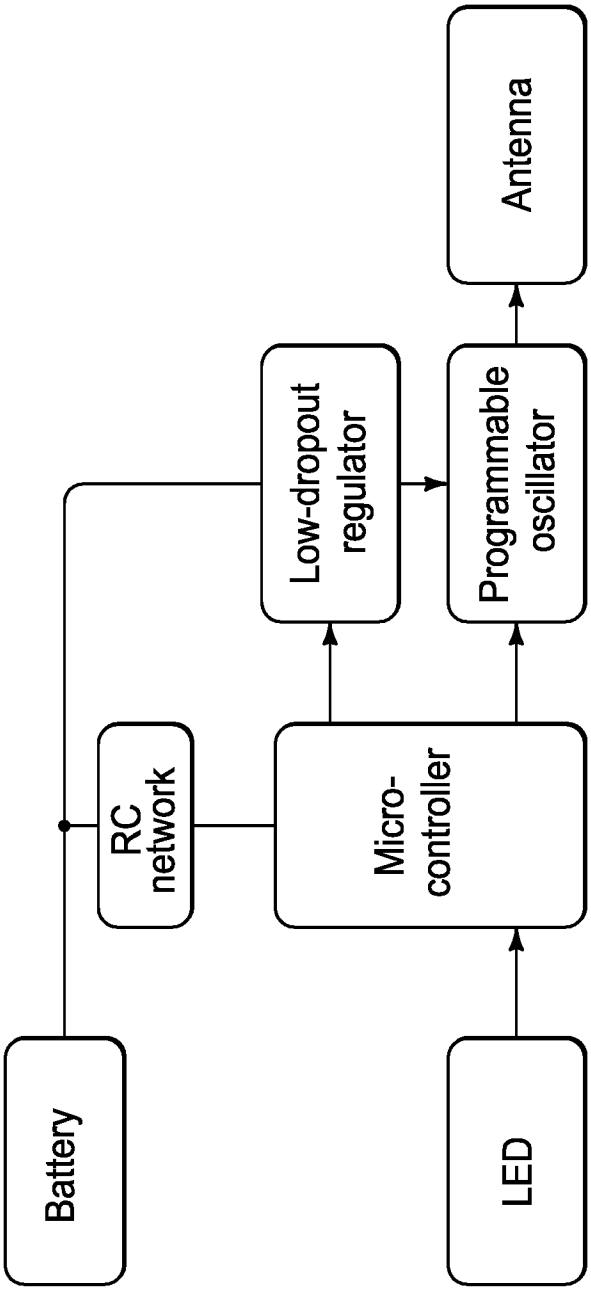

Antenna
Low-dropout regulator
Programmable oscillator
RC network
Micro-controller
Battery
LED

FIG. 6

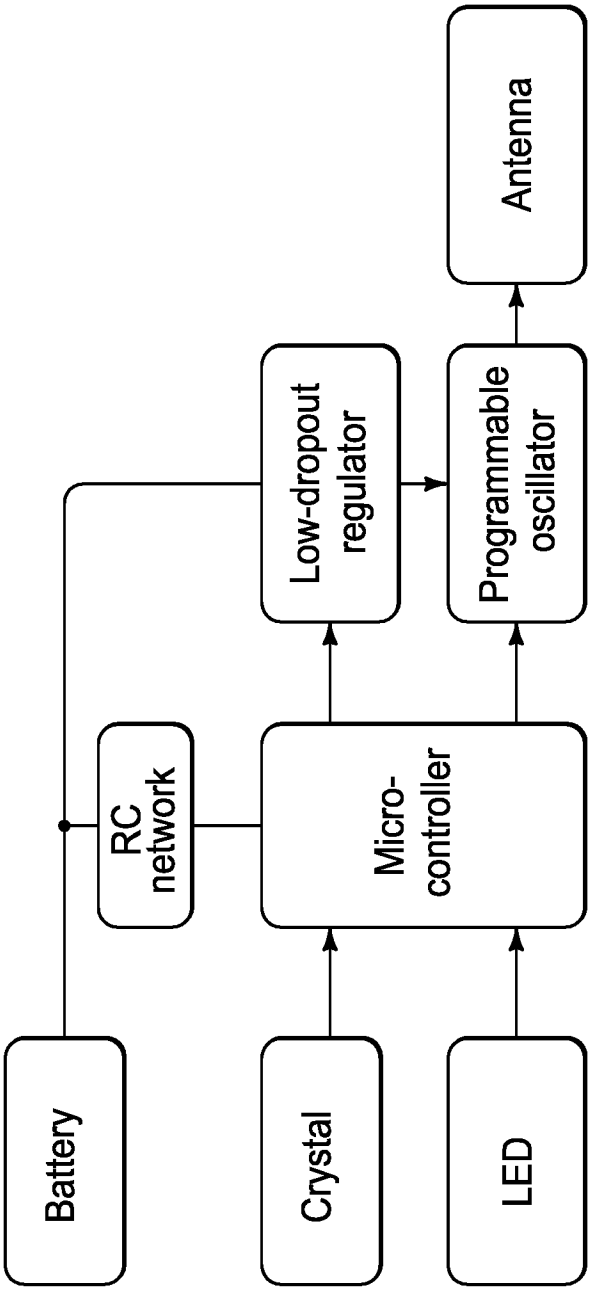

Battery
Crystal
LED
RC network
Micro-controller
Low-dropout regulator
Programmable oscillator
Antenna

FIG. 7

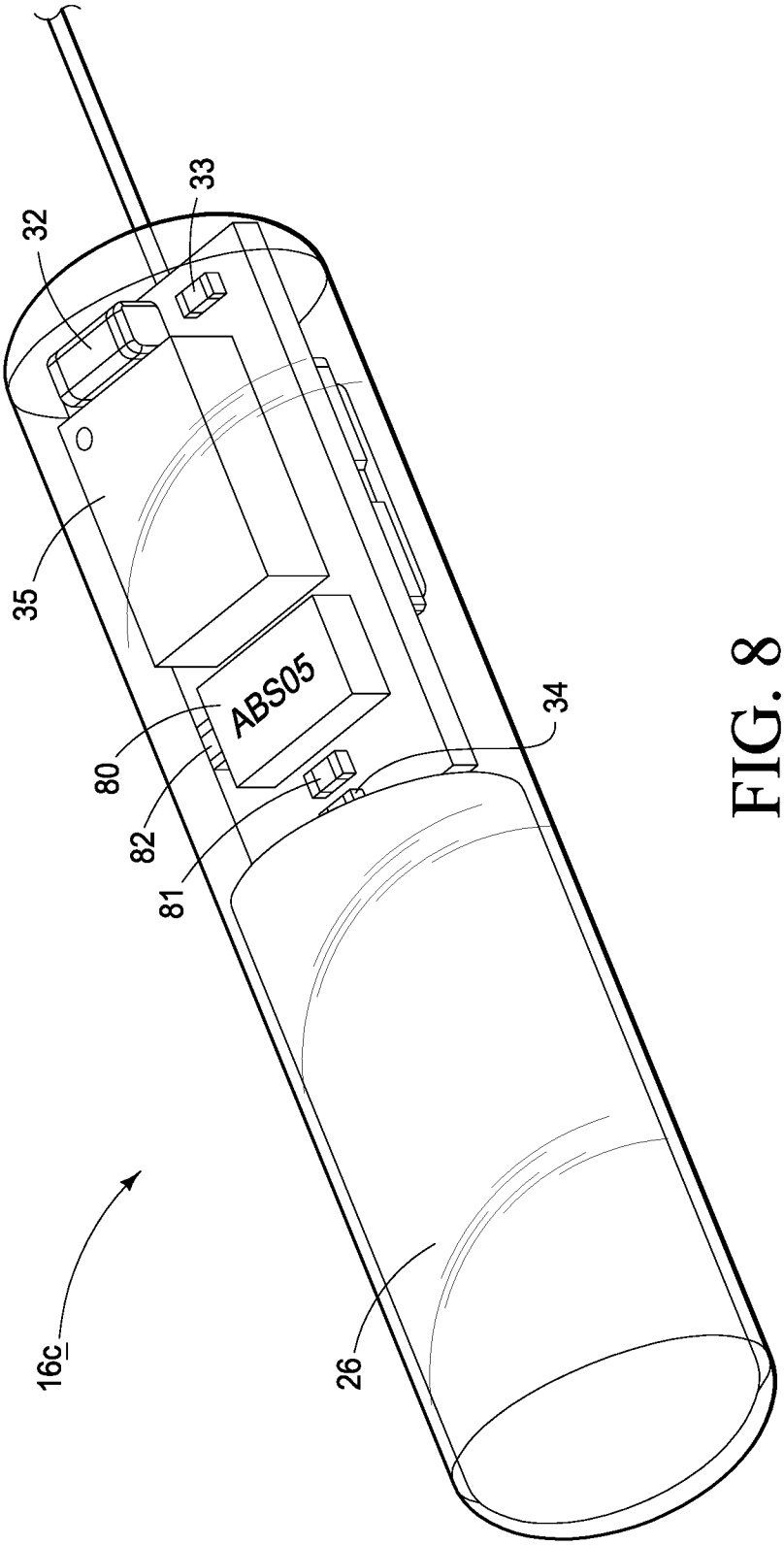


FIG. 8

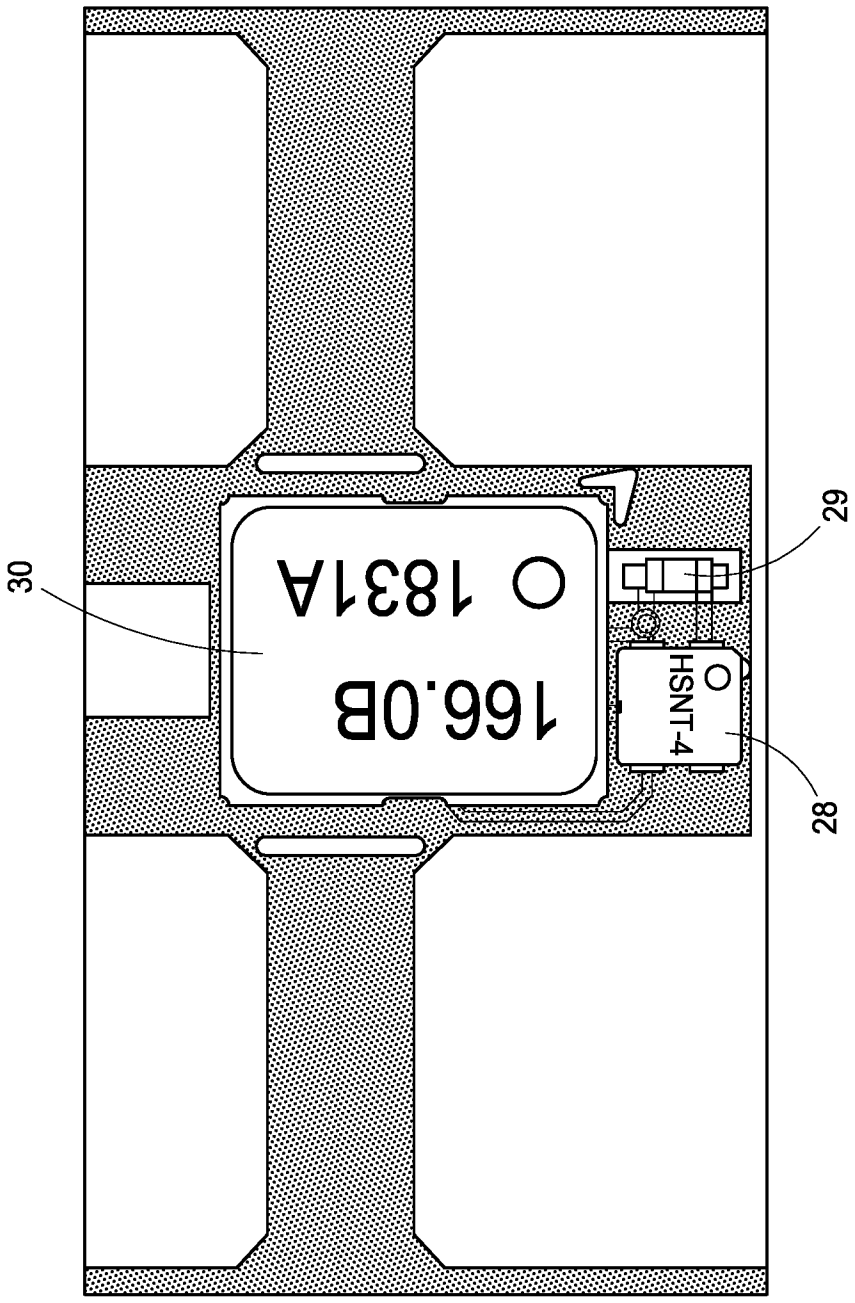


FIG. 9

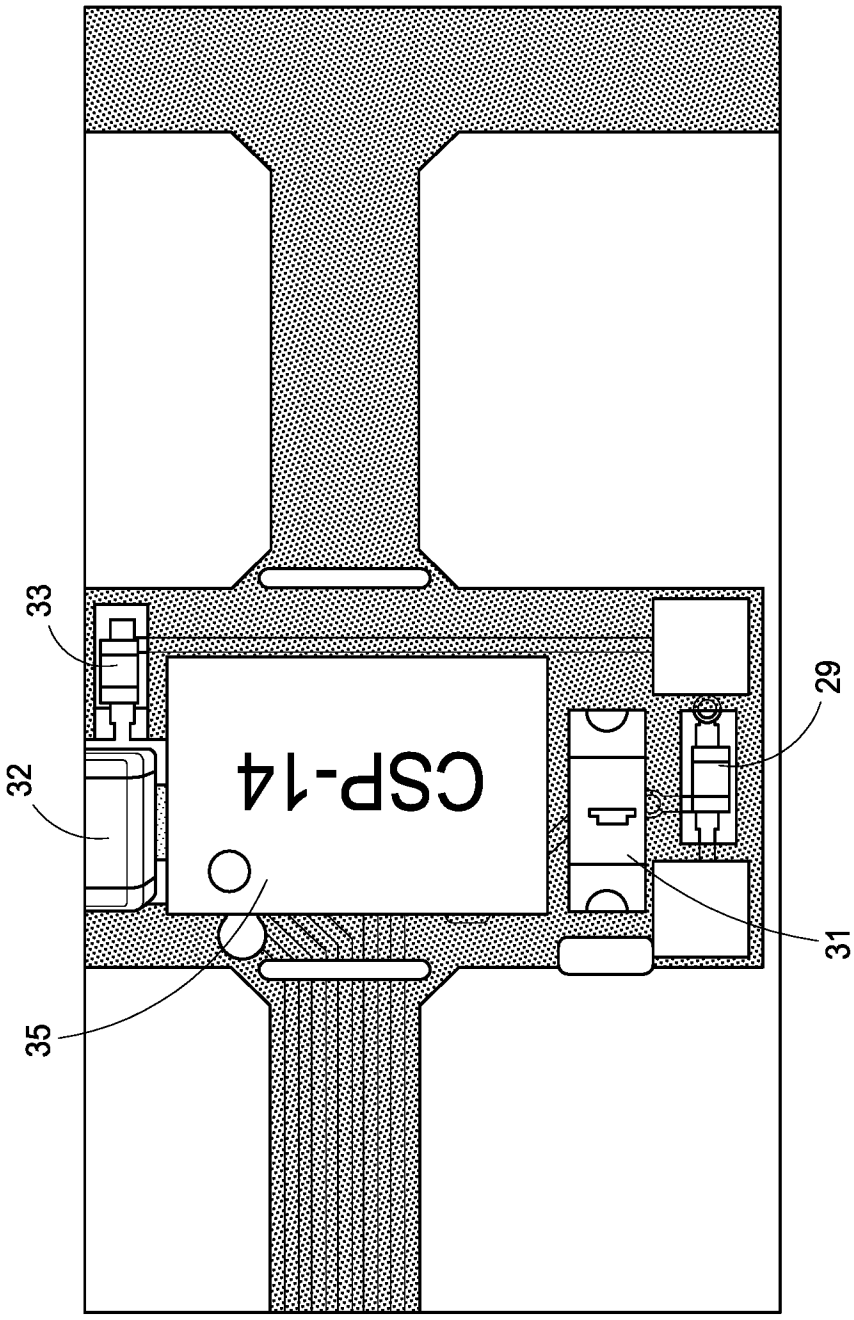


FIG. 10

Description	Designator	Manufacturer
CAP CER 10UF 10V 20% X5R 0402	C10	Samsung Electro-Mechanics America, Inc
CAP CER 0.1UF 6.3V 20% X5R 01005	C11, C12	Samsung Electro-Mechanics America, Inc
LED BLUE RECTANGLE SMD 0402	D10	Panasonic Electronic Components
CONN HEADER 6POS PIC PRGM INTF	J10	MOLEX
RES SMD 33K OHM 5% 1/32W 01005	R10	Panasonic Electronic Components
MCU 8BIT 8K FLASH 14CSP	U10	Microchip Technology
Seiko Instruments 200MA VOLT REG ULTRA LOW IQ	U11	Seiko Semiconductors
OSC CMOS PROG 1.8V OE SMD	Y10	EPSON

Manufacturer Part Number	Quantity	VALUE	
CL05A106MP5NUNC	1	10µF	10 µF capacitor
CL02A104MQ2NNNC	2	0.1µF	0.1 µF capacitor
LNJ947W8CRA	1		blue LED
22-05-2061	1		programming header
ERJ-XGNJ333Y	1	33k	33K ohm resistor
PIC16F1823T/CL	1		microcenter
S-1313D18-A4T1U3	1		voltage regulator
SG-8003CG-PEB	1		oscillator

FIG. 11

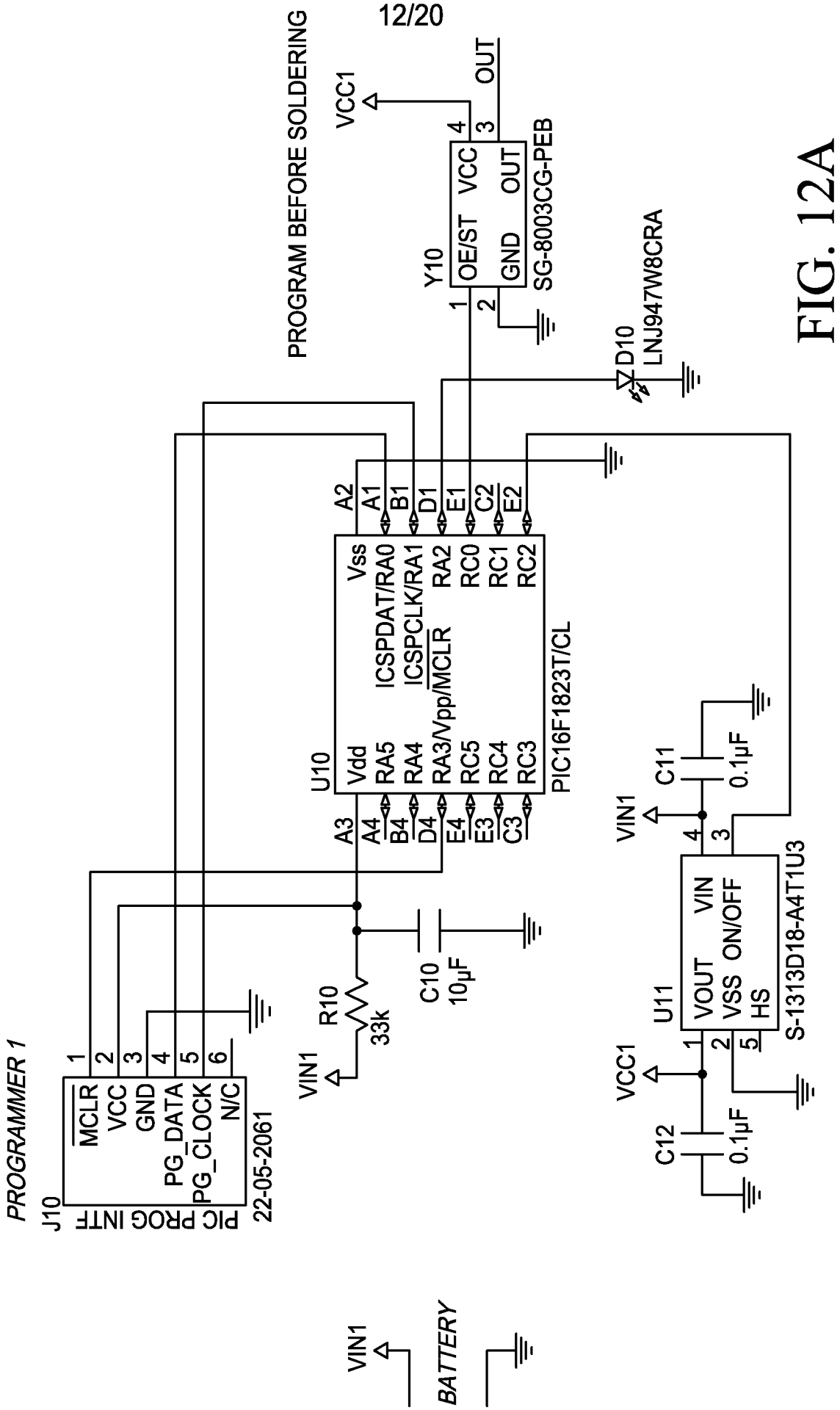

PROGRAM BEFORE SOLDERING
VCC1
OUT
Y10
OE/ST
VCC
GND
OUT
SG-8003CG-PEB
D10
LNJ947W8CRA
U10
Vdd
RA5
RA4
RA3/Vpp/MCLR
RC5
RC4
RC3
Vss
ICSPDAT/RA0
ICSPCLK/RA1
RA2
RC0
RC1
RC2
PIC16F1823T/CL
A2
A1
B1
D1
E1
C2
E2
A3
A4
B4
D4
E4
E3
C3
PROGRAMMER 1
J10
PIC PROG INTF
MCLR
VCC
GND
PG_DATA
PG_CLOCK
N/C
22-05-2061
VIN1
R10
33k
C10
10µF
U11
VOUT
VSS
VIN
ON/OFF
HS
S-1313D18-A4T1U3
C11
0.1µF
C12
0.1µF
VCC1
VIN1
BATTERY

FIG. 12A

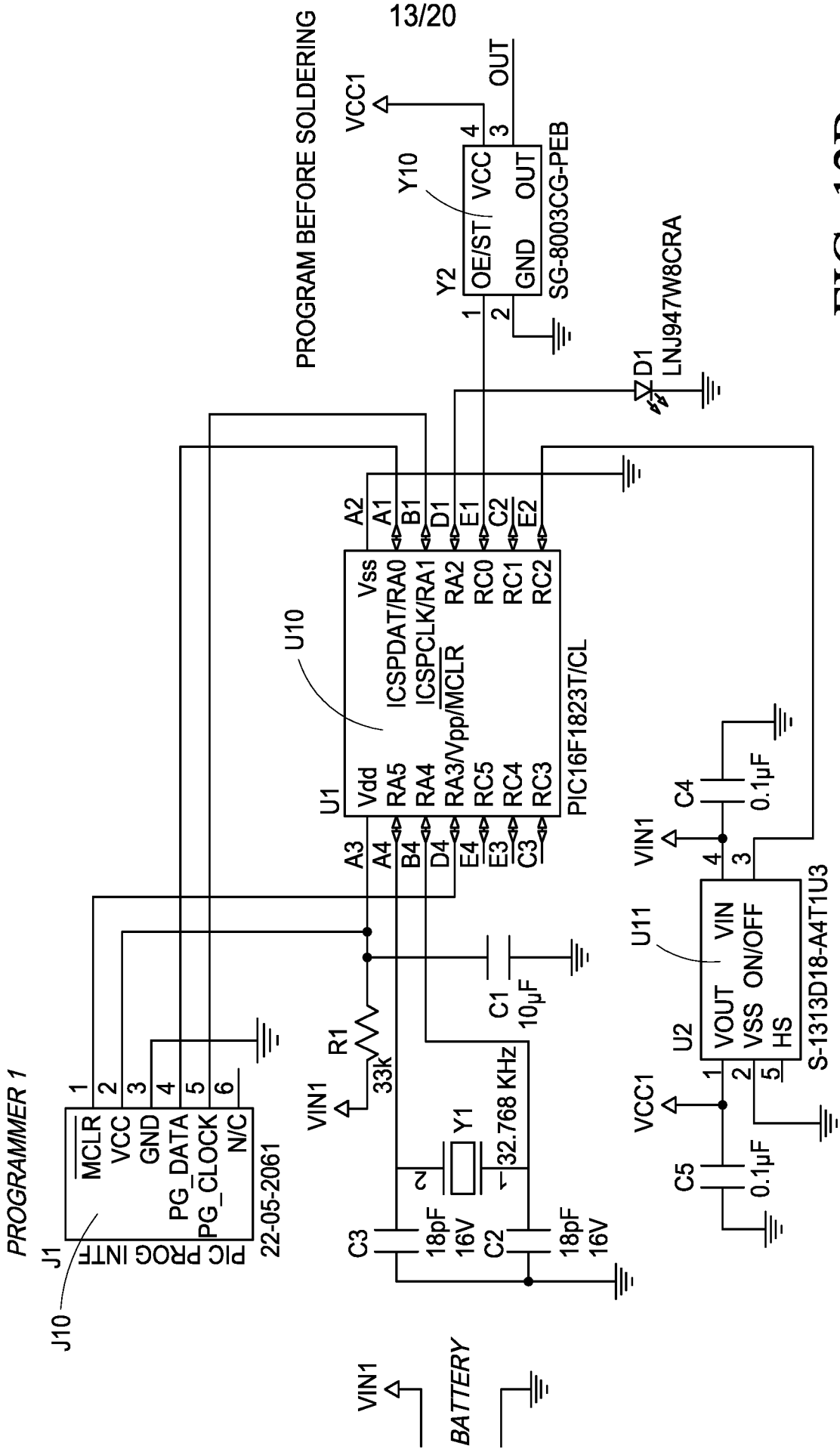

PROGRAM BEFORE SOLDERING
VCC1
Y10
OUT
VCC
OE/ST
GND
OUT
Y2
SG-8003CG-PEB
D1
LNJ947W8CRA
U10
U1
Vss
ICSPDAT/RA0
ICSPCLK/RA1
RA3/Vpp/MCLR
RA2
RC0
RC1
RC2
Vdd
RA5
RA4
RC5
RC4
RC3
PIC16F1823T/CL
A2
A1
B1
D1
E1
C2
E2
A3
A4
B4
D4
E4
E3
C3
PROGRAMMER 1
J1
J10
MCLR
VCC
GND
PG_DATA
PG_CLOCK
N/C
PIC PROG INTF
22-05-2061
R1
33k
VIN1
C1
10µF
Y1
32.768 KHz
C3
18pF
16V
C2
18pF
16V
U11
U2
VIN
ON/OFF
VOUT
VSS
HS
S-1313D18-A4T1U3
C4
0.1µF
C5
0.1µF
VIN1
VCC1
BATTERY

FIG. 12B

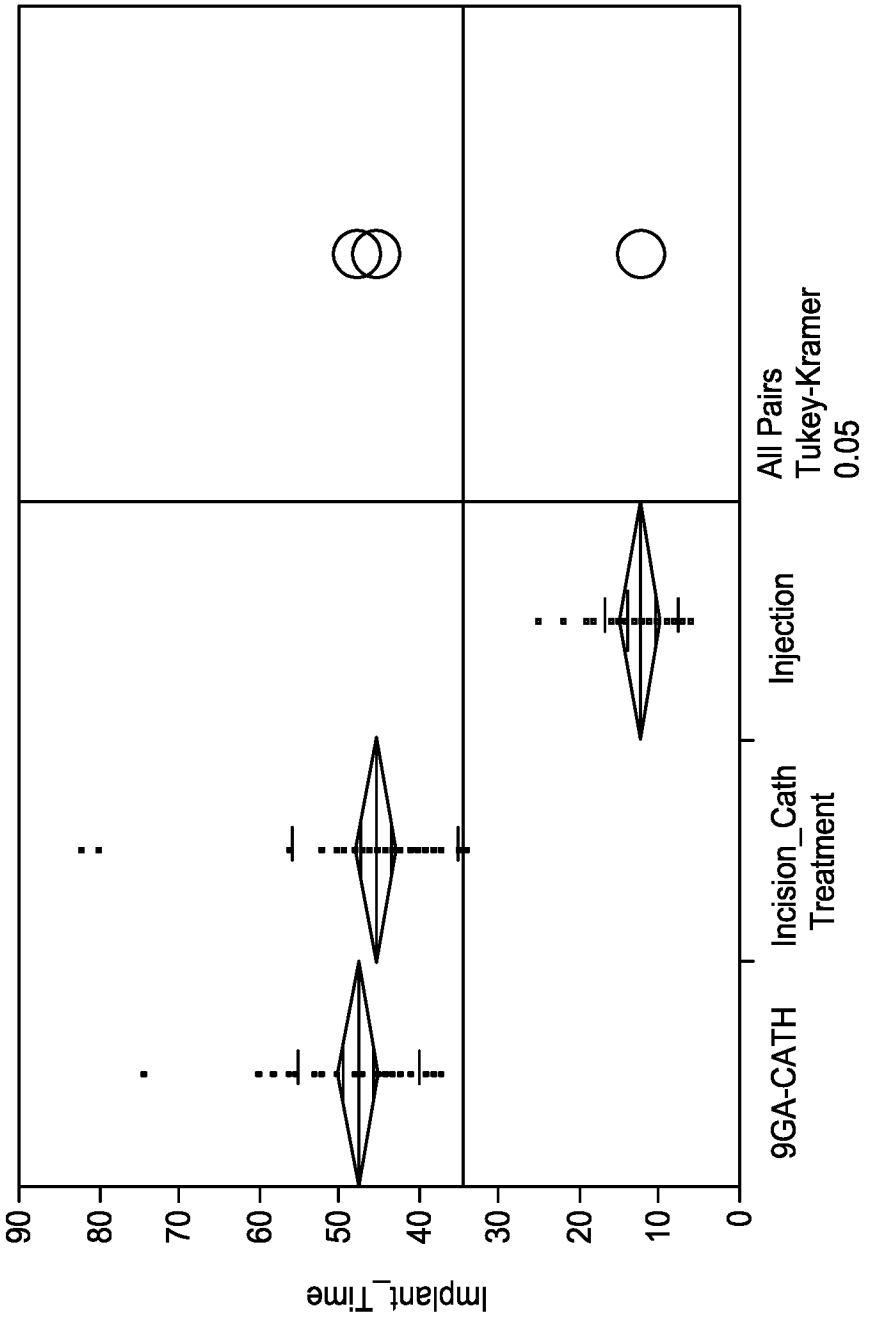

Implant_Time
90
80
70
60
50
40
30
20
10
0
9GA-CATH
Incision_Cath
Injection
Treatment
All Pairs
Tukey-Kramer
0.05

FIG. 13

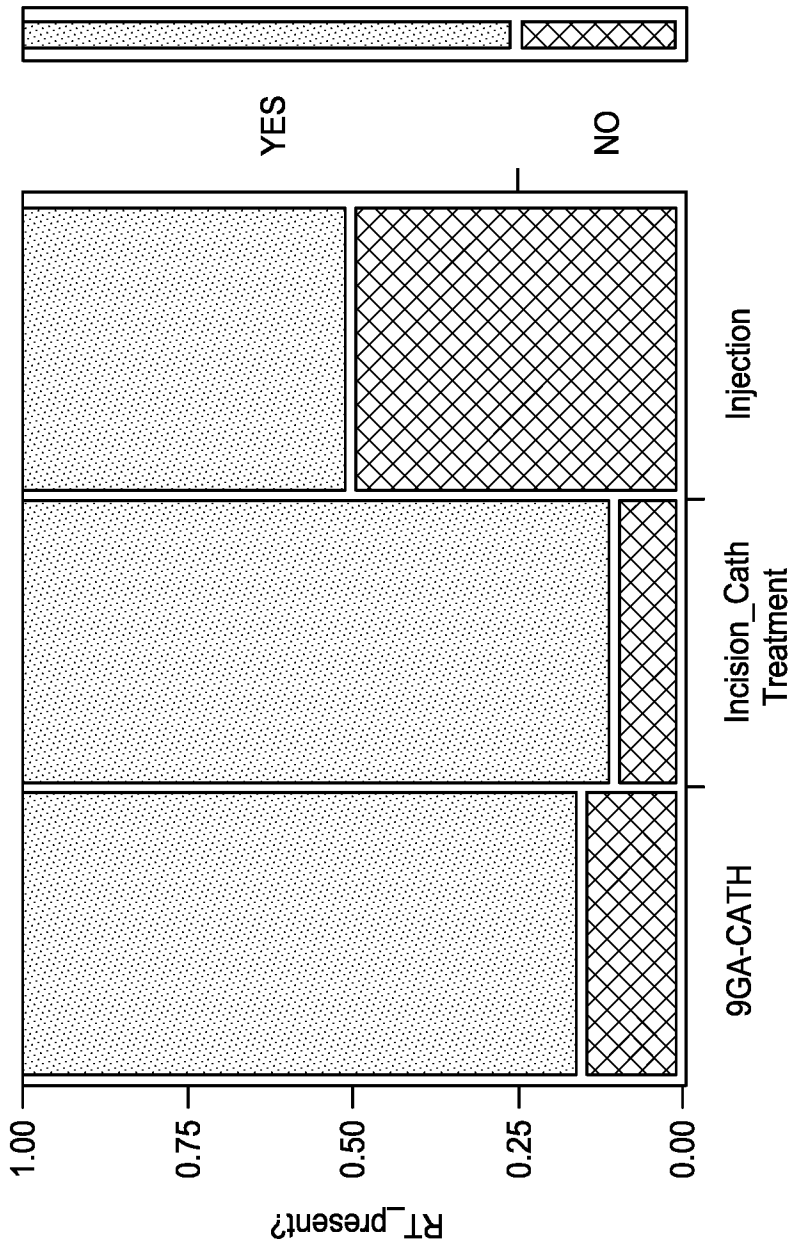

RT_present?
1.00
0.75
0.50
0.25
0.00
9GA-CATH
Incision_Cath Treatment
Injection
YES
NO

FIG. 14

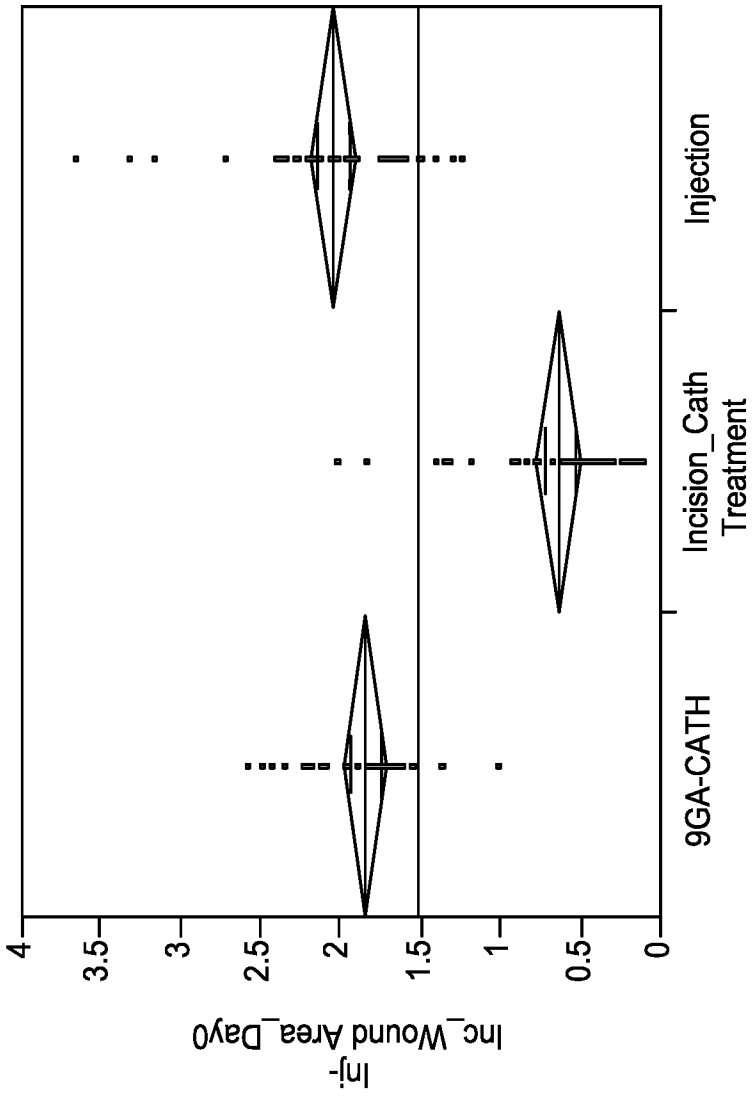

Inj-
Inc_Wound Area_Day0
4
3.5
3
2.5
2
1.5
1
0.5
0
9GA-CATH
Incision_Cath
Injection
Treatment

FIG. 15

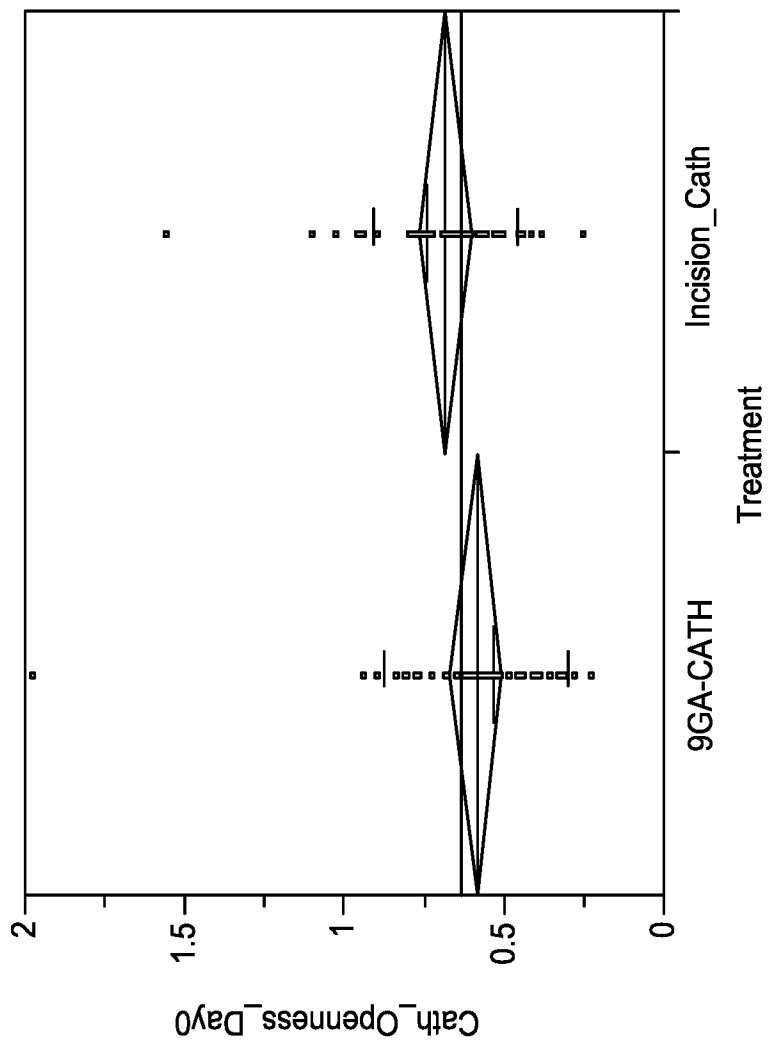

Cath_Openness_Day0
2
1.5
1
0.5
0
9GA-CATH
Incision_Cath
Treatment

FIG. 16

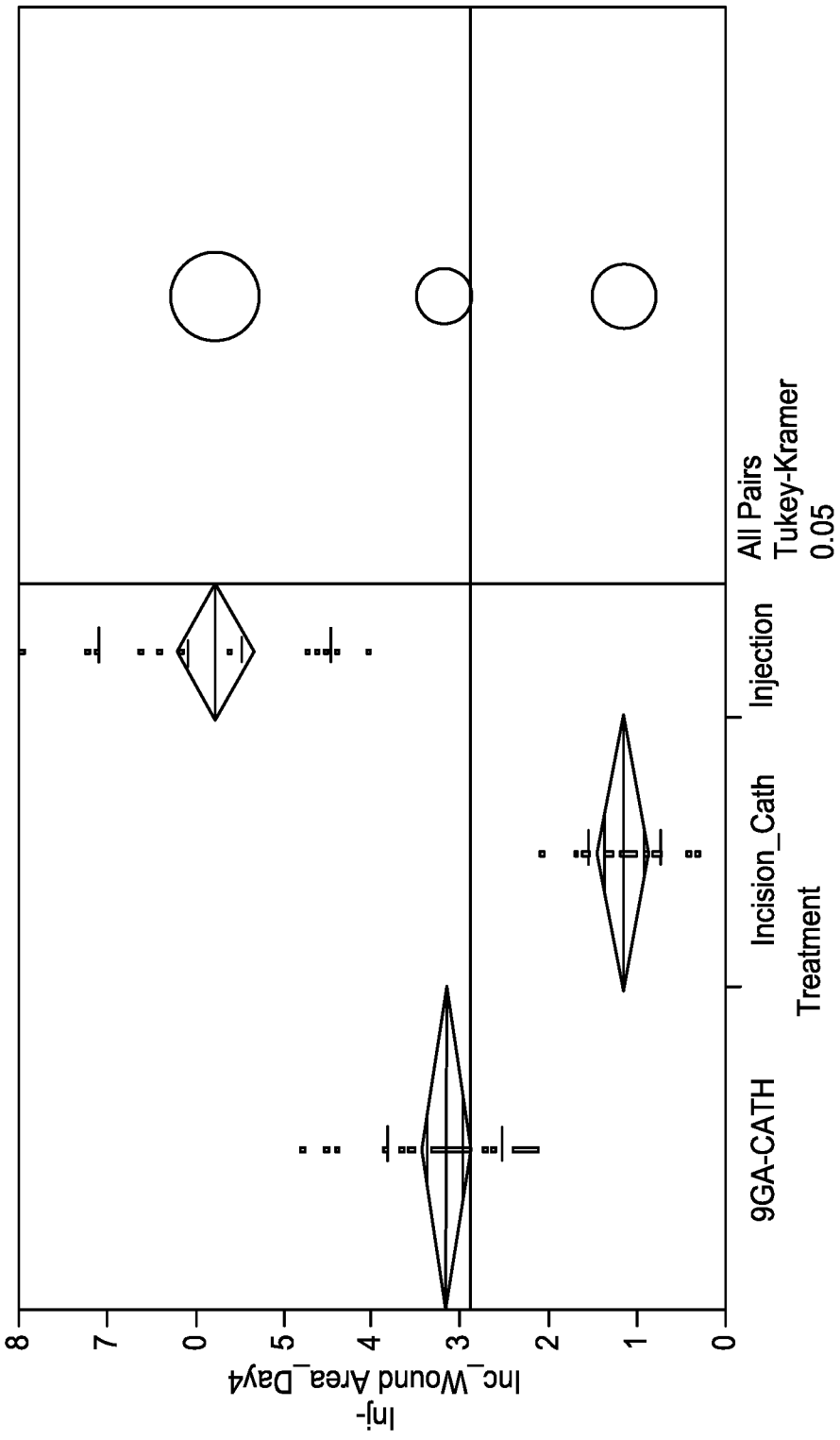

Inj-
Inc_Wound Area_Day4
8
7
0
5
4
3
2
1
0
9GA-CATH
Incision_Cath
Injection
Treatment
All Pairs
Tukey-Kramer
0.05

FIG. 17

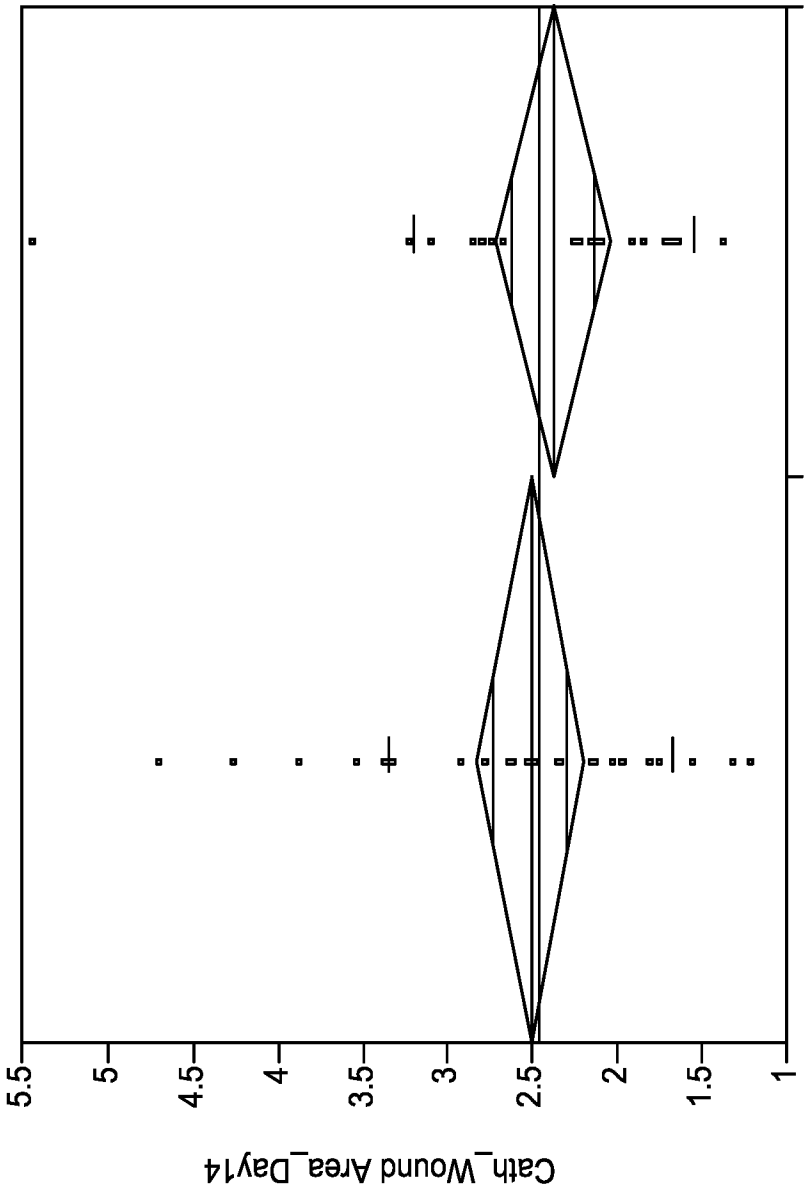

Cath_Wound Area_Day14
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
9GA-CATH
Incision_Cath
Treatment

FIG. 18

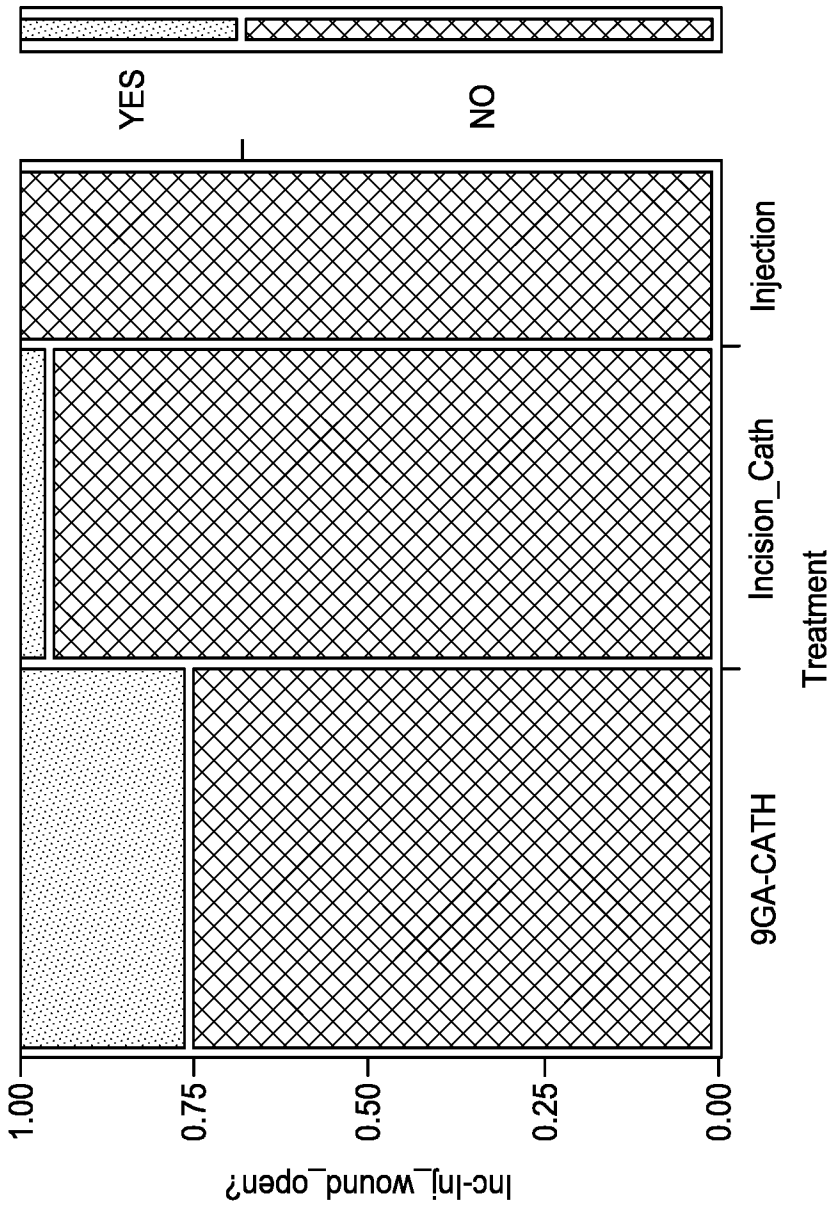

Inc-Inj_Wound_Open?
1.00
0.75
0.50
0.25
0.00
9GA-CATH
Incision_Cath
Injection
Treatment
YES
NO

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055045

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K99/00 A01K11/00 A01K61/90 G01S1/02
ADD. A01K35/00

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)
G06K A61B A01K G01S

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2007/103314 A1 (GEISSLER RANDOLPH K [US]) 10 May 2007 (2007-05-10) abstract paragraph [0040] paragraphs [0121] - [0127] -----	1,2,4-7 3,8-14
X	GB 2 188 028 A (BRIEN HUGH O) 23 September 1987 (1987-09-23) the whole document -----	1,4-7
X	EP 2 037 396 A1 (DATAMARS SA [CH]) 18 March 2009 (2009-03-18) paragraphs [0002] - [0003] -----	1,4-7
Y	US 2015/063072 A1 (DENG Z DANIEL [US] ET AL) 5 March 2015 (2015-03-05) abstract paragraph [0020] -----	3
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 2017	07/02/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Loveniers, Kris

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055045

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 697 384 A (MIYAWAKI YUTAKA [JP] ET AL) 16 December 1997 (1997-12-16) column 4, line 10 - column 7, line 52; figures 8B, 9,11	8-14
A	WO 2011/079338 A2 (SMAXTEC ANIMAL CARE SALES GMBH [AT]; ROSENKRANZ STEFAN [AT]; FALLAST M) 7 July 2011 (2011-07-07) page 7, line 3 - page 9, line 13	8-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/055045

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. [X] Claims Nos.: 15-20
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 15-20 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) PCT, because their subject-matter relates to a method for treatment of the human body by surgery.

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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/055045

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