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- (54) **Title:** DEVICE AND METHOD FOR GROWING A BIOFILM AND MEASURING AN ELECTRICAL PROPERTY THERE-OF

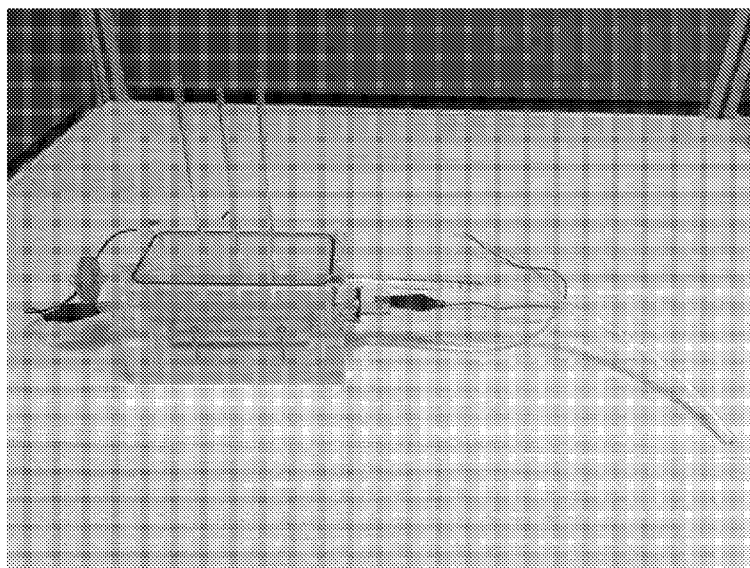


FIGURE 3B

- (57) **Abstract:** The invention relates generally to a device and a method for monitoring the growth of a biofilm and measuring a property of the biofilm, and in particular, to an integrated device wherein the growth and measurement of the conduction of the biofilm are performed in the same integrated device. The integrated device comprises a flow cell biofilm growth chamber entirely housed within an electrochemical impedance spectroscopy (EIS) chamber. The flow cell biofilm growth chamber comprises a working-electrode-supported substrate and the EIS chamber comprises a counter-electrode-supported substrate, wherein the two substrates are adapted for electrical connection to an external impedance measurement apparatus.



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DEVICE AND METHOD FOR GROWING A BIOFILM AND MEASURING AN ELECTRICAL PROPERTY THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[001] This application claims the benefit of priority of Singapore Patent Application No. 10201505981X, filed July 30, 2015, the contents of which being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[002] The invention relates generally to a device and a method for monitoring the growth of a biofilm and measuring a property of the biofilm, and in particular, to an integrated device wherein the growth and measurement of a property of the biofilm are performed in the same integrated device. Preferably, an electrochemical impedance spectroscopy (EIS) is carried out to measure the conduction of the biofilm.

BACKGROUND

[003] A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Biofilms grown on solid surfaces have increasingly gained more attention in recent years because of its wide range of applications, such as industrial, maritime, medical and environment.

[004] In order to investigate the structure of a biofilm in relation to its electrical properties, it is important to be able to implement a practical work flow growing the biofilm and analyzing it. The need to grow biofilms for microscopic analysis has previously been addressed via amongst others Danish Technical University (DTU) in flow cells from a biological perspective.

[005] Electrochemical impedance spectroscopy (EIS) is a common tool to measure conduction in materials under controlled conditions, i.e. in an atmosphere where the

surroundings of the sample, such as gas, temperature or pressure, is known and can be altered and controlled.

[006] The immediate problem with the commercially available biofilm growth flow cells is that they are difficult to seal, introducing air bubbles in the growth chambers, causing leakage or contamination. In addition, the flow cell itself is primarily suited for microscopy analysis and hence common analytical techniques within materials science (e.g. X-ray Diffraction (XRD), Infrared spectroscopy (IR), Raman spectroscopy, thermal gravimetric analysis (TGA), Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy (SEM/EDX), Transmission Electron Microscopy (TEM), etc.) cannot be readily used for characterizing the biofilm.

[007] Other ways of growing biofilm involves doing so in tubing, where the problem of removing the biofilm from the growth capillary is solved by squeezing it out, disturbing the biofilm structure.

[008] Biofilms are moreover highly sensitive to foreign substances possibly causing contamination and hence the practical set-up of a conventional EIS experiment, introducing amongst others wires and clamps to the biofilm which are not easily sterilized, is not convenient.

[009] Carrying out work in a glove box is one way around contamination, while fine tuning of practical issues become complicated.

[0010] In addition, the EIS measurement needs to be carried out through the biofilm (i.e. across its thickness), and not along the length of the biofilm as is commonly practiced currently, since the biofilm will operate as a membrane or electrolyte in a future electrical device.

[0011] Therefore, there remains a need to provide for a device and a method that overcome, or at least alleviate, the above problems.

SUMMARY

[0012] According to a first aspect of the invention, there is disclosed a device for growing a biofilm and measuring an electrical property of the biofilm. The device may include an electrochemical impedance spectroscopy (EIS) chamber. The device may further include a flow cell biofilm growth chamber. The flow cell biofilm growth chamber is entirely housed within the EIS chamber.

[0013] The flow cell biofilm growth chamber may include an inlet for introducing a flow of nutrient into the flow cell biofilm growth chamber. The flow cell biofilm growth chamber may further include an outlet for purging a flow of nutrient out of the flow cell biofilm growth chamber. The flow cell biofilm growth chamber may also include a working-electrode-supported substrate located in a flow channel of the flow cell biofilm growth chamber. The working-electrode-supported substrate is adapted for the biofilm growth thereon.

[0014] The EIS chamber may include an inlet for introducing a flow of inert gas into the EIS chamber. The EIS chamber may further include an outlet for purging a flow of inert gas out of the EIS chamber. The EIS chamber may also include a counter-electrode-supported substrate, wherein the counter-electrode-supported substrate and the working-electrode-supported substrate are adapted for electrical connection to an external impedance measurement apparatus.

[0015] In various embodiments, the EIS chamber may further include one or more openings for insertion of one or more sensors for sensing the environment condition in the EIS chamber.

[0016] In various embodiments, the EIS chamber may further include one or more sensors inserted into the one or more openings. In preferred embodiments, the one or more sensors may include a pH, temperature, pressure, humidity or gas sensor.

[0017] In various embodiments, the flow cell biofilm growth chamber may further include one or more openings for insertion of one or more sensors for sensing the environment condition in the flow cell biofilm growth chamber.

[0018] In various embodiments, the flow cell biofilm growth chamber may further include one or more sensors inserted into the one or more openings. In preferred embodiments, the one or more sensors may include a pH, temperature, pressure, humidity or gas sensor.

[0019] Preferably, the one or more openings of the EIS chamber may correspond respectively to the one or more openings of the flow cell biofilm growth chamber.

[0020] In various embodiments, the working-electrode-supported substrate may include a material selected from the group consisting of a carbon-supported microscope slide, a silver-supported microscope slide, a gold-supported microscope slide, a fluorinated titanium oxide (FTO) glass, a magnesium, alumina, titanium, copper, silver or gold foil, mesh, tape, plaster, or grid, a paper or plastic printed with carbon, magnesium, titanium, copper, gold, silver or alumina electrode, and a glass microscope cover slip.

[0021] In various embodiments, the counter-electrode-supported substrate may include a material selected from a carbon-supported microscope slide, a silver-supported microscope slide, a gold-supported microscope slide, a fluorinated titanium oxide (FTO) glass, a magnesium, alumina, titanium, copper, silver or gold foil, mesh, tape, plaster, or grid, a paper or plastic printed with carbon, magnesium, titanium, copper, gold, silver or alumina electrode, and a glass microscope cover slip.

[0022] According to a second aspect of the invention, there is provided a method of growing a biofilm and measuring an electrical property of the biofilm. The method may include providing a device of the first aspect mentioned above. The method may further include introducing a flow of nutrient into the flow cell biofilm growth chamber. The

method may also include allowing sufficient time for the growth of a biofilm on the working-electrode-supported substrate located in a flow channel of the flow cell biofilm growth chamber. The method may additionally include electrically connecting the counter-electrode-supported substrate and the working-electrode-supported substrate to an external impedance measurement apparatus to obtain an EIS reading.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The drawings are not necessarily drawn to scale, emphasis instead generally being placed upon illustrating the principles of various embodiments. In the following description, various embodiments of the invention are described with reference to the following drawings.

[0024] **Figure 1** shows some examples of substrates of various materials onto which biofilms are grown: fluorinated titanium oxide (FTO) glass (top left) can be cut to any desired size and sold in commercially available sheets; various metallic foils, meshes tapes, plasters and grids substrates (top right); printed carbon, silver and alumina electrodes on commercial paper and plastic substrates with biofilms directly grown on the electrode surfaces (mid right); sputtered carbon, silver and gold on microscope slides (bottom right); and microscope cover slips in various sizes (bottom left).

[0025] **Figure 2A-2L** show photographs of the EIS chamber and the flow cell biofilm growth chamber according to one embodiment.

[0026] **Figure 2A** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the short side of the device.

[0027] **Figure 2B** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the long side of the device.

[0028] **Figure 2C** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the top of the device.

[0029] **Figure 2D** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the short side of the device.

[0030] **Figure 2E** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the long side of the device.

[0031] **Figure 2F** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the top of the device.

[0032] **Figure 2G** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the short side of the device.

[0033] **Figure 2H** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the long side of the device.

[0034] **Figure 2I** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the top of the device.

[0035] **Figure 2J** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the short side of the device.

[0036] **Figure 2K** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the long side of the device.

[0037] **Figure 2L** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the top of the device.

[0038] **Figure 3A-3L** show photographs of the present device according to another embodiment.

[0039] **Figure 3A** shows a sealed device when viewed from the top of the device.

[0040] **Figure 3B** shows a sealed device when viewed from one long side of the device.

[0041] **Figure 3C** shows a sealed device when viewed from another long side of the device.

[0042] **Figure 3D** shows a sealed device when viewed from one short side of the device.

[0043] **Figure 3E** shows a sealed device when viewed from another short side of the device.

[0044] **Figure 3F** shows the disassembled device whereby the gas inlet and the gas outlet are removed.

[0045] **Figure 3G** shows the disassembled device whereby the working-electrode-supported substrate (including the associated wire clip) and the counter-electrode supported substrate (including the associated wire clip) are removed.

[0046] **Figure 3H** shows the disassembled device whereby the sensors and the nutrient inlet and outlet are removed.

[0047] **Figure 3I** shows the disassembled device whereby the lid of the EIS chamber is removed.

[0048] **Figure 3J** shows the disassembled device whereby the EIS slide adapters are removed.

[0049] **Figure 3K** shows the disassembled device whereby the flow cell biofilm growth chamber is removed.

[0050] **Figure 3L** shows the disassembled device whereby the lid of the flow cell biofilm growth chamber is removed.

[0051] **Figure 4A-4B** show composition and spatial organization of populations for *Pseudomonas aeruginosa* wildtype PAO1 and its over- (+FAP) and non-amyloid expressing (DFAP) mutants, communities of *P. protegens* Pf5 tagged with CFP (cyan), *P. aeruginosa* tagged with YFP (yellow) and *K. pneumoniae* KP1 tagged with DsRed (red) triple-, dual- and single species biofilms.

[0052] **Figure 4A** shows a diagram of the flow cell system and chamber (A: growth media, B: peristaltic pump, C: bubble traps, D: flow cell, E: effluent tubes, entering the waste-bottle). The middle section shows a schematic of a flow cell with three channels, tubes connected to both ends of each channel. The micrograph on the right shows a typical confocal laser scanning microscopy (CLSM) image of a biofilm.

[0053] **Figure 4B** shows the biofilm architecture of the *P. aeruginosa* +FAP strain in medium A shows layers of live and dead (green and red respectively, stained via conventional Syto9 kit) cells (x1000 magnification). The images provide unique 3D fingerprints of the cell composition. The middle images show the triple species biofilms *P. protegens*-CFP (blue), *P.aeruginosa*-YFP (yellow) and *K. pneumoniae*-DsRed (red) in medium A, representing species composition of the multispecies community.

[0054] **Figure 5A-5C** show EIS data collected in the EIS chamber of different embodiments of the present device. Nevertheless, it can be concluded that with the embodiment shown in **Figure 2A-2L**, valuable and consistent conductivity data (**Figure 5A**) were collected. This was confirmed with the embodiment shown in **Figure 3A-3L** at two different time points (24h, (**Figure 5B**) and 48h, (**Figure 5C**)).

DESCRIPTION

[0055] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the invention may be practised. These embodiments are described in sufficient detail to enable those skilled in the art to practise the invention. Other embodiments may be utilized and structural, logical, and electrical changes may be made without departing from the scope of the invention. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

[0056] According to a first aspect of the invention, there is disclosed a device for growing a biofilm and measuring an electrical property of the biofilm. The device may include an electrochemical impedance spectroscopy (EIS) chamber. The device may further include a flow cell biofilm growth chamber. The flow cell biofilm growth chamber is entirely housed within the EIS chamber.

[0057] In other words, the device of the present invention is an integrated device that enables the growth of a biofilm and the characterization of the thus-grown biofilm via EIS in a single device.

[0058] To protect the EIS chamber from contamination and influence from external environment, the EIS chamber may be covered or sealed (e.g. via screws) with a lid.

[0059] Similarly, to protect the flow cell biofilm growth chamber from contamination and influence from external environment, including the EIS chamber environment, the flow cell biofilm growth chamber may be covered or sealed (e.g. via screws) with a lid.

[0060] The flow cell biofilm growth chamber may include an inlet for introducing a flow of nutrient into the flow cell biofilm growth chamber. The flow cell biofilm growth chamber may further include an outlet for purging a flow of nutrient out of the flow cell

biofilm growth chamber. The flow cell biofilm growth chamber may also include a working-electrode-supported substrate located in a flow channel of the flow cell biofilm growth chamber. The working-electrode-supported substrate is adapted for the biofilm growth thereon.

[0061] The EIS chamber may include an inlet for introducing a flow of inert gas into the EIS chamber. The EIS chamber may further include an outlet for purging a flow of inert gas out of the EIS chamber. By providing a flow and circulation of inert gas into and out of the EIS chamber, the risk of contamination during the biofilm growth can be minimized. The risk of contamination may be further reduced by sealing tightly the EIS chamber via screws and O-rings, as will be illustrated in the drawings.

[0062] The EIS chamber may also include a counter-electrode-supported substrate, wherein the counter-electrode-supported substrate and the working-electrode-supported substrate are adapted for electrical connection to an external impedance measurement apparatus.

[0063] To accommodate the counter-electrode-supported substrate in the EIS chamber, EIS slide adapters may be provided inside the EIS chamber. A slid, corresponding to the dimension of the cross-section of the counter-electrode-supported substrate, may be provided on one side and an opposing side of the EIS chamber such that the EIS slide adapters and the respective slid on the two opposing sides of the EIS chamber are aligned to allow the insertion of the counter-electrode-supported substrate therethrough.

[0064] With the realization of the device of the present invention, direct growth of a biofilm on any substrate in the flow cell biofilm growth chamber is made possible and therefore the undisturbed biofilm structure can be analysed immediately in the EIS chamber. This is in contradistinction to commercially available techniques where biofilm is first grown in a flow cell reactor before transferring the grown biofilm to an

EIS apparatus for analysis, thereby incurring the risk of contamination during the transfer.

[0065] Furthermore, due to the small and compact volume of the device of the present invention, small amounts of gas are used during the growth and analysis processes, thereby decreasing the operating costs compared to carrying out the same processes in a large glove box.

[0066] In addition, there is no specialized equipment needed to scale up the biofilm growth and this technology can hence be readily integrated into a product line for larger volume production. The biofilms can be scaled up for large volume production by simply increasing the size of growth vessel. As the bacteria are alive, they will form homogeneous solution which can be integrated to the current battery production line when the electrolyte is applied to battery shell.

[0067] As mentioned above, a biofilm can be directly grown on any substrate located in a flow channel of the flow cell biofilm growth chamber. Commercially available systems for biofilm growth provide non-practical growth channels, besides for microscopic analysis. The device of the present invention allows a support or substrate of various materials to be placed in the growth chamber and taken out after the growth process is completed. On the other hand, existing known growth chambers are sealed shut at the beginning of the growth and it is not possible to open them after the growth is completed, thereby limiting the biofilm analysis to a great extent.

[0068] **Figure 1** shows some examples of substrates of various materials onto which biofilms are grown: fluorinated titanium oxide (FTO) glass (top left) can be cut to any desired size and sold in commercially available sheets; various metallic foils, meshes tapes, plasters and grids substrates (top right); printed carbon, silver and alumina electrodes on commercial paper and plastic substrates with biofilms directly grown on

the electrode surfaces (mid right); sputtered carbon, silver and gold on microscope slides (bottom right); and microscope cover slips in various sizes (bottom left).

[0069] **Figure 2A-2L** show photographs of the EIS chamber and the flow cell biofilm growth chamber according to one embodiment.

[0070] **Figure 2A** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the short side of the device.

[0071] **Figure 2B** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the long side of the device.

[0072] **Figure 2C** shows an arrangement (from left to right) of an EIS chamber lid, the EIS chamber, the growth chamber and the growth chamber lid viewed from the top of the device.

[0073] **Figure 2D** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the short side of the device.

[0074] **Figure 2E** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the long side of the device.

[0075] **Figure 2F** shows, from left to right, the respective individually assembled EIS chamber and the growth chamber both with their respective lids screwed when viewed from the top of the device.

[0076] **Figure 2G** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the short side of the device.

[0077] **Figure 2H** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the long side of the device.

[0078] **Figure 2I** shows, from left to right, the EIS chamber lid, the growth chamber inside the EIS chamber and the growth chamber lid when viewed from the top of the device.

[0079] **Figure 2J** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the short side of the device.

[0080] **Figure 2K** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the long side of the device.

[0081] **Figure 2L** shows the assembled growth chamber including its lid inside the EIS chamber including its lid when viewed from the top of the device.

[0082] **Figure 3A-3L** show photographs of the present device according to another embodiment, whereby the lid of the EIS chamber is provided with three openings for insertion of three sensors therethrough.

[0083] As mentioned in earlier paragraphs, sensors are added to the device of the present invention in order to carry out an even more controlled measurement in the growth chamber and the EIS chamber. As illustrated in the figures, the sensors are placed close to the inlet with regard to the nutrients, in the middle of the EIS chamber and growth chamber, and close to the outlet of the nutrient flow in order to fully comprehend the internal atmosphere in the device throughout the growth or analysis. There are two sensor holes in parallel to each other at the three positions, so that it is possible to get an average of the environmental conditions in the device and thereby securing the results further. For convenience, a standard diameter of the sensor holes common in today's commercially available pH, temperature and gas (H₂, O₂, N₂ etc.)

microsensors is chosen, in order to accommodate any or several environment uptakes to increase flexibility of use.

[0084] **Figure 3A** shows a sealed device when viewed from the top of the device.

[0085] **Figure 3B** shows a sealed device when viewed from one long side of the device.

[0086] **Figure 3C** shows a sealed device when viewed from another long side of the device.

[0087] **Figure 3D** shows a sealed device when viewed from one short side of the device.

[0088] **Figure 3E** shows a sealed device when viewed from another short side of the device.

[0089] **Figure 3F** shows the disassembled device whereby the gas inlet and the gas outlet are removed.

[0090] **Figure 3G** shows the disassembled device whereby the working-electrode-supported substrate (including the associated wire clip) and the counter-electrode supported substrate (including the associated wire clip) are removed.

[0091] **Figure 3H** shows the disassembled device whereby the sensors and the nutrient inlet and outlet are removed.

[0092] **Figure 3I** shows the disassembled device whereby the lid of the EIS chamber is removed.

[0093] **Figure 3J** shows the disassembled device whereby the EIS slide adapters are removed.

[0094] **Figure 3K** shows the disassembled device whereby the flow cell biofilm growth chamber is removed.

[0095] **Figure 3L** shows the disassembled device whereby the lid of the flow cell biofilm growth chamber is removed.

[0096] Based on the above-described device of the present invention, biofilm preparation and data collection for confocal laser scanning microscopy (CLSM) analysis are carried out in the flow cell biofilm growth chamber as follows.

[0097] The composition of biofilms on glass was determined with CLSM directly from the flow cell channels. A multi-track mode was used (CFP, YFP and DsRed λ_{ex} : 458, 514 and 561 nm and λ_{em} : 476, 527 and 584, respectively) to avoid crosstalk between different fluorescent channels.

[0098] For each sample, an average of five images were collected around the center channel positions, spaced approximately 1×10^{-3} m apart over approximate areas of $1.6 \times 10^5 \mu\text{m}^2$, above the required level of representative data. Image analysis was carried out on 10 biological replicates with the Imaris software (Bitplane).

[0099] **Figure 4A-4B** show composition and spatial organization of populations for *Pseudomonas aeruginosa* wildtype PAO1 and its over- (+FAP) and non-amyloid expressing (DFAP) mutants, communities of *P. protegens* Pf5 tagged with CFP (cyan), *P. aeruginosa* tagged with YFP (yellow) and *K. pneumoniae* KP1 tagged with DsRed (red) triple-, dual- and single species biofilms.

[00100] **Figure 4A** shows a diagram of the flow cell system and chamber (A: growth media, B: peristaltic pump, C: bubble traps, D: flow cell, E: effluent tubes, entering the waste-bottle). The middle section shows a schematic of a flow cell with three channels, tubes connected to both ends of each channel. The micrograph on the right shows a typical confocal laser scanning microscopy (CLSM) image of a biofilm.

[00101] **Figure 4B** shows the biofilm architecture of the *P. aeruginosa* +FAP strain in medium A shows layers of live and dead (green and red respectively, stained via conventional Syto9 kit) cells (x1000 magnification). The images provide unique 3D fingerprints of the cell composition. The middle images show the triple species biofilms

P. protegens-CFP (blue), *P. aeruginosa*-YFP (yellow) and *K. pneumoniae*-DsRed (red) in medium A, representing species composition of the multispecies community.

[00102] **Figures 4A-4B** are results of the CLSM analysis described for the invention in [0065]-[0067]. The experimental description on the growth process using the growth chamber of the present device is as follows:

Biofilm generation. *Pseudomonas protegens* Pf5 and *Pseudomonas aeruginosa* POA1 from the American Type Culture Collection (ATCC) BAA-477 and BAA-47 respectively and environmental isolate *Klebsiella pneumoniae* KP1 were mixed by inoculating a single colony of bacteria species into minimal medium A (M9): (M9: 48×10^{-3} M Na_2HPO_4 ; 9×10^{-3} M NaCl ; 19×10^{-3} M NH_4Cl ; 2×10^{-3} M MgSO_4 ; 0.1×10^{-3} M CaCl_2 ; 0.4% w/v $\text{C}_6\text{H}_{12}\text{O}_6$), or medium B (M9; 0.04% w/v $\text{C}_6\text{H}_{12}\text{O}_6$; 0.2% w/v casamino acids). Mixed species were formed in ratio *P. protegens*: *P. aeruginosa*: *K. pneumoniae*, 5:5:1 to account for *K. pneumoniae*'s faster growth rate. Over night cultures were shaken (24 h, 200 rpm, 25°C) and growth was determined via optical density (UV-spectrophotometer) over a 12 h period, to normalize the colony forming units (CFU) prior to inoculation. Cultures were diluted (1×10^8 cfu ml^{-1}), stained with 4', 6-diamidino-2-phenylindole (DAPI) (Yu, W. et al. *Optimal staining and sample storage time for direct microscopic enumeration of total and active bacteria in soil with two fluorescent dyes. Appl. Environ. Microbiol.* 61, 3367-3372 (1995)), detected with CLSM and analysed with standard image quantification software (Imaris (Bitplane AG, Belfast, UK)). The biofilms were cultured in Media A and B under continuous flow conditions in three-channel flow cells (Sternberg, C. et al. *Growing and analyzing biofilms in flow cells. Curr. Protoc. Microbiol. Chapter 1, Unit 1B.2* (2006)) or directly in tubing (9×10^{-3} l h^{-1}). The channels were injected with the bacterial cultures and approximately 10 biological replicates were used for experimental characterization.

[00103] Further, biofilm preparation and data collection with EIS analysis were carried out in the EIS chamber of the present device.

[00104] The conduction features were investigated with EIS, carried out over a frequency sweep (from 1MHz to 0.1 Hz) with an Autolab frequency response analyser using standalone mode and on a VSP five channel potentiostat (Bio-Logic, France). The sine wave amplitude was 25 mV and to ensure good ohmic contact and non-invasive handling, the biofilm samples were directly attached to needle electrodes in the tubing. The experiments were carried out at room temperature under a flow of air after initial dwelling (30 min) to enable the electronic component to be identified. For each sample, an average of five runs were collected on eight biological replicates with standard software (ECLab, Bio-Logic, France; Nova, Metrohm Autolab, The Netherlands; ZView, Scribner Associates Inc., USA). Constant phase elements in series with resistances in parallel were used to fit the time constants in an empirical element model.

[00105] **Figure 5A-5C** show EIS data collected in the EIS chamber of different embodiments of the present device. Nevertheless, it can be concluded that with the embodiment shown in **Figure 2A-2L**, valuable and consistent conductivity data (**Figure 5A**) were collected. This was confirmed with the embodiment shown in **Figure 3A-3L** at two different time points (24h, (**Figure 5B**) and 48h, (**Figure 5C**)).

[00106] While the above-described device is directed to an integrated device comprising an EIS chamber and a flow cell biofilm growth chamber, the same teaching may be easily extended to other types of electronic analyses of the biofilms.

[00107] Table 1 below shows techniques which are suitable for use in the measurement of a property or analysis of the composition of the biofilm after the growth has completed.

Techniques	General usage materials science		
	Elemental composition	Structure	Electric properties
Infrared Spectroscopy (IR) ql	x	x	
Thermogravimetric Analysis (TGA) qn	x	x	
Mass Spectroscopy (MS) qn	x		
X-ray Diffraction (XRD) ql	x	x	
Scanning Electron Microscopy (SEM) ql		x	x
Energy Dispersive X-ray Spectroscopy (EDX) qn	x		
Transmission Electron Microscopy (TEM) ql		x	
Confocal Scanning Laser Microscopy (CSLM) qn		x	
Atomic Force Microscopy (AFM) qn		x	x
Fluorescent In Situ Hybridization (FISH) qn	x	x	
Electrochemical Impedance Spectroscopy (EIS) qn		x	x

Qualitative: ql

Quantitative: qn

Table 1

[00108] By “comprising” it is meant including, but not limited to, whatever follows the word “comprising”. Thus, use of the term “comprising” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

[00109] By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of”. Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

[00110] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including”, “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been

specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00111] By “about” in relation to a given numerical value, such as for temperature and period of time, it is meant to include numerical values within 10% of the specified value.

[00112] The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00113] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

1. A device for growing a biofilm and measuring an electrical property of the biofilm, the device comprising:

an electrochemical impedance spectroscopy (EIS) chamber; and

a flow cell biofilm growth chamber, wherein the flow cell biofilm growth chamber is entirely housed within the EIS chamber,

wherein:

the flow cell biofilm growth chamber comprises:

an inlet for introducing a flow of nutrient into the flow cell biofilm growth chamber;

an outlet for purging a flow of nutrient out of the flow cell biofilm growth chamber; and

a working-electrode-supported substrate located in a flow channel of the flow cell biofilm growth chamber, wherein the working-electrode-supported substrate is adapted for the biofilm growth thereon, and

the EIS chamber comprises:

an inlet for introducing a flow of inert gas into the EIS chamber;

an outlet for purging a flow of inert gas out of the EIS chamber; and

a counter-electrode-supported substrate,

wherein the counter-electrode-supported substrate and the working-electrode-supported substrate are adapted for electrical connection to an external impedance measurement apparatus.

2. The device of claim 1, wherein the EIS chamber further comprises one or more openings for insertion of one or more sensors for sensing the environment condition in the EIS chamber.

3. The device of claim 2, wherein the EIS chamber further comprises one or more sensors inserted into the one or more openings.
4. The device of any one of claims 1 to 3, wherein the flow cell biofilm growth chamber further comprises one or more openings for insertion of one or more sensors for sensing the environment condition in the flow cell biofilm growth chamber.
5. The device of claim 4, wherein the flow cell biofilm growth chamber further comprises one or more sensors inserted into the one or more openings.
6. The device of claim 5, wherein the one or more openings of the EIS chamber correspond respectively to the one or more openings of the flow cell biofilm growth chamber.
7. The device of claim 3 or 5, wherein the one or more sensors comprise a pH, temperature, pressure, humidity or gas sensor.
8. The device of any one of claims 1 to 7, wherein the working-electrode-supported substrate comprises a material selected from the group consisting of a carbon-supported microscope slide, a silver-supported microscope slide, a gold-supported microscope slide, a fluorinated titanium oxide (FTO) glass, a magnesium, alumina, titanium, copper, silver or gold foil, mesh, tape, plaster, or grid, a paper or plastic printed with carbon, magnesium, titanium, copper, gold, silver or alumina electrode, and a glass microscope cover slip.
9. The device of any one of claims 1 to 8, wherein the counter-electrode-supported substrate comprises a material selected from a carbon-supported microscope slide, a silver-supported microscope slide, a gold-supported microscope slide, a fluorinated titanium oxide (FTO) glass, a magnesium, alumina, titanium, copper, silver or gold foil, mesh, tape, plaster, or grid, a paper or plastic printed with carbon, magnesium, titanium, copper, gold, silver or alumina electrode, and a glass microscope cover slip.

10. A method of growing a biofilm and measuring an electrical property of the biofilm, the method comprising:

providing a device of any one of claims 1 to 9;

introducing a flow of nutrient into the flow cell biofilm growth chamber;

allowing sufficient time for the growth of a biofilm on the working-electrode-supported substrate located in a flow channel of the flow cell biofilm growth chamber; and

electrically connecting the counter-electrode-supported substrate and the working-electrode-supported substrate to an external impedance measurement apparatus to obtain an EIS reading.



FIGURE 1

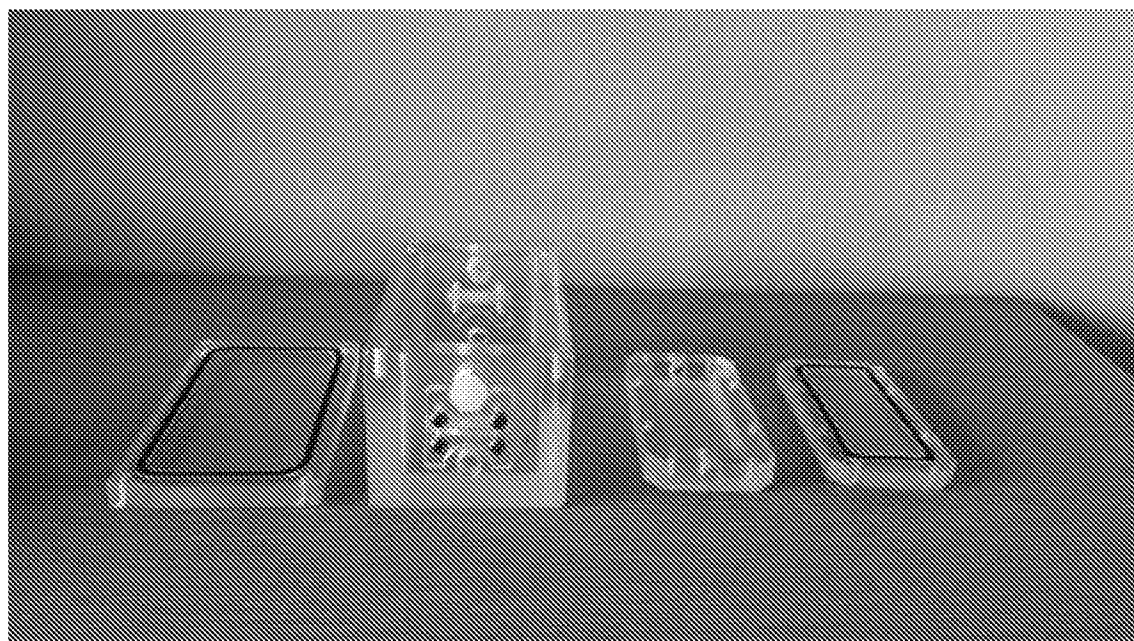


FIGURE 2A

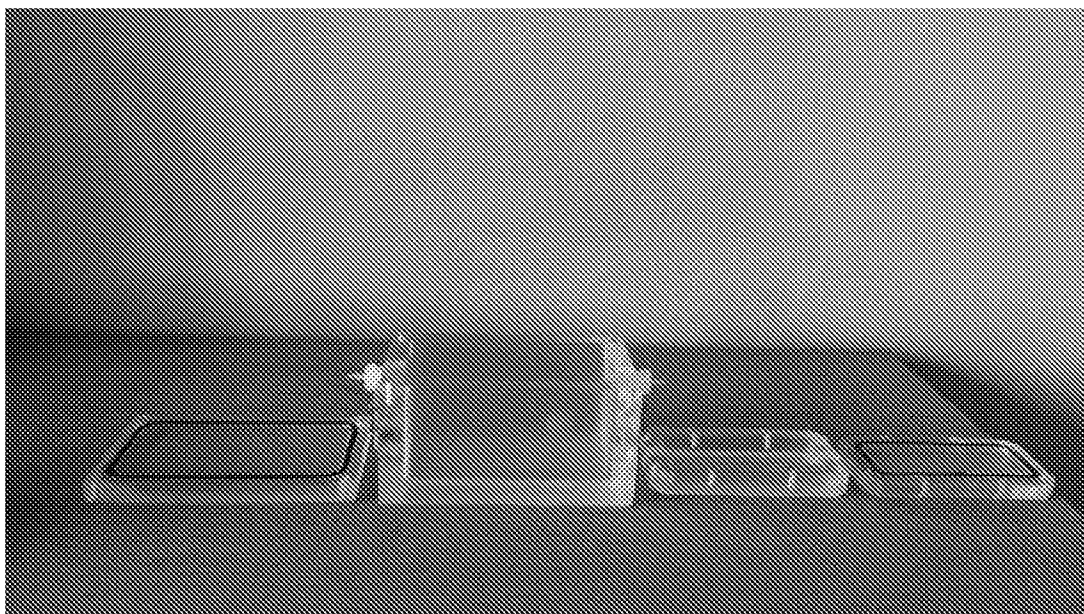


FIGURE 2B

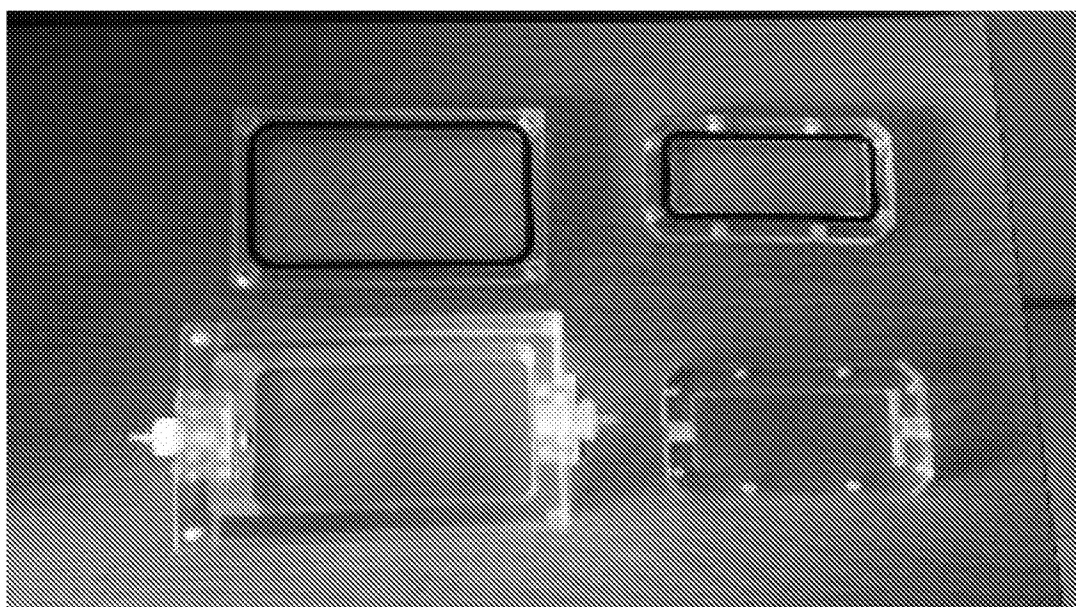


FIGURE 2C

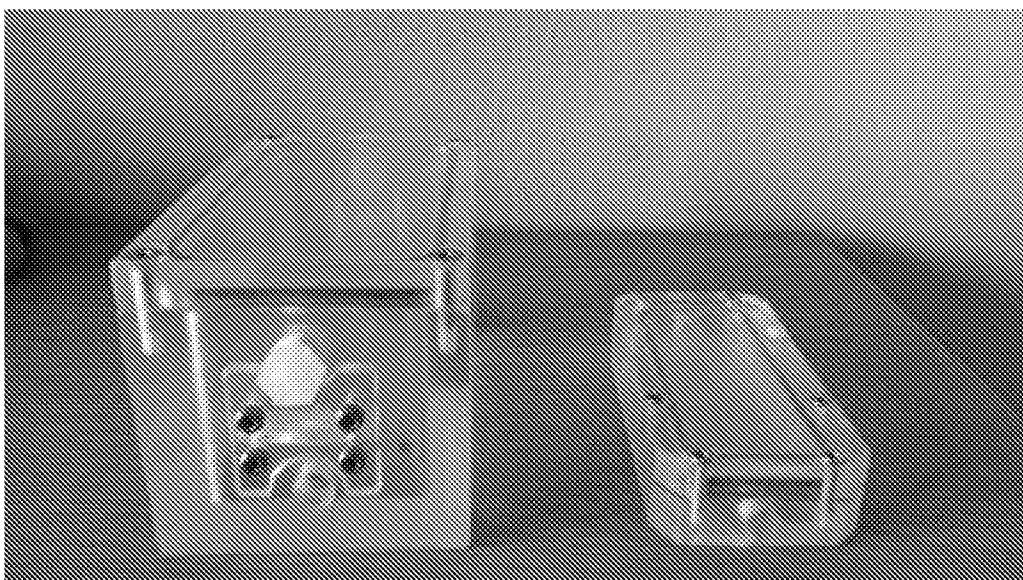


FIGURE 2D

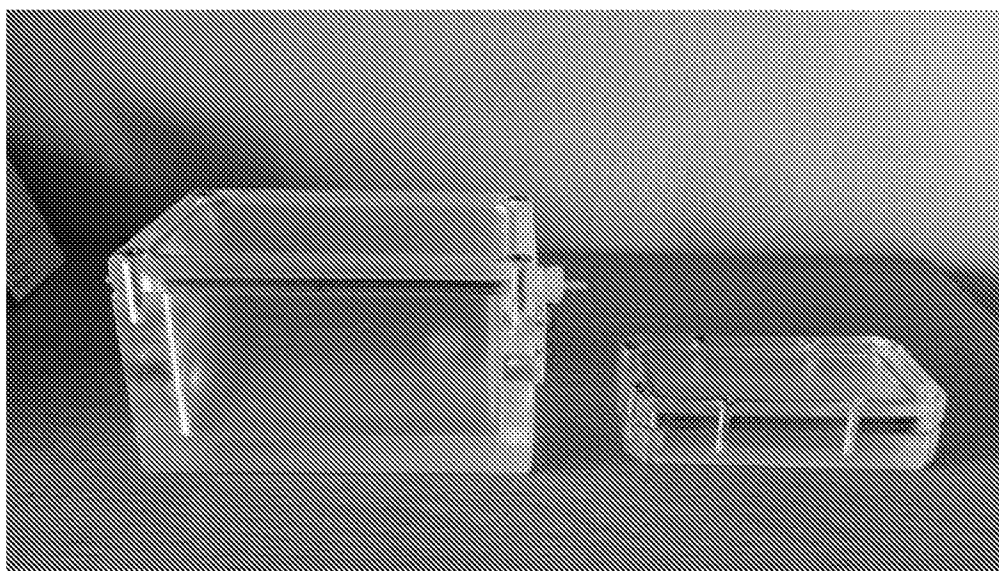


FIGURE 2E

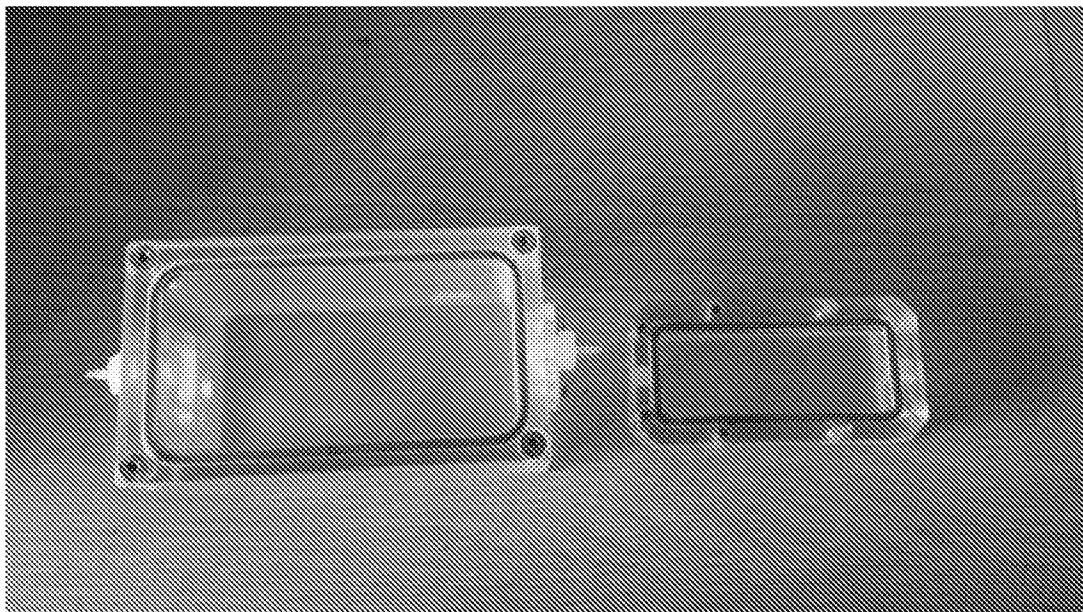


FIGURE 2F

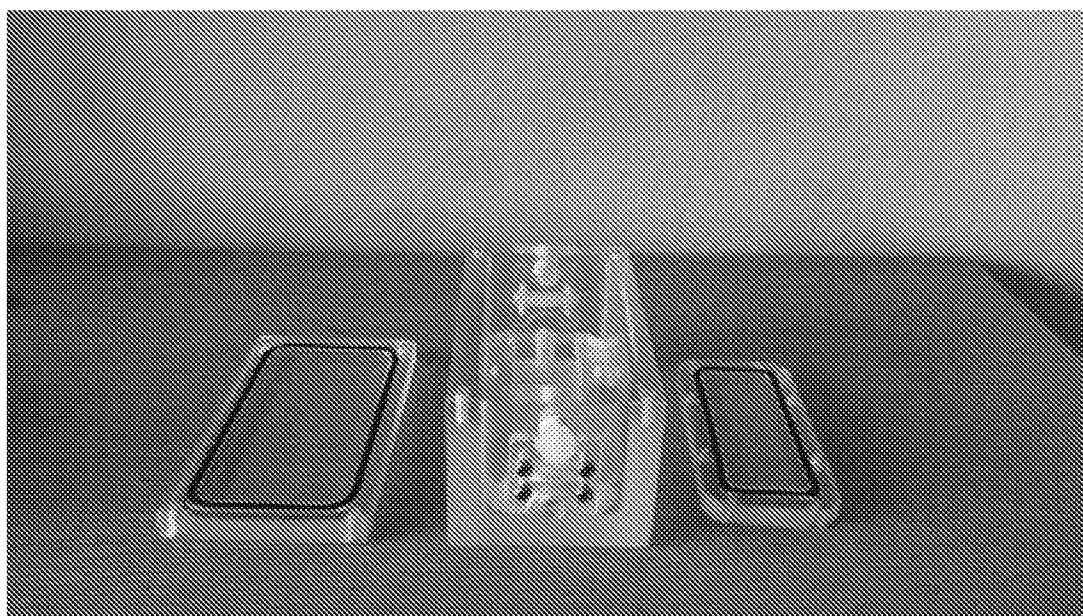


FIGURE 2G

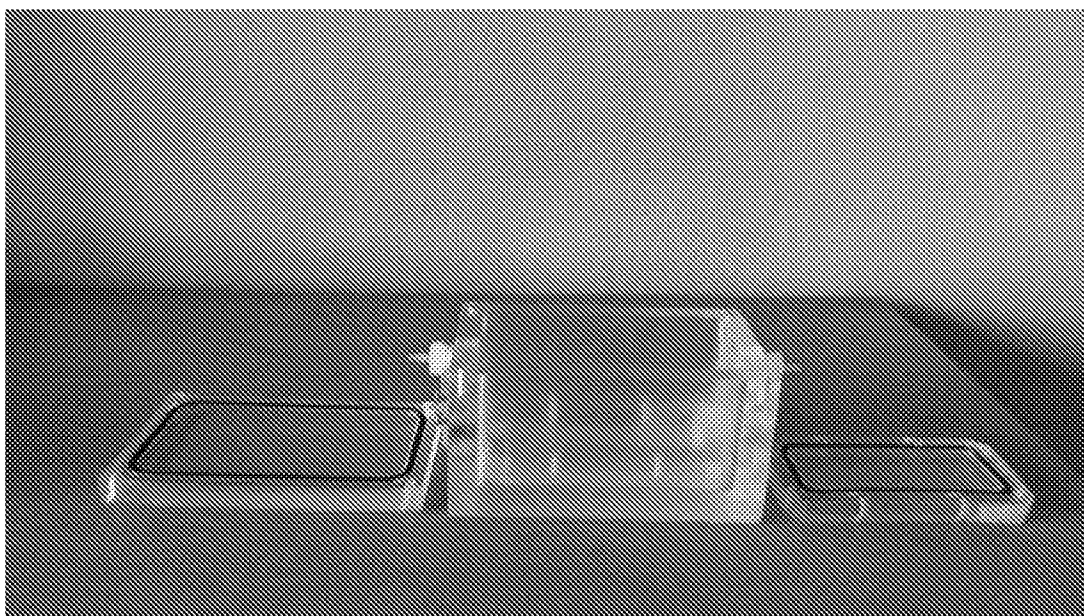


FIGURE 2H

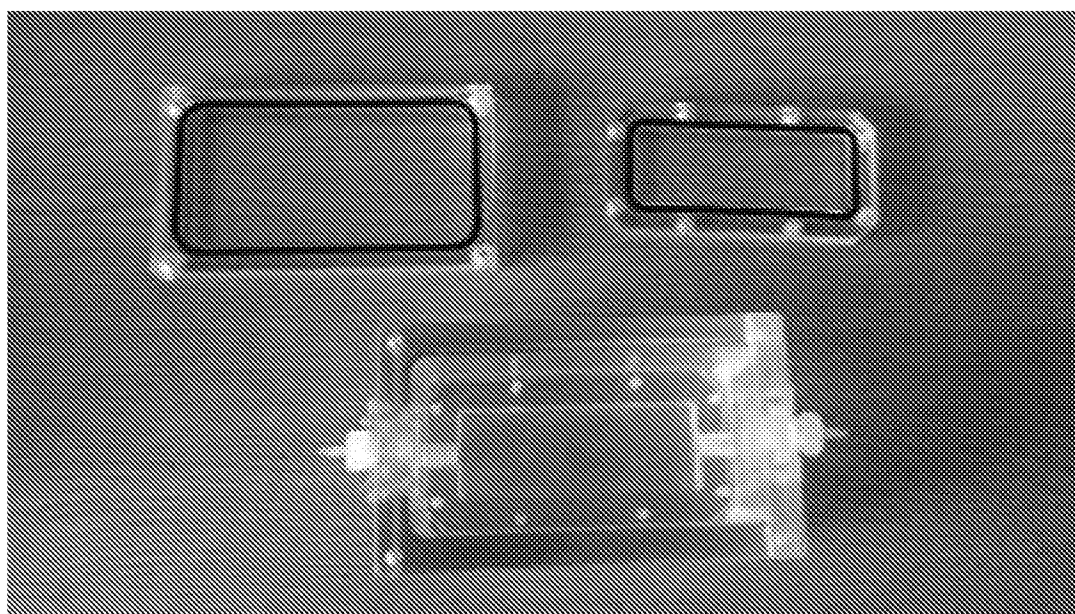


FIGURE 2I



FIGURE 2J



FIGURE 2K



FIGURE 2L

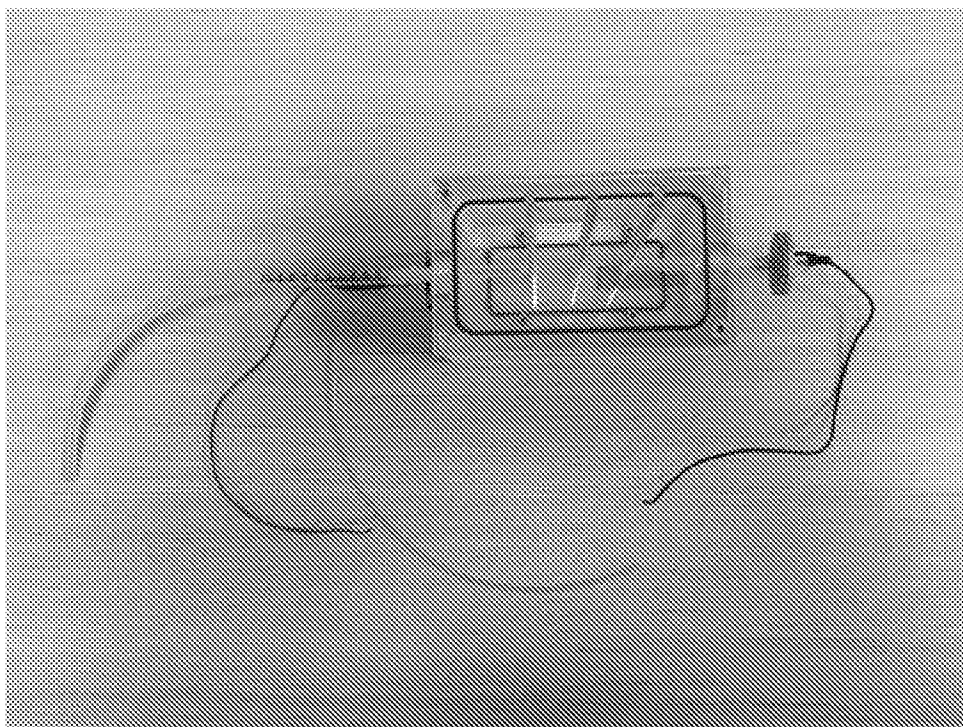


FIGURE 3A

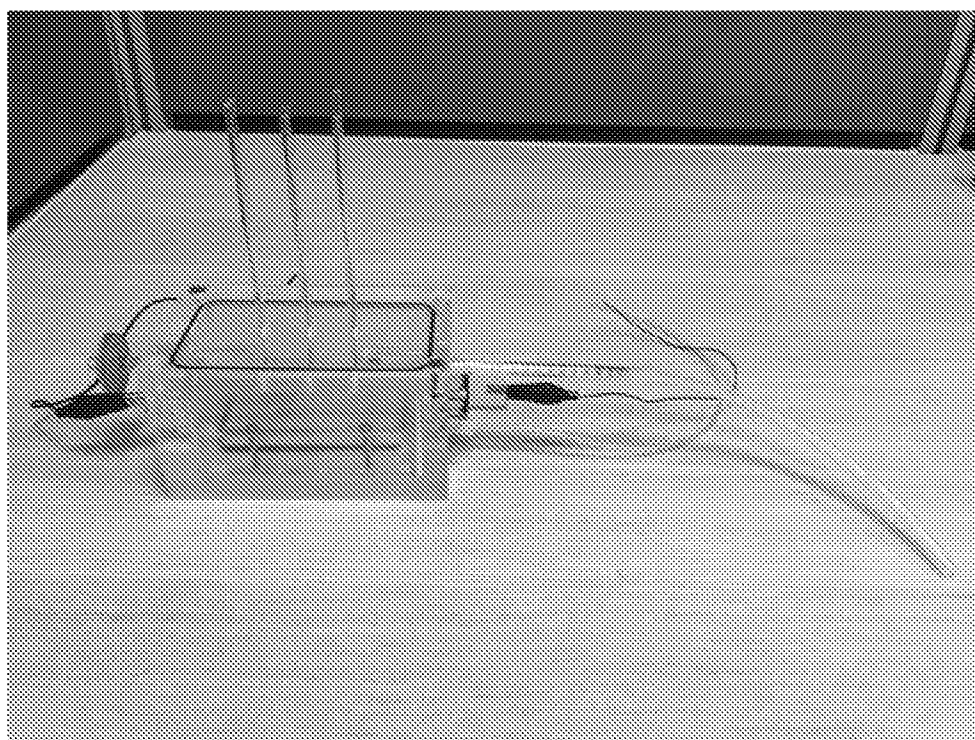


FIGURE 3B

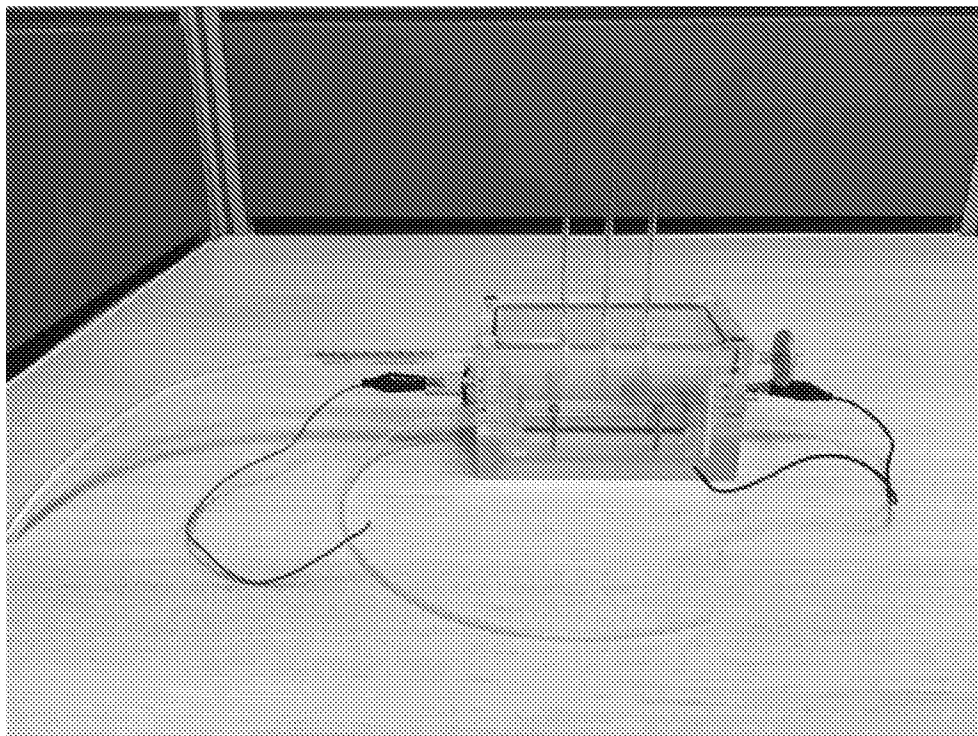


FIGURE 3C

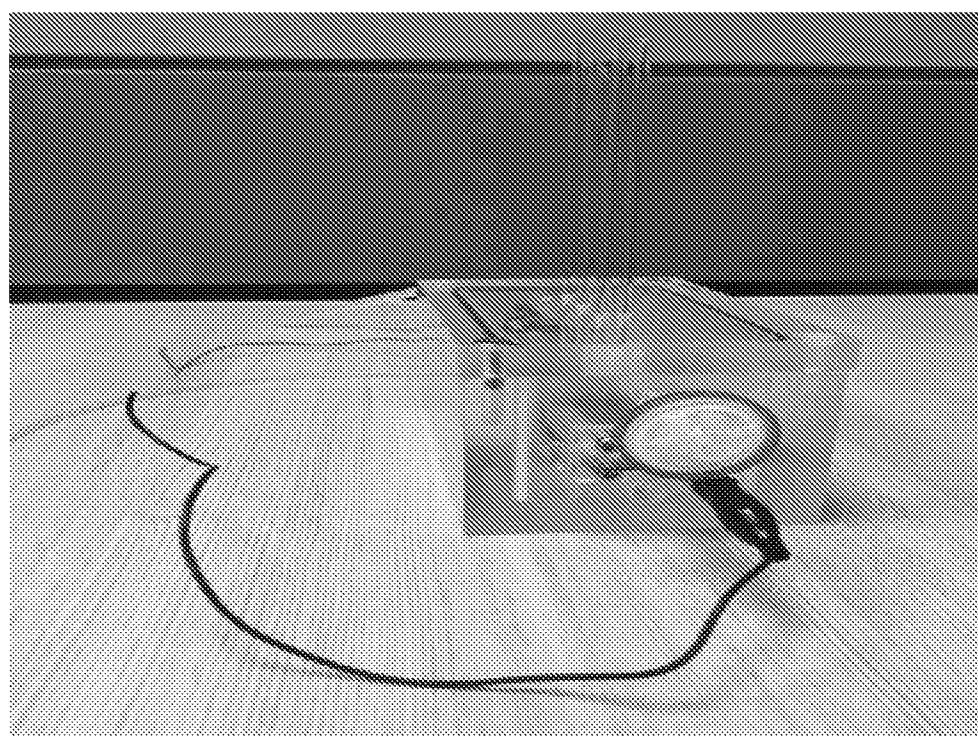


FIGURE 3D

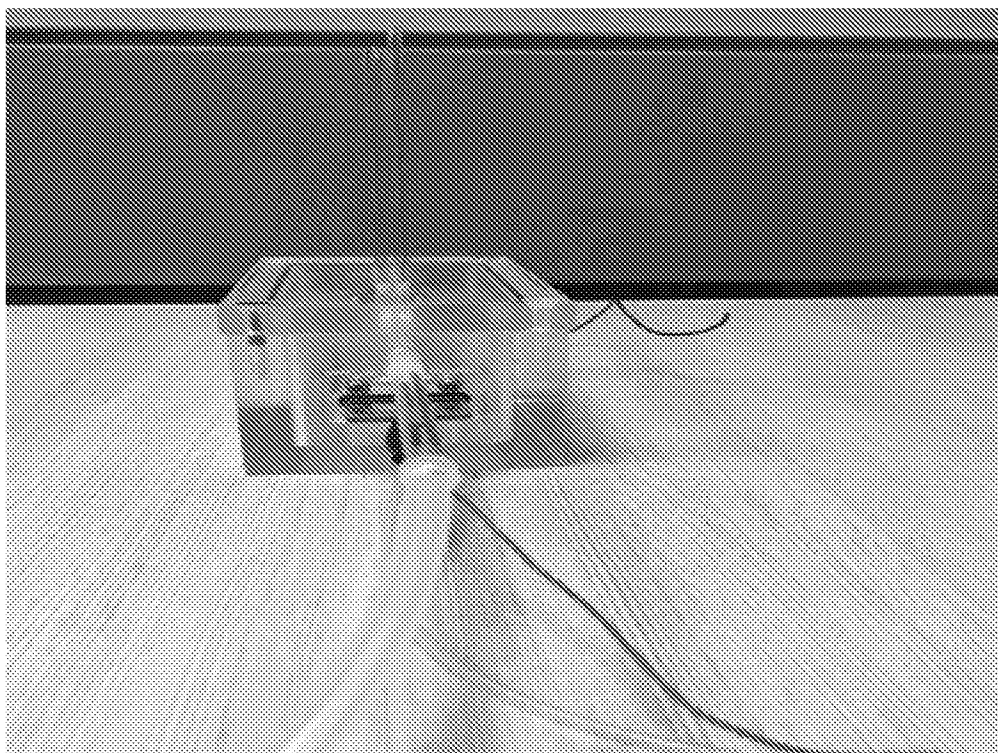


FIGURE 3E

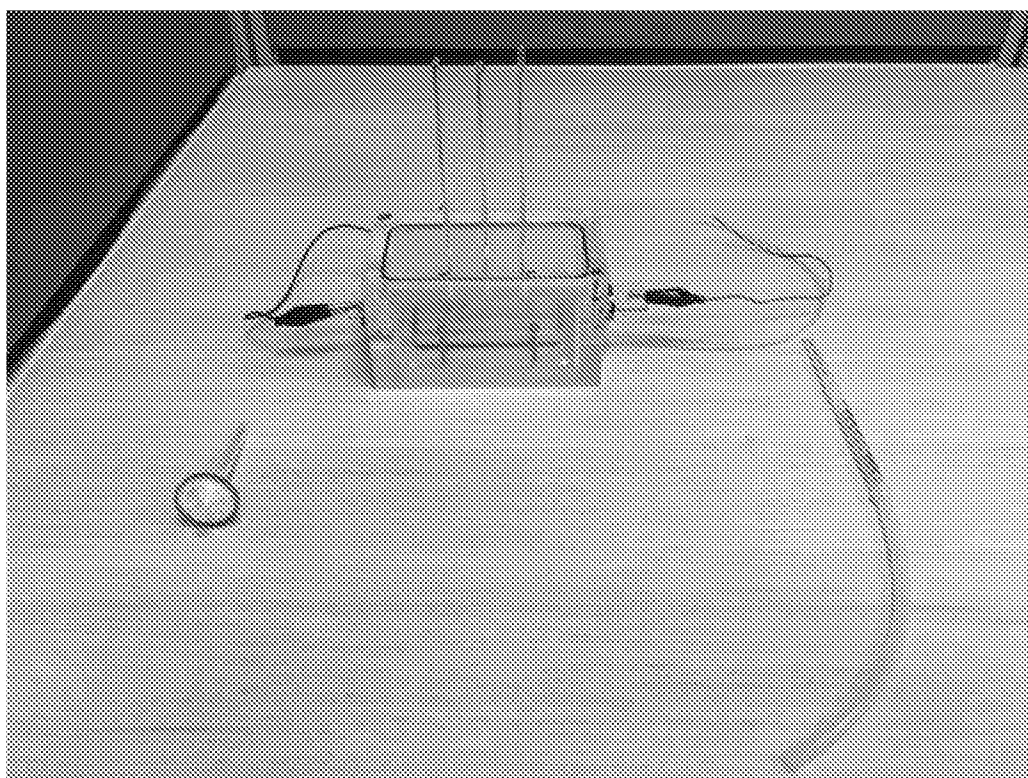


FIGURE 3F

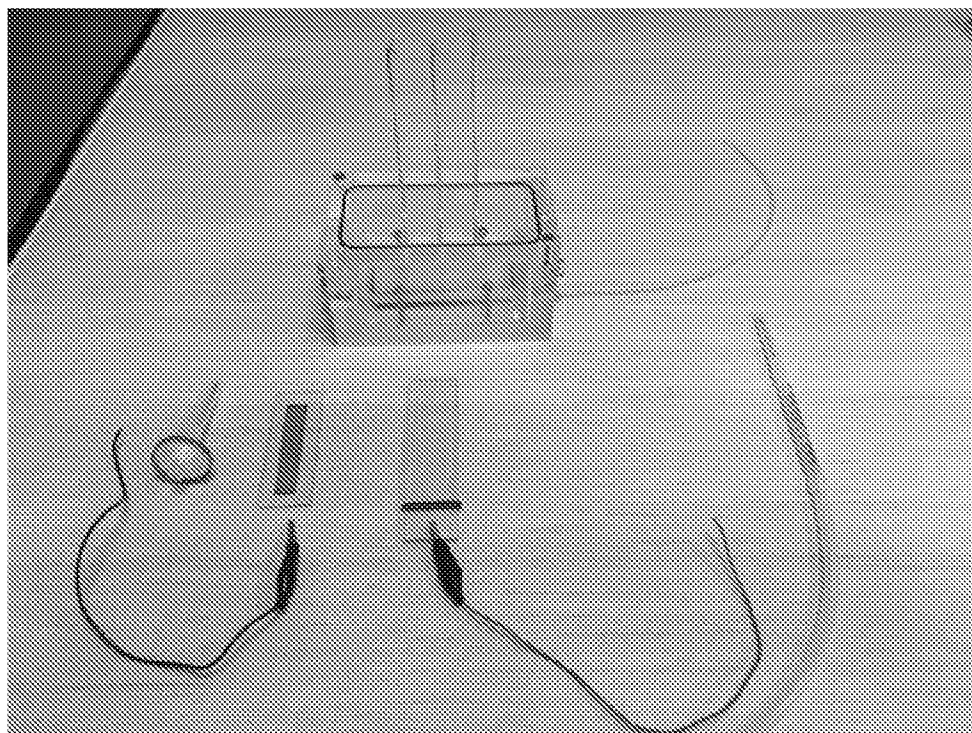


FIGURE 3G

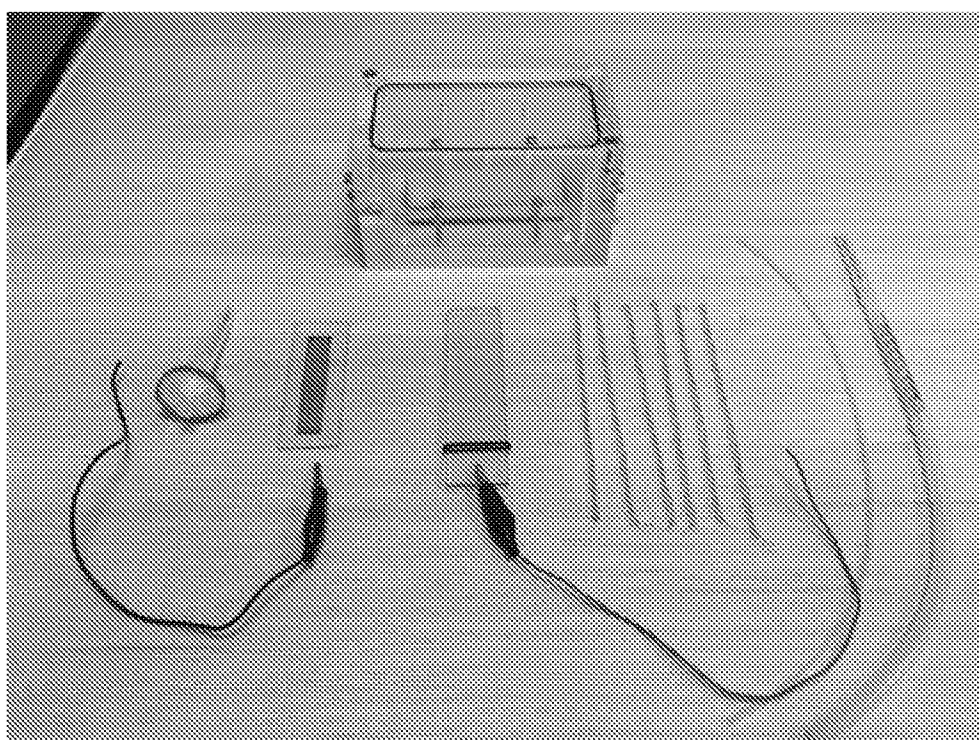


FIGURE 3H

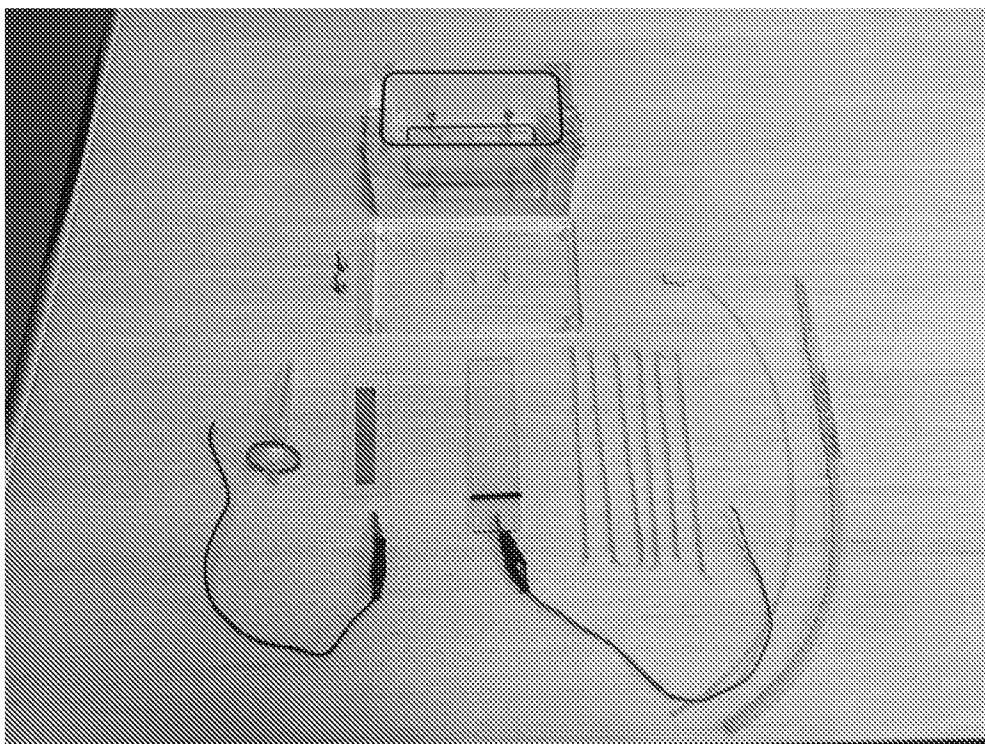


FIGURE 3I

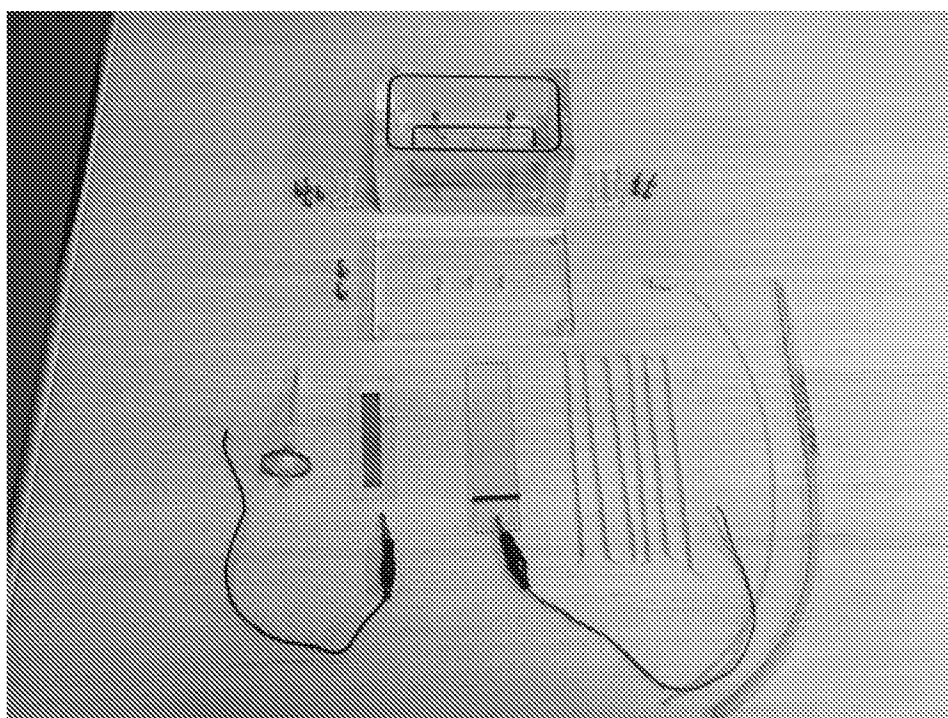


FIGURE 3J

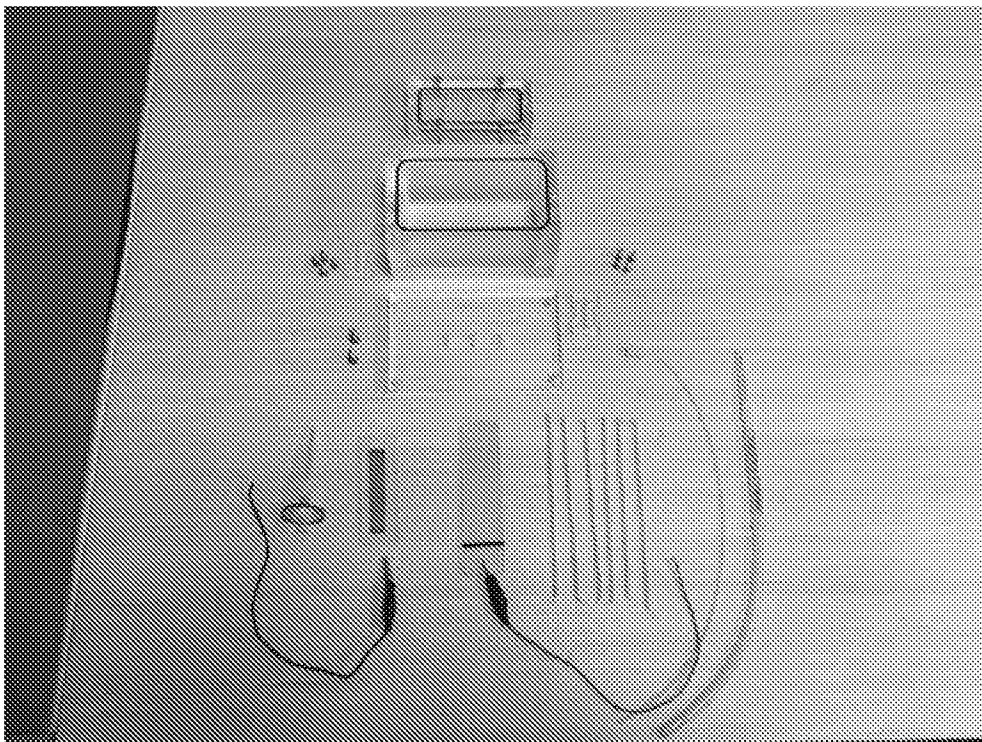


FIGURE 3K

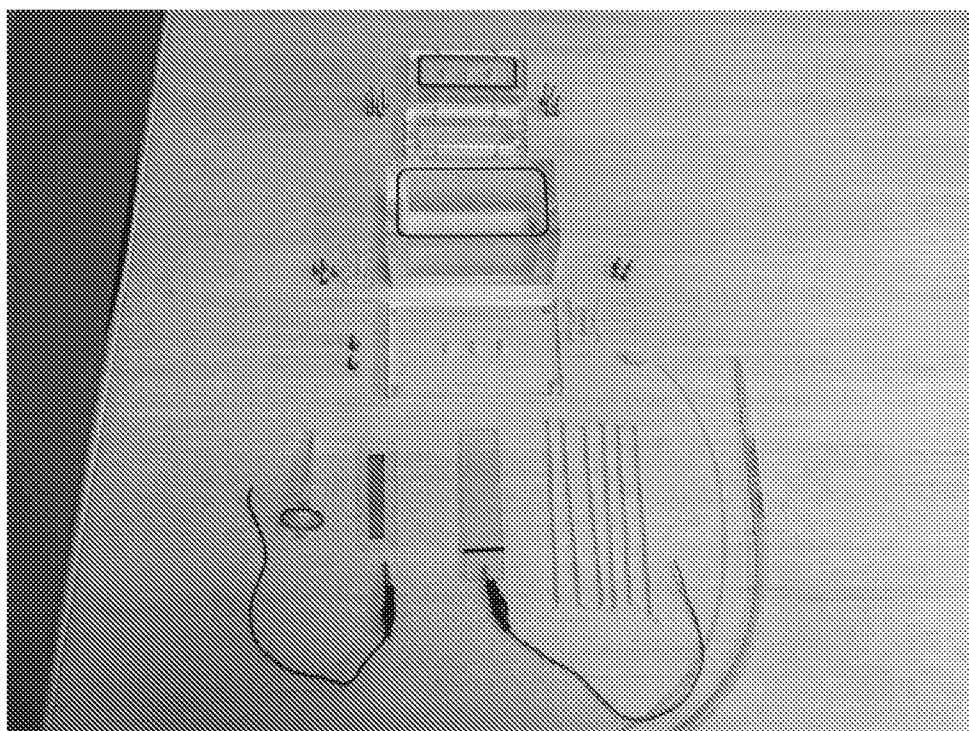


FIGURE 3L

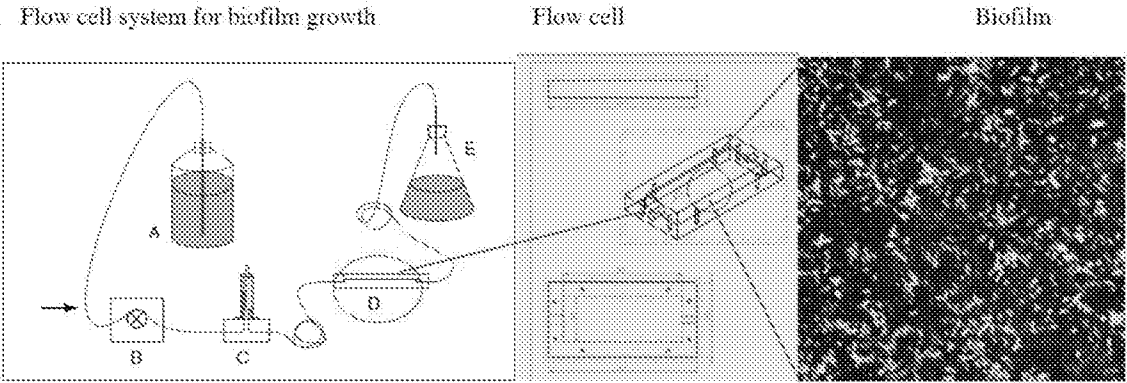


FIGURE 4A

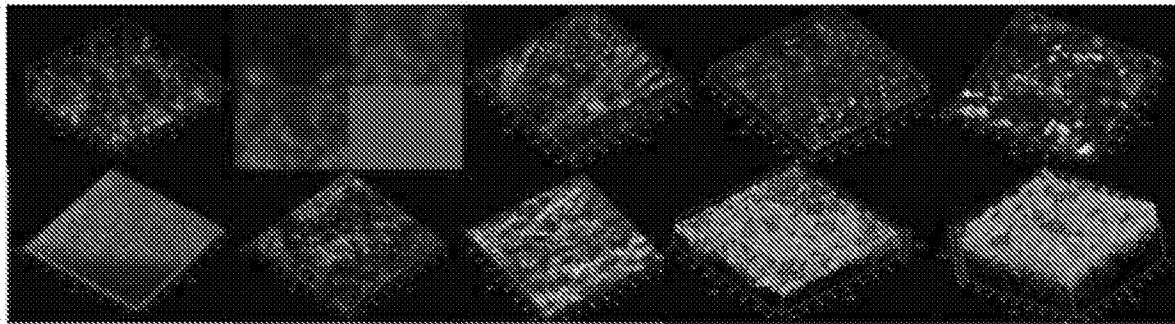


FIGURE 4B

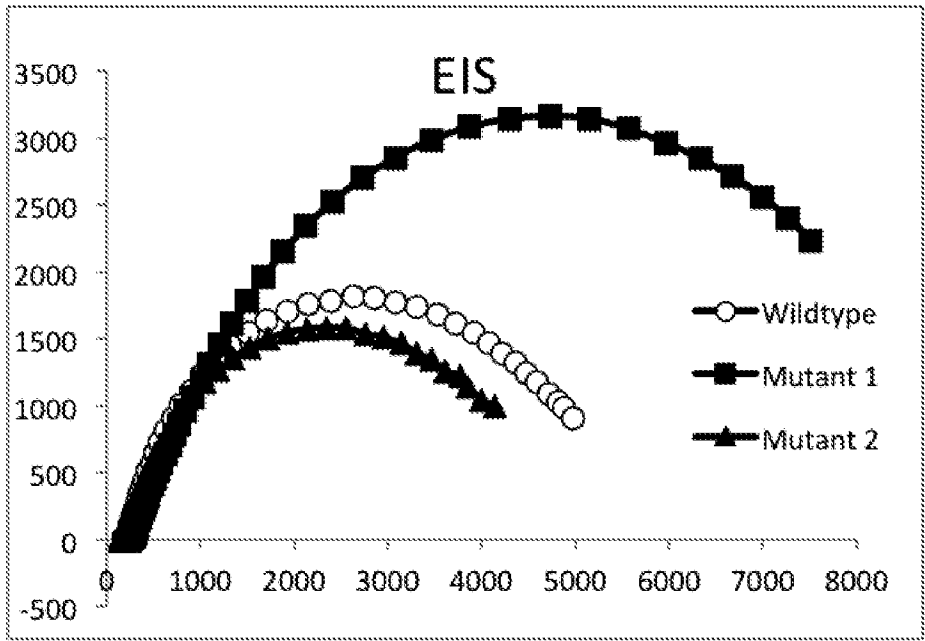


FIGURE 5A

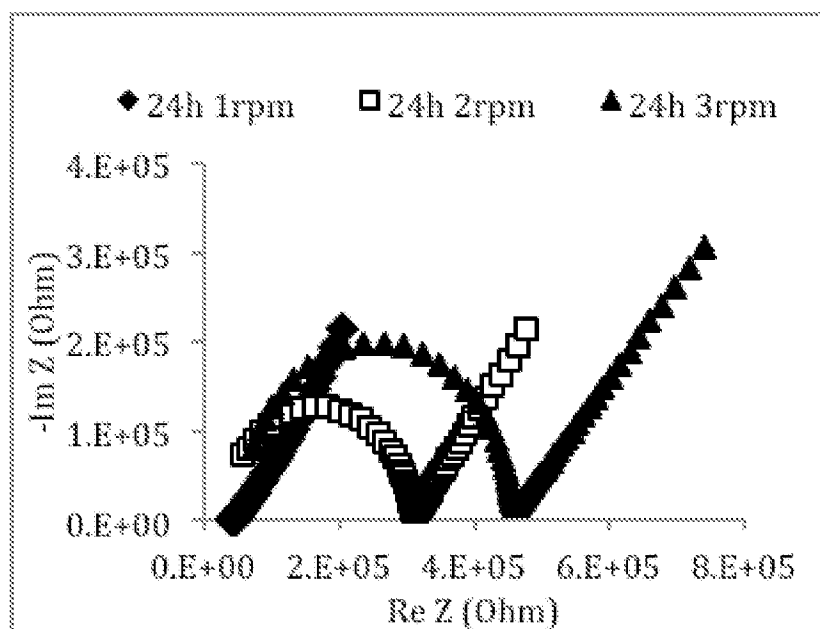


FIGURE 5B

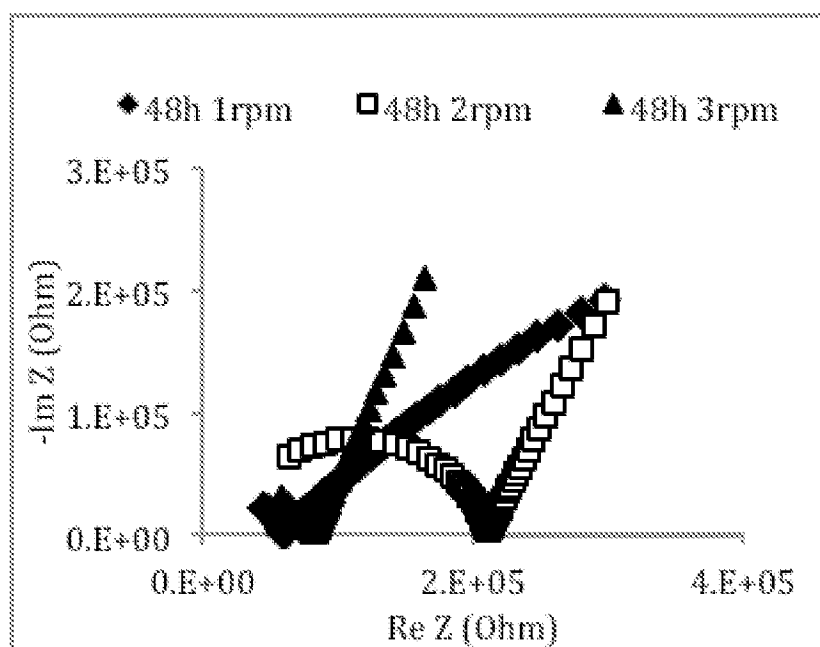


FIGURE 5C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2016/050345

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/483 (2006.01) C12M 3/04 (2006.01)

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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)

FamPat/Scopus: biofilm, growth, electrochemical impedance spectroscopy, inert gas

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 2006/0011491 A1 (LOGAN, B. & GROT, S.) 19 January 2006 para [0008]-[0015], [0042]-[0043], [0091], [0096]-[0098], [0108], [0135], fig 9	1, 8-10 2-7
Y	US 2010/0178530 A1 (MIN, B. & ANGELIDAKI, I.) 15 July 2010 para [0034]-[0035], [0104]-[0105], [0129], [0131], fig 1	2-7
A	WO 2010/117844 A2 (UNIVERSITY OF MARYLAND BIOTECHNOLOGY INSTITUTE) 14 October 2010 Whole document	
A	US 2011/0236769 A1 (XIE, X. ET AL.) 29 September 2011 Whole document	
A	FRAIWAN, A. ET AL., Microbial Power-Generating Capabilities on Micro-/Nano-Structured Anodes in Micro-Sized Microbial Fuel Cells. <i>Fuel Cells</i> , 15 September 2014, Vol. 14, No. 6, pages 801-809 [Retrieved on 2016-08-25] <DOI: 10.1002/FUCE.201400041> Whole document	

☐ 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

"E" earlier application or patent but published on or after the international filing date

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"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
25/08/2016 (day/month/year)

Date of mailing of the international search report
09/09/2016 (day/month/year)

Name and mailing address of the ISA/SG
Intellectual Property Office of Singapore
IP OS 51 Bras Basah Road
INTELLECTUAL PROPERTY #01-01 Manulife Centre
OFFICE OF SINGAPORE Singapore 189554
Email: pct@ipos.gov.sg

Authorized officer
Jie Xiao (Dr)
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2016/050345

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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