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Miniaturized biological and electrochemical fuel cells: challenges and applications

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Jie Yang,[a] Sasan Ghobadian,[b] Payton J. Goodrich,[a] Reza Montazami[a] and Nastaran Hashemi*[a]

This paper discusses the fundamentals and developments of miniaturized fuel cells, both biological and electrochemical. An overview of microfluidic fuel cells, miniaturized microbial fuel cells, enzymatic biofuel cells, and implanted biofuel cells in an attempt to provide green energy and to power implanted microdevices is provided. Also, the challenges and applications of each type of fuel cell are discussed in detail. Most recent developments in fuel cell technologies such as novel catalysts, compact designs, and fabrication methods are reviewed.

1. Introduction

There is an increasing demand for portable renewable energy generators with a small footprint and high power density. It is desirable for these devices to operate continuously for long periods of time without the need to recharge on a regular basis.[1–4] This has inspired the development of microscale fuel cells. These types of fuel cells could be used in a number of applications including powering cell phones, laptops,[5–8] automobile batteries,[9,10] and small integrated biosensors. The microscale fuel cells have a faster start-up and mass transfer rate compared to their macroscale counterparts.[11] Also since the battery technology has not yet met the increasing demands for portable power generators,[1,12] microscale fuel cells have been identified as the potential alternative technology to satisfy the growing need for energy.

Microfluidic fuel cells represent a relatively new type of microscale fuel device, which have a lower cost and satisfy the demand of power generation.[2] In these devices, fuel and oxidant streams introduced into a microchannel proceed in parallel laminar flow without turbulent mixing. However, diffusion happens across the interface between the two streams transverse to the flow streams. Laminar flow occurs at low Reynolds numbers where viscous forces are dominant over inertia forces. Microfluidic devices have been used in many applications such as clinical diagnostics,[13–17] environmental monitoring,[18] and most recently energy generation.[3]

A proton exchange membrane (PEM) has been used in fuel cell designs to separate the fuels in the anodic and cathodic chambers. However, microfluidic fuel cells often run without the membrane to eliminate the problems associated with the operation of the fuel cells. These problems include fuel crossover and anode dry out.[19–21] Microfluidic fuel cells have higher efficiency once running at higher temperatures. However, the PEM tends to dry out at these temperatures resulting in a reduction of protons exchanged.[3] Also, the laminar nature of the flows prevents turbulent mixing throughout the entire channel. For these reasons, the physical barrier has been removed from most microfluidic fuel cell designs. PEMs can be modified by addition of thin-films to enhance their ionic, electrical, and mechanical properties.[22–24]

Microchannels are commonly fabricated using soft lithography[25] with a T-shaped or Y-shaped geometry. The microfluidic fuel cells are typically made of polydimethylsiloxane (PDMS). Electrodes are either placed in the inner wall of the main channel, or immersed in an electrolyte and placed in the main chamber. The performance of MFCs can be affected by the efficiency of chemical reactions as well as size and geometry of the chamber. In the absence of PEM fuel and oxidant can be chosen independently to obtain higher power efficiency. Also regarding the chamber size, the small size of the chamber efficiently improves the power density. This is due to the larger surface-to-volume ratio which shortens energy production start-up time and allows for faster power generation recovery. It is demonstrated that a high aspect ratio (width/height) microchannel could improve the fuel utilization and consequently the power density of the fuel cells.[26]

Microbial fuel cells have been seen as a means of wastewater treatment[27–31] and as a new type of electricity generator.[32]

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[a] *Department of Mechanical Engineering, Iowa State University, Ames, IA, USA. E-mail: nastaran@iastate.edu*

[b] *Department of Civil and Environmental Engineering, Tarbiat Modares University, Tehran, Iran*

Specifically, microbial fuel cells have received increased attention as a means of producing "green" electricity. A large amount of energy contained in biological wastewater remains unexploited and is being wasted in most cases.[33] The development of microbial fuel cells makes it possible to reuse and extract energy from what was considered as waste before. In this type of fuel cells, microbes (as an alternative to chemicals) are employed to convert chemical energy into electricity under environmentally friendly and mild conditions.

Microscale microbial fuel cells have received increasing attention because they have shorter start-up time and faster power generation recovery after refilling.[34–36] They can also greatly improve the density of power generation because the density essentially depends on the surface-to-volume ratio in such reactors. Microbial fuel cells are commonly fabricated by placing electrodes and PEM between two PDMS chambers. The choice of electrodes and biocatalysts (microorganism) are the two main factors that affect the power output. Carbon cloth,[11,37] silver chloride reference electrode,[33] Toray cloth,[38] and foam are several electrodes that have been extensively used. *Shewanella*, *Pseudomonas*, and *Geobacter* have been widely used as biocatalysts in microbial fuel cells.[27,37,39] Like microfluidic fuel cells, the chambers are fabricated using soft lithography methods. Bacteria[40–42] or algae[43] are used as the electrogenic microbes. Some microbial fuel cells use pure culture, meaning only one strain of bacteria or algae is allowed to grow in the chamber. However, there are also some examples of binary[44] or mixed-culture fuel cells that obtain results better or similar to pure-culture counterparts.

Latest developments in the microscale power generator technologies have been inspired by our recent advances towards understanding the biochemical activities. In enzymatic fuel cells, enzymes are used as catalysts to produce electricity.[45–47] Compared to a conventional fuel cell, an enzymatic biofuel cell has a simpler design and is more cost-efficient. This is due to the abundance of the enzymes in nature. Typical fuel for an enzymatic biofuel cell is glucose, which is easily obtained.[48] Like other fuel cells, fuel is oxidized in the anode chamber of enzymatic biofuel cells causing electrons to move from the anode to the cathode through an external wire and consequently generating electricity. Although enzymatic biofuel cells have a number of advantages, their power output is currently lower than traditional fuel cells and the technology has plenty of room for improvement.[49]

Implanted biofuel cells are enzymatic fuel cells which are implanted into animals and plants such as snails,[50] clams,[51] grapes[52] and insects[53] in order to operate as *in vivo* microscale power sources. Chemical reactions in living organisms are used to generate electricity, which is based on the same theory applied in enzymatic fuel cells. It is more difficult to implant biofuel cells into living animals because of the lower concentration of oxygen in blood compared to the circulatory system of arthropods.[50] Implanted biofuel cells are relatively new in the area of fuel cell technology[50,51,53] and still require a more complete understanding of the host to produce power *in vivo.* Here, several successful implantations of such fuel cells are reviewed.

2. Microfluidic fuel cells

A microfluidic fuel cell is a device that includes all of the fundamental components of a fuel cell incorporated into a single microfluidic channel with fluid delivery and removal.[2,54] There are many applications using the concept and technology of microfluidic fuel cells; ranging from small portable electronic devices to large power generation plants.[55] Although there have been a lot of developments and improvements in the field of microfluidic fuel cells in the last decade, this technology has not gone far beyond laboratory testing.[54,56] Only a few commercialized fuel cells have been used for power generation purposes. However, fuel cell technology is expected to grow, as it is unlikely that batteries will be able to keep pace with increasing portable power generation demands. Since microfluidic fuel cells have larger power densities, they are being considered as a potential replacement for conventional batteries.

The behavioral studies of fluids in microstructures, where one of the characteristic dimensions is in the range of 1–1000 μm, can be done in microfluidic platforms.[54,57] Microfluidic fuel cells typically employ a platform with co-laminar flows. The separation of these flows is based on the laminar nature of streams,[2] and is usually done without a physical membrane. In the most common configuration of microfluidic fuel cells two streams, fuel and oxidant, are introduced into the channel from different inlet channels and then come into contact in the main channel. Electrodes are typically placed on the side or bottom walls of the channel. The most common types of channel geometries are Y-shaped[58] and T-shaped.[59] The laminar flow characteristic eliminates convection mixing of fuel and oxidant, thus making it possible to design a fuel cell without the use of a separating membrane. The only way the fluids mix is through diffusion, and it is restricted to the center of the channel. This mixing area is variable, and can be adjusted by changing the channel dimensions and flow rate to optimize energy production. The laminar flow nature also allows the anolyte and catholyte composition to be chosen independent of each other. The liquid–liquid interface of microfluidic fuel cells eliminates some of the disadvantages of the PEM-based fuel cells such as membrane degradation, water management, and fuel crossover. However, the distance from the anode to the cathode limits the performance of membraneless fuel cells since it is a longer distance for a proton to travel. Many groups have reported using methanol,[55,60] ethanol, hydrogen, glucose, and vanadium redox species as the fuel in their microfluidic fuel cell designs. Oxygen, in both gas and liquid form, is the most commonly used oxidant, but there have also been some designs based on hydrogen peroxide, potassium permanganate, and vanadium redox species as the oxidant. An electrolyte such as a base or a strong acid is added to support the ionic charge transportation within the fuel and oxidant.

Microfluidic fuel cells are lower in cost compared to PEM-based fuel cells, as a PEM is a relatively expensive material used in the fabrication of fuel cells. Microfluidic fuel cell design, on the other hand, is able to overcome the disadvantages of using the PEM. The operating conditions of microfluidic fuel cells are also easy to achieve. A microfluidic fuel cell only requires room temperature and has no specific desired humidity.

Microfluidic fuel cells can be applied in clinical, genetic, and energy fields, making this technology a potential future option for energy generation. However, improvements are needed in microfluidic fuel cells in order to make them commercially successful.

2.1. Materials and methods

Microfluidic fuel cells are typically made using standard photolithography techniques.[2,3] In soft lithography, a solid mold carrying a negative pattern of the microchannel is prepared by microfabrication techniques. A piece of solid substrate such as glass or silicon wafer is spin-coated with a photoresist. Then a desired pattern is printed on a photomask, followed by UV exposure. Then the photoresist exposed to UV light is removed and is etched down to the preferred thickness. Microfluidic fuel cells are commonly fabricated in PDMS and oxygen plasma treatment is used to covalently bond PDMS to itself or to glass.

Recently, a fast and non-photolithography method of microfabrication has been reported which employs a standard laser printer.[61–63] The microfluidic channel is printed onto commercially available thermoplastic Shrinky-Dinks, polystyrene thermoplastic sheets that shrink after being heated in an oven. This could be used as a substitute to the photolithography method of making a mold with microfluidic channels on it. It is reported that after heating the Shrinky-Dink for several minutes at approximately 160 °C, the printed pattern shrank equally in all directions by about 63% from the original dimensions while the height of the pattern increased significantly.[61] This method is significantly less expensive than conventional photolithography and micromachining methods, and can generate patterns of various heights by changing the number of prints. The main disadvantage associated with this method is that the quality of the pattern, especially at the edges, is not as precise as if it were generated by standard photolithography techniques.

In a microfluidic fuel cell, electrodes are typically positioned in parallel on the side or the bottom wall of the channel for a side-by-side streaming microfluidic fuel cell. Gold, carbon cloth, carbon paper, and graphite are all commonly used electrodes. The position of the electrodes influences the ohmic resistance in the channel; electrodes spaced close to each other allow lower internal resistance and are consequently more desirable. However, the design must be optimized to avoid the inter-diffusion area in the center of the channel as well.

2.2. Membraneless microfluidic fuel cells

In a Y-shaped membraneless microfluidic fuel cell presented by the Kenis group, the electrodes were placed in the inner wall of the main channel.[3] The channel was 3 cm long with a cross sectional area of 1 mm × 1 mm. The anode and cathode materials were deposited on the inner support polymer. The fuel was either formic acid or potassium permanganate and the oxidant was oxygen dissolved in sulfuric acid, both in 18.3 Ω cm Millipore water. The flow rates were 0.3–0.8 ml min^{-1} per stream. With the same fuel cathode (10% formic acid), a fuel anode of oxygen dissolved in sulfuric acid can provide a potential of 0.4 V with a current density of 0.4 mA cm^{-2}, while an anode of potassium permanganate can provide a potential of 0.55 V with a current density of 4 mA cm^{-2}. It was found that by decreasing the size of the channel, the current density at the same volumetric flow rate is increased (Fig. 1a and b). The disadvantage of the membraneless microfluidic fuel cell design is the limited proton conductance. This is because of the further distance from the anode to the cathode that a proton needs to travel.

It is possible to focus a stream on one side of the channel, by changing the ratio of volumetric flow rates (Q_2/Q_1) in a microchannel. In a microreactor with buffer and reagent streams flowing in parallel, this can be used in reversing an unwanted reaction; that is crucial for enzyme/cofactor regeneration; by focusing the reagent stream close to the electrode.[64] It is shown that pumping buffer and ferricyanide at a flow rate of 12 into a microchannel with a length of 3 cm and width of 250 μm, the conversion of ferricyanide to ferrocyanide was 30% higher. By changing the Q_2/Q_1 to 1, a large fraction of the ferricyanide leaves the microchannel without reacting (Fig. 1c and d).

2.2.1. Porous electrodes. Sinton's group has demonstrated that in a microfluidic vanadium redox fuel cell, using a high aspect ratio (width/height) cross-sectional channel geometry, a fuel utilization of only 50% per single pass was achieved at low flow rates. While the geometry of the channel limits the fuel utilization, operating at low cell voltages has proven to facilitate higher fuel utilization.[56] In an attempt to improve the power density of the fuel cell, they employed flow-through porous electrodes which resulted in a power density 72% higher than planar counterparts. This is due to the increase in the effective active area as well as the rate of species transport. A portion of flow penetrates into the porous medium.[2] Porous carbon strip electrodes with a thickness of approximately 300 μm and a porosity of 78% were used in this experiment. With a volume of 3.6 μL, the theoretical cell potential was calculated to be 1.246 V, with the possibility of increasing the potential beyond 1.50 V by using high purity vanadium solutions. Overall, the performance of the porous electrode fuel cell was improved significantly with power densities as high as 131 mW cm^{-2} and nearly complete fuel utilization.

The limitations of this fuel cell are that the concentration of the reactant is zero at the surface, and a concentration boundary layer is formed in the channel that limits the flux of the reactant to the surface depending on the flow characteristics. Additionally, to have a practical level of energy conversion efficiency, the cell must operate at high voltages.

Another example of using porous electrodes in a radial membraneless fuel cell was demonstrated by Posner's group.[65] In their convective microfluidic fuel cell design, the fuel stream was introduced into the center of the disc and flowed radially outward as shown in Fig. 2(a). The oxidant stream was introduced into the gap between the anode and the cathode and

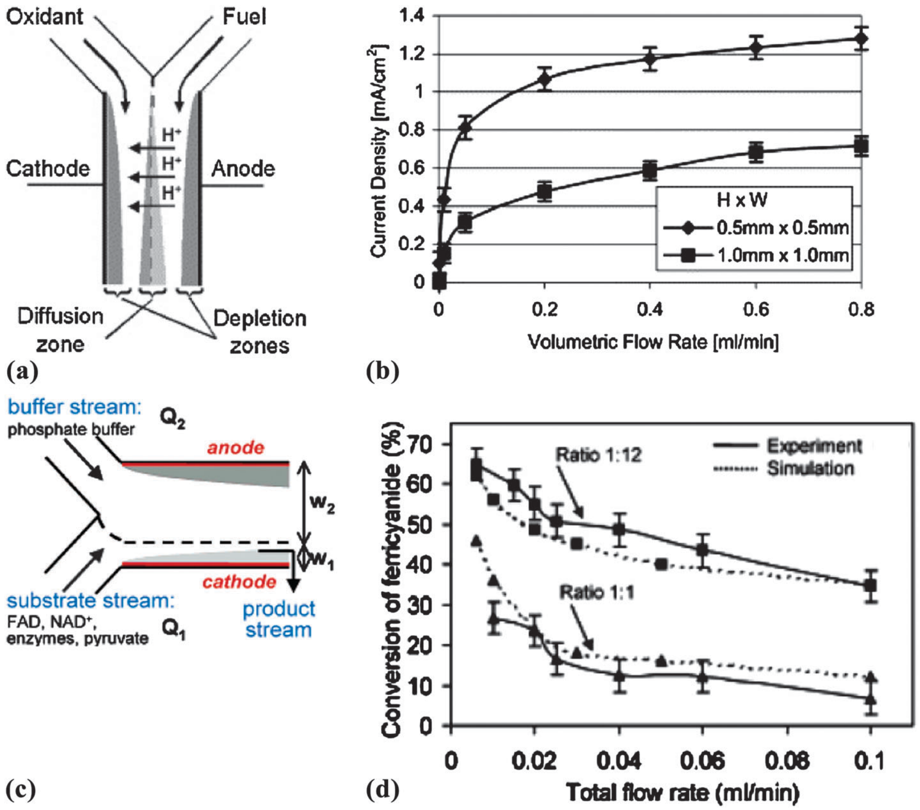

Fig. 1 (a) A schematic of the membraneless microfluidic fuel cell shows the fuel and oxidant streams running in parallel in the microchannel. (b) Current density was found to be higher for a smaller channel.[3] (c) A schematic of the microreactor used in cofactor regeneration and biocatalytic conversion. (d) Comparing the effect of flow rate ratios on conversion of ferricyanide to ferrocyanide.[64]

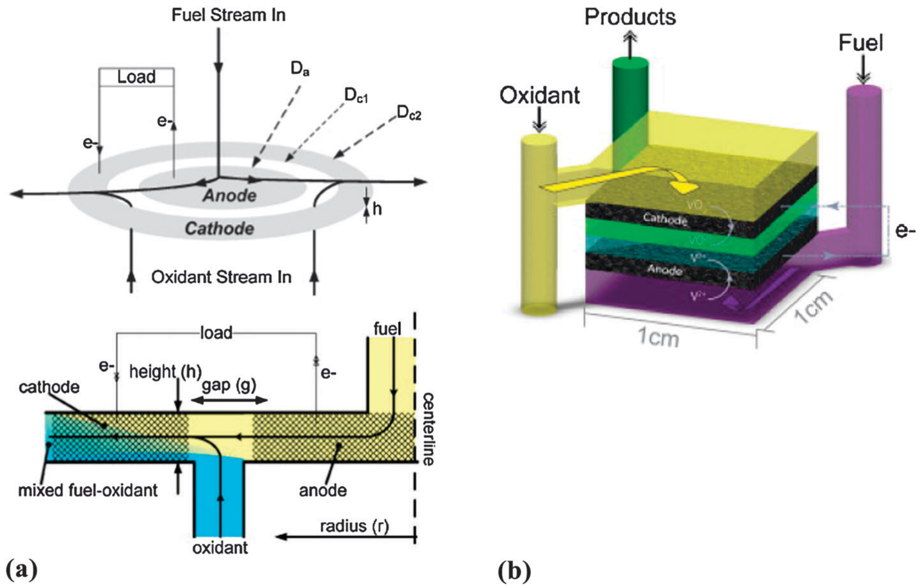

Fig. 2 (a) Schematics of a radial membraneless fuel cell. Fuel flows radially outward from the center of the anode disk while being oxidized. The oxidized species then mixes with the oxidant stream before flowing through the cathode.[65] (b) Internal geometry of the plate-frame microfluidic fuel cell. The oxidant and fuel are pumped through the porous cathode and anode respectively. The two streams meet in the center at a horizontal liquid–liquid interface before being pumped out in the product stream.[66]

then crossed a gap to a ring shaped cathode. This design provides control over the flow rates of fuel and oxidant, and the electrode areas independently. Higher maximum currents and peak power densities were achieved by increasing the flow rate. Maximum power densities of 2.8 mW cm^{-2} and fuel utilization of 58% (at a flow rate of 100 μL min^{-1}) were obtained.

Recently, Erickson's group[66] presented a microfluidic fuel cell with a plate-frame architecture that used vanadium redox species and porous flow-through electrodes. This vertical stacking geometry is volumetrically efficient with little dead volume and takes advantages of using flow-through electrodes. In this design, the electrodes are separated by the electrolyte and are arranged horizontal to each other while the fuel and oxidant flow is introduced vertical to the electrodes (Fig. 2(b)). This fuel cell had peak power densities of 5.8 mW cm^{-2}. The size of the electrode and the pore size are important in determining the performance of the cell. Smaller pore sizes result in a higher power gain, but also increase pumping loss slightly. In this experiment, a 140% increase in power density and a six fold increase in fuel utilization were observed by replacing commercial carbon paper with porous electrodes.

2.2.2. Air-breathing laminar flow-based fuel cells. In an air-breathing laminar flow-based fuel cell, a stream of liquid fuel such as methanol and a stream of oxidant such as hydrogen peroxide are introduced into a microchannel, where the anode and cathode form the sidewalls.[4] This design is called multi-stream laminar flow-based fuel cells (LFFCs). One of the main advantages of LFFCs is that the composition of fuel and oxidant can be selected independently, allowing it to be used with a variety of media.

To provide higher oxygen concentration and consequently higher power generation rate, a porous gas diffusion electrode (GDE) as the cathode was used to allow direct oxygen transport from the air into the cathode. This makes air-breathing microfluidic fuel cells convenient in many applications, as the oxygen that is used as the oxidant in the microfluidic channel can be taken directly from the surrounding atmosphere.

A formic acid containing sulfuric acid stream entered the channel. An electrolyte stream containing sulfuric acid (without formic acid) entered the channel and prevented formic acid from reaching the GDE cathode (see Fig. 3). The maximum current density of 130 mA cm^{-2} and the maximum power density of 26 mW cm^{-2} were obtained when formic acid concentration was 1 M, five times the power density that could be obtained from the same LFFC using an oxygen-saturated aqueous stream instead of an air breathing cathode. This is due to the higher diffusion coefficient of oxygen in air. The performance and fuel utilization can be altered by adjusting electrode-to-electrode distances, electrode designs, and fuel concentrations.

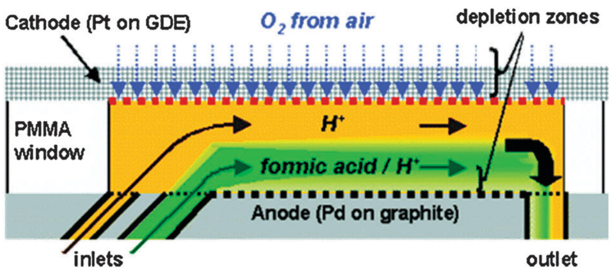

Fig. 3 The microfluidic channel is made of poly(methyl methacrylate) (PMMA). The anode is a graphite plate covered with Pd black nanoparticles. A sheet of Toray carbon paper with a platinum loading of 0.35 mg cm^{-2} is used as the gas diffusion cathode.[4]

Nguyen's group[67] invented a new design of microfluidic fuel cell with porous electrodes to overcome disadvantages of the older designs. An anode of Toray carbon-fiber based paper with a thickness of approximately 280 μm and a porosity of 78% was used with a catalyst layer of Pd black, while the cathode consisted of simple carbon-fiber based paper. A microfluidic channel was made using 0.5 mm thick silicon rubber with a channel length of 30 mm and width of 3 mm. Electrodes were machined into the channel with a cathodic catalytic active surface area of 0.9 cm^2. The fuel used was 1 M and 3 M formic acid with 0.5 M sulfuric acid as an electrolyte stream.

With this design, the fuel never comes into direct contact with the electrolyte stream, which allows a higher fuel concentration to be used. Additionally, low ohmic losses were achieved using low anode-to-cathode spacing. Finally, mass transport is more efficient in this design due to a uniform supply of fuel over the anode and bubble removal from the anode active sites. At atmospheric pressure, they obtained a current density of 50 mA cm^{-2} and a power density of 14 mW cm^{-2}. When performed at a gauge pressure of 3 mbar, a limiting current density of 140 mA cm^{-2} was found along with a maximum power density of 29 mW cm^{-2}.

2.3. Microfluidic fuel cells operating with a PEM

Microfluidic fuel cells operating with the PEM are rarely used nowadays due to the disadvantages that they pose, such as water management and fuel cross-over. However, as it is a type of microfluidic fuel cells, it will be briefly discussed in this section. Designs of microfluidic fuel cells operating with the PEM were introduced by Nuzzo[68] and Besser groups.[69]

The performance of a microfluidics-based H_2–O_2 fuel cell with thin film Pt electrodes was studied. The array of electrodes was then fully immersed in a liquid electrolyte. The device had a maximum power density of 700 mW cm^{-2} while operating at room temperature. The maximum power densities were obtained using rough Pt electrodes, with a roughness factor of approximately 90 relative to smooth Pt film. It was observed that the use of PEMs would eliminate the instability problems associated with the dehydration of the ionomer at higher temperatures. It is also suggested that using an elastomer membrane of higher reactant solubility enables a higher power output.

Shah *et al.* introduced a hydrogen–air micro proton exchange membrane fuel cell (PEMFC) on silicon and PDMS substrates. This fuel cell generated a peak power of 0.282 mW at about 0.3 V.[69]

3. Miniature biofuel cells

The term biofuel cell refers to a type of fuel cells in which biocatalysts such as enzymes or microorganisms are used to convert chemical energy into electrical energy. While in conventional microfluidic fuel cells the fuel is oxidized using metal catalysts at the anode, in biofuel cells inexpensive biocatalysts are used for this purpose.[70] The two subtypes of biofuel cells are microbial fuel cells and enzymatic fuel cells. A biofuel cell is considered microbial if living microorganisms are involved, and enzymatic if not.[71]

Human cells are also being used as biocatalysts. Sakai *et al.*[72] have shown the possibility of using human macrophages in a biofuel cells application. NADPH oxidase is an enzyme that enables electron transport across the plasma membrane. Human macrophages were employed in their fuel cells to activate NADPH oxidase and generate current. These devices could be used as batteries for small medical devices implanted in human body such as pacemakers.[73]

3.1. Microbial fuel cells

Microbial fuel cells employ microorganisms as a biocatalyst to generate electricity. It is reported that microbial fuel cells have a high potential of use in the areas of portable devices, wastewater management, and biosensors.[70,74] Compared with enzymatic fuel cells, microbial fuel cells are often more desirable due to the fact that enzymes existing in living cells perform more stably and at a lower cost than purified enzymes.

Microorganisms are inoculated in the anode chamber to generate electricity through their metabolism, while chemical solution in the cathode chamber acts as an electrolyte. The electrons are transferred through a proton exchange membrane between these two chambers. *Shewanella*, *Pseudomonas*, *Proteobacteria*, and *Geobacter* families are the most common electrogenic bacteria employed in microbial fuel cells.[39] Also, *C. butyricum* and *C. beijerinckii* have been reported to successfully generate electricity where starch is the fuel.[33] Multiple culture is used in wastewater based microbial fuel cells.[75] Bacterial reactions have been observed in a wide range of temperature: low temperatures (<15 °C),[76] room temperatures (15–35 °C),[11,40] and high temperatures (50–60 °C).[77]

Microbial fuel cells can operate under mild conditions (room temperature and neutral pH value) with a considerably high power density, long running period without recharging,[1,3,4] environmentally friendly reaction processes,[41] and low cost. It is demonstrated that this type of fuel cell could be miniaturized to the microscale, which is highly useful for medical applications.[11,34,40] Microscale microbial fuel cells are new to this field. The advantages of miniaturized fuel cells include larger power density, shorter start time, and faster power generation recovery after refilling.[34–36]

Recent developments in the field of microscale power devices have been inspired by concepts and technologies from the chemical and biological fields.[1] Since the density of power generation of such reactors depends heavily on the surface-to-volume ratio, miniaturizing fuel cells improves the power density greatly. Microbial fuel cells are usually fabricated by sandwiching electrodes and PEM within two PDMS chambers.[78] Electrodes are one of several factors that affect the power density. Toray carbon paper, carbon cloth,[11] carbon paper,[38,79] graphite rod,[80] graphite fiber brush,[81] graphite paper,[82] Ag/AgCl, sat. KCl, and thin film Pt are among the materials that have been used as electrodes. Nafion 117 is the most used PEM in microbial fuel cell applications.

The PDMS chambers are commonly fabricated using the soft lithography method[25] the same as microfluidic fuel cells. Most microbial fuel cells apply bacteria[40–42] or algae[43] as the electrogenic microbes. Microbial fuel cells employ both pure culture – meaning only one kind of bacterial or algae strain is allowed to grow in a chamber – as well as binary[44] or multiple culture. Both methods of developing cultures derive similar results, however are used in different applications. Multiple culture bacteria, for example, are used in wastewater based microbial fuel cells.[39,75]

The field of miniaturized microbial fuel cells is of interest because of its potential in small scale applications[34–36] such as powering implantable medical devices.[83] A design configuration introduced by Morse's group has a 1.5 μL anode chamber and a 4 μL cathode chamber, making it one of the smallest MFCs created.[40] *Shewanella oneidensis* MR-1 was cultured in the anode chamber as the electrogenic bacteria, while buffered ferricyanide was continuously pumped into the cathode chamber. Gold was used as the anode, while carbon cloth was used as the cathode. For a period of two weeks, a maximum current density of 1300 A m^{-3} and a power density of 15 W m^{-3} were obtained. A newer example of the *S. oneidensis* MR-1 microbial fuel cell introduced by Qian *et al.* had several advantages such as lower cost, easier fabrication process, and reusable configuration.[11] The PDMS chamber was 10 mm × 4 mm × 100 μm (length × width × height) which provided a volume of 4 μL. Carbon cloth was used for both the anode and cathode. Both carbon cloths were fitted into PDMS chambers that were separated by a PEM. The ferricyanide catholyte ($K_3Fe(CN)_6$ in a sodium phosphate buffer) ran through the cathode chamber at a rate of 50 μL h^{-1} using a syringe pump. The nutrient medium was introduced once the current generation dropped to the background value (typically about 10 hours). A 10 kΩ resistor was wired into the system using titanium wires to measure the cross voltage drops, so that current could be measured accurately. The peak current obtained using *S. oneidensis* MR-1 was in the range of 0.6–2.2 μA with a 10 kΩ resistor. The power density of this microbial fuel cell was recorded to be 62.5 W m^{-3} (Fig. 4a and b).

One of the smallest microbial fuel cells with a total volume of 0.3 μL was reported to generate a maximum current density of 92 A m^{-3} using *Geobacter sulfurreducens* and 127 A m^{-3} using *Shewanella oneidensis*.[84]

Choi and Chae have recently presented a high power density microscale microbial fuel cell. This design facilitates an optimum biofilm formation and a minimum oxygen penetration into the anode chamber (Fig. 4c).[85] This fuel cell design had a PEM between two glass chips that were coated with Cr/Au of 20 nm/200 nm. The volume of the cathode chamber was 25.4 μL. Two holes were drilled near the middle of each layer to lay tubing for the transport of fuel and oxidant. Additionally, four holes were drilled through all layers near the border so that the MFC could be screwed tightly using nuts and bolts. The thickness of the PDMS layer was adjusted to obtain the

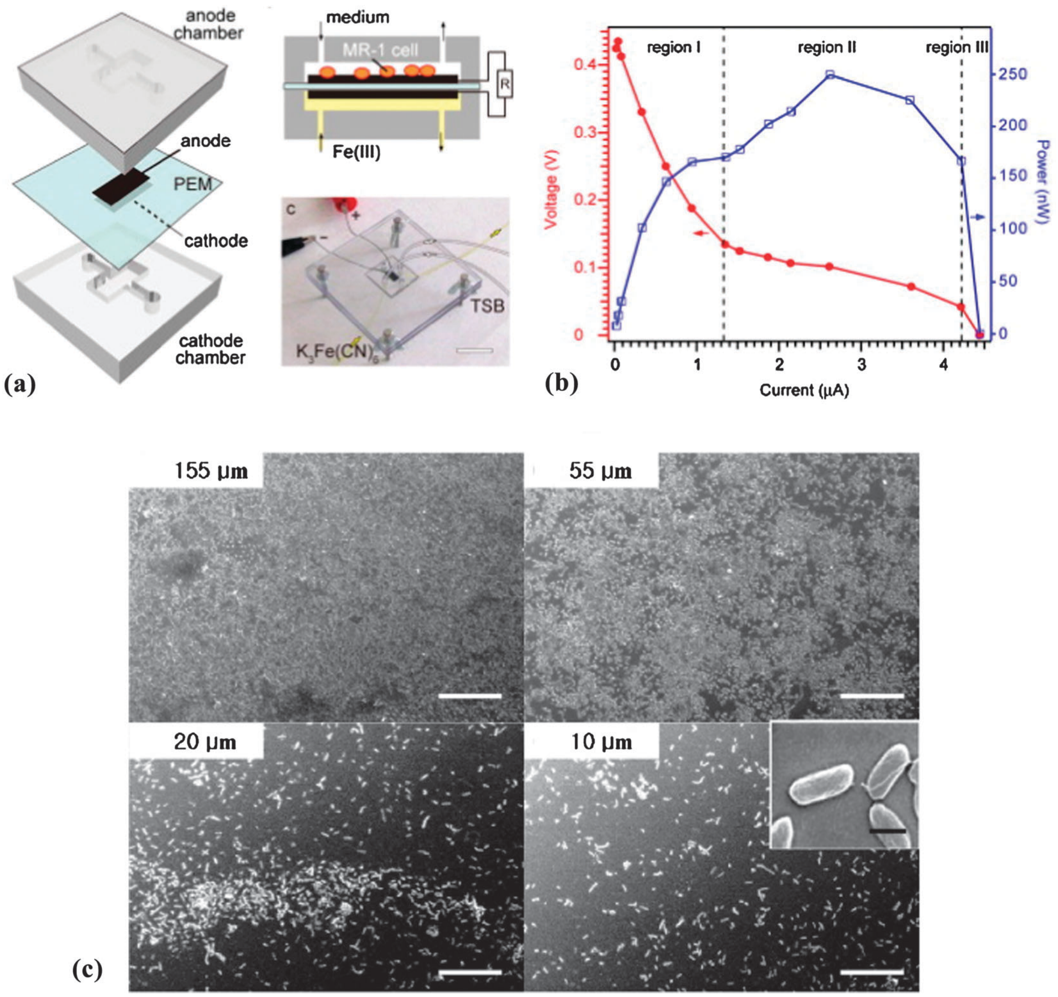

Fig. 4 (a) Schematic, principle, and photograph of the microbial fuel cell. Bacteria in the anode chamber were fed each time the current dropped to the base line, and ferricyanide was pumped into the cathode chamber continuously at a rate of 50 μL h^{-1} using a syringe pump. (b) Polarization curve and output power measured as a function of current for the 4 μL microbial fuel cell.[11] (c) SEM images of the anode surface covered by *Geobacter* sp. for different thicknesses of PDMS spacers for the 25.4 μL MFC. This fuel cell optimized biofilm formation.[85]

maximum power density. Although this fuel cell was relatively expensive to create, with all other fuel cells utilizing gold electrodes, the fuel cell generated a maximum power density of 95 μW cm^{-2} once the thickness of the PDMS layer was 155 μm.

Cooney's group[86] has reported a hybrid lactate–air biofuel cell. The flow-through anode chamber was filled with a porous chitosan–multiwalled carbon nanotube material. *Shewanella* MR-1 was inoculated into the anode chamber. The cathode was air-breathing and enzymatic, and a PEM was used to separate the chambers from each other. A maximum power density of 26 W m^{-3} was reported. One of the main advantages of this type of fuel cell is the flexibility it has in choosing different combinations of anodic bacteria, cathodic enzymes, and fuel (Fig. 5).

3.1.1. Microorganisms and medium. The nutrition provided to the bacteria in microbial fuel cells is an important factor that influences the power output. Most microbial fuel cells require their fuel to be low molecular. This pre-requirement puts macromolecular compounds, such as complex carbohydrates, out of the list of usable biofuels.

To show the feasibility of generating electricity from natural carbohydrate sources, a fuel cell that operates with starch has been presented by Schroder's group.[33] *C. butyricum* and *C. beijerinckii* were used as the biocatalysts to accelerate the reaction due to their ability to digest various substrates and their high hydrogen production rate. The versatility of these bacteria makes them excellent choices for biocatalysts in microbial fuel cells. In this particular work, starch had been used as the fuel for the device, and a three-electrode arrangement which consisted of an additional reference electrode was applied. 25–50 mL of culture was mixed with 100 mL fresh growth medium. The solution needed to be purged with nitrogen for 10 min to remove oxygen before the inoculation. The maximum current generation of 1.3 mA cm^{-2} was reported with starch as the fuel. The current dropped after five hours, and recovered nearly instantaneously when 80% of the bacterial medium was substituted by fresh medium.

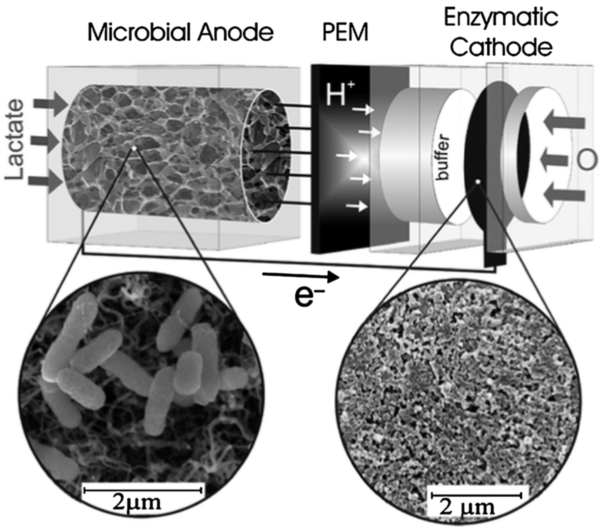

Fig. 5 Schematic of the hybrid fuel cell. *Shewanella* MR-1 on multiwalled carbon nanotubes and the porous carbon black gas-diffusion electrode are shown. Advantages of a hybrid fuel cell include cost reduction with increased volume, flexibility in design, biodegradability, and relatively high stability under small (<5 A m^{-3}) loads.[86]

The pH can also affect the performance of microbial fuel cells. For instance, an acidic medium is beneficial for cathodic oxygen reduction.[87] However, it decreases anodic substrate oxidation. Studying the fuel cell performance at medium pH from 6 to 8 shows that the maximum open circuit voltage and power density are achieved at neutral pH levels of 7 and 6.5, respectively.[88]

3.1.2. Geometry and configuration. A U-shaped microbial fuel cell configuration allows a shorter distance between the anode and cathode electrodes by housing the cathode chamber inside the anode chamber. In a U-shaped MFC introduced by He *et al.*, contact between the biomass and the substrate was enabled by the influent flow and the cathode chamber was constructed by gluing two PEM tubes into a plastic base connector.[42] The cathode was positioned inside of the anode chamber and both chambers were filled with granular activated carbon. A copper wire connected to a graphite rod in the anode chamber and a carbon fiber in the cathode chamber completed the circuit. This particular fuel cell was built with an intention of using it for wastewater treatment, so the fuel it ran on was ground up granular anaerobic sludge from a bioreactor treating brewery wastewater. The fuel was introduced into the fuel cell at a rate of 0.5 mL min^{-1} at 35 °C. This system was able to produce a maximum volumetric power of 29.2 W m^{-3} when fed continuously. One of the advantages of this fuel cell is a relatively lower internal resistance due to the shorter distance between the anode and cathode electrodes as well as the larger surface area of the PEM.

Logan's group has demonstrated a comparison of an air-cathode microbial fuel cell operating with and without a PEM.[38] The MFC had a single cylindrical chamber with a length of 4 cm and a diameter of 3 cm. The anode and cathode were placed opposite to each other in the chamber. Toray carbon paper without wet proofing was used as an anode. The carbon electrode–PEM cathode (CE-PEM) was made by bonding a PEM onto a flexible carbon-cloth electrode covered with a Pt catalyst. After being treated, the PEM was then hot-pressed directly onto the cathode. The cathode used in the absence of the PEM was a rigid carbon paper that contained 70% of the concentration of Pt as the cathode with the PEM. A platinum wire connected the circuit.

Bacteria that are present in wastewater were introduced into the fuel cell as biocatalysts for electricity production. The wastewater had a pH of 7.3–7.6 and a chemical oxygen demand of 200–300 mg L^{-1}. A glucose medium containing minerals and vitamins was used as the fuel to feed the bacteria. Power density in the absence of PEM was reported to be much higher than what was commonly found using aqueous-cathode MFCs. This suggests that the air cathode (rather than an aqueous one) caused an increase in the power output. With the PEM, a maximum power density of approximately 262 mW m^{-2} was obtained while removing the PEM resulted in an increase in the maximum power density to approximately 494 mW m^{-2}. Coulombic efficiency was 40–55% with the PEM and 9–12% without the PEM. This difference was due to the diffusion of oxygen into the anode chamber when the PEM is removed. In summary, comparing the two systems showed that removing the PEM enabled an increase in the amount of maximum power density and a decrease in the Coulombic efficiency of the microbial fuel cells.

3.1.3. Electrode materials and configuration. The power output was found to significantly vary with the choice of electrodes. Many researchers have investigated different electrode materials in order to maximize power density. Logan's group has recently studied the requirements needed in scaling up microbial fuel cells.[89] They set up an air-cathode microbial fuel cell with a volume of 130 mL. Each reactor had three anodes and a cathode. Electrode spacing was minimized using a separator electrode assembly (SEA). The maximum power density was observed to be 975 mW m^{-2} with a maximum Coulombic efficiency of 53%. This shows that SEA type microbial fuel cells can produce a stable power output with a high Coulombic efficiency, making it viable for continuous flow wastewater treatment (Fig. 6).

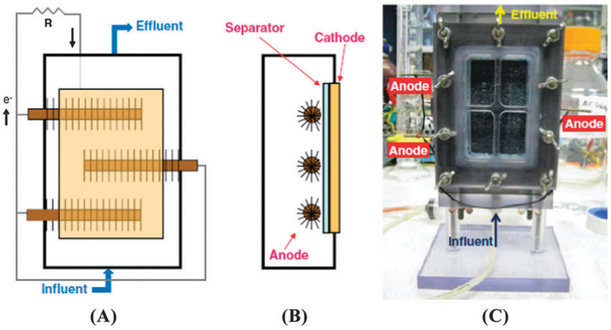

Fig. 6 Schematic of the multi-electrode continuous flow microbial fuel cell with a separator electrode assembly (A) front view, (B) side view, and (C) photograph. Performance of this fuel cell was comparable to that of small-scale MFCs, however the lower ohmic resistance makes it a more viable option to treat low conductivity solutions (such as wastewater).[89]

Table 1 Summary of microbial and microfluidic fuel cells

Volume			Electrodes								
Anode (μL)	Cathode (μL)	Author	Anode	Cathode	Microbe	Anolyte	Catholyte	PEM	P_{max}	V_{max} (V)	I_{max} (mA cm^{-2})
3.6	3.6	Kjeang *et al.*, 2008[2]	Porous carbon strip	Porous carbon strip	—	Vanadium	Oxygen	—	131 mW cm^{-2}	1.246	326
7.5	7.5	Choban *et al.*, 2004[3]	Platinum black	Platinum black	—	O_2/H_2SO_4 or $KMnO_4$	10% formic acid	—	—	0.4/0.55	8
18 000	—	Jayashree *et al.*, 2005[4]	Graphite plate covered with Pd	Toray carbon paper GDE covered with Pt	—	O_2	Formic acid-containing 1 M sulfuric acid	—	26 mW cm^{-2}	—	130
9.9	9.9	Kjeang *et al.*, 2007[56]	Graphite rod	Graphite rod	—	Vanadium redox (VO^{2+})	Vanadium oxidant (VO_2^+)	—	35 mW cm^{-2}	—	92
2800	2800	Mitrovski *et al.*, 2004[68]	Thin film Pt	Thin film Pt	—	0.1 M H_2SO_4/ 0.1 M NaOH/ 1.0 M NaOH	0.1 M H_2SO_4/0.1 M NaOH/1.0 M NaOH	Yes	0.7 mW cm^{-2}	—	—
4	4	Qian *et al.*, 2011[11]	Carbon cloth	Carbon cloth	*S. oneidensis* MR-1	TSB medium	Ferricyanide catholyte	Yes	0.0625 mW cm^{-3}	—	0.01
100 000	100 000	Niessen *et al.*, 2004[33]	Ag/AgCl, sat. KCl	Ag/AgCl, sat. KCl	*C. butyricum* and *C. beijerinckii*	Starch, glucose, molasses	50 mM ferricyanide in pH 7 buffer	Yes	—	0.759	1.3 – starch; 1.1 – molasses; 1.33 – glucose
440 000	210 000	He *et al.*, 2006[42]	Graphite rod	Reticulated vitreous carbon rod and platinum coated carbon paper	Anodophilic microbes	Sucrose	Hexacyanoferrate	Yes	0.0292 mW cm^{-3}	—	—
28 000 (single chamber)	—	Liu *et al.*, 2004[38]	Toray carbon paper	CE-PEM	Wastewater	Glucose medium	Oxygen	Yes	0.0494 mW cm^{-2}	0.52	—
28 000 (single chamber)	—	Kiely *et al.*, 2011[30]	Ammonia-treated graphite fiber brushes	Platinum and four diffusion layers to 30 wt% wet-proofed carbon cloth	Mixed microbial community (wastewater)	Potato processing waste water/animal wastewater	Oxygen	Yes	0.0217/0.0189 mW cm^{-2}	0.4	—
350 000 (single chamber)	—	Ishii *et al.*, 2012[37]	Carbon cloth	Air-cathode	Mixed microbial community (sewage)	Sterilized anaerobic basal medium	Oxygen	—	0.14 mW cm^{-2}	—	1.12
1.5	4	Qian *et al.*, 2009[40]	Gold	Carbon cloth	*S. oneidensis* MR-1	TSB medium	Ferricyanide catholyte	Yes	0.015 mW cm^{-3}	—	—

A microbial fuel cell with an open-pore vitreous carbon foam as both the anode and cathode was introduced by Perrier's group.[90] This fuel cell is a continuous flow-through reactor and has been used for wastewater treatment. This design showed a solid microbial biofilm formation on the carbon surface and provided a maximum power density of 40 W m^{-3}.

Microbial fuel cells have been very successful as lab-scale designs, but similar to microfluidic fuel cells, this type of fuel cells has not been commercially used in real world applications. An effort of making these fuel cells more practical is needed to extend this technology beyond the laboratory. Table 1 summarizes some of the microfluidic and miniature microbial fuel cell designs.

3.2. Enzymatic biofuel cells (*in vitro*)

Enzymatic fuel cells convert chemical energy into electricity through the use of isolated enzymes that metabolize a chemical or compound, inducing a current.[45,91–94] Unlike microbial fuel cells, however, these enzymes are not located inside of living cells. Enzymatic fuel cells can be used as both an implanted power source and a general power generating source, but these fuel cells are not as competitive as conventional power generating devices because of their low power output and poor long-term stability and fuel utilization.[45] An enzymatic fuel cell is also easy to miniaturize[95,96] which makes it an appropriate choice as an implantable power source for biomedical applications. For instance, they can be used as body function monitors or glucose sensors. Compared to conventional fuel cells, enzymatic biofuel cells have simpler designs and are lower in cost, since enzymes exist everywhere in nature. A typical fuel for this type of fuel cell is glucose, which is easily obtained.

The underlying principle of how enzymatic biofuel cells generate power is the same as other fuel cells. Fuel is oxidized in the anode chamber; causing the electrons to move from the higher potential anode to the lower potential cathode. Unlike traditional fuel cells, however, the PEM is not necessary for most enzymatic biofuel cells to operate because enzymes at the anode and cathode are selective with what substrates they react with – eliminating the need for a membrane separator.[97] The types of fuels that can be used are vast, considering this kind of biocatalyst involves chemical reactions that happen every day in living organisms. As the reactions normally take place in living cells, enzymatic biocatalyst reactions happen under mild conditions: near-body temperature and neutral pH value.[98]

Enzymatic biofuel cells have large potential to be an efficient power source in the future, but the disadvantages of low voltage, low current, and lower power density limit their application area for now. Since enzymes are not used for electricity generation in nature, it is difficult to establish electrical communication between proteins and electron surfaces, thus it is not stable enough. Although enzymatic biofuel cells have a number of advantages, their power output is lower than traditional fuel cells, though they still have room for improvement.

Glucose[49,99–101] is the most commonly used fuel for enzymatic biofuel cells; since it is abundant in nature and is often used as an energy source for living organisms, it is the likely first choice. Glucose may be used in aerobic respiration, anaerobic respiration, and fermentation. After releasing energy, it breaks down to CO_2 and H_2O.

Bolan and Leech[96] presented a glucose–oxygen enzymatic fuel cell based on a redox polymer. The difference between using planar or spherical electrodes was investigated. The enzymes were either glucose oxidase at the anode or *Melanocarpus albomyces* laccase at the cathode with a buffer at a pH of 7.4. The electrodes were ordered macroporous, prepared by electro-disposition of gold. It was found that the power density approximately doubled from 17 μW cm^{-2} to 38 μW cm^{-2} when changing from planar electrodes to 2.5 sphere macroporous electrodes.

Fructose[97,102] is another sugar that is widely applied as a fuel in enzymatic fuel cells. Studying the effect of using different temperatures and concentrations of both glucose and fructose, it was found that 0.2 M glucose in 1 M KOH created a maximum power density of 1.38 mW cm^{-2} while 0.2 M fructose in 1 M KOH resulted in a maximum power density of 0.57 mW cm^{-2}. The performance of the cell began to decrease rapidly when above either 0.2 M glucose/fructose or 40 °C. Although in this case glucose appears to be the only logical choice for a fuel, there is still promise for fructose based fuel cells.

Kamitaka *et al.* presented a one-compartment fructose/dioxygen biofuel cell without a separator.[97,103] In this direct electron transfer model of the fuel cell, a maximum power density of 850 μW cm^{-2} was achieved. In addition, it was reported that carbon particle treated electrodes were effective in trapping active enzymes without affecting the mass transfer of the fuel.

The selection of enzyme and electrode is highly important in determining the current–potential characteristics. Ethanol,[104,105] methanol,[106,107] and lactose[89,108] are other fuels that are usually applied in enzymatic fuel cells.

Minteer's group[104] presented a fuel cell with a thirty day lifespan and a power density of 0.46 mW cm^{-2} running on ethanol with an alcohol dehydrogenase enzyme. It was found that using ethanol in enzymatic fuel cells is more efficient than in typical fuel cells, as the particular enzymes are better in breaking it down than noble metal catalysts. In a different experiment, the possibility of using a hydrogen peroxide/methanol fuel cell was studied by Rusek and Prater.[106] Initially, it was attempted to catalyze the reaction with several noble metal catalysts (in this case Ir, Os, Pt, and Pd) however they eventually found that enzymes which behave like peroxidase (which turns hydrogen peroxide into water) created a higher power density – albeit at the cost of requiring a specific environment to operate and with a shorter lifespan of the fuel cell.

In a miniature glucose/O_2 biofuel cell reported by Mao *et al.*, single-walled carbon nanotubes are used to support stability.[109] Under ambient conditions, a power density of 58 μW cm^{-2} was obtained at 0.4 V. Pan *et al.* have investigated the use of nanoscale enzymatic fuel cells.[110] In this fuel cell glucose oxidase was immobilized at the anode using carbon nanotubes, allowing direct electron transfer to the anode with the oxidation of glucose. A proton-conductive nanowire is necessary to transfer protons. In many other cells, a bioelectrolytic solution often serves this purpose, however in this case a Nafion nanowire product is used. A fuel cell of this description can easily be converted into a self-powered glucose or pH sensor with a ZnO nanowire, as well as other *in vivo* nanodevices.

As glucose is the most widely used fuel, glucose oxidase is usually employed as the enzyme. Another enzyme being applied in many enzymatic fuel cells is laccase.

3.3. Implanted biofuel cells operating *in vivo*

Enzymatic fuel cells are a good choice for implanted fuel cells which operate *in vivo.* Development of the enzymatic fuel cell has been slow due to operational difficulties – despite the fact that the concept of implanting fuel cells *in vivo* was suggested long ago.[50] Implanted biofuel cells *in vivo* are still a challenging area of development today. This type of fuel cell has a great advantage in that it can use glucose in the body to generate electricity and could provide long term power for implantable medical devices such as miniature pacemakers or artificial organs.[48,50] Such designs have not been tested in a real human body, however they have been tested in human serum solution.[111]

Electrified clams were introduced by Katz's group[51] as biotechnological living devices. Clams can generate electrical power with biological glucose as fuel. Using this battery (three clam-biofuel cells), a maximum power of 5.2 μW and 37 μW was generated when connected in series and in parallel, respectively (Fig. 7).

These electrodes were inserted between the body wall and the heart of the clam through holes that were cut in the dorso-posterior part of their shells. This experiment was not optimal, however, as the clams weren't operating in a biological environment that is optimal for the enzyme-biocatalyzed reactions. This technology is more environmentally friendly than other fuel cells in that it uses less harmful chemicals. Living batteries such as this one could one day be used in environmental monitoring as well as several security applications.

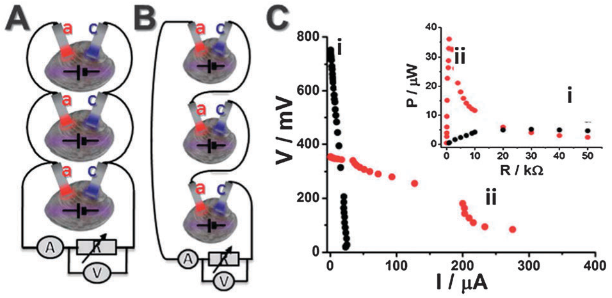

Fig. 7 Biofuel cells operating *in vivo* in clams. Circuitries of three clam-biofuel cells connected in (a) parallel and (b) series. (c) Polarization curves for cells connected in (a) series and (b) parallel. Power as a function of load resistance for the (i) series and (ii) parallel battery connections is also shown. Low resistance micro-power electronic devices such as sensors and wireless information transmitters have potential to be powered in a similar manner, especially with the help of a capacitor to store energy.[51]

Heller's group[52] has reported a compartment-less miniature glucose–O_2 grape biofuel cell, which had two bioelectrocatalyst-coated carbon fibers. The fibers were 7 μm in diameter and 2 cm in length. The electrocatalysts were electrostatic adducts of redox enzymes (polyanions at neutral pH) and electron-conducting redox polymers (polycations). By implanting the fibers in the grapes, the biofuel cell generated a power density of 2.4 μW mm^{-2} at 0.52 V. The power density of this fuel cell was found to be higher than the previous generation[112] of compartment-less glucose–O_2 cell operating under physiological conditions. With further development, a disposable cell such as this one could be used in sensor–transmitter applications as well as monitoring the temperature of a site following a surgery to detect inflammation.

Scherson's group[53] has presented a biofuel cell which has a trehalose–glucose oxidase bienzymatic anode and a bilirubin oxidase cathode. This particular set of enzymes was used to oxidize trehalose present inside an insect with oxygen from the surrounding air. The biofuel cell was implanted into the abdomen of a cockroach. A maximum power density of 55 μW cm^{-2} at 0.2 V was achieved, with a very small decrease over two and a half hours. Unfortunately, unless it is operating intermittently, the lower power output would not meet the requirement of any high power microscale device. Still, efforts are being made to create a more compact and versatile Tr–O_2 biofuel cell.

Cinquin *et al.*[113] have studied an enzymatic fuel cell implanted in the abdomen of rats. They surgically implanted the fuel cell into the retroperitoneal area of a rat. After the rat recovered from anesthesia and was allowed to roam freely, the fuel cell obtained a maximum power of 6.5 μW which slowly decreased over time. For powering small implanted medical devices, such as pacemakers which require about 10 μW, the biofuel cell would have to provide constant and effective power over extended periods of time. There is clearly improvement to be made in this area. However the success of this type of biofuel cells is quite promising for the future.

4. Future directions

Microscale biological and electrochemical fuel cells could be used to meet the increasing demand for high power density energy storage devices with small footprints. In particular, biological microfluidic fuel cells can be designed to be completely biodegradable so they could be used as non-permanent power sources in the environment without pollution. Microbial fuel cells also offer a means of extracting energy from the otherwise untapped source of wastewater. Microbial fuel cells that are inoculated with appropriate bacteria can oxidize the particles in wastewater and produce green energy and clean water without contaminants simultaneously.

Fuel cells implanted into human or animal bodies could power devices from the glucose in their blood. Pacemakers, for example, require replacement surgery every five to fifteen years. Further progress to increase the stability and lifespan of glucose-based microbial or enzymatic fuel cells could make them a viable solution to eliminate the need for replacement surgeries for this and other medical devices.

Finally, developments in the area of fuel cells could ultimately result in replacing standard batteries with miniaturized fuel cells that are capable of storing more energy in the same volume. Laboratory fabricated MFCs already surpass the energy density of common batteries. For this reason, it is only a matter of time before MFCs are standardized and begin to replace batteries as power demands for portable devices rise. These devices may be applied to power *in vivo* sensors in remote areas or implanted medical devices.

However, the realization of miniaturized fuel cells faces several challenges that must be overcome to find market applications. At this time, the amount of power supplied by fuel cells of this size is not adequate to satisfy realistic energy demands. This is primarily limited by the anodic surface area, and requires further research in device architecture and novel catalysts. Miniature biofuel cells also struggle with long term and consistent power supply, which would require the addition of capacitors to store charge or further investigation of novel catalysts.

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