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Recent Development of Miniatured Enzymatic Biofuel Cells

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1. Introduction

The global energy demands have increased significantly every year and current reliance on fossil fuels is unsustainable due to finite supplies from environment. In addition, the products from using fossil fuels cause pollution and global warming. Fuel cells offer an alternative solution to this issue. A fuel cell is an electrochemical cell that converts chemical energy from a fuel to electrical energy. In a fuel cell, an oxidation reaction occurs at the anode generating electrons that transfer to the cathode through the external circuit and a reduction reaction occurs at the cathode. Conventional fuel cells, for example, can be operated by using hydrogen or methanol (MeOH) as fuels to produce energy, releasing water and carbon dioxide as by-products. However, hydrogen is gaseous which gives rise to safety issues in storage and transport. Besides, many of the alternative fuels that can be used for fuel cells still rely on petroleum products. Therefore, it is well recognized that alternative sources of renewable energy are urgently required. Numerous efforts have been made to develop different power sources alternatives that are capable of performing in physiological conditions for prolonged lifetime without recharging. More recently, miniaturized medical implants such as pacemaker, defibrillator, insulin pumps, sensor-transmitter systems for animals and plants, nano-robots for drug delivery and health monitoring systems gain increasing attention which led to an upsurge in research and development in micropower source, especially, biofuel cells (Ramanavicius, 2005; Liu & Dong, 2007; Zhu et al., 2007; Moehlenbrock & Minteer, 2008 and Wang et al., 2009). Biofuel cell is a particular kind of fuel cell, which converts biochemical energy to electrical energy by using biocatalysts (Palmore & Whitesides, 1994). The two major types of biofuel cells are microbial fuel cells and enzymatic biofuel cells. Microbial fuel cells employ living cells such as microorganism as the catalyst to convert chemical energy into electricity while enzymatic biofuel cells use enzymes to catalyze the redox reaction of the fuels. In this chapter, we will first introduce both kinds of biofuel cells along with the type of catalysts used, electron transfer mechanism, electrode materials and cell performance. Then we will briefly review recent progress in miniaturized biofuel cells, which offer possibilities for implantable devices within the human body. Carbon-microelectromechanical system (C-MEMS) based miniaturized enzymatic biofuel cells are also highlighted in the chapter.

1.1 History

The earliest discovery between biology and electrical energy was demonstrated by Galvani in 1791 showing the frog leg twitching from an electric current (Galvani, 1791). The first fuel

cell, which involved electrolysis of water, was discovered by Grove in 1839. An electrical stimulation can induce a biological reaction and vice-versa a biological process can also generate electricity. The first half-cell using microorganism (E.coli) was demonstrated by Potter at University of Durham (Potter, 1910). Further development of half-cell by Cohen from University of Cambridge led to one of the major types of biofuel cells, i.e., microbial fuel cells. Cohen applied a number of microbial half-cells connected in series, which generated over 35 volts (Cohen, 1931). In the late 1950s and early 1960s, the interest in development of biofuel cells received a boost by the USA space program, which led to the application of microbial fuel cells as an advanced technology for waste disposal treatment in space flights. Also, in the late 1960s, a biofuel cell using cell-free enzyme systems was discovered aiming to permanently power medical implants by utilizing specific body fluids as fuel (Yahiro et al., 1964). The concept of using microorganism as a biocatalyst in microbial fuel cells was widely applied since the 1970s (Suzuki, 1976 and Roller et al., 1984) and in the 1980s it was found out that power output could be greatly improved by using electron mediators (Vega & Fernandez, 1987; Habermann & Pommer, 1991 and Allen & Bennetto, 1993). However, the toxicity and instability of mediators limited the cell performance. A breakthrough was made when some microorganisms were found to transfer electrons directly to the electrode which led to the mediator-less microbial fuel cells first used in wastewater treatment and electricity generation (Kim et al., 1999; Chaudhuri & Lovley, 2003). These microorganisms are stable and yield a high Coulombic efficiency which facilitates the direct electron transfer (Scholz & Schroder, 2003). Shewanella putrefaciens (Kim et al., 2002), Geobacteraceae sulferreducens (Bond & Lovley, 2003), Geobacter metallireducens (Min et al., 2005) and Rhodoferax ferrireducens (Chaudhuri & Lovley, 2003) are all bioelectrochemically active microbes and can transfer electrons directly through the membrane. On the other hand, since the first enzymatic biofuel cell was reported in 1964, noticeable developments have been made in the terms of the power density, cell lifetime, operational stability (Bockris & Srinivasan, 1969; Govil & Saran, 1982 and Palmore & Whitesides, 1994). However, the output potential generated from enzymatic biofuel cells was still far beyond the demand of commercial application. Therefore, instead of considering enzymatic biofuel cells as a conventional power source, most of the researches on enzymatic biofuel cells have been aimed toward special applications such as implantable medical devices (Katz & Willner, 2003; Barton et al., 2004 and Heller, 2004). In the past ten years, cell performances on both types of biofuel cells have been improved significantly and we will discuss the detailed development in the following sessions.

1.2 Microbial fuel cells

A microbial fuel cell (MFC) converts chemical energy to electrical energy by the catalytic processes of microorganisms. Microorganisms in the MFC oxidize organic substrates and generate both electrons and protons on the anode. Electrons transfer from the anode to the cathode through an external circuit and simultaneously the protons migrate to the cathode and reduce the oxygen with the electrons available at the cathode surface. Various kinds of microorganisms are reported in association with electrodes in MFC systems. For example, brevibacillus sp. PTH1 is one of the most abundant microorganisms in a MFC system. Pure cultures used for generating current in a MFC include firmicutes, acidobacteria, proteobacteria and yeast strains Saccharomyces cerevisiae and hansenula anomala (Allen & Bennetto, 1993). These microorganisms interact with fuels through a variety of direct and indirect processes to generate energy. Microbial biofuel cells have major advantage of

thorough oxidation of the fuels due to the use of microorganism as catalyst system and they can be typically operated for long lifetimes. Besides, a MFC has no intermediated processes thus it is a very efficient energy producing process. In addition, as a fuel cell, a MFC does not need charging during operation (Willner et al., 1996; Katz et al., 2003 and Calabrese et al., 2004). However, the bottlenecks of MFC still remain. Power generation of a MFC is affected by many factors including microbe type, fuel biomass type and concentration, ionic strength, pH, temperature, and reactor configuration.

The principle cell performance of MFCs lies in the electron transfer from microbial cells to the anode electrode. The direct electron transfer from the microorganism to electrodes is hindered by overpotential due to transfer resistance. The overpotential lowers the potential of a MFC and significantly affects the cell efficiency. In this case, the practical output potential is less than ideal because the electron transfer efficiency from the substrate to the anode varies from microbe to microbe. Microorganism species do not readily release electrons and hence the redox mediators are needed. A desirable mediator should have a whole range of properties: Firstly, its potential should be different from the microorganism potential to facilitate electron transfer. Secondly, it should have a high diffusion coefficient in the solution. Lastly, it is suitable for repeatable redox cycles in order to remain active in the electrolyte. Widely used Dye mediators such as neutral red (NR), methylene blue (MB), thionine (Th), meldola's blue (MelB) and 2-hydroxy-1,4-naphthoquinone (HNQ) can facilitate electron transfer for microorganism such as Proteus, Enterobacter, Bacillus, Pseudomonas and Escherichia coli. In the electron transfer process, these mediators are reduced by interacting with electron generated within the cell then these mediators in reduced form diffuse out of the cell to the anode surface where they are electrocatalytically oxidised. The oxidised mediator is then capable to repeat this redox cycle.

Better performing electrodes can improve the cell performance of a MFC because different anode materials can result in different activation of a polarization loss, which is attributed to an activation energy that must be overcome by the reactants. Carbon or graphite based materials are widely used as electrodes due to their large surface area, high conductivity, biocompatibility and chemical stability according to Table 1. Also, platinum and gold are popular as electrode system although they are expensive. Compared with carbon based electrode materials, platinum and gold electrodes are superior in the performance of the cells based on the Table 1. Besides, they have a higher catalytic kinetics towards oxygen compared to carbon based materials and hence the MFCs with Pt based cathodes yielded higher power densities than those with carbon based cathodes (Moon et al., 2009).

Electrode modification is another way to improve MFC performance of cells. (Park & Zerkeis, 2003) reported an increase of 100-folds in current compared to the previous results by using (neutral red) NR-woven graphite and Mn^{4+}-graphite anode instead of the woven graphite anode alone. Four times higher current was reported in 2004 using the combination of Mn^{4+}-graphite anode and Fe^{3+}-graphite cathode (Niessen et al., 2004). NR and Mn^{4+} doping ions serve as mediators in their MFC systems and also catalyze the cathodic reactions to facilitate electricity generations. Electrodes modifications including adsorption of AQDS or 1,4-naphthoquinone (NQ) and incorporation with Mn^{2+}, Ni^{2+}, Fe_3O_4 increased the cell performance of MFCs in their long-term operations (Lowy et al., 2006). In addition, the fluorinated polyanilines, poly (2-fluoroaniline) and poly (2, 3, 5, 6-tetrafluoroaniline) outperformed polyaniline were applied for electrode modification (Niessen et al., 2006). These conductive polymers also serve as mediators due to their structural similarities to conventional redox mediators.

Fuel	Organism	Electrode (cm^2)	Electron transfer	OCV (V)	Current density ($\mu A\ cm^{-2}$)	Reference
Sugar/ferricyanide	Suspended Proteus vulgaris/anaerobic	RVC anode (30.4), platinum cathode (16)	MET	0.52	5.26	Kim et al. (2000)
Glucose/ferricyanide	Suspended E. coli/ anaerobic	Woven graphite	MET	0.85	5.3	Park & Zeikus (2000)
H_2/O_2	Desulphovibrio vulgaris,	Carbon felt mat (5.1)	MET	1.17	176	Tsujimura et al. (2001)
Lactate/O_2	Suspended Shewanella putrefaciens and E. coli , anaerobic	Graphite felt (56)	DET	0.5	0.02	Kim et al. (2002)
Marine sediment con-stituents/ seawater constituents	Mixed natural bacteria	Drilled graphite discs	DET & MET	0.75	3.2	Tender et al. (2002)
Lactate/O_2	Suspended Shewanella putrefaciens/ anaerobic	Mn^{4+} graphite plate (80) anode, Fe^{3+} modified graphite plate cathode (50)	MET	0.6	0.94	Park & Zeikus (2002)
Glucose/ferricyanide	Rhodoferax ferrireducens/ anaerobic	Graphite rod (0.65)	DET	N/A	N/A	Chaudhuri & Lovley (2003)
Glucose /O_2	Suspended E. coli/ anaerobic	NR-woven graphite (80) or Mn^{4+}graphite plate anode (80), woven graphite or Fe^{3+} graphite plate (80) cathode	MET	N/A	N/A	Park & Zeikus (2003)
Glucose /O_2	Mixed culture	Graphite plate electrodes (50)	MET and DET	N/A	36	Rabaey et al. (2003)
Glucose/ ferricyanide	Suspended E. coli/ anaerobic	Woven graphite cloth	DET	0.895	120	Schroder et al. (2003)
Glucose/ ferricyanide	Clostridium butyricum	Woven graphite cloth	MET	0.759	200	Niessen et al. (2004)

Fuel	Organism	Electrode (cm^2)	Electron transfer	OCV (V)	Current density ($\mu A\ cm^{-2}$)	Reference
Glucose/ ferricyanide	Mixed culture	Graphite plate electrodes (50)	MET & DET	N/A	231	Rabaey et al. (2004)
Glucose/ ferricyanide	Shewanella oneidensis DSP-10	Graphite Felt (610)	DET	N/A	110	Ringeisen et al. (2006)
Glucose /O_2	Saccharomyces cevevisiae	Gold (0.51)	MET	N/A	15	Chiao et al. (2006)
Glucose /O_2	Mixed bacterial culture	Carbon cloth (7)	MET	N/A	90	Fan et al. (2007)
Glucose/ ferricyanide	Geobacter sulfurrenducens	Pt (7.8)	DET	N/A	688	Richter et al. (2008)
Glucose /O_2	Shewanella oneidensis MR-1	Pt (1.2)	MET	N/A	302	Siu & Chiao (2008)
Glucose/ ferricyanide	Shewanella oneidensis MR-1	Pt (0.49)	MET	N/A	370	Hou et al. (2009)
Glucose/ ferricyanide	Shewanella oneidensis MR-1	Gold (0.15)	MET	N/A	130	Qian et al. (2009)

Table 1. Summary of microbial biofuel cells

Proton exchange membrane (PEM) can also significantly affect a MFC system's internal resistance and concentration polarization loss because the internal resistance of MFC decreases with the increase in the PEM surface area (Oh & Logan, 2006). Nafion (DuPont, Wilmington, Delaware) is the most popular proton exchange membrane material due to its highly selective permeability of protons (Min et al., 2005). Compared with the performance of MFC using a PEM or a salt bridge, the power density using the salt bridge MFC was 2.2 mW/m^2 that was an order of magnitude lower than that attained using Nafion. However, side effect is unavoidable with the use of PEM. For example, the concentration of cation species such as Na^+, K^+, NH^{4+}, Ca^{2+}, Mg^{2+} is much higher than that of proton so that transportation of cation species dominates. In this case, Nafion used in the MFCs is not an efficient proton specific membrane but actually a cation specific membrane (Rozendal et al., 2006). Subsequent studies have implied that anion-exchange or bipolar membranes has better properties than cation exchange membranes regarding to cell performance (Zhang et al., 2009).

Two promising applications of MFCs in the future are wastewater treatment and electricity generation (Feng et al., 2008 and Katuri & Scott, 2010). Although some noticeable development has been made in the MFC research, there are still a lot of challenges to be overcome for large-scale applications. The primary challenge is how to improve the cell performance in terms of power density and energy efficiency. In addition, catalytic effect of bioelectrodes needs to be further enhanced to solve the problems caused by enzyme activity loss and other degradation processes. Moreover, the lifetime of the MFC must be significantly improved.

1.3 Enzymatic biofuel cells

Enzymatic biofuel cells (EBFCs) utilize redox enzymes such as glucose oxidase (GOx), laccase as the catalysts that can facilitate the electron generation between substrates and electrode surface, hence generating the output potential. There are two types of electron transfer mechanisms which are direct electron transfer (DET) and mediator electron transfer (MET). In DET based EBFCs, the substrate is enzymatically oxidized at the anode, producing protons and electrons which directly transfer from enzyme moleculars to anode surface. At the cathode, the oxygen reacts with electrons and protons, generating water. However, DET between an enzyme and electrode has only been reported with several enzymes such as cytochrome c, laccase, hydrogenase, and several peroxidases (Schuhmann, 2002 and Freire et al., 2003). Some enzymes have nonconductive protein shell so that the electron transfer is inefficient. To overcome this barrier, MET was used to enhance the transportation of electrons. The selection and mechanism of MET in EBFCs are quite similar to those of MFCs that are discussed before. Similarly, there are still some challenges in using MET in EBFCs, such as poor diffusion of mediators and non-continuous supply. Therefore, modification of bioelectrodes to realize DET based EBFCs attracted most attention. In EBFCs system, power density and lifetime are two important factors which determine the cell performance in the application of EBFCs. Significant improvements have been made during the last decade (Katz et al., 2003; Calabrese et al., 2004; Zhu et al., 2007; Moehlenbrock & Minteer, 2008; Wang et al., 2009; Lee et al. 2010 and Saleh et al. 2011). Noticeably, these advancements have been mostly achieved by modification of electrode with better performance, improving enzyme immobilization methods as well as optimizing the cell configuration. Recent development of enzymatic biofuel cells is shown in Table 2.

The performance of electrodes for EBFCs mainly depends on: electron transfer kinetics, mass transport, stability, and reproducibility. The electrode is mostly made of gold, platinum or carbon (Katz & Willner 2003). Besides these conventional materials, biocompatible conducting polymers are widely used because they can facilitate electron transfer and co-immobilize the enzymes at the same time (Schuhmann & Muenchen, 1992; Haccoun et al., 2006 and Nagel et al. 2007). In order to maximize the cell performance, mesoporous materials have been applied in many studies because of their high surface areas thus high power density could be achieved. Moreover, many attempts using nanostructures such as nanoparticles, nanofibers, and nanocomposites as electrode materials have also been made to fabricate electrodes for EBFCs. The large surface area by using these nanostructures leads to high enzyme loading and enables to improve the power density of the cells. Recently, one of the most significant advances in EBFCs is electrode modification by employing carbon nanotubes. (Wang et al.2009; Lee et al. 2010; Tanne et al. 2010 and Saleh et al. 2011.) Several research activities have addressed the application of single wall carbon nanotube hybrid system. The oriented assembly of short SWNT normal to electrode surfaces was accomplished by the covalent attachment of the CNT to the electrode surface. It was reported that surface assembled GOx is in good electric contact with electrode due to the application of SWNT, which acted as conductive nanoneedles that electrically wire the enzyme active site to the transducer surface. Other studies have been reported on improving electrochemical and electrocatalytic behavior and fast electron transfer kinetics of CNTs. Improved enzyme activity was observed in comparison to similar enzyme-containing composites without using SWNTs. It was discussed that the application of SWNTs, which

Fuel	Enzyme	Electrode	Electron transfer	OCV (V)	Current density ($\mu A\ cm^{-2}$)	Reference
Glucose/ O_2	GOx/laccase	Carbon fiber electrodes	MET	0.8	64	Chen et al. (2001)
Glucose/ O_2	GOx/BOx	Carbon fiber electrodes	MET	0.84	432	Mano et al. (2002)
Glucose/ O_2	GDH/BOx	Glassy carbon disc electrodes	MET	0.44	58	Tsujimura et al. (2002)
Glucose/ O_2	GOx/COx	Gold electrodes coated with Cu	MET	0.12	4.3	Katz & Willner (2003)
Glucose/ O_2	GOx/BOx	Carbon fiber electrodes	MET	0.68	50	Kim et al. (2003)
Glucose/ O_2	GOx/BOx	Carbon fiber electrodes	MET	0.8	440	Mano et al. (2003)
Glucose/ O_2	GOx/BOx	Carbon fiber electrodes	MET	0.63	244	Mano & Heller (2003)
Glucose/ O_2	GOx/laccase	Carbon fiber electrodes	MET	1.0	350	Heller (2004)
EtOH to CH_3CHO to CH_3COOH	ADH, ADH +AldDH, formaldehyde dehydroge-nase + FDH	Carbon coated with poly(methylene)	MET	0.62	1160	Akers et al. (2005)
Glucose/ O_2	PLL-VK3 / PDMS	Pt	MET	0.55	130	Togo et al. (2007)
Ethanol/H_2O_2	QH-ADH/AOx	Pt	DET	0.24	30	Ramanavicius et al. (2008)
Glucose/ O_2	GDH/PDMS	Pt	DET	0.80	11000	Sakai et al. (2009)
Glucose/ O_2	GOx/laccase	Silicon/ SWNTs	DET	N/A	30	Wang et al. (2009)
Glucose/ O_2	GOx/laccase	Au/SWNTs	DET	0.46	960	Lee et al. (2010)
Glucose/ O_2	PQQ-GDH/BOD	Au/MWNTs substrates	DET	0.60	200	Tanne et al. (2010)
Glucose/O_2	GDH/NB	Glass carbon/ SWNTs	DET	0.35	100	Saleh et al. (2011)

Table 2. Summary of enzymatic biofuel cells.

possesses a high specific surface area, may effectively adsorb enzyme molecules and retain the enzyme within the polymer matrix, whereas other forms of enzyme-composites may suffer from enzyme loss when they were placed in contact with aqueous solutions. Although recent advancement in modification of electrodes appears to be promising due to the improvement of cell performance obtained, biocompatibility and nanotoxicity need to be further studied and addressed.

Successful immobilization of the enzymes on the electrode surface is considered as another critical factor that affects cell performance. The immobilization of enzyme can be achieved physically or chemically. There are two major types of physical methods, physical absorption and entrapment. The first one is to absorb the enzymes onto conductive particles such as carbon black or graphite powders. For example, hydrogenase and laccase were immobilized by using physical absorption on carbon black particles to construct composite electrodes and the EBFCs could continuously work for 30 days. Another physical immobilization method is based on polymeric matrices entrapment, which usually shows more stabilized enzyme immobilization (Mano et al., 2002; Mano et al., 2003, Heller, 2004 & Soukharev et al., 2004). For example, Soukharev utilized redox polymers to fabricate enzymatic biofuel cells system. The electrodes were built by casting the enzyme-polymer mixed solution onto 7 μm diameter, 2 cm length carbon fibers. It showed that the glucose–oxygen biofuel cell was capable of generating a power density up to 0.35 mW/cm^2 at 0.88 V (Soukharev et al., 2004). Compared with the physical immobilization which is unstable during the operation, the chemical immobilization methods with the efficient covalent bonding of enzymes and mediators are more reliable. Katz et al. reported a biofuel cell using co-immobilized enzyme-cofactor-mediator composites on metal electrodes to functionalize the electrode surface with a monolayer then integrate with enzymes via bioaffinity (Willner et al., 1998; Katz et al., 2001 and Katz et al., 2003). Another example is that a redox monolayer was covalently grafted with pyrroloquinoline quinone (PQQ) to Au-electrode. Then GOx-FAD electrode was assembled with PQQ as mediators (Willner et al., 1996). Other widely used materials to functionalize electrode surface have also been reported, such as nitrospiropyran (Blonder et al., 1998), rotaxane (Katz et al., 2004), C-60 (Patolsky et al., 1998) and Au nanoparticles (Xiao et al., 2003).

Rapid development on EBFCs has been achieved in the past decade with the arised demands for reliable power supplies for implantable medical device. It has shown particular advantages over conventional batteries because of the specific biocatalysts and the possibility of miniaturization. However, there are still challenges for further development of long term stability of the enzymatic bioelectrodes and efficient electron transfer between enzymes and electrode surfaces. Recent efforts have been given to protein engineering, reliable immobilization method and novel cell configuration.

2. Miniature biofuel cells

Miniature power systems using biocatalysts have received increased attention associated with demand for micro-scale power supplies for implantable medical devices. Development of miniature biofuel cells offers a great opportunity to serve as long-term power sources in implantable device where frequent replacement of battery is not practical. The ability of biocatalyst in converting available indigenous fuels into electrical energy makes miniature biofuel cells attractive to enable long-term and self-sustained power system. The success of medical implants is akin with the effective miniaturization of power sources. This can be

achieved by miniaturization of different functional components such as electrodes, power supply, and signal processing units. Some of the effective techniques for miniaturization involve fabricating microfluidic systems using photolithography, etching, polymer molding, and metal deposition (Kim et al., 2008). For example, Siu and Chiao (Siu & Chiao, 2008) applied photolithography and polymer molding to fabricate polydimethylsiloxane (PDMS) electrodes. It was also used by Hou et al. (Hou et al., 2009) to fabricate gold electrode arrays for the microbe screening. Besides polymer molding, etching can also be used to transfer micro-patterns onto device-building substrate. Chiao (Chiao et al., 2006) applied wet etching to construct silicon-based chambers containing serpentine channels. Additionally, C-MEMS microfabrication technique for 3D microstrustures, involving the pyrolysis of patterned photoresist has been developed which can be used as microelectrodes for miniature biofuel cells (Wang & Madou, 2006). With current microfabrication processes, the miniature biofuel cells offer unique advantages such as large surface area to volume ratio, short distance between the electrode, fast response time and low Reynolds number. In the following section, we will discuss the developments of both miniature MFCs and EBFCs.The experimental demonstration of miniature biofuel cells, along with the discussion of the key challenges and opportunities for realizing the practical potential of miniaturized biofuel cells for medical implants will be discussed.

2.1 Miniature microbial biofuel cells and its state of the art

One of the early efforts on miniature microbial biofuel cells reported a surface power output of 0.023 mW/m^2 and current density of 150 mA/m^2 based on the 10μm diameter circular anodic electrode (Chiao et al., 2006). The miniature microbial biofuel cells were limited by relatively low volumetric power density and coulombic efficiency due to their high internal resistances. Compared with macro scale microbial biofuel cells using the same microbes (Ringeisen et al., 2006), miniature microbial biofuel cells generated similar volumetric current density but significantly lower volumetric power density, which is insufficient for the anticipated applications. It was pointed out that the internal resistances of miniature microbial biofuel cells were around 40 fold higher than that in the macro scale microbial biofuel cells. The ohmic loss was higher in the micropillar devices with the same catholyte and anolyte; however, they generated higher volumetric power density (32 A/m^3) than the serpentine-channel devices (0.5 A/m^3) (Ringeisen et al., 2006). The high surface area-to-volume ratio and good microbe adaptivity of the micropillar electrodes decreased the anode resistance and resulted in higher volumetric power output. Carbon based anodes are known for high surface area-to-volume ratio and easy adaptation of microorganism and they are widely used in macro scale microbial biofuel cells. The recent investigations using carbon nanotubes (CNT) as electrodes (Qiao et al., 2007 and Timur et al., 2007) provide promising solutions for constructing carbon-based anodes in miniature microbial biofuel cells. The CNT based electrodes showed great improvement in the electricity generation and biocompatibility. Its maximum power density was 42 mW/m^2 using E.coli as the microbial catalyst.

In the pursuit to improve the miniature microbial biofuel cell performance, different strategies were employed such as increasing the anode surface area, improving coupling of microorganism to anode surface, developing electrochemically active microbes and decreasing proton diffusion resistance. In summary, the enhancement strategies resulted in enhanced mass transport, improved reaction kinetics, and reduced ohmic resistance. Based

on these developments, the ability to generate sufficient current and power from miniature devices was realized, thus breaking the conventional concept that small scale microbial biofuel cells would perform unsatisfactorily due to limited amount of substrates and microorganism. Since the development of the first miniature microbial biofuel cells in 2006, the volumetric power density and coulombic efficiency have been increased over 5 times. Although the output potential from the miniature MFCs is still insufficient for powering conventional equipment, they are promising options for on-chip power sources, especially for medical implants, which only require several millivolts to operate. Given the evidence that volumetric current density of the miniature MFC was achieved to be 2400 mA/m^3 and required power from the cell was therefore 960 mW/m^3, which is sufficient for existing devices (Wang & Lu, 2008). However, higher current density can result in excessive ohmic heating and electrolysis during the operation. Therefore, study in optimizing current density, overall output voltage and stability of the miniature MFCs as well as electrode design and device configuration for implantation rejection, microbe leakage, and analysis of the composition and distribution of internal resistances is necessary before further implementation in practical applications.

2.2 Miniature enzymatic biofuel cells and its state of the art

The first micro-sized enzymatic biofuel cells reported in 2001 (Chen et al., 2001). A glucose/O_2 biofuel cell consisted of two 7 μm diameter, 2 cm long electrocatalyst-coated carbon fibers operating at ambient temperature in a pH 5 aqueous solution. The areas of the anode and the cathode of the cell were about 60 times smaller than those of the smallest reported fuel cell and 180 times smaller than those of the previously reported smallest biofuel cell. The power density of the cell is 64 $\mu W/cm^2$ at 23 °C and 137 $\mu W/cm^2$ at 37 °C, and its power output is 280 nW at 23 °C and 600 nW at 37 °C. The results revealed that the miniature enzymatic biofuel cells could generate sufficient power for small power-consuming CMOS circuit. Later, a miniature enzymatic biofuel cell with the same carbon fibers operating in a physiological buffer was reported (Mano et al., 2002). In a week operation the cell generated 0.9 J of electrical energy while passing 1.7 C charge. Based on this result, Mano developed a miniature compartment-less glucose/O_2 biofuel cell operating in a living plant. Implantation of the fibers in the grape leads to an operating biofuel cell producing 2.4 μW at 0.52 V, which is adequate for operation of low-voltage CMOS/SIMOX integrated circuits. The performance of the miniature enzymatic biofuel cell was upgraded to 0.78 V operating at 37 °C in ph 5 buffer later on (Mano et al., 2003). In 2004, a miniature single-compartment glucose/O_2 biofuel cell made with the novel cathode operated optimally at 0.88 V, the highest operating voltage for a compartmentless miniature fuel cell (Soukharev et al., 2004). The enzyme was formed by "wiring" laccase to carbon through an electron conducting redox hydrogel, its redox functions tethered through long and flexible spacers to its cross-linked and hydrated polymer, which led to the apparently increased electron diffusion coefficient. The latest report on miniature glucose/O_2 biofuel cells demonstrated a new kind of carbon fiber microelectrodes modified with single-wall carbon nanotubes (CNTs) (Li et al., 2008). The power density of this assembled miniature compartment-less glucose/O_2 BFC reaches 58l Wcm^{-1} at 0.40 V. When the cell operated continuously with an external loading of 1 M resistance, it lost 25% of its initial power in the first 24 h and the power output dropped by 50% after a 48 h continuous work. Although from the practical application point of view, the performance and the stability of the current

emzymatic biofuel cells remain to be improved, the miniature feature and the compartment-less property as well as the tissue-implantable biocapability of enzymatic biofuel cell essentially enable the future studies on in vivo evaluation of the cell performance and stability in real implantable systems.

In an effort to miniaturize the EBFCs, we have developed a versatile technique based on C-MEMS process for the miniaturization of electrodes. Our research focuses on the fabrication of 3D microelectrodes for miniature enzymatic biofuel cells. First, the functionalization methods for EBFCs enzyme immobilization were studied. Then we apply finite element approach to simulate the miniature EBFCs to attain the design rule such as electrode aspect ratio, configuration as well as orientation of the chip. Building an EBFC based on the design rule we obtained is on-going.

3. C-MEMS 3D architecture electrodes

The surface area of biofuel cells determines its amperage, meaning that cell power is directly proportional to the electrode surface area. A conventional 2D power system is typically a parallel arrangement of a planar cathode and an anode separated by a solid or liquid electrolyte. More recently, carbon-microelectromechanical (C-MEMS) fabrication technology has offered the flexibility to fabricate complex carbon-based EBFCs, with 3D dense microelectrode arrays. C-MEMS, describes a manufacturing technique in which carbon microstructures are fabricated by baking UV sensitive polymers at high temperatures in an inert environment. It has been demonstrated that 3D high-aspect-ratio carbon structures can be made from carbonizing (pyrolysis) patterned NANO™ SU-8 negative photoresist (Wang et al., 2005). The four steps involved in converting organic polymer to pyrolytic carbon is shown as schematic in Fig. 1. Positive photoresist (AZ4620, AZ1518) as well as negative photoresist (SU-8) can be converted to carbon by pyrolysis depending on the application. Electrodes based on 3D microstructures are expected to offer higher surface area and significant advantages in comparison to thin-film devices for powering MEMS and miniaturized electronic devices.

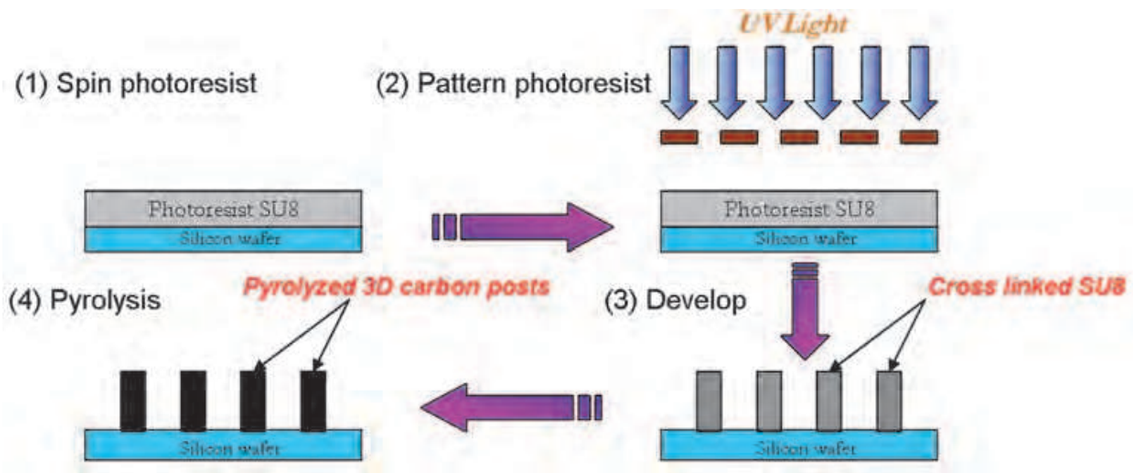


Fig. 1. Schematic showing the typical C-MEMS process.

3.1 C-MEMS microelectrodes

Fig. 2. shows the various carbon architectures possible using C-MEMS technique. The versatility in the technique gives us the opportunity to integrate nanofeatures such as suspended carbon nanowires, carbon nanofibers with microelectrodes (Wang et al., 2005; Malladi et al., 2006).

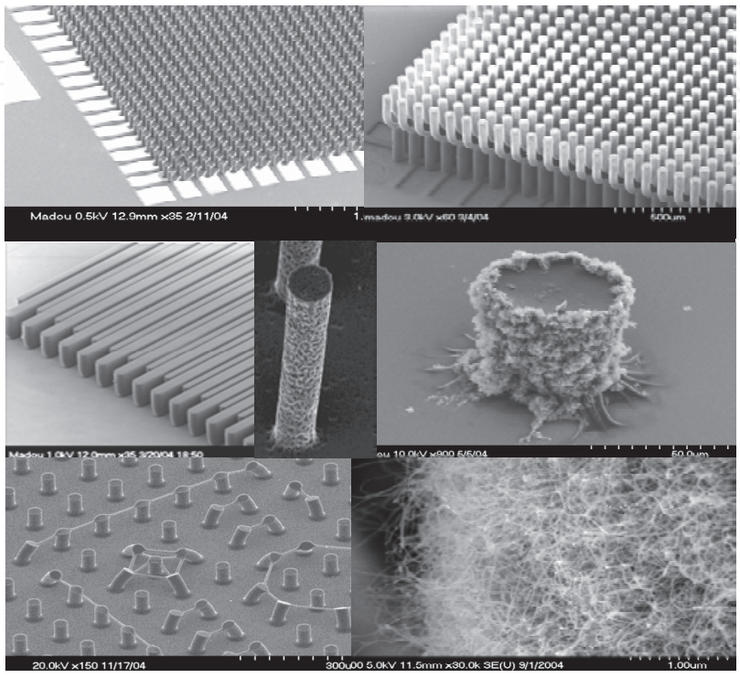


Fig. 2. SEM images of different carbon architectures possible by C-MEMS technique.

Besides, in order to increase the surface area, we reported a modified C-MEMS process using a block copolymer F127 as porogen capable of producing porous carbon microelectrodes (Penmatsa et, al., 2010). These results indicated that porous carbon thin film electrodes derived from 10% F127 mixed in SU-8 had an A_{eff} 185% compared to the conventional photoresist derived carbon electrode. This fabrication approach can be employed to produce reproducible high aspect ratio carbon microelectrodes with different shapes for various electrochemical devices.

Although the 3D structures compared to 2D planar electrodes or thin films have advantages, such as an increase in the surface area and power density for same foot print area, there are yet certain important issues which need to be addressed in order to use these structures effectively. Anandan and Godino have studied the mass transport phenomenon in micro and nano-electrodes by finite element analysis approach. They suggest that in order to accommodate the specific analyte species in terms of reaction kinetics and mass transport, it is necessary to optimize the geometry of nanopillars (their diameter, spacing and height), to reap the true benefit of using micro-nanostructured electrodes for enhancing the performance of biosensors. They reveal that the glucose immediately react with the top portions of the nanopillars due to higher reaction rate of enzymes and hence the bottom portion of the pillars lack the diffusion of glucose, which may not be favorable to improve the performance of EBFC. Jeffrey suggests that in contrast to the 2D electrodes, in which uniform current density is naturally obtained over the surface of the cathodes and anodes, the current density in the 3D

microelectrode array suffer from a non-uniform primary current distribution. These non-uniform currents result in utilization of the electrode materials and are thus associated with lower cell efficiencies, reduced electrodes stability due to non-uniform stresses, and non-uniform heat dissipation. Therefore, it is essential to optimize the geometries of electrodes to homogenize the current density distribution around microelectrodes surfaces.
In the later section, we introduced finite element method based simulative approach to understand the effect of 3D design rule and spatial distributions of the microelectrodes in the arrays with respect to the mass transport of glucose, enzymatic reaction rate and open circuit output potential.

4. Surface functionalization of C-MEMS

There are several factors regulating the lifetime of biofuel cells, which has always been a concern for their practical application. In most cases, the stability of enzyme determines the lifetime of biofuel cells. Immobilization of enzyme through covalent bonding on solid surface has attracted great attention for applications in catalytic processes. Therefore, in our research, covalent attachment of enzyme on supports was studied to promote rigidification of enzyme structure of the immobilized enzyme.
We have studied three different types of covalent surface functionalization for enzyme immobilization in EBFCs – (1) Direct amination; (2) Diazonium grafting; (3) Diamine grafting. In all these methods, the functional groups are realized on the pyrolyzed carbon surface.

4.1 Direct amination on pyrolyzed carbon

Direct amination was conducted by functionalizing its surface with ultraviolet (UV) irradiation under ammonia gas (Yang et, al., 2009). Quantified amino groups on the carbon surface were estimated by X-ray photoelectron spectroscopy in Fig. 3. It is found out that the amino

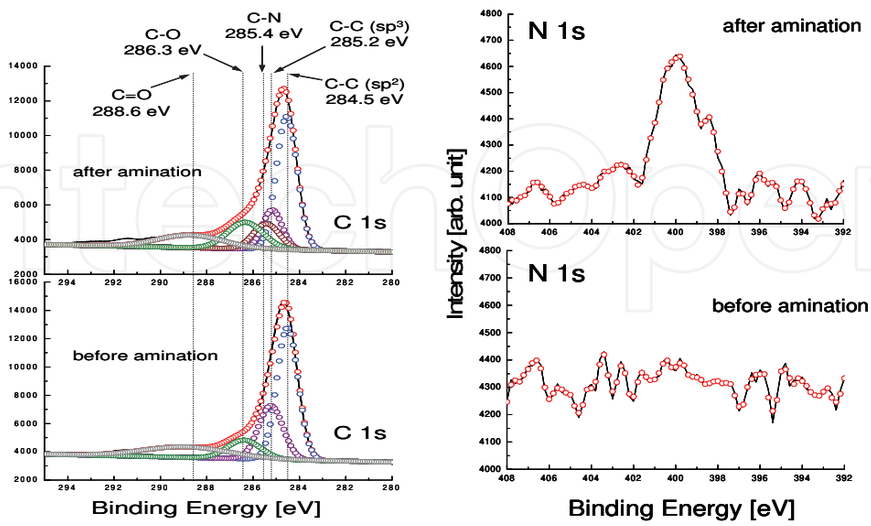


Fig. 3. X-ray photoelectron spectra of pyrolyzed carbon surface. (a) High-resolution scan of the C 1s peak and (b) high-resolution scan of the N 1s peak are compared before and after direct amination.

groups exist at surface by amination processes due to the high density of carbon. Ammonia gas forms as C-NH_2 on carbon substrate because C-H bonds react easily with ammonia gas and undergo photochemical reaction on exposure to UV irradiation although steric limitations will limit the amine group coverage on the surface. The results showed that the amino groups were successfully formed on pyrolyzed carbon surface by direct amination.

4.2 Surface functionalization of carbon surface by diazonium grafting

Diazonium grafting is a promising alternative to conventional electrode functionalization method. In this approach the electrochemical reduction of diazonium forms an aryl centered radical. The resulting aryl radical can then form a covalent bond with conducting and semiconducting surfaces. The CV results shown in Fig. 4. indicate the first cycle of electro-reduction process from NO_2 to NH_2 at different diazonium concentrations. The reduction of NO_2 to NH_2 occurs on the first negative-going sweep in a range of potential from -1.0 V to -1.1 V forming a clear irreversible anodic peak. From the results, it was confirmed that the amino groups are successfully grafted on the carbon surface for further immobilization.

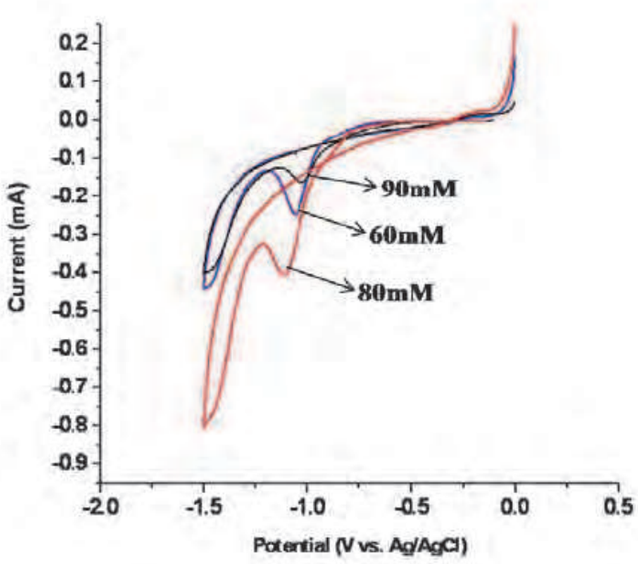


Fig. 4. CV curves showing the electroreduction from NO_2 to NH_2 at different concentrations of diazonium. The irreversible peak indicates the electroreduction process.

4.3 Functionalization of carbon surface by diamine grafting

Ethylene diamine grafting on the carbon surface was conducted using the electrochemical reduction. As the potential sweeps to negative direction during the first cycle, the oxidation of amino group in ethylene diamine is reduced on pyrolyzed carbon surface at potentials between -0.8 V and -1.4 V, leading to a clear irreversible anodic peak. It also shows that any peaks by oxidation and reduction do not exist after first reduction sweep even though we notice that the anodic current of electrode is decreased. This result indicates that the ethylene diamine was grafted on the surface of pyrolyzed carbon by applying potential and pyrolyzed surface is successfully functionalized by the electrochemical method.

We have introduced three types of functionalization on carbon surface in this session. In the future work, we will immobilize different biomolecules based on these functionalization methods for EBFCs device. Work on building a prototype EBFC consisting of glucose oxidase immobilized anode and a laccase immobilized cathode using C-MEMS based interdigitated electrode arrays is underway.

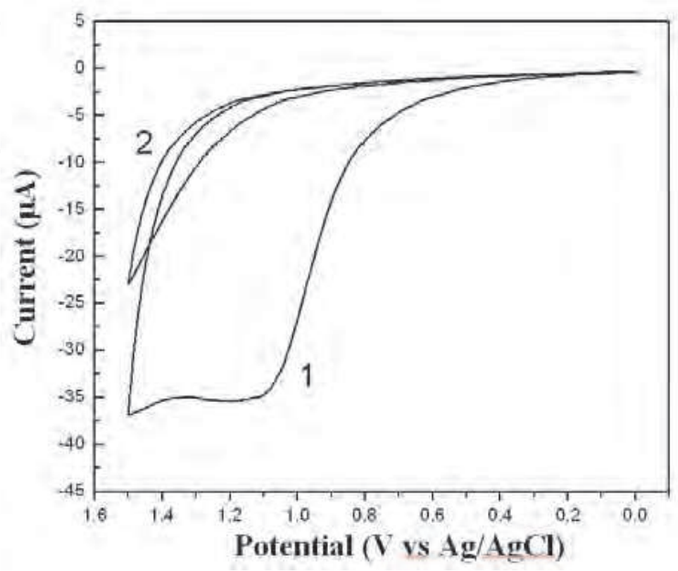


Fig. 5. Cyclic voltammograms showing the first and second cycle confirming the surface functionalization completed in the first irreversible cycle.

5. Simulation of C-MEMS based EBFCs

5.1 Finite element approach for optimization of electrodes design

For our simulation approach, we used commercially available COMSOL 3.5 software multiphysics software, which solves partial differential equations (PDEs) by finite element technique. In the model we assume that 3D carbon microelectrode arrays were uniformly immobilized with glucose oxidase and laccase on anode and cathode respectively with out the use of any mediators. The proposed implantable membraneless EBFC is assumed to be placed inside a blood artery of the human body thus utilizes the glucose extracted from blood as a fuel. In principle, glucose oxidase reacts with glucose and produces gluconolactone and hydrogen peroxide. This hydrogen peroxide oxidizes on the anode to generate electron and hydrogen ions. The hydrogen ions travel from electrolyte to cathode, while electrons flow through an external load and generate electricity. On cathode, dissolved oxygen is reduced via laccase enzyme and by combining with electrons and hydrogen ions forms water.

We applied Michaelis-Menten theory in our 2D model to analyze phenomenon between enzyme kinetics on the electrode surface and glucose diffusion and thus optimize the electrode microarray design rule according to the enzyme reaction rate In order to determine the output potential in developing biofuel cell, we also incorporated Nernst equation. The numerical simulations have been performed with various electrodes heights and well widths (distance between any two electrodes) to obtain the relation between design

rule and EBFCs performance. Various 2D models are investigated for same foot print length (600 µm) of SiO_2, with fixed electrode diameter of 30 µm and fixed enzyme layer thickness of 10 µm. The height of electrodes is chosen as 60 µm, 120 µm and 240 µm for different well widths (WW-distance between any two electrodes) of 10µm, 20 µm, 40 µm, 60 µm, 80 µm, 100 µm, 120 µm, 140 µm, 160 µm, 180 µm and 200 µm.
The quantification of reaction rates of enzymes on anode and cathode is showed in Fig. 6.

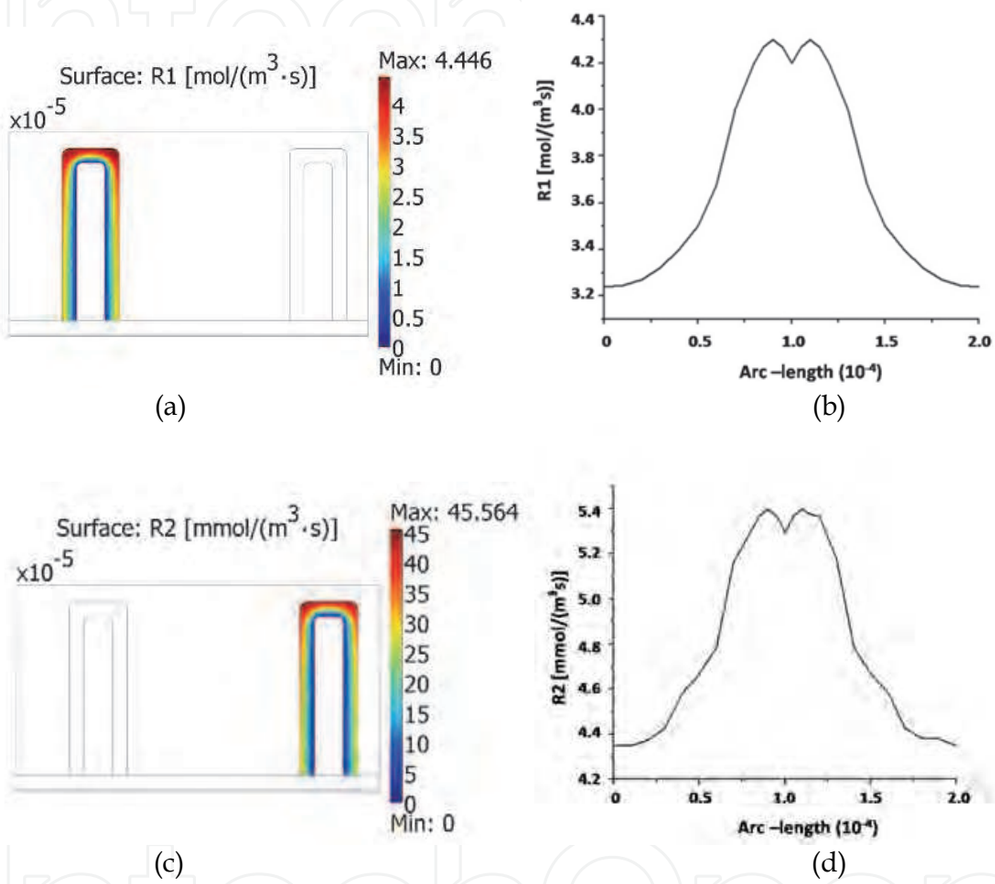


Fig. 6. (a) Subdomain plot of anode reaction rate (R1); (b) reaction rates from the whole surface of anode. (c) Subdomain plot of cathode reaction rate (R2); (d) reaction rates from the whole surface of cathode.

From the results, we observe that the reaction rate decreased from the top to the bottom along the surface of both electrodes due to the lack of diffusion of the substrate as we go towards the bottom; also the outer surfaces of the electrodes have the larger reaction rate in the enzyme layer. The reaction rate along the surface of both electrodes is plotted in Fig. 6. The reaction rate is increased from the bottom to the top along the electrode surface and reached the maximum at edge of the top due to the edge effect. The maximum reaction rates of GOx enzymes vs. different well widths is shown in Fig. 7. for three different heights of electrodes: 60 µm, 120 µm and 240 µm, with 10 µm, 20 µm, 40 µm, 60 µm, 80 µm, 100 µm and 120 µm well widths, respectively. In the case of 60 µm height of electrodes, the maximum reaction rate is obtained when the well width is about 30 µm. For the height of 120 µm and

240 μm, reaction rate reached the highest at the well width of 60 μm and 120 μm respectively. From all these three sets of models both in anode and cathode, we can conclude that the reaction rates of one pair of electrodes reach the maximum when the well width is half as the height of electrodes.

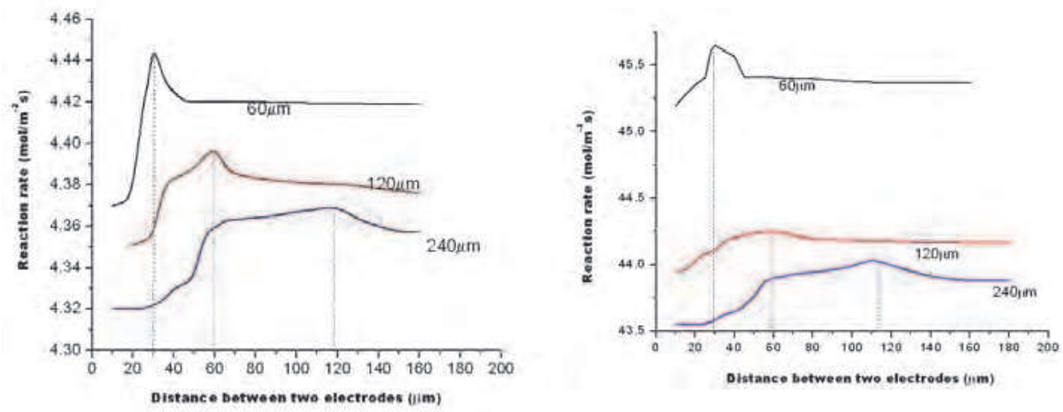


Fig. 7. (a) Anode reaction rate curves vs. well width at different ratio of electrode dimensions; (b) Cathode reaction rate curves vs. well width at different ratio of electrode dimensions.

The open circuit output potential also has been simulated for the same heights and well widths of electrodes by applying the Nernst equation. The current collectors are assumed at the bottom of the electrodes and hence these potentials are calculated from the bottom. Fig. 8. shows the open circuit output potential vs. well width of electrodes at different height of electrodes. From the results of simulation, we could find out an empirical relationship between electrodes height and well width to achieve optimized output potential is when height of electrodes is twice than that of well width which is in agreement to the results we obtain for the diffusion of the substrate.

5.2 Finite element approach for optimization of orientation of microelectrodes chip for enzymatic biofuel cells

Until now, majority of the research was focused on in-vitro experiments by mimicking physiological conditions. The additional complex problems may arise when a BFC chip is placed inside a blood artery. The first is with implantation process itself, which involves a surgery for the insertion of a BFC, and other necessary electronics components. The second is the stability of this chip inside an artery and how/where this chip can be fixed such that it can survive against the blood flow. Third problem is the clotting of the blood. The goal is to put this EBFC chip in such a way that it does not obstruct the flow of blood and lead to substantial pressure drop inside an artery. The fixation of this chip with the blood artery also should not harm the blood vessel walls (Parikh et al., 2010).

In order to improve mass transport around microelectrodes by optimizing the positioning of an EBFC chip, we have adopted the finite element analysis approach to look into the stability of an EBFC inside a blood artery. On the initial stage, we have analyzed only two orientations: horizontal position (HP) and vertical position (VP). The stability of the chip in these positions, diffusion and convectional fluxes around microelectrodes has been finely

investigated. We have proposed a novel chip design, with holes in between all electrodes on the substrate, which can drastically improve the diffusion in between microelectrodes.

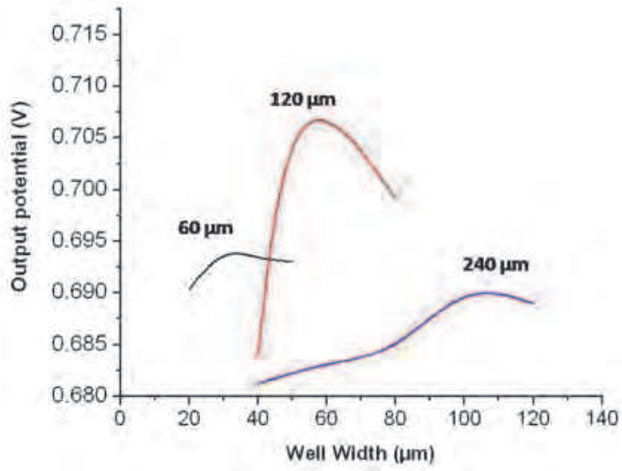


Fig. 8. Output potential vs. well width for different ratio of electrode dimensions.

The diffusion between the microelectrodes has shown in Fig. 9, where Fig. 9a and b shows the simulation profiles for diffusive flux along with the streamlines around microelectrodes in HP and VP, respectively. In HP, it is observed that the diffusive flux is less near the central electrodes and increases when going towards outer electrodes. However, the diffusive flux is almost same on top of all electrodes in VP. It is observed that in both the positions, the diffusive flux is following laminar pattern. The diffusive flux from bottom of an electrode to top of an electrode is investigated in HP and VP as shown in Fig. 9c and d, respectively. The flux is not uniform from the central to outer electrodes. The electrodes located at the circumference of a chip are having more flux compared to those located in the centre of the chip. The variation of the diffusive flux distribution around inner to outer electrodes is high in HP. The flux is not constant at every instance, but it is oscillating as shown in inset figures. The diffusive flux profiles in these figures are considered at the time, when the flux reaches its maximum value. This is also evident from Fig. 9e and f, the flux is higher exactly at the top of electrodes while lesser in the vicinity between any two electrodes. In comparison of HP and VP, the diffusive flux is 8 orders larger in case of VP than in HP.

Total flux is the combination of a diffusive flux and a convective flux. Fig. 10 depicts the total flux data for (a) HP and (b) VP of a chip. In HP, flux is negligible up to almost 275 µm height of electrodes and then increasing at the top. Total flux is highest at the top of outer most electrodes and then reducing to the central electrodes. In case of VP, the flux is almost uniform on top of all electrodes, with negligible value in between electrodes up to 200 µm height and then gradually increasing to about 2000–3500 mmol m^{-2} s^{-1} at the top of all electrodes.

Based on the results, the new design with the holes in between all microelectrodes has been inspected precisely and compared with the prototype design. The diffusive flux (Fig. 11a, c, e) and convective flux (Fig. 11b, d, f) profiles for the new design are compared with diffusive flux and convective flux profiles of the prototype model, respectively. The streamlines present the lines of motion of glucose at a particular instance.

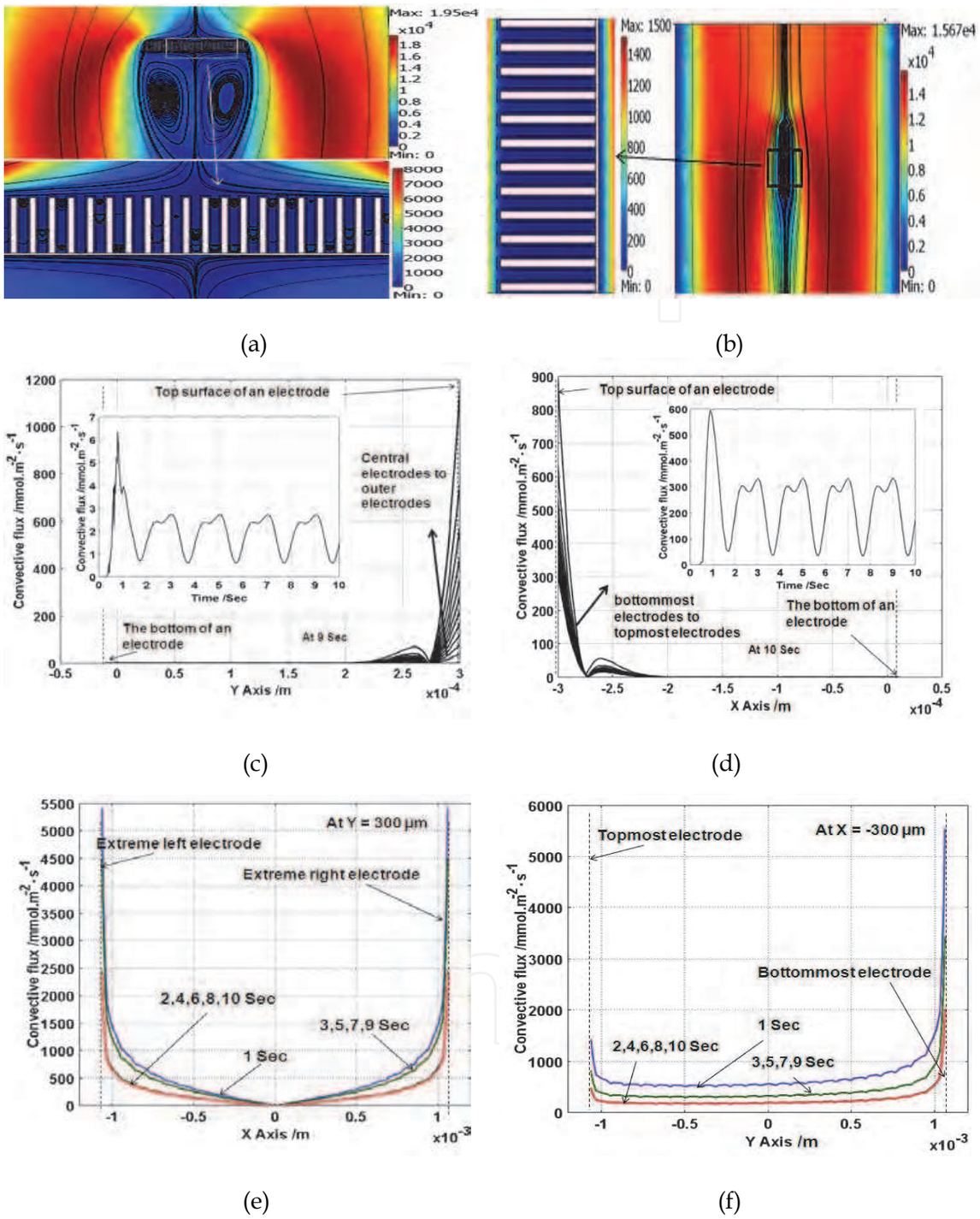
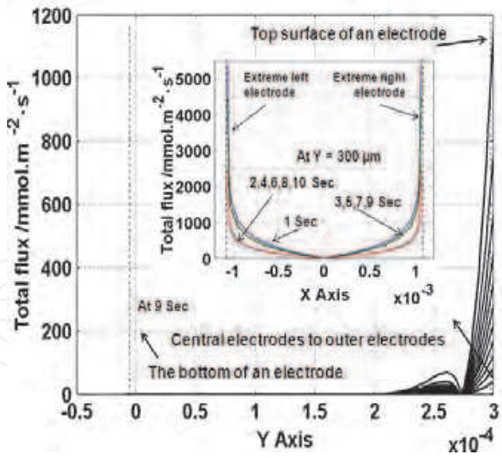
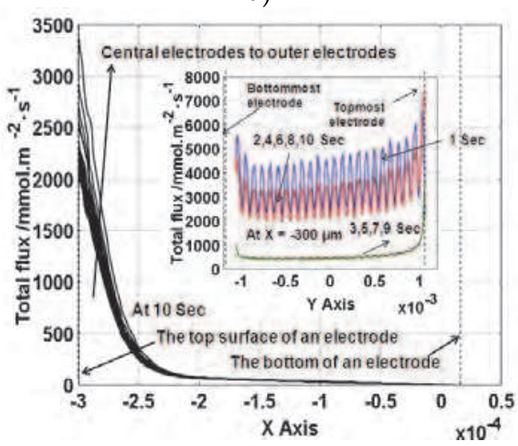


Fig. 9. Surface plot with streamlines for convective flux of glucose around microelectrodes for a) HP, b) VP, convective flux in between all 24 electrodes from bottom of electrodes to up to 300 µm height is shown for c) HP and d) VP, convective flux at top of all the electrodes from leftmost to right most electrodes for 0 – 10 secs in e) HP and f) VP.



a)



b)

Fig. 10. Total fluxes in between micro-electrodes for a) HP and b) VP. Insets provide the total flux on top of all electrodes.

From Fig. 11 it is inferred that the total flux (combined diffusive and convective flux) has been improved between all microelectrodes in terms of values and their uniformity for the chip with the holes. This enhanced mass transport around microelectrodes is significantly important for an EBFC performance. This proposed design could also be advantageous to prevent blood clotting. Human blood is mainly consisted of red blood cells and white blood cells. The sizes of all these cells such as red blood cells (6 µm), lymphocyte (7–8 µm), neutrophil (10–12 µm), eosinophil (10–12 µm), basophil (12–15 µm), and monocytes (14–17 µm) are mostly smaller than 20 µm, the size of the holes provided in the chip. So these cells can pass through the holes in between microelectrodes without blocking the way in between micro-electrodes. These holes can be made bigger depending on the requirement. The improved convection in between microelectrodes may also be forceful enough to eliminate the bubble formation. However, the biomechanical process and hemodynamic process are more complex than convection and diffusion, especially on the micro-scale level. Cell growth and clotting phenomenon are related to many aspects, such as: biocompatibility, bending of blood artery, platelet and protein components. More detailed research needs to be done with biologists in order to obtain more sufficient and helpful information and further reach the applicable level of the EBFCs.

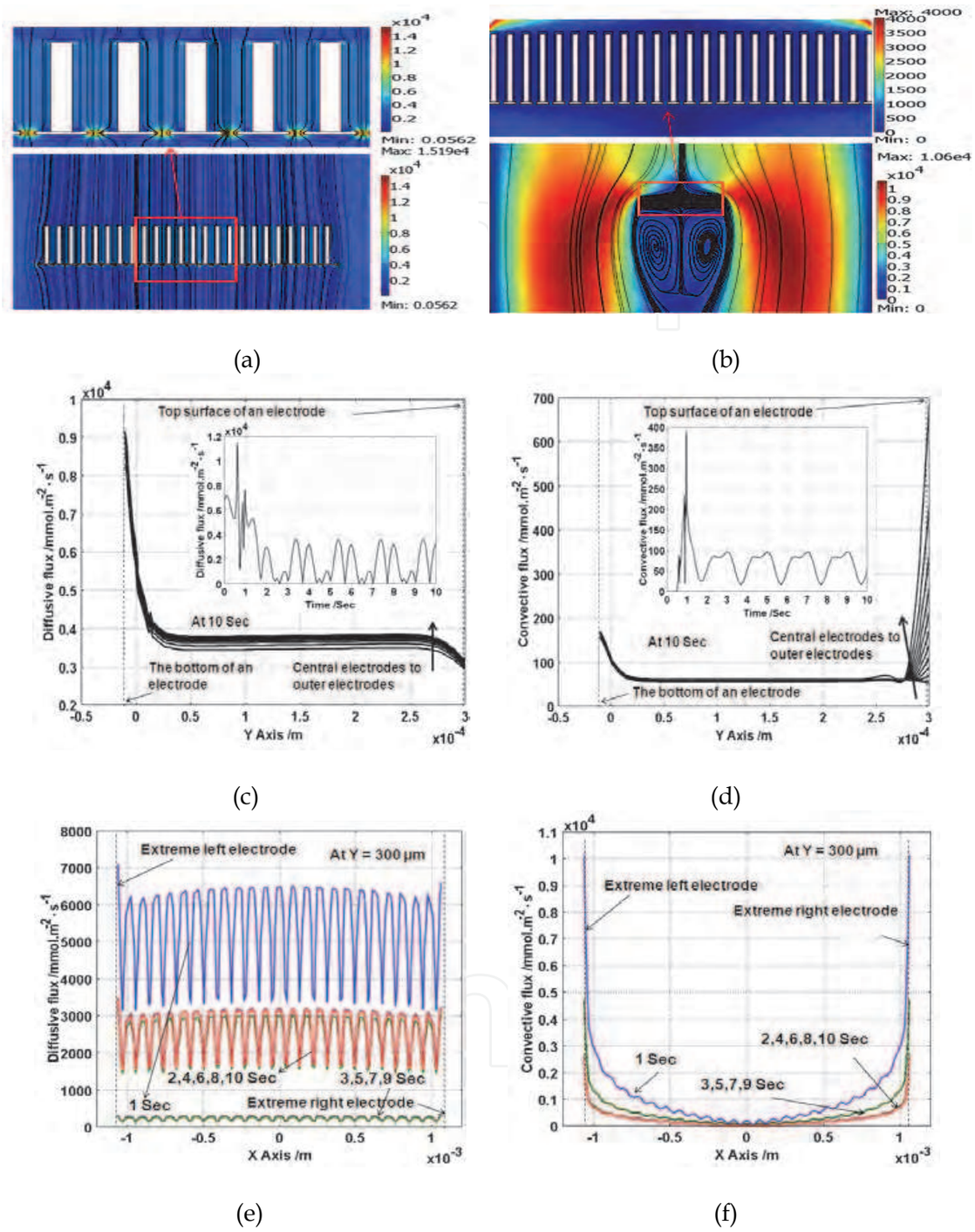


Fig. 11. Surface plot with streamlines for (a) diffusive flux and (b) convective flux of glucose around microelectrodes; (c) diffusive flux and (d) convective flux in between all 24 electrodes from bottom of electrodes to up to 300 µm height; (e) diffusive and (f) convective flux at top of all the electrodes from leftmost to right most electrodes for 0 – 10 secs.

6. Conclusion

In this chapter, we have introduced the two major kinds of biofuel cells-microbial fuel cells and enzymatic biofuel cells. Significant development on both biofuel cells has been achieved in the past decade. With the demands for reliable power supplies for medical devices for implantable applications, great effort has been made to make the miniaturized biofuel cells. The past experiment results revealed that the enzymatic miniature biofuel cells could generate sufficient power for slower and less power-consuming CMOS circuit. In addition, we have also presented simulation results showing that the theoretical power output generated from C-MEMS enzymatic biofuel cells can satisfy the current implantable medical devices. However, there are some challenges for further advancements in miniaturized biofuel cells. The most significant issues include long term stability and non-sufficient power output. Successful development of biofuel cell technology requires the joint efforts from different disciplines: biology to understand biomolecules, chemistry to gain knowledge on electron transfer mechanisms; material science to develop novel materials with high biocompatibility and chemical engineering to design and establish the system.

7. Acknowledgements

This project is supported by national Science Foundation (CBET# 0709085).

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Biofuel's Engineering Process Technology

Edited by Dr. Marco Aurelio Dos Santos Bernardes

ISBN 978-953-307-480-1
Hard cover, 742 pages
Publisher InTech
Published online 01, August, 2011
Published in print edition August, 2011

This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Yin Song, Varun Penmasta and Chunlei Wang (2011). Recent Development of Miniatured Enzymatic Biofuel Cells 657, Biofuel's Engineering Process Technology, Dr. Marco Aurelio Dos Santos Bernardes (Ed.), ISBN: 978-953-307-480-1, InTech, Available from: http://www.intechopen.com/books/biofuel-s-engineering-process-technology/recent-development-of-miniatured-enzymatic-biofuel-cells-657

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