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## Challenges for successful implantation of biofuel cells

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### ABSTRACT

There is a growing interest in the design and engineering of operational biofuel cells that can be implanted. This review highlights the recent progress in the electrochemistry of biofuel cell technologies, but with a particular emphasis on the medical and physiological aspects that impact the biocompatibility of biofuel cells operating inside a living body. We discuss the challenge of supplying power to implantable medical devices, with regard to the limitations of lithium battery technology and why implantable biofuel cells can be a promising alternative to provide the levels of power required for medical devices. In addition to the challenge of designing a biofuel cell that provides a stable level of sufficient power, the review highlights the biocompatibility and biofouling problems of implanting a biofuel cell that have a major impact on the availability of the substrates inside body that provide fuel for the biofuel cell. These physiological challenges and associated ethical considerations are essential to consider for biofuel cells that are designed to be implanted for long-term operation inside a living animal and eventually to human clinical applications.

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## 1. Introduction

An implanted medical device (IMD) is one that is intended to aid or deliver the functions of certain malfunctioning organs. IMDs have also been variously utilized for diagnosis, prognosis and treatment. An IMD can either be active or passive depending on whether it requires a power source or not. A passive IMD, by definition, operates free of an electrical power source; examples include bone prostheses, artificial bones, stents, nano- or micocapsular drug delivery systems and contrast agents. An active IMD does require an electrical power source, with examples including the pacemaker, cochlear implant, implanted bladder stimulator, insulin pump, implantable wireless pressure sensor and neurostimulators (Fig. 1) [1].

An IMD can be a powerful weapon for mass healthcare development, when utilized to assist or replace the functions of certain malfunctioning organs [2]. For example, 8–10% of the population in America and 5–6% of people in industrialized countries have experienced an IMD for restoring body functions, achieving a better quality of life, or increasing longevity [2]. According to the American Stroke Association 60,000 pacemakers are implanted each year in United States [3] and the European Society of Cardiology estimates for 2016 that 590 pacemakers were implanted per million of the population across 56 countries [4]. According to the US Food and Drug Administration in December 2010, over 219,000 cochlear implants are already in use worldwide [5]. The world's population continues to expand and the sharp rise in life expectancy has resulted in an increase in the average age of the world's population [2]. Maintaining the health and quality-of-life of this aging population will present new demands for health care services in the future, and perhaps the need for a new bioinspired power supply to enable the IMD to assist in such mass healthcare applications.

There are several issues that need to be resolved for the successful generation of power for an IMD. The first is that of the choice between a closed or open electric cell. An electric (electrochemical, voltaic) cell is a device that converts chemical energy directly into electrical energy. The two major groups of closed electric cells are primary (one-time usage) and secondary (rechargeable) electric cells. Rechargeable electric cells are often called accumulators. Although the term battery, in strict usage, designates an assembly of two or more electric cells connected in series to increase the voltage, the term is commonly applied to a single electric cell. Open electric cells are also called fuel cells (FCs). A fuel cell resembles a battery in many respects, but it can provide electrical energy over a much longer period of time since the device is continuously supplied with fuel and oxidant from an external source,

whereas a battery contains only limited amounts of reducing and oxidizing components that become depleted with use.

An implanted battery will either have a single-use defined life-time or will need a system for recharging, such as through the use of transcutaneous energy transfer. For a single-use battery, enough stored energy needs to be provided to supply power to the IMD for a sufficiently long duration (Table 1). Balancing the power requirements with a long duration of function and the size of the battery creates a dilemma, since device integrated batteries need to be as small as possible [6], yet increasing battery life-time requires an increase in volume. A fuel cell has the advantage of continually utilizing an external source of fuel for energy conversion. However, this presents a challenge when used for an IMD, since the fuel needs to be readily available from inside the body. Such a biofuel cell (BFC) needs specific bioelectrodes that are able to function continuously and efficiently in a physiological fluid environment. The essential difficulties to overcome are that the bioelectrodes may be unstable, not be biocompatible, may have toxic components or become biofouled. Furthermore, the need for the bioelectrodes to communicate with the physiological fluid environment raises issues of interconnectivity with the IMD, which in turn can induce the more standard electrical engineering problems of corrosion and of physical interconnect fidelity.

Numerous reviews have been written in the area of the electrochemistry of biofuel cells. However, there is a growing interest in the design and engineering of operational, biocompatible implantable biofuel cells. In this review we focus on the issue of incorporating a power supply with the IMD that could be long-lasting and ideally become symbiotic with the IMD and the body. Achieving this goal would fulfill the long held ambition of replacing and/or restoring organ function, allowing the associated active IMD to truly become a powerful weapon for mass healthcare application.

## 2. Limitations of existing batteries for IMDs

Since the earliest usage of practical implantable cardiac pacemakers in the late 1950s, batteries have been used to supply power to IMDs. Initially, IMDs were powered by Zn/HgO batteries, but following the introduction of lithium/iodine batteries for cardiac pacemakers in 1972 [7], the usage swung rapidly toward electrochemical power sources based on lithium technology, which continues to be the first choice for powered IMDs [2, 7].

There are two types of implantable batteries: primary (single-use) and secondary (rechargeable) [8]. The primary batteries are based on

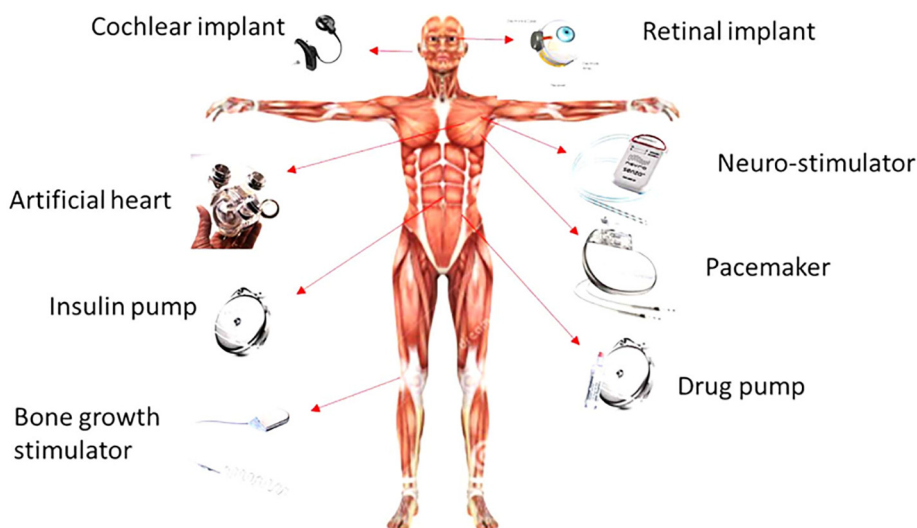


Fig. 1. Examples of IMDs.

**Table 1**

Power requirement of different IMDs in comparison with main characteristics of modern batteries.

IMD	Power	Battery life time
Pacemaker	10 $\mu$ W–30 W	5–7 years
Insulin pump	70 $\mu$ W	Up to 5 years
Neurological stimulator	0.03 mW–3 mW	Up to 3 years
Cochlear implant	0.02 W–1 W	–
Artificial Organs	30 W	Several hours

lithium metal anodes and on cathode systems variously comprising iodine, manganese oxide, carbon monofluoride, silver vanadium oxide and hybrid cathodes using both carbon monofluoride and silver vanadium oxide. Single-use batteries are used to supply IMDs such as pacemakers, implanted stimulators, and cardiovascular defibrillators, and they need to be changed surgically (or *via* vascular intervention for the latest generation of lead-less pacemakers that are implanted directly inside the heart) when the electrical power is no longer sufficient to properly operate the IMD. Moreover, the issue is not always that of power consumption, but rather of power management. For example, the resynchronization pacemaker suffers from significantly greater power failure than the standing monopolar pacemaker. The remaining capacity of the power in a non-rechargeable battery is regularly checked by wireless inductive telemetry [9].

A secondary, or so called lithium-ion ( $\text{Li}/\text{I}_2$ ), battery contains a graphite anode (e.g. mesocarbonmicrobeads, MCMC) and a cathode formed by a lithium metal oxide ( $\text{LiMO}_2$ , e.g.  $\text{LiCoO}_2$ ). During operation of the battery, Li ions transport back and forth between cathode and anode. These batteries are used for IMDs that require high power, such as cochlear implants and retinal prostheses. The secondary batteries can be recharged transcutaneously using external signals such as radio frequency (RF), ultrasound, infrared light, or low-frequency magnetic field [9, 10, 11]. The main reason why  $\text{Li}/\text{I}_2$  batteries have a

dominant role in powering implantable cardiac pacemakers is their high discharge voltage and high energy density. The discharge voltage of  $\text{Li}/\text{I}_2$  batteries can reach 3.6 V, which allows for their use in place of three nickel cadmium cells or three nickel-metal hydride cells in series [12]. Their energy density can reach 1000 W h/kg [13], which is good enough to power a cardiac pacemaker for several years.

Although there are advantages of lithium batteries in flexible electronics and wearable consumer devices, further implementation for IMDs has been limited due to issues of potential toxicity and the remaining obstacle of battery size. In practice, the volume of the pacemaker batteries fills 75% of the total volume of the classical pacemaker IMD. If an IMD requires high power, for example  $>20$  mW, then the volume of a lithium battery would be close to 1 L with total weight of  $>1$  kg (Table 1), which is clearly medically unacceptable. For these reasons there is a barrier to the development of IMDs that require large amounts of power, such as artificial organs, since such IMDs typically require supplementary power in addition to an implanted  $\text{Li}/\text{I}_2$  battery.

### 3. Energy harvesting from biofuels inside the body

The human body contains or releases many forms of energy, including heat, the chemical energy of organic molecules such as glucose, and physical forms of energy such as breathing and the motion of the limbs (Fig. 2). Energy harvesting systems are devices that can convert these types of physical and chemical body energies to electrical energy, to assist the recharging of implanted batteries or even their replacement.

Research on this topic has examined various techniques of passive energy harvesting for portable devices. In the case of vibration energy harvesting, there are three main mechanisms for converting motion or vibration to electrical energy: electromagnetic, electrostatic and piezoelectric [14]. The disadvantage of these mechanisms for generating electrical energy is that they are dependent on the non-continuous nature of

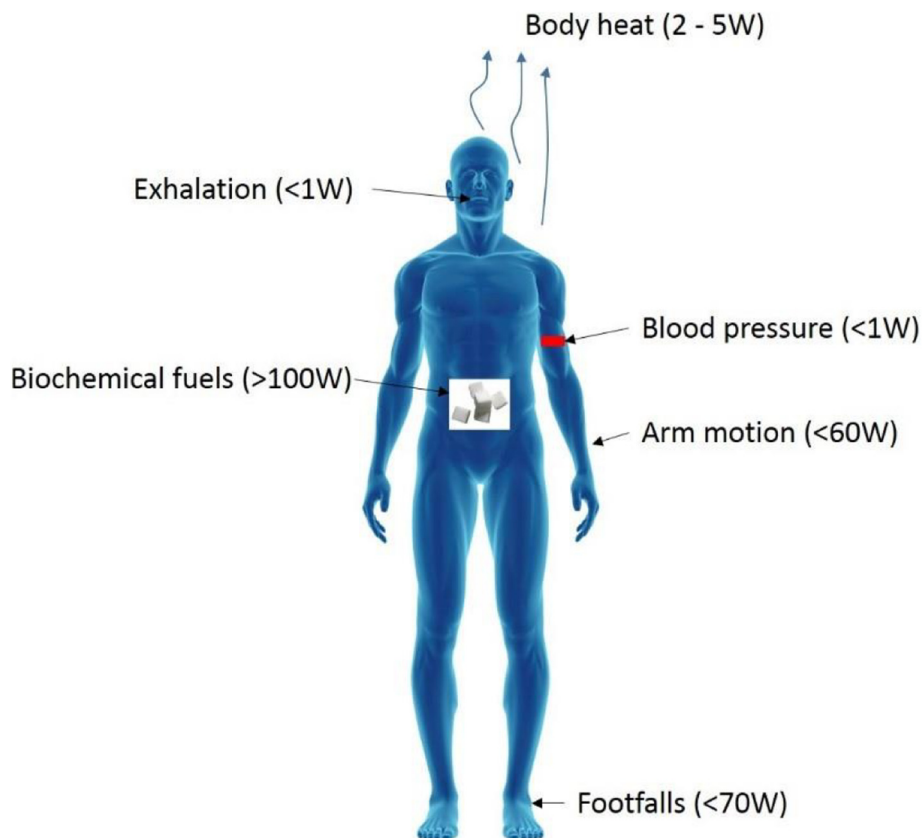


Fig. 2. Energy in the human body.

the vibrations. Nonetheless, respiratory motion based system are under development [15].

Thermal energy harvesters exploit the Seebeck effect to transform available temperature gradients within the human body. Due to the limited temperature gradient inside the human body, the output voltage of implantable thermal energy harvesters is expected to be very low, and typically in the range of a few tens of  $\mu\text{W}$  [16]. Vullers et al. [17] suggest that the combination of an energy harvester with a small-sized rechargeable battery (or with another energy storage system like a thin-film rechargeable battery or a super capacitor) is the best approach to enable energy autonomy of the IMD over the entire lifetime. (Fig. 3).

Biochemical harvesters, such as Biofuel Cells (BFCs), are promising since there is  $>100$  W of power contained as chemical energy in our body [18]. These devices transform chemical energy into electrical energy from molecules presents in a living organism. The difference between biofuel cells and classical batteries is that in BFCs the concentration of the reactants is continually re-established by the body fluids. The constant presence and availability of the fuel directly from the body makes external recharging mechanisms or replacement unnecessary and provides a theoretical capability for operating indefinitely, as long as there is a constant supply of fuel [19]. For example, glucose is the most commonly used fuel for BFCs due to its availability in most body fluids. Significantly, biofuel cells can be miniaturized.

#### 4. Availability of substrates in the living body: physiological aspects

##### 4.1. Glucose

Glucose is an essential nutrient for the human body and the major energy source for many cells. The human body is not a closed system and the glucose level is subject to contributions from three independent pathways (i) prandial glucose intestinal absorption, (ii) glycogenolysis, and (iii) gluconeogenesis. Intestinal glucose absorption occurs following food digestion and it is a discontinuous process [20] which relies on mixing of ingested food with fluids (acids and enzymes) in the stomach and small intestine to break down the food carbohydrates (sugars and starches) into hexoses, of which D-glucose is the most efficiently absorbed due to the presence of several passive and active intestinal transporters [21]. The final products of carbohydrate digestion in the alimentary tract are almost entirely glucose, fructose, and galactose, with glucose providing about 80% of these hexoses [22]. After absorption from the intestinal tract, most of the fructose and galactose is rapidly converted into glucose in the liver. Thus, the outcome from carbohydrate breakdown is that glucose is the predominant, common molecule available for transport to the cells. By comparison, endogenous glucose production (glycogenolysis and gluconeogenesis) are continuous processes, although modulated by the body's need for blood glucose

stability. Thus, endogenous glucose production is upregulated or down-regulated in fasting and fed conditions, respectively. For example, for a body surface area of  $1.73\text{m}^2$  the rate of endogenous glucose production is  $0.69$  mmol/min [23]. Plasma glucose homeostasis is maintained in a healthy body by balancing glucose utilization with endogenous glucose production and dietary glucose delivery. Liver, muscle, adipose tissue, brain and the endocrine organs work together to achieve this homeostasis. Several studies in dogs and humans have shown that the liver is responsible for  $\sim 30\%$  of glucose uptake and storage following glucose ingestion [24, 25].

The basal metabolic rate of a 70 kg person is usually within the range from 1200 to 2100 kcal/day (500–900 kJ/day) depending on such factors as fat content, sex and age [18]. Assuming a normal energy utilization from carbohydrates of 50% and since the enthalpy of glucose combustion is about 2803 kJ/mol or 3.72 kcal/g [26], then glucose intake would need to be between 161 g to 270 g per day for a 70 kg person. The important question is whether an implanted GBFC will consume too much of that glucose requirement and hence cause a detrimental effect on health. In the case of a GBFC, the maximum work is determined by the change in Gibbs free energy ( $\Delta G$ ) of complete glucose oxidation (maximally produces 24 electrons). However, a practical GBFC that utilizes the enzyme glucose oxidase (GOx) leads to an incomplete oxidation of glucose and only 2 electrons are produced:



The basic thermodynamic equation for a reversible electrochemical reaction is:

$$\Delta G = \Delta H - T\Delta S$$

Where  $T\Delta S$  is the heat associated with the organization/disorganization during the reaction, with  $T$  being the absolute temperature and  $\Delta S$  the variation in entropy. Theoretically,  $\Delta G$  is converted into electrical power by the GBFC, whereas  $T\Delta S$  is released in the form of thermal energy during the electrochemical reaction so the electrical work ( $W_{\text{el}}$ ) by the GBFC electrochemical system is:

$$W_{\text{el}} = Q \cdot \Delta E$$

where  $\Delta E$  is the operating voltage of the GBFC and  $Q$  is the transported charge given by the equation:

$$Q = n \cdot F \cdot N$$

where  $n$  is the number of electrons concerned in the reaction,  $F$  is the Faraday constant, and  $N$  the number of moles of oxidized glucose.

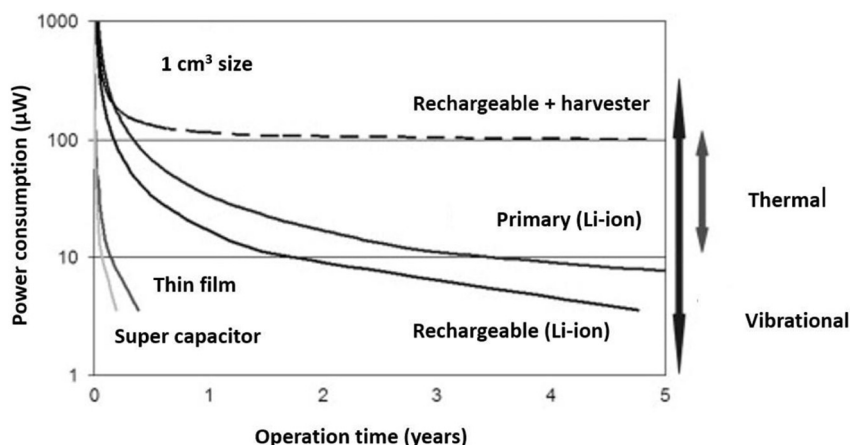


Fig. 3. Comparison of lifetime versus power consumption for several energy storage systems ( $1\text{ cm}^3$ ), including one with an energy harvester [17].

If we assume that the GBFC consumes only 2% of the body intake of glucose, then the GBFC would likely consume between 1.78 and 3 mol (3.22 g to 5.4 g) of the glucose that might be required each day by a 70 kg person at resting states. Such glucose consumption would produce a power output from such a GBFC of 11 to 20 mW (mJ/s). Since, enzymatic glucose oxidation involves only 2 electrons in comparison the 24 electrons for total oxidation, then the quantity of charge produced per day varies between 343.488 kC to 578.912 kC. For a GBFC operating at 0.3 V, these charge quantities can produce an electrical energy that is between 103.046 kJ to 173.674 kJ. For a person at rest, an IMD consuming between 1 mW to 100 mW, the glucose requirement for the GBFC will be between 0.1% to 10% of total body carbohydrates intake. Clearly for the non-resting states the percentage value will be substantially lower, with a little or no impact on body metabolism.

#### 4.2. Oxygen

Oxygen is an essential component for energy metabolism in the body, so the major questions concerning the function of an implanted GBFC are (i) whether the GBFC will have a detrimental impact on the level of oxygen that is available for general metabolism in the body, and (ii) whether the level of available oxygen impacts operating limits of the GBFC. The average adult at rest inhales and exhales about 7 to 8 L of air per minute. From the inhaled air, oxygen moves down a pressure gradient from a relatively high level in air (160 mm Hg) eventually to the mitochondria where the partial pressure of oxygen ( $pO_2$ ) reaches the lowest level (7.5–11.2 mm Hg) [27], as shown in the oxygen cascade (Fig. 4).

Oxygen diffuses from the high partial pressure in the alveoli (97 mm Hg) to the lower partial pressure of the blood in the pulmonary capillaries (32 mm Hg) [28]. Oxygen in the blood is carried mostly combined with hemoglobin (Hb), although some dissolves directly in the plasma [29]. When carried by Hb, the oxygen capacity of blood is 20.8 mL per 100 mL of blood, since 1 g of Hb can combine with 1.39 mL oxygen and the concentration of Hb in normal blood is 15 mg of Hb per 100 mL of blood. Taking into account the 97.5% oxygen saturation of the arterial blood ( $pO_2 = 100$  mm Hg) and the 75% of the venous blood ( $pO_2 = 40$  mm Hg) [32], we find that there is 1000 mL of oxygen delivered to the body in 1 min in arterial blood [29, 30, 31]. Furthermore, because Hb serves as a reservoir for oxygen, its supply is sustained despite local consumption. About 25% of the arterial oxygen

content is used by a conscious person at rest, which implies a resting oxygen consumption of 250 mL to 300 mL every minute [27, 30]. Thus, our body consumes approximately 0.18 mmol to 0.22 mmol of oxygen per second. At rest, oxygen delivery to the cells of the body exceeds oxygen consumption.

In the distal parts of the capillary the prevailing  $pO_2$  may be as high as 30–40 mm Hg [27]. This value is in agreement with the estimation of Rapoport et al. [32] that the  $pO_2$  in cerebrospinal fluid is 25–50 mm Hg, which corresponds to an oxygen concentration of 70  $\mu\text{mol/L}$ . Based on a mathematical model developed by Lu et al. [33], Rapoport et al. calculated that there was a minimal fractional change in oxygen concentration in the cerebrospinal fluid due to the implantation of a GBFC delivering a power of 1 mW. The presence of the GBFC added an additional rate of oxygen consumption from the cerebrospinal in addition to the usual background physiological factors of (i) the oxygen partial pressure  $P_c$  in the choroid plexus capillaries, whose content is filtered and transported through the choroid ependymal cells to become cerebrospinal fluid; (ii) the oxygen partial pressure  $P_i$  within the brain tissue interstitial fluid; (iii) the formation rate of cerebrospinal fluid; and (iv) the drainage rate of cerebrospinal fluid. Nonetheless, the authors estimated that in the case of a GBFC implanted in cerebrospinal fluid and delivering 1 mW, the fractional change in oxygen concentration in the cerebrospinal fluid due to the presence of the GBFC was only a few parts per million.

The parameters presented in Rapoport et al. [32] allow an estimation of maximal current density that is limited by  $O_2$  diffusion, for a biocathode operating in the cerebrospinal fluid using the following equation [34].

$$i = nFADC/\delta$$

where  $n$  is the number of electrons (4),  $F$  is the Faraday constant (96,485C/mol),  $A$  is the electrode area ( $10 \text{ cm}^2$ ),  $D$  is the diffusion coefficient of oxygen in cerebrospinal fluid ( $3 \times 10^{-5} \text{ cm}^2/\text{s}$ ),  $C$  is  $O_2$  concentration in the fluid (ca. 50 nmol/ $\text{cm}^3$ ), and  $\delta$  is the thickness of the Nernst diffusion layer ( $8 \times 10^{-3} \text{ cm}$ ). The calculated maximal current density was approximately 40  $\mu\text{A}/\text{cm}^2$ . This is quite a small current density produced by the GBFC. Thus, it is quite probable that implanted BFCs will be limited by the cathodic reaction of oxygen bioelectroreduction rather than by glucose bioelectrooxidation due to the quite low molar concentration of the  $O_2$  that is directly available in the human body.

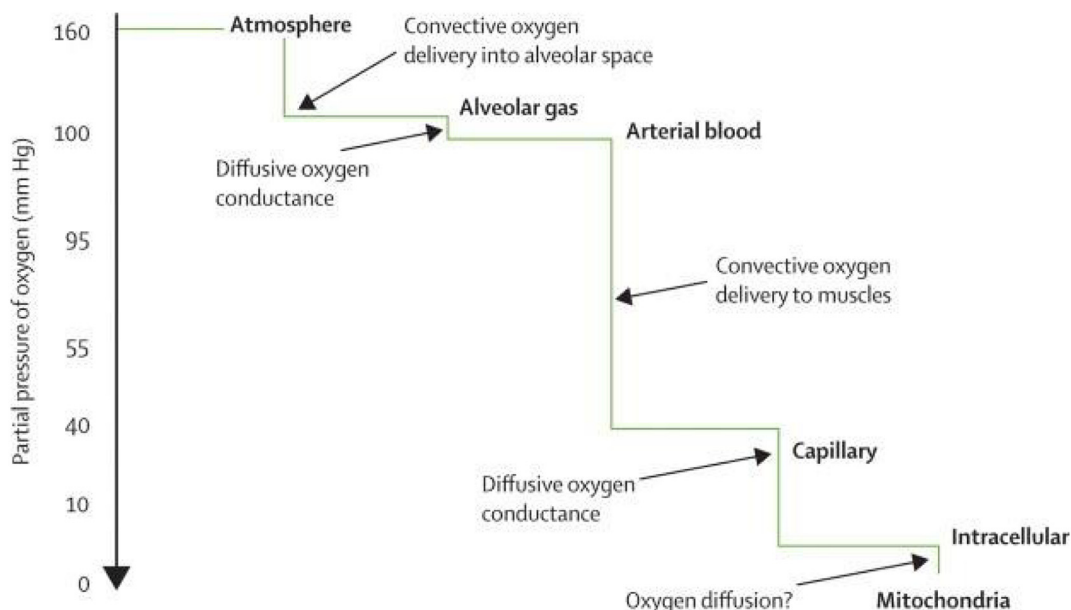


Fig. 4. The sequential reduction in the partial pressure of oxygen throughout the oxygen cascade, from the air to mitochondria in muscle cells.

It is likely that the impact of an implantable GBFC would not significantly affect the level of oxygen that is available for general metabolism inside the body. Nonetheless, there remain three major issues to consider for the BFC electrodes to be able to produce a current from the available supply of oxygen. The first issue is the oxygen mass transfer to reach the surface of the BFC biocathode. The maximum theoretical current is very difficult to calculate, but it is likely to be around the order of a few tens of  $\mu\text{A}/\text{cm}^2$ , as was calculated by considering the parameters reported by Rapoport [32]. The constraints of fibrosis (see section 7) will also impact that maximum theoretical current. Moreover, one should also consider possible local hypoxia for the tissue surrounding the biocathode. The second issue is related to the total volume of an implanted BFC that can be tolerated by the body, ca. 100 mL, which would subsequently influence the BFC surface area available to generate an electrical current. Of course, it is a difficult task of practical engineering to optimise bioelectrodes of different volumes and surface areas so as to provide the appropriate threshold of power output and for a sufficient lifetime (>1 year, for example). Such laboratory-based experimental demonstrations of thresholds for power and lifetime should be a priority for ongoing work in this field of implantable biofuel cells. The third issue is related to the maximal voltage of an implanted BFC. It seems possible to enhance the current or voltage by connecting BFCs in-parallel or in-series. However, the practical problem to overcome is that such BFCs connected in-series when implanted inside the same host all utilize the same electrolyte for the generation of the voltage from each BFC.

## 5. The implantable BFC

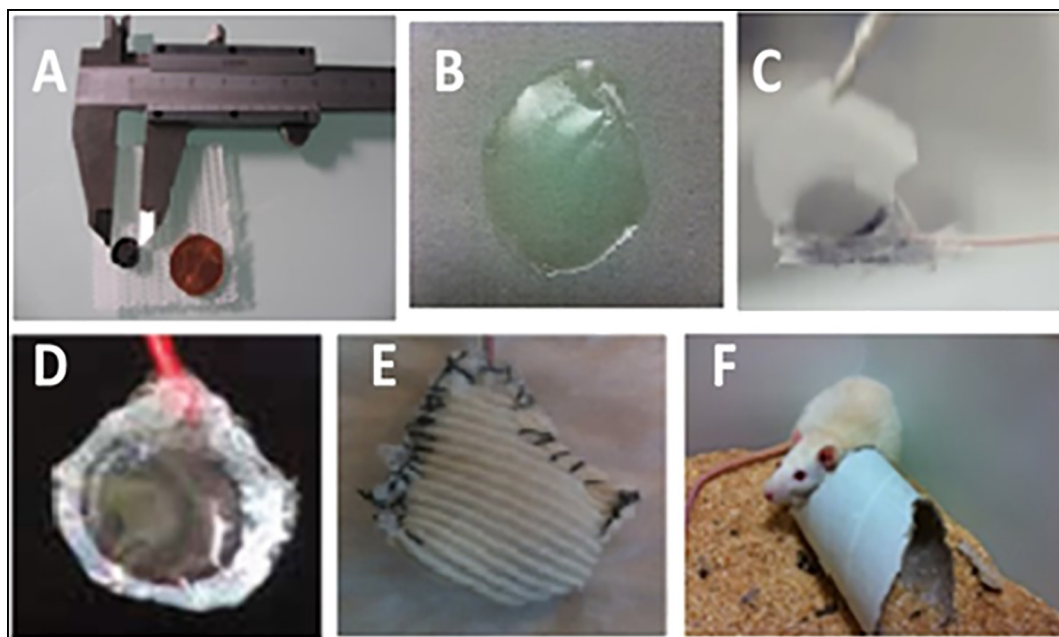
Enzymatic biofuel cells have a rich research history over the last 50 years. The field was started in the early 1960s when researchers first realized that oxidoreductase (redox enzymes) could be used to catalyze fuel oxidation at the anode of the biofuel cell, albeit with quite low current densities produced by the early devices [35]. However, it was not really until the late 1990s and early 2000s that researchers starting developing prototypes that could produce close to  $\text{mA}/\text{cm}^2$  under *in vitro* conditions [36, 37].

Although there were several early attempts at implantation, performance and stability showed that biofuel cells had not yet developed to

the state where implantable prototypes were feasible. After decades of subsequent research and development in the field, a demonstrator glucose enzymatic BFC was implanted in a grape by Heller et al. [38] in 2003. The first decade of the twenty-first century has resulted in major technical developments in the field, so it is not surprising that this has led researchers back to prototyping for *in vivo* use. From 2010 onwards, there have been multiple examples of implantable biofuel cells in the literature. One of the major advances came in 2010 when researchers implanted a glucose biofuel cell (GBFC) in a rat [39]. This was followed in 2012 by a trehalose biofuel cell in a cockroach [40] and biofuel cells in snails [41], clams [42], and lobsters [43].

Research in this field has now achieved considerable advances in the development of GBFCs to overcome the three major problems that could reduce the GBFC performance and limit the capability for their long-term implantation [17]. These problems include (i) the life-time of the enzymes and their low yield, (ii) biocompatibility of their components in the living body, and (iii) bio-fouling phenomena of biological molecules on the surface of the implanted biofuel cell. Research to address such biocompatibility issues was published by El-Ichi et al. [44], who studied the stability of an enzymatic biocathode when implanted for 167 days in a rat. The biocathode was fabricated from a 3D nanofibrous network of compressed chitosan in the presence of genipin as cross-linker, carbon nanotubes and laccase (Fig. 5). To ensure the biocompatibility of the biocathode, the authors covered its surface with a thin film made of chitosan cross-linked with genipin. Prior to implantation, the biocathode was capable under *in vitro* conditions (pH 7.4, NaCl 140 mM) to deliver a current density of  $-0.3 \text{ mA mL}^{-1}$  for 20 days in physiological buffer under continuous discharge.

This biocathode design minimized the inflammatory response in the first two weeks after implantation. After several months, the growth of macrophages was observed (see section 7). After explantation, the biocathode remained operational in subsequent *in vitro* measurements. Those explanted *in vitro* measurements of OCP (0.45 V to 0.48 V) and delivered current ( $-0.6 \text{ mA mL}^{-1}$ ) under optimal conditions (pH 5, under oxygen saturations conditions) confirmed that the electrical connections and the catalytic activity of the enzyme entrapped in the biocathode were retained during the previous almost 6 months of implantation. Those results are an important confirmation of the maturity of the design of bioelectrodes for implantable BFCs applications, since



**Fig. 5.** Chit-MWCNT-laccase electrode, (B) Plastic chitosan-based film, (C) Film coating of the surface of the electrode, (D) Chit-MWCNT-laccase electrode coated with the chitosan film, (E) implantable electrode inside a Dacron bag, (F) Rat with implanted biocathode [35].

they constitute the first demonstration of biocompatibility in addition to the importance of bioelectrode design in preventing enzyme activity loss within the body.

In addition to the ongoing development to improve the life-time of GBFC enzymes, it is important for research in this field to address the issues of biocompatibility and the effect of biofouling on the stability of GBFCs. For example, recently a bioelectronic device comprising an enzymatic biofuel cell (GBFC) connected to a wireless tele-transmission system was implanted in a rabbit and its function was monitored and controlled *in vivo* for a period of 2 months [45]. This recent use of a GBFC provides an encouraging basis to justify the further development of implantable BFCs for long-term use in patients and for other *in vivo* applications.

### 5.1. Classification of BFCs

Several fundamentally different classifications of BFCs can be found in the literature. The first classification is based on the definition of a BFC, *i.e.* a device utilizing a bio-fuel and/or bio-oxidant independently of any catalyst used in the construction of the device [46, 47]. The second classification is based on the definition of a BFC as a device utilizing biocatalysts (redox molecules, organelles, and living cells) [46]. This review is focused on BFCs that incorporate biocatalysts to accelerate fuel oxidation and oxidant reduction, and, more precisely, on biological BFCs, which are designed for implantation and utilization of human biofuels (*glucose*) and biooxidant (*molecular oxygen*) to power the

IMD. Most BFCs (using a biocatalyst) are classified by the mechanism used for electron transfer (ET) and the type of fuel.

The two main mechanisms for ET are direct electron transfer (DET) and mediated electron transfer (MET) [48]. DET is the process by which a biocatalyst can transfer electrons to/from the electrode without the use of another redox moiety. MET utilizes a separate redox moiety to shuttle the electrons between the biocatalyst and the electrode. The redox moiety could be a small molecule in solution, a redox active protein, or a redox polymer that can utilize self-exchange instead of physical diffusion to transport electrons to/from the current collector (electrode) and the biocatalyst [49]. The last, but not least, classification is based on the capacitive abilities of BFC electrodes, *i.e.* both conventional and charge-storing biodevices. Recently, a new kind of BFC comprised a hybrid electric power biodevice (Fig. 6) [50], which were self-charging biosupercapacitors [51, 52] that function as charge-storing biofuel cells [53]. These biodevices can operate in both continuous and pulse modes and are based on double-function electrodes, which are discrete electrodes that have simultaneous electrocatalytic and charge-storage features [54]. When operating in a pulse mode hybrid electric power biodevices deliver much higher power outputs, albeit for a shorter period, compared with conventional BFCs.

### 5.2. The sites for BFC implantation: Physiological and medical aspects

In order to provide optimum operation and good tolerance of BFCs in the body of an organism, the implantation site has to fulfill a number of criteria. First, it has to allow adequate supply of substrates and efficient

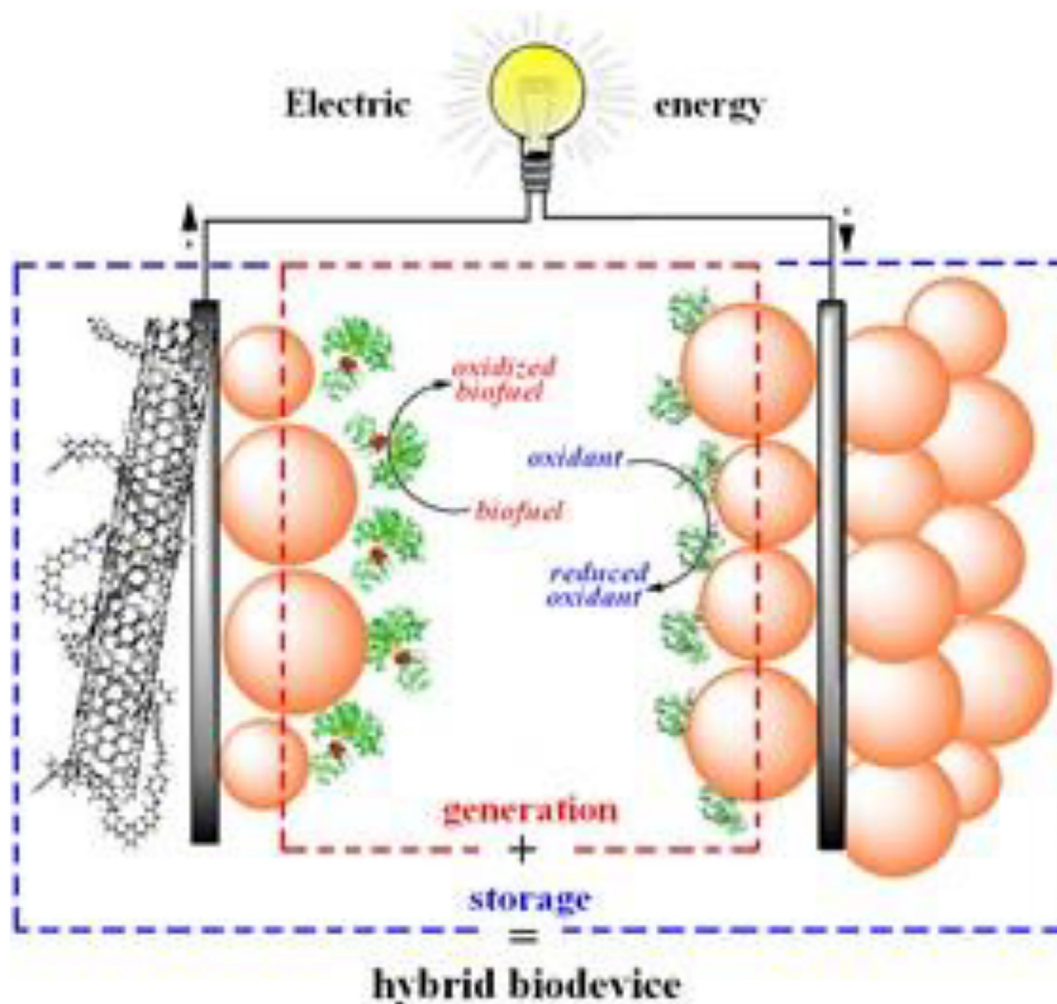


Fig. 6. Schematic illustration of an enzymatic hybrid electric power biodevice. Adapted from [50] with permission. Copyright 2014 Wiley-VCH.



removal of catabolites to avoid accumulation and so prevent local toxicity. Secondly, the presence of the BFC must not cause mechanical constraints likely to exert adverse physiological outcomes such as through compression of important structures or obstruction of lumen or cavities. Finally, the implantation site should not augment biofouling processes at the BFC. Importantly, biofouling processes may restrict exchanges between the device and the body, with the effect to compromise energy production. A first consideration of the above criteria could suggest that the blood vascular system may be an ideal site for implantation, since blood glucose and oxygen concentrations are relatively high and there is efficient catabolite washout. However, BFCs implanted inside blood vessels only function for short periods, are easily biofouled and induce local blood flow disturbances that may promote thromboembolism. The first attempt to place a biobattery in contact with blood was described in 1973 by Giner et al. [55] in a sheep model. In this study, an abiotic biobattery was placed on an extracorporeal arteriovenous shunt for a short period of time (90 min). More recently, enzymatic biofuel cells have been directly implanted in the vascular system of animals but these experiments have also been of short duration: 1 h in the marginal ear vein of a rabbit [56] (Fig. 7A) and 1 day in the jugular vein of a rat [57] (Fig. 7B). In the first experiment, only the negative electrode was introduced in the vessel in the form of a needle, the cathode being in contact with ambient air allowing good oxygen supply [58]. The anode was plated with a deposit of 2-methacryloyloxyethyl phosphorylcholine (MPC) to avoid adhesion of serum proteins and platelets. This polymer has again recently been the object of research to increase the biocompatibility of polysulfone fibers used in dialysis [59] and stent type implantable devices [60]. Nevertheless, the technical challenges that are associated with the introduction of an implantable micro-needle network in the vasculature to produce energy are far from being solved especially because of short-term biofouling.

In order to limit the phenomenon of biofouling, the group of Rapoport [32] have proposed implantation in the cerebrospinal fluid of humans, basing their analysis on an abiotic biobattery. Mathematical calculations have shown that such a device could develop an electrical power of 1–10  $\mu\text{W}/\text{cm}^2$  if implanted in this body compartment. The main benefits expected for this site in terms of biocompatibility are the limited cell content and low protein concentration (0.02 to 0.04%) a major contrast with plasma (8%). These arguments further highlight the importance of good design and integration of an implantable device to minimize biofouling of the electrode surface. However, the study by Rapoport remains theoretical and does not take into account possible inflammatory reaction, the recruitment of antibodies, the relatively low glucose content, representing only 50 to 75% of glycemia level, or the difficulty of miniaturizing a device for implantation in this compartment. Furthermore, the cerebrospinal fluid is less well buffered and production of gluconic acid at the anode of a biotic battery could generate a local acidic change affecting the central regulation of breathing, an effect that could have supply side effects on the consumption of oxygen by the cathode. An *in vivo* experimental implantation of a DET based cellobiose dehydrogenase/bilirubin oxidase cell in the brain of a rat using microelectrodes structured with gold nanoparticles (electrodes surface area 0.5  $\text{mm}^2$ ) produced an electrical power of 2  $\mu\text{W}/\text{cm}^2$  [61] (Fig. 7C). However, continuous discharge of the battery for 2 h induced a large decay of the potential, reducing activity by >50%.

Other implantation sites have been tested with the aim to increase the supply of substrate and oxygen to the BFC. Highly vascularized regions of the body, such as skeletal muscle, have been suggested as good candidates. In this respect, a biofuel cell with buckypaper electrodes (sheets of carbon nanotubes) has been implanted directly in contact with rat cremaster muscle [62] (Fig. 7D). Nevertheless, the electrical

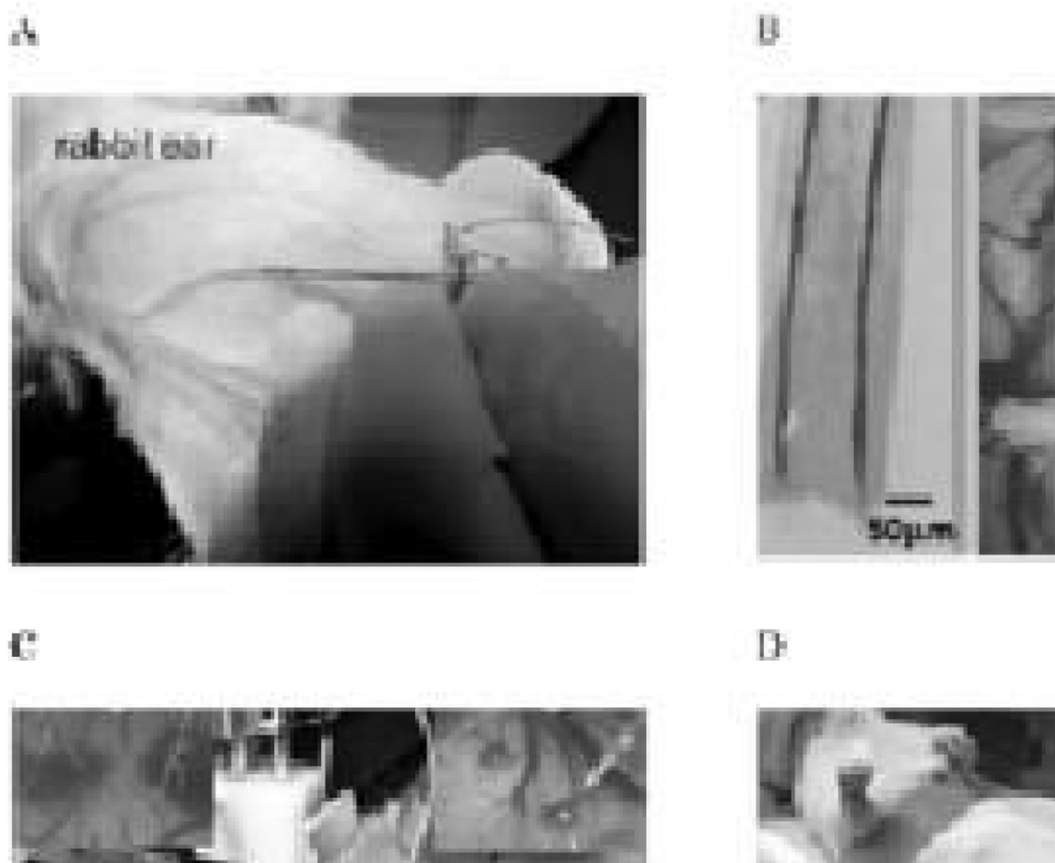


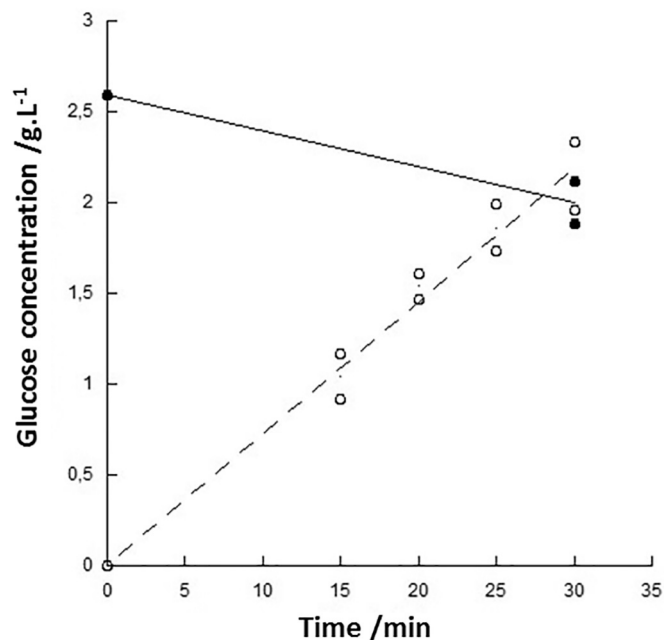
Fig. 7. Examples of recent implantation of BFCs in animals, A - in a rabbit marginal ear vein [56], B - in an art jugular veins [57], C - in a rat brain [61], D - at the surface of the cremaster muscle of a rat [62].

power produced by this cell remained disappointing (ca.  $0.2 \mu\text{W}/\text{cm}^2$ ). Moreover, the duration of the experiment was again very short.

Because of the large size of the first prototypes of BFCs, the intraperitoneal compartment was chosen for long-term implantation in animals. In the first study of this type, the electrical leads were tunneled subcutaneously up to the nape of a rat to allow connection to a potentiostat for electrical characterization of the BFC localized in the abdomen. In this experiment, the biofuel cell, which consisted of two electrodes each  $100 \mu\text{L}$  in volume, delivered  $25 \mu\text{W}/\text{ml}$ , with a daily discharge of  $5 \mu\text{A}$  for 10 min for 11 days (power  $1.8 \mu\text{W}$  and OCV  $200 \text{ mV}$ ) [39]. This proof of concept has opened the way for the optimization of this system to produce more energy and make the device more suitable for long-term implantation. The abdominal cavity is a compartment where extracellular fluids are present, and the movement of the organs also facilitates the diffusion of oxygen and substrates. Previous studies have shown, more than two decades ago, that glucose concentrations equilibrate between blood and intraperitoneal fluid under physiological conditions in rats [63]. More recently, additional experiments from the TIMC-IMAG laboratory have allowed the quantification of the kinetics of glucose exchange between the circulating compartment and the peritoneal cavity. For this purpose,  $5 \text{ ml}$  of glucose-free saline were injected intraperitoneally *via* a catheter to isoflurane-anesthetized rats. A large laparotomy was performed 15 min later and the edges of the surgical incision were kept lifted to avoid blood seepage into the abdominal cavity. Intra-abdominal glucose concentration was then assessed every 5 min for 15 min using a glucometer (Accu-Chek Performa, Roche). All measurements were performed in duplicate. Fig. 8 shows the rapid diffusion of glucose from the vascular compartment to the extracellular fluid and complete equalization of the concentration between the two compartments within 30 min. This experiment confirms the relevance of the abdominal cavity as implantation site, at least regarding glucose delivery.

## 6. Electrochemical practicalities for implantable BFCs

The main task of an implantable BFC is to provide enough power to supply stable electric energy to an active IMD for a period of time that should be longer than its expected operational lifetime. Such a lifetime



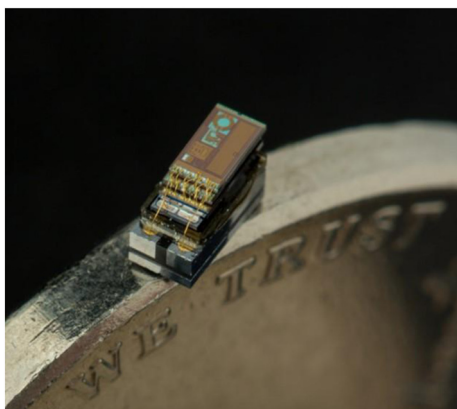
**Fig. 8.** Kinetics of diffusion of glucose to the intraperitoneal space of a rat. Full circles (solid line): glucose concentration in blood; open circles (dashed line): glucose concentration in intraperitoneal fluid. Dashed and solid lines are least squares regression lines fitted to the data.

for an IMD can be several years, which presents quite a challenge for an implantable BFC that needs to operate in human physiological fluids. Nonetheless, there are certain types of BFCs that have design advantages that mitigate their performance drawbacks, particularly by matching the particular BFC design to the specific requirements of the IMD.

In broad terms, an active IMD needs electric power either for continuous or periodic operation [64], which allows one to consider an optimized design for a BFC that is either conventional (devoted to the highest output, when continuously providing stable electric power) or charge-storing (optimized for fast and efficient self-charging along with a long-term charge storage and fast discharge abilities). On more practical terms, an active IMD such as an implantable cardioverter-defibrillator (ICD) [65] requires electric power for a combination of continuous and periodic operation. An ICD continuously monitors the heart's electrical signals and senses when the heart works abnormally, e.g. when it is beating dangerously fast. Within about 5–10 s, the ICD delivers one or more electric shocks (with up to 10 W total power consumption) to return the heart to a normal rhythm [66]. ICDs are also capable of delivering continuous low energy stimuli like cardiac pacemakers. Since self-charging biosupercapacitors/charge-storing biofuel cells are able to operate in both continuous (providing a low electric power on  $\mu\text{W}$  level [51]) and pulse (up to  $0.5 \text{ J}/\text{cm}^2$  even for non-optimized biodevices [51]) modes they seem to be suitable candidates to power ICDs. To the best of our knowledge, currently designed hybrid electric power biodevices have been neither optimized for actual implantation nor studied in human physiological fluids even *in vitro*. Such studies are in the scope of further investigations by our laboratories.

Conventional BFCs are devices that rely on direct (DET) or mediated (MET) electron transfer. Usually, most DET based glucose/ $\text{O}_2$  BFCs provide quite low current and power densities compared to MET based biodevices [67]. This is because of the usage of specific anodic bioelements to design DET enzymatic anodes, such as cellobiose and glucose dehydrogenases [68, 69]. Those redox enzymes have quite low catalytic activity toward glucose compared with glucose oxidase (GOx) and the highly active and selective oxidoreductase now used in most of MET based glucose oxidizing bioanodes [70]. There are, however, some exceptions: highly active mediator-less bioanodes based on mechanically compressed carbon nanotube (CNT) disks with incorporated GOx have been developed [71]. Nevertheless, MET based BFCs usually provide much higher power outputs compared with most of DET based biodevices, even though, theoretically, it should be the other way around. Usage of mediators leads to voltage losses arising from the potential difference between the active site of the enzyme and the mediator. Thus, under identical conditions (catalyst choice, electrode design, etc.) the operating voltage of a DET based device should be higher than that of MET *a priori*. Moreover, mediators are potentially toxic compounds, which makes utilization of DET based BFCs for implantable applications less risky. Taking into account the rapid development of electronics during the last few decades toward the micro and even the nano-scale, when one talks about both size and power (Fig. 9), even BFCs providing electric energy on  $\mu\text{W}$  level can be quite useful devices [72].

The use of oxidoreductases in a DET based enzymatic FCs (EFCs) can achieve high performance due to the biocatalytic turnover numbers of  $10^5$ – $10^6 \text{ s}^{-1}$ , which are close to the diffusion-controlled rates of redox reactions [73]. Moreover, EFCs that contain oxidoreductases also have potentially good biocompatibility. So at least in theory, redox enzymes could be used to create the most powerful FCs, compared with all other devices based on either non-biogenic or biogenic compounds [74]. Moreover, oxidoreductases are natural renewable catalysts, which can be produced at low cost [75]. Furthermore, unparalleled selectivity makes enzyme utilization in BFCs highly advantageous not only technologically, but also scientifically and commercially, by eliminating problems regarding cross-reactions and catalyst poisoning [67].



**Fig. 9.** 1 mm size self-contained device (wireless and self-powered) with M3 computer and temperature sensor. Adapted from [www.eecs.umich.edu/eecs/about/articles/2015/Worlds-Smallest-Computer-Michigan-Micro-Mote.html](http://www.eecs.umich.edu/eecs/about/articles/2015/Worlds-Smallest-Computer-Michigan-Micro-Mote.html).

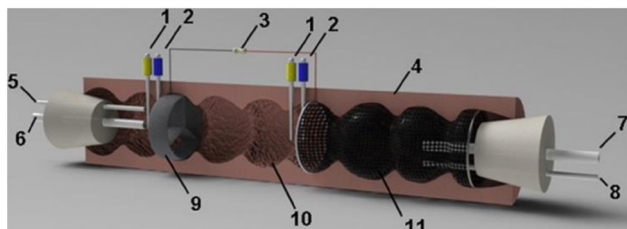
Last but not least, many oxidoreductases are highly active at close to neutral pH values and at room temperature [73], which are conditions for operation of an active IMD.

But what about their long-term and operational stabilities? Even though the fundamental question concerning an intrinsic stability of enzymes in general, and oxidoreductases in particular, has not been properly answered yet [76], problems with stability issues are obvious in practical tests of EFCs both *in vitro* and *in vivo*. Thus, even though this review emphasizes enzymatic devices, other types of BFCs that are based on whole living cells based have the advantage of better operational stability compared to EFCs [47]. Thus, there is a large effort to develop microbial macroscale biodevices as potential power sources for remote objects. To the best of our knowledge there are only few reports in the literature in which human cells have been exploited to fabricate separate bioanodes (human leukocytes/lymphocytes [77]) or biocathodes (red blood cells [78]) of BFCs. Because the reported onset and operating voltages of cathodes based on red blood cells were lower compared with the voltages of leukocyte/lymphocyte based anodes, one can suggest that human blood cells are not very good candidates to design functional BFCs.

### 6.1. Implantable microbial FCs

There are two reports in the literature concerning microbial FCs (MFCs) (Fig. 10) as electric power supplies for IMDs [79, 80].

The authors proposed to place a MFC in human large intestine, utilizing intestinal contents and microorganisms to generate electricity. Because of the large intestinal surface area, even a small power density of a developed MFC (few tens of  $\mu\text{W}/\text{cm}^2$ ) would theoretically allow generation of up to 10 mW electricity, which should be enough to power low and medium energy-consuming IMDs. However, in practical *in vitro* tests the electric power obtained was >5 times lower. Moreover,



**Fig. 10.** Continuous-flow single-chamber membraneless MFC configuration design simulated colonic environment as power supply for IMDs. 1 – redox potential transducer; 2 – pH transducer; 3 – external resistance; 4 – simulated transverse colon; 5 – feed inlet; 6 – sampling port of cathodic area; 7 – sampling port of anodic area; 8 – liquid outlet; 9 – cathodic plate; 10 – simulated colonic haustra; 11 – anodic plate. Adapted from [79] with permission. Copyright 2013 Elsevier B.V.

it is unclear how to achieve the electric connection to an IMD located far from the transverse colon. Furthermore, the long-term consequences of implantation of such a massive device inside the body are not fully understood. Obviously, miniature high performance enzymatic FCs (with power density up to several  $\text{mW}/\text{cm}^2$ ), are a much better option, if sufficient stability of these biodevices can be ensured.

### 6.2. Stability of chemical components of the enzymatic BFC

The major challenges of enzymatic BFCs are long term and operational stability. In general, the instability of an implantable enzymatic BFC is due to at least (i) the instability of the enzyme, (ii) the instability of the cofactor, (iii) the instability of the mediator, (iv) the physical deterioration of the electrodes, or (v) the biofouling of the electrodes (Fig. 11) [81]. There is a widely held notion that enzymes are relatively labile with active lifetimes in solution of hours to days at body temperature [82, 83, 84]. Nonetheless, enzymes can be stabilized *via* genetic engineering, removal of proteases from the system, addition of protease inhibitors, immobilization within a protective polymer structure, crosslinking into aggregates, or encapsulated within a biomineral [85]. There are reports of bioelectrodes with entrapped enzymes, which have retained bioelectrocatalytic activity *in vivo* after almost 6 months of implantation [44].

Many enzymes require additional unbound cofactors, for example  $\text{NAD}^+$  and  $\text{NADP}^+$ , which are not particularly stable in either of their oxidation states. There have been three strategies for dealing with this issue. The first has been to engineer the enzyme to use a more stable cofactor [86]. Secondly, many oxidoreductase enzymes are  $\text{NAD(P)}$ -dependent dehydrogenases, so it is preferable to use alternative dehydrogenase enzymes like PQQ-dependent and FAD-dependent dehydrogenases [87, 88, 89], because they have stable and bound cofactors. Finally, there has been an effort to immobilize the cofactor for eliminating the need to add cofactor to the fuel solution [90], but, to our knowledge, there is minimal experimental evidence that immobilization leads to long term stability of the cofactor.

Instability of the mediator is a large issue in enzymatic fuel cells. When choosing a mediator, researchers will study the reversibility of the mediator in response to many cyclic voltammetric scans. However, even if mediators appear reversible over hundreds of voltammetric scans, organometallic redox mediators frequently lose ligands during long term cycling and organic redox mediators commonly have issue with dimerization or cause electrode passivation.

In an effort to counter physical deterioration of the electrodes, an approach is to add crosslinkers and polymers that bind the electrocatalytic systems more tightly [91, 92, 93]. This does result in increasing the stability, but activity/current density typically decreases because crosslinkers decrease enzyme activity and polymers provide a layer that impedes solute transport to/from the enzyme. Another common issue is fouling of bioelectrodes, which causes a decrease in performance over time. This can be due to non-specific adsorption of proteins and other components in biological samples, as well as microbial attack or immune response attack.

The final concern regarding the chemical stability of BFCs is the chemical and physical complexity of the overall device. To successfully implant a complete BFC, the issue of sterilisation remains the key roadblock to experimental and clinical uses. This is despite the possibility of entirely sealing the device and the partitioning of non-sterile components away from the tissue contacting surface. However, in the special case of a complex device such as a BFC in direct contact with the organism's fluids for an extended period, complete sealing is not possible. Furthermore, it contains biocomponents which would be destroyed by heat and high oxidative agents. Also, the nature of the polymers used in the BFC to entrap any enzymes has to be taken into account. Low toxicity disinfectants or vaporized hydrogen peroxide offer a credible route to sterilisation. Recently, we proposed the use of gamma radiation for the sterilisation of the BFC, and results showed that this method also

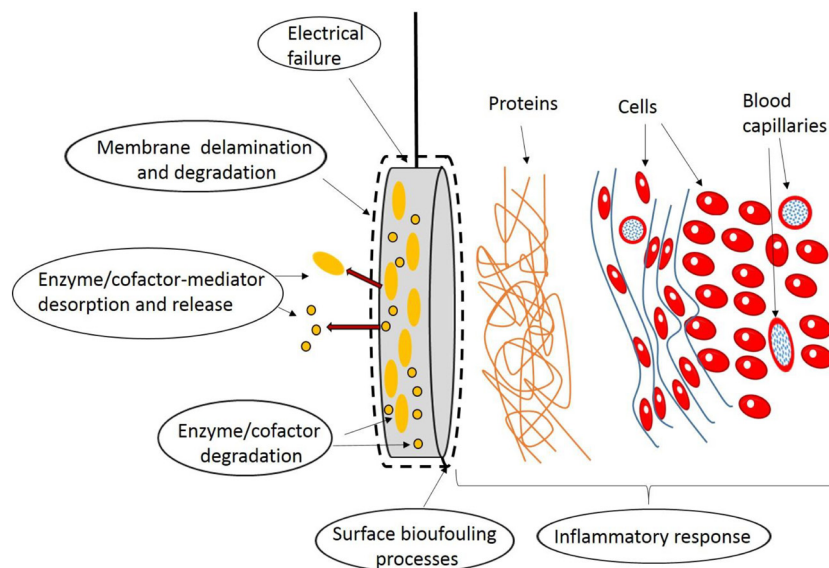


Fig. 11. Illustration of the several potential sources of malfunction for bioelectrodes after implantation.

meets the Pharmacopoeia requirements of sterility with the high degree of sterility assurance level (SAL) of  $10^{-6}$  [94].

## 7. Physiological practicalities for implantable BFCs

In this section the more basic processes occurring at colloidal and cellular level, which might be loosely covered under the term 'biofouling', will be summarized together with some of the mitigating materials strategies that might be used in the future to minimize adverse risk to performance.

### 7.1. Biocompatibility and biofouling

Irrespective of tissue location, medical purpose or functional attributes, an implant constitutes a highly tissue disruptive medical intervention. This has direct adverse consequences for its intended function, and much research effort is directed to optimise the design of the implant to minimize adverse outcomes both for the implant and the patient. A particular difficulty is the unpredictable nature of long term *in vivo* outcomes from short term evaluation. At the core of the material requirement is that the constitutive material is not toxic, teratogenic, carcinogenic nor antigenic. This constraint clearly would apply to both the material itself and its degradation products, which is a particular concern for polymeric implants. One outcome of this has been a high degree of conservatism in applied material development for clinical use, which is quite distinct from the voluminous literature on experimental biomaterials. For example, established materials are the hard tissue support structures as used in classic hip/knee replacement prostheses [95] and 'designer' stents used in coronary artery repair where, for example, a therapeutic agent is released to counter local vascular tissue inflammatory changes in response to local trauma [96].

Independent of the level of engineering sophistication for an implant material nature and its overall design, the body generally recognizes it as just another foreign body and a cause of distorted or disrupted tissue organization. There is a sequence of programmed tissue inflammatory responses and in the case of blood a host of free proteins and cellular components act together to establish a coagulation cascade that eventually forms a surface coagulum made up of fibrinogen and platelets [97]. In some cases, for example for weight-bearing implants, if such surface-induced reactivity does not cause biocorrosion processes, the bulk properties of the implant are not degraded and the mechanical support

function is retained. This is particularly true for implanted batteries, where provided there is effective battery component encapsulation and near-hermetic sealing [98], local tissue reactions have no discernible effects on power generation. At the opposite end of the vulnerability scale are material and devices that require a maintained, stable interface for their function, examples are filtration and dialysis membranes, biochemical sensing devices and drug release systems.

The implantable electrochemical sensor, most frequently embodied in the glucose electrode concept, is based on redox enzymes. As such, their signal output is critically linked to glucose and co-substrate flux to the sensing surface. Any distortion of this due either to changes in the surrounding tissue matrix or the scale of adherent biological deposit has a direct effect on both response dynamics and the steady state response. During the time of implantation this can change in entirely unpredictable ways, with profound consequences for the stability and fidelity of the generated signal. Even the most elaborate of devices, therefore, needs regular recalibration [99]. Similarly, a biofuel cell needs a stable and sufficient solute flux to a reactive surface in order to maintain optimum function and thus good power output. With long-term implantation the encapsulation processes effectively partition the BFC from the available solutes with the consequence of a decay in energy output that could reach sub-critical levels. The design of a BFC needs to consider the cumulative nature of the chronic tissue response and so, for example, the value of protective, permeable barriers has been explored recently [100].

### 7.2. The biomatrix as a colloid system

Immediately on contact with a protein loaded solution, a surface becomes coated with a protein layer. The effect of interfacial forces on the proteins is to distort the native structure and a largely denatured layer is formed which subsequently begins to grow. The key attributes of this are that, regardless of the nature of the engineered surface, denaturation is unavoidable with minimal possibilities for effective desorption or 'self-cleaning'. This initial biofouling layer thereby presents a 'non-self matrix' that, if anything, promotes reactivity to the surface rather than to provide a surface passivating layer. Certainly, the composition of adsorbed protein layer can have an effect on subsequent deposits [101]. Albumin can help retard further deposition or proteins as compared with fibrinogen at least in the context of blood, but the achievement of a fully non-protein coated interface remains elusive. The analysis of such adventitious layers has tended to be morphological,

but in regard to solute dependent devices clearly there needs to be so greater quantitative analysis of their diffusional resistance. It is the latter that will determine the short term power output performance of a biofuel cell. Operation in serum, for example, has given useful indications of performance [102] and design needs, and is of relevance to deployment in the tissue matrix because, whilst interstitial tissue is normally poor in protein, this ceases to be the case following implantation trauma and the later sequence of inflammatory changes are in any case effected through an influx of protein mediators and a plasma and cellular influx through a permeabilized local capillary bed.

### 7.3. The tissue response

Following implantation of any device, there is firstly a capillary response to the induced trauma, extravasation of blood and a millisecond timespan dynamic of a primary protein coating layer. The proximity of the implant is a high theatre of inflammatory mediator turnover manifest as high concentrates of cytokines and chemoattractants for cell unregulated inflammatory cell recruitment, independent of the fact that the implant is microbiologically sterile.

The first cells on the site are phagocytic polymorpholeucocytes ('white cells') which characterize the early phase of any inflammatory response. Subsequently, mast cells, variously releasing potent chemicals (e.g. histamine, heparin, serotonin) together with extravasated fibrinogen are seen. The latter coats the implant, and in contrast to an albumin coating, promotes the inflammatory milieu around any foreign body [103]. The effects of this physiological response cannot leave the movement and metabolism of putative biofuel molecules unaffected. This has been examined quantitatively with regard to the implantable electrochemical glucose sensor by Reichert's group [104, 105], who analyzed and modelled the effect of inflammatory changes on glucose accessibility to a tissue embedded sensor. Surprisingly, they did not consider macromolecular surface biofouling to cause a loss of glucose flux, but found instead that it was inflammatory cell components of such biofouling layers that actually compromised glucose responsiveness. The likelihood is that with cellular inflammation a biofuel cell would have to compete for glucose and  $O_2$  with high metabolic activity white cells. It is also relevant in this regard that inflammation activated leucocytes

(now tissue macrophages) have an upregulated metabolic activity likely to act as an intensified sink for glucose/ $O_2$ . The transport model used appeared to indicate that the barrier effect of leucocyte layers was also not a major factor in transport limitation. Furthermore, the less active metabolizing red blood cells that washed into the region, whatever their local prevalence, did not create a significant pathway for glucose losses. An implanted biofuel cell will be subject to identical initial environments. However, in contrast to glucose sensors where a barrier membrane is integral to *limiting* diffusion in the first place, the flux of glucose is to be maximized for a biofuel cell. Hence, a protein/cellular adventitious layer will likely have a substantial effect on performance and power output; high barrier membrane packaging for biofuel cells does not seem to be a rational strategy.

What is less clear are the potential effects of the foreign body giant cell, which is another player in the implant response that does not feature in normal inflammation. This end stage multinucleated cell results from the fusion of phagocytic tissue monocytes and releases potent moieties that can degrade materials and promote inflammation (Fig. 12) [105]. So the inflammatory change around the implant is actually qualitatively different to that in normal inflammation. A further special aspect is the binding of macrophages to foreign material mediated through the various surface covering protein layers. Such binding is made possible through integrin proteins of the cell membrane and functional motifs on the proteins, such as the RGD amino acid sequence. In the case of polyurethane, which is a candidate for biofuel cell packaging, this proximity, cell mediated degradation and the local generation of free radical can lead to serious degradative change culminating in stress cracking. The facilitator of the cellular profile is local blood flow. The modulation of local capillary density, through new growth and increased blood flow, is a key counterpart of the response [106, 107]. Such neovascularisation is a constant part of an evolving inflammatory process, and indeed, agents promoting the growth of new blood vessels have been tried as active device components to generate local delivery of metabolites such as glucose. Their biological metastability, however, makes it unlikely that there would be benefit for long term implants, though they are worth considering as test systems for biofuel cell evaluation under different local blood flow conditions.

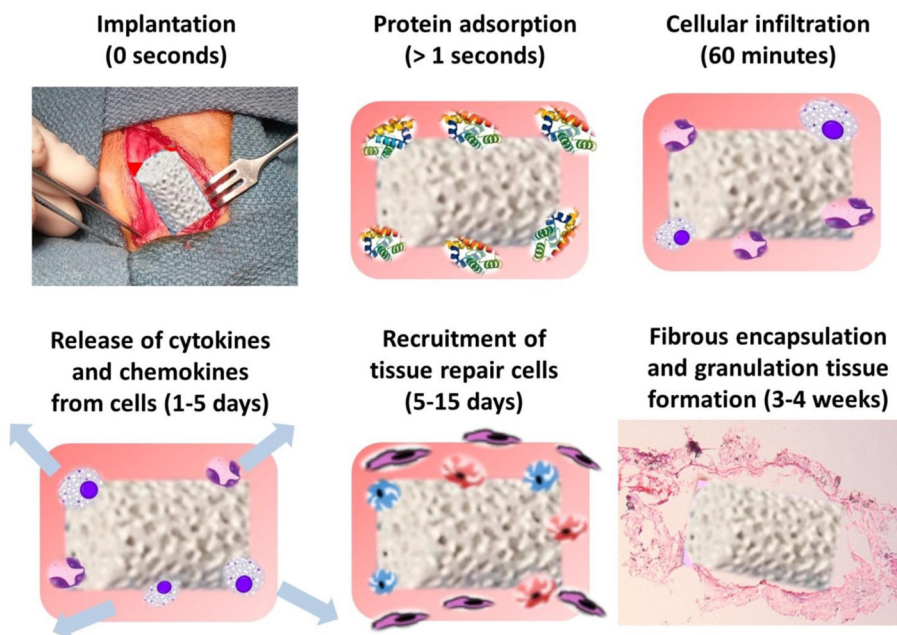


Fig. 12. Sequence of surface interactions immediately following biomaterial implantation, commencing with protein adsorption followed by cellular recruitment and the inflammation end stage of fibrous encapsulation (From Ref. [14] with permission).

The end result of the acute cell proliferative stage of inflammation is an excess tissue matrix. This is not simply the original edema fluid, but a sustained fibrous and glycosaminoglycan phase that bulks up the interstitial tissue (Fig. 13). This combines with the growth of granulation tissue, which is an ultra-high vascular regenerative tissue that in normal circumstance promotes tissue repair. However, in the presence of a non-resorbable implant, granulation tissue culminates in fibrous tissue deposition around the device and its encapsulation since the body attempts to wall-off the implant that it cannot degrade. It is also the case that early-stage macrophages, especially through hydrolases, hypochlorite and free radical generation, will have initiated surface degradative changes on any protective packaging of the fuel cell. The long-term influence of macrophages and their behavior will most likely be influenced by subtle interactions such as device micro motion in the tissue, and surface physical properties such as stiffness, profile and pore structure. The behaviour of macrophages has also been recognized to function as mobile chemo- and mechanosensors [106].

At the conclusion of the acute phase a collagen dominated fibrous capsule forms around the implant. Although this does not have a direct surface biofouling action and may in fact have limited adherence to the implant, its action as an outer barrier has profound implications for biofuel cells targeted to operate with similarly long lifetimes as for pacemaker batteries. The collagen phase is a dense polymeric membrane material that has an anisotropic structure, is micro/nanoporous, is cell free and is capable of charge, size and polarity based molecular discrimination. This final avascular capsule as it thickens and densifies and remodels will have barrier properties that are clearly different to that of normal tissue interstitial, with a consequent reduction in the transport of a micro solute such as glucose [108, 109].

In addition to the immune and tissue responses in the immediate environment of a biofuel cell, it is possible that there will also be local changes in biochemistry including to pH,  $pO_2$  and  $pCO_2$ . Such biochemical changes will be driven by the balance between vascularity and leucocyte density, which will also be influenced by the inflammatory responses. The data are as yet incomplete, but in one study of chronic

wound surfaces there was clear evidence of hypoxia with sustained pH gradients measured between pH 6.5 and 8.0 [110]. Within the sequestered fibrous capsule site, the environmental influence of the bio-cathode/anode reaction is clearly complex. The so called constancy of the *in vivo* environment is perhaps over stated, and the traditional approach to assess *in vitro* responses of biofuel cells using isotonic phosphate buffered saline less fit for purpose than might initially appear. What is necessary now is to model and assess biofuel cell performance under better suited physiological model conditions.

#### 7.4. Materials strategies

For *in vivo* biosensors, extreme low permeability barrier membranes impose a dominant diffusive resistance such that any additional impact of surface biofouling or tissue change becomes secondary. However, this comes at the price of a suppressed solute flux, and would evidently not be appropriate for a high fuel flux dependent biofuel device. Nevertheless, it should be possible to engineer surface modifications to the device package so as to retain high permeability at the same time as retaining a bioadapted surface that reduces both physical fouling and the inflammatory outcome. A simple way of achieving this could be to use hydrophilic and bioinert polymers, since less protein denaturation occurs on these as compared with hydrophobic surfaces. The latter in any case pose a barrier to polar molecules unless they have a built in microporosity. The latter is an engineering option only provided the pore structure could be made to be stable over time and resist colloid blocking. However, it is likely that a continuous barrier layer will be needed as a buffer phase to partition leachable and antigenic fuel cell components from the body. Chemically grafted bioactive molecule coatings including heparin, collagen and cell interactive peptide sequences, such as RGD, have all been reported [111]. However, their lifespan on a surface is limited and long term effectiveness uncertain. Non-biologics may have a place here in that poly(ethylene glycol) (PEG), brush polymers and copolymers employing PEG derivatives could provide longer sustained biocompatible systems. Though synthetic methods are well established for all these, and such materials would likely be clinically acceptable, the biophysical levers for biocompatibility are as yet uncertain. What the optimum might be for functional group density, surface mobility and need for surface nanoscale distribution patterning remains unknown. However, the use of zwitterionic molecules has been proved effective as a design feature [112]. The early pioneering work of Chapman's group adopted a biomimetic strategy in using zwitterionic phosphoryl choline groups in a bioinspired strategy that modelled the biocompatibility of the outer cell membrane surface [113].

The surface of a polymer can be nanostructured so as to engineer feature sizes above 50 nm, which are known to influence cell attachment, motility and activity quite independently of surface chemistry [114]. As a final remark, it is reported that late stage capsule density and thickness [115, 116] may well be conditioned by the early stage interactions, and so a shorter-term tissue model studied with the biofuel cell can have important heuristic implications for the rationalisation of the design of biofuel cells.

#### 8. Ethical considerations

If we consider that a major application for enzymatic BFCs is to provide an implantable power supply for medical devices, then the major challenges remain as being primarily physiological rather than purely electrochemical. Such physiological challenges include finding solutions to the biocompatibility and biofouling problems of implanting a biofuel cell, which then also have a major impact on the availability of the substrates inside body that provide fuel for the biofuel cell. These physiological challenges are essential to consider for biofuel cells that are designed to be implanted for long-term operation inside a living animal and eventually to human clinical applications. Such a clinically-oriented direction for the application of BFCs necessarily requires consideration

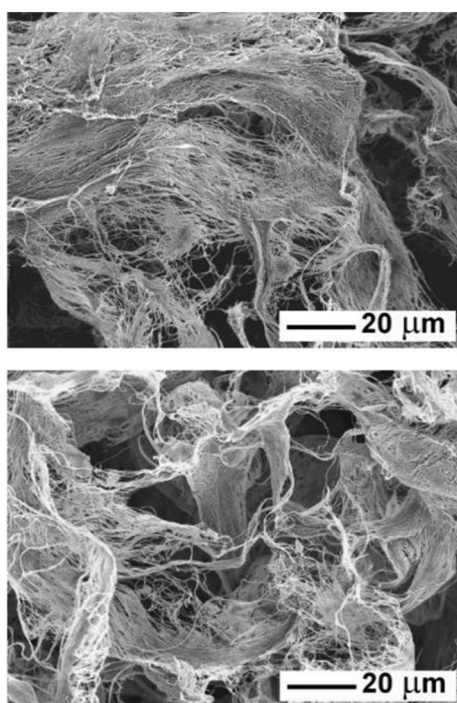


Fig. 13. SEMs of two collagen-glycosaminoglycan composite samples showing residual collagen fibers after partial collagenase treatment.

of the ethics of using animals and humans in research that leads to developing clinically useful medical devices. Of course, the use of a GBFC in plants has advantages for environmental monitoring and applications, such as in wireless transmission using the power supplied from a BFC implanted in an orange growing on a tree [117]. Such applications for environmental monitoring do not raise the same type of ethical considerations as for implantable BFCs in animals.

Experiments for BFCs that function in invertebrates and arthropods, such as clams [42], snails [41] and cockroaches [40], do not raise the same level of ethical concerns as for animal experimentation. Another advantage of experiments using invertebrates and arthropods is the existence of an haemolymph, which has a higher concentration of non-fixed (directly available) oxygen for diffusion to the electrode. That directly available oxygen increases the yield of the BFC as compared to implantation in animals, where oxygen is fixed to hemoglobin in the blood. Furthermore, the surgery for the invertebrates and arthropods is quite rudimentary since the GBFC is literally driven in the living organism without any thought of sterility and biocompatibility. Although there is electrochemical information to be gained, the risk is that the experiments are too far from physiological conditions in humans even if the ethical concerns are less for experiments on invertebrates and arthropods compared to animals closer to humans.

At this stage, the ethical considerations for animal and human applications require that all the physico-chemical properties of the BFC must be tested under appropriate *in vitro* conditions before any *in vivo* testing in an animal experiment. Such *in vitro* testing is performed using conditions that mimic the human (or animal) *in vivo* physiology. Indeed, to mimic the *in vivo* environment the BFC can be tested in plasma or serum at an appropriate temperature (using 37 °C instead of 25 °C or RT) and pH (7.4). Also, the *in vitro* experiments can be performed in physiological salt buffer solutions, with or without added proteins, and under controlled concentrations of gas (CO<sub>2</sub> and O<sub>2</sub>). Even the presence of enhancers and potential inhibitors for the bioelectrode reactions can be tested in this way. A typical example is the use of different concentrations of chloride ions to assess laccase activity [118]. Also, an accelerated aging test for the BFC can be performed using an elevated temperature to increase the Q10 (thermodynamics) of the electrochemical and polymeric reactions in the BFC bioelectrodes. Although such *in vitro* conditions can be designed to closely mimic the *in vivo* physiological conditions, additional animal experimentation is required to properly assess the complex biocompatibility of the BFC. This includes the influence of the implanted BFC on the immune response, the inflammatory response, and the long-term physiological response of the host. Indeed, it is compulsory to use animals at the pre-clinical stage of developing implantable medical devices and the animal is the only existing integrated model that is available.

Animal testing follows strict protocols in accordance with animal welfare legislation, notably by applying the “three Rs” rule of “Replace, Reduce and Refine” [119]. When it is not possible to use an alternative method (“Replace”), then we choose the least sensitive animals from the point of view of neurophysiology. If an animal has a functional nervous system, then that animal is highly sensitive and the experimental constraints imposed on the animal must be perfectly justified. The number of animals used in the study must be “Reduced” while ensuring that valid and reproducible data can be accessed. The use of anesthetics and analgesics is mandatory (“Refine”) in order to reduce animal suffering. Since 2012, in addition to considering the ethics of the “three Rs”, the enrichment of the environment was considered to play an increasingly important role in the overall health of the animal and hence to the relevance and success of experiments. Indeed, transportation, handling, care, and housing conditions are all sources of stress that can affect laboratory animals. Those sources of stress can profoundly change not only the behaviour of the animals but also the physiological and biochemical processes. One of the first experiments that demonstrated the importance of environmental enrichment was concerned with the thickening of the rat cerebral cortex [120]. This fact is now well established and

there is a very well-documented literature on the influence of the physical and social environment on research outcomes [121, 122].

The ethical approach is not only about compliance with regulations. An important part involves the personal will of the experimenter. In this context, the three Rs rule becomes the four Rs with the responsibility of the experimenter, who must carry out an ethical study prior to the protocol being put in place. To assist the experimenter, there are quantitative scales that measure the degree of relevance of using animal testing [123, 124] with the goal of achieving the highest possible score in all categories. The correlation between animal welfare and good quality experimentation results is well established. In this context, all actions lowering the level of stress of the animal used in the experimentation are of benefit to a successful implantation. This is also partly due to the fact that the animals would also be in good physical and psychological health. The biological responses to stress include the response of the immune system, and it is essential to promote good healing and a state of well-being avoiding opportunistic pathologies. In practice, environmental enrichment covers many ingenious and imaginative technical initiatives to provide opportunities for activity and retreat, and also taking the social behaviour of the animals into consideration.

## 9. Conclusion

In recent years, the research in enzymatic BFCs has made a great deal of progress and several strategies have been proposed to overcome the problems related to their performance and stability. In parallel, several teams had published their work concerning implanted BFCs, and demonstrated the feasibility to produce electric power from substrates present in life body. Much of the essential electrochemistry for sub-component operation and efficiency for BFCs has been determined under *in vitro* conditions. Nonetheless, the issues of implanting an electrochemical device such as a BFC require an approach that takes a strong account of the problems of biocompatibility. There are two essential challenges for the design of a successful implantable BFC, which are (i) to overcome the engineering challenges at the laboratory-level to design and optimise bioelectrodes, with an acceptable surface area and volume, that are capable to deliver the required threshold power density for a sufficient lifetime. Unfortunately, to date little work has been done to optimise the BFC geometry and to demonstrate the lifetime of the BFC, under laboratory-based physiological conditions, to deliver sufficient thresholds of electrical power; and (ii) to overcome the problems of biocompatibility in order to achieve long-term *in vivo* function of the BFC. In particular, the presence of the implanted BFCs must be well tolerated by the body and cause no chemical or biological undesired reactions. Moreover, the living body must provide sufficient substrate to reach the BFC for operational viability in the first place. Today, except for our own recent studies, there are no specific reports on the interactions between the glucose/O<sub>2</sub> requiring BFCs and the physiology of the living organism, and it is this specific dynamic that will remain the real challenge to the development of future clinical implantable BFCs. Furthermore, even if there has been recent progress towards overcoming the biocompatibility challenges, at the fundamental basis of enhancing the performance of the BFC, there remains the need to improve the control and stability of the enzymatic bioelectrode to bring implantable BFCs closer to real-world medical applications.

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