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► **To cite this version:**

Sufia Ul Haque, Mohammad Yasir, Serge Cosnier. Recent advancements in the field of flexible/wearable enzyme fuel cells. *Biosensors and Bioelectronics*, 2022, 214, pp.114545. 10.1016/j.bios.2022.114545 . hal-03797703

**HAL Id: hal-03797703**

**<https://hal.science/hal-03797703v1>**

Submitted on 7 Oct 2022

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## **Recent Advancements in the field of flexible/wearable enzyme fuel cells Cells**

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### **Abstract**

This review article focusses on new advances in the field of enzymatic biofuel cells (EFCs), especially, on flexible materials which can be used to make flexible EFCs for wearable devices, three-dimensional (3D) printed structures for electrodes, EFCs preparation via 3D printing techniques, and micro/nano electromechanical structures (MEMS/NEMS) to fabricate micro-EFCs. Particular attention is given to newly developed approaches to obtain efficient electrodes for harvesting energy via EFCs. This review article explains the various attributes of these recently developing technologies and their ability to mitigate the energy requirements of flexible/wearable bioelectronic devices. Besides discussing key milestones achieved, this perspective review article also emphasizes the main hurdles that are currently impeding the realization of flexible/wearable EFCs. Particular emphasis has been given to the major hurdles related to the flexible materials required to fabricate wearable EFCs, suitable 3D printing techniques required, and MEMS and NEMS to fabricate micro-EFCs. In all the recent developed techniques, there are some common issues like stability, high power output, mechanical strength and flexibility. Also, this review article provides various routes to mitigate these issues. The main aim of this perspective article is to develop curiosity among the researchers of various fields to team up in order to address the huge challenges that restrict the real-world application of flexible/wearable EFCs. Such collaboration is important to harness the full potential of EFCs.

**Keywords:** Flexible/wearable EFCs; 3D printing; MEMS and NEMS

## **1. Introduction**

Biofuel cells (BFCs) harness electricity from biologically catalysed reactions using enzyme, bacteria, or algae as the catalysts for the oxidation and reduction of fuel and oxidant, respectively. The compliance of nature to provide a wide range of organic substrates extends the capability of BFCs to derive electrical power. BFCs are categorized into those that utilize bacteria and algae, known as microbial fuel cells (MFCs), and those that use enzymes, enzymes cascades, and mitochondria, known as enzyme fuel cells (EFCs). The remaining detail of EFCs is provided in supplementary document under heading S1.

This review is intended to provide a selective overview on new insights for the advancement of EFCs. The wide range of reviews available have focused on EFCs and their aspects (Brito and Turner, 2010; Chen et al., 2020; Wang et al., 2021; Ivanov et al., 2010; Osman et al., 2011; Willner et al., 2009; A. Karim and Yang, 2021). We focus here on new advancements in the field like flexible materials which can be used to make flexible EFCs for wearable devices such as those that allow a monitoring process on soft surfaces like human skin as an ideal platform for personalized wearable devices. Also, flexible EFCs are unique in properties such as light weight, low cost, high flexibility and great conformability. Further in the list of advancement are 3D printed structures to fabricate electrodes for EFCs via 3D printing techniques, and micro electromechanical structures (MEMS) to fabricate micro-EFCs.

## **2. Flexible EFCs for wearable devices**

The on-body energy scavenging wearable bioelectronic devices have been gaining huge attention in this power growing era. The modern on-body wearable EFCs in the form of patches, tattoo, textiles, and contact lenses open the way for bioenergy harnessing and self-powered biosensing. These non-invasive BFCs were employed to utilize bioenergy or monitoring biomarkers available in the biofluids like tears, saliva, fitness, sweat, and interstitial fluid (Zhang et al., 2021). But, the real-world application of potent wearable EFCs needs an efficient and excellent enzyme-electronic interface having a good electrochemical system and mechanical flexibility. Also, the knowledge of the mechanisms and kinetics involved in the electron transfer and enzyme immobilization techniques, are important for the development of stable bioenergy harvesting devices under various mechanical strains, fluctuating operating conditions, and in a diverse outdoor activity (Li et al., 2021). Such bioelectronic devices development is dependent upon body-compliant soft electrochemical

systems made of various flexible and stretchable materials. The further information is provided in supplementary document under heading S2.

For instance, a temporary tattoo-based BFCs was made of an elastomeric thin film utilized as a flexible underlying primary material based on ecoflex and polyurethane (PU) (Figure 1A) (Bandodkar et al., 2016b). It is important to maintain a slight gap between the skin and tattoo to enhance the flow of biofluid over the layer of enzyme as well as keeping the BFC safe from detachment due to the built-up pressure (Figure 1B) (Jia et al., 2013). The screen-printed BFC made of stress-enduring nano carbon tube-based ink, formulated to provide the desirable flexibility for skin-worn applications. The significance of realizing this flexible BFC was the free-standing serpentine electrode assembly carrying the combination of mechanical and electrochemical properties of carbon nanotubes (CNTs) having intrinsic flexibility offered by the PU binder. The combination of serpentine electrode structures made of stress-enduring materials enhanced the degree of device flexibility up to 500%.

Free standing buckypaper functionalized with a pyrene-polynorborene homopolymer was used as electrode material for flexible lactate/O<sub>2</sub> BFC (Chen et al., 2019). A stretchable screen-printed current collector with an island bridge configuration was used to ensure conformal contact between the wearable BFC and the human body under stretching condition. The stretchability of the device was maintained by the coupling of an island bridges design and strain enduring inks. This device retained its performance stability under multiple stretching cycles. This research work demonstrated the promising potential of the buckypaper-based EFC for next-generation wearable bioelectronics. Also, a textile based flexible nylon/polyurethane material support used for the bioanode and biocathode immobilized with fructose dehydrogenase and bilirubin oxidase, respectively, as shown in Figure 1C (Ogawa et al., 2015). The CNTs dispersed in deoxycholate or Triton X-100 were used for the coating of the fabric which showed high conductivity of 200 mScm<sup>-1</sup>. In between these textile-based electrodes (anode and cathode), fructose fuel stored in a double network hydrogel sheet was placed for the oxidation of fructose at the anode and reduction of oxygen at the cathode. This BFC provided a power density of 0.2 mWcm<sup>-2</sup> with a stretching stability of 50% for 30 cycles.

Also, textiles and electronic yarns are promising materials to be used for wearable devices, capable of performing electronic functions and offering large geometric areas for the integration of body-compliant wearable electronic devices, but posing various material challenges (Shi et al., 2020). Such textile-based energy conversion with energy storage

devices have already confirmed their potential for wearable bioelectronic applications. In the beginning, efforts were made to integrate the conformal electrodes onto textiles by using screen printing techniques as shown in Figure 1E (Jeerapan et al., 2016; Lv et al., 2021) or the deposition of conductive materials on non-conductive textiles (Andrew et al., 2018; Ogawa et al., 2015). Still, the wearable electronic devices based on electrically conductive fibres or yarns that transmitted electrical signals via a garment have grabbed much more attention for the manufacturing of on-body bioelectronic devices due to their direct attachment with normal clothing (Zeng et al., 2014).

The main challenges of textile-based wearable bioelectronic devices are the need for repeated washing while maintaining their existing properties and the effective wiring of biocatalysts with the electrodes. Recently, several studies demonstrated washing resistant bioelectronic devices (Van Lam et al., 2016). Numerous approaches utilized modified conductive yarns and printing techniques with modified inks like CNT based inks comprising gel-based nanoparticles and ionic liquids (Lee et al., 2018). Yet, the construction of washable BFCs is a huge challenge as the fabrication of conductive yarn for the construction of bioelectronic devices is very complicated. However, carbon-based nanomaterials seem to be very attractive as a host for the entrapment of biocatalysts due to their outstanding electrical conductivity, electrochemical properties, and high surface area. That was the reason encouraging the idea to synthesize conductive yarn made of vertically aligned multiwalled carbon nanotube (MWCNT) sheets coated with poly(3,4-ethylenedioxythiophene) (PEDOT) for the immobilization of enzymes (Kwon et al., 2016). But then again, the loading of enzymes onto a small support in a linear profile/fashion poses another major issue. For instance, the construction of self-assembled PEDOT-MWCNT yarn electrodes loaded with enzyme depended on the surface tension of the drying enzyme solution. The electrode showed direct electron transfer (DET) between aligned MWCNTs and GOx enzyme. Thus, the yarn-based BFC generated  $236 \mu\text{Wcm}^{-2}$  in glucose solution (0.03 M) without any mediators or cross-linkers.

Conductive metallic yarns were prepared by layer-by-layer assembly of gold nanoparticles onto the fibres of cotton with the help of organic linkers (Kwon et al., 2018). These metallic yarns provided an excellent electrical communication with glucose oxidase enzyme loaded on the anode. Also, these conductive metallic yarns could also function for oxygen reduction reaction (ORR) at the cathode without enzyme immobilization. The gold nanoparticles coated cotton fibres were utilized to construct the glucose/O<sub>2</sub>-based BFC with enhanced DET between conductive yarns and glucose oxidase. The power density obtained from this

metallic yarn-based BFC was  $3.7 \text{ mWcm}^{-2}$ . An alternative yarn-based stretchable, stable, and soft fibre BFC was constructed by utilizing a rubber fibre wrapped with MWCNT sheets (Sim et al., 2018). This MWCNTs wrapped rubber fibre provided stretchability (100% in the tensile direction) and was used as an effective support to immobilize Os-based redox polymeric mediators, enzymes and a cross linker. This diameter of the BFC fibre was approximately  $380 \text{ }\mu\text{m}$ , which could provide stable power density even under external stretching. This BFC used GOx at the anode and BOx at the cathode with mediated electron transfer processes. The constructed fibre-based BFC could bear up to 100% stretching strain while producing sufficient power. On applying 1000 stretch-release cycles, only a 5% reduction in power density was observed compared to the initial power density.

The environmental conditions anticipated in real-life by the EFCs poses more challenges for their employment in energy-harvesting wearable devices. It has inspired the development of new approaches aimed at the enhancement in the stability of the enzyme's catalytic activity, which contributes directly to excellent BFC performance. The further text is provided in supplementary document under sub-heading S2.1.

Further, a stretchable wearable skin-based BFC was fabricated, which showed high power output ( $1.2 \text{ mWcm}^{-2}$ ) for long duration obtained from human sweat. The enhanced performance of BFC was due to three-dimensional (3D) anodes and cathodes immobilized with enzyme (Bandodkar et al., 2017). The power was obtained for long duration even under repeated stretching, which was due to the stretchable island-bridge architectural design integrated with serpentine bridges (Figure 1D). The better performance of this wearable BFC was due to the enhancement in the loading of enzyme, mediator, and conductive base through packing as pellet. Here, the enzyme and mediator are involved in electrode composition rather being superficially covered by drop casting. With the assistance of a DC/DC converter, this BFC was able to power a light emitting diode and a Bluetooth Low Energy Radio.

The fabrication of buckypaper is straightforward still some factors create challenges regarding material reproducibility and functionality. The further text is provided in supplementary document under sub-heading S2.2. Cosnier's group developed the lab-made flexible buckypaper by combining CNTs with polynorbornene linear polymers having pyrene groups (Cosnier et al., 2015). They demonstrated that polynorbornene-pyrene functionalized buckypaper was an excellent support material for redox mediator and enzyme immobilization (Fritea et al., 2019; Gross et al., 2016a). The main advantage of buckypapers compared to other 3D carbon electrodes is that they are free-standing and do not require additional support for current collection (Elouarzaki et al., 2015). Although exhibiting high CNT densities,

buckypaper electrodes, in contrast to CNT pellet electrodes, are lightweight, very thin, and can have large surface areas. The mechanochemical properties of buckypaper can be modified for the construction of effective EFC (Lalaoui et al., 2016). The strength and Young's modulus of buckypaper electrodes can be modified by integrating polynorbornene copolymers and molecular cross-linkers (Choi, 2018; Gross et al., 2016b). The integration of polynorbornene copolymers gave the insight to incorporate many other functionalities to the buckypaper, like pyrene groups (as cross-linkers) and N-hydroxysuccinimide ester (for covalent wiring of enzymes) (Gross et al., 2016b; Yin and Tang, 2018). These research work showed that buckypaper based materials have high mechanical stability and flexibility. This material is considered as a promising alternative to design flexible BFCs. The further text is given under sub-heading S2.3.

Several challenges impeding the development of the wearable EFCs have been mitigated through the utilization of advanced materials and innovative approaches. All the designing parameters of high-performance wearable EFCs are intrinsically linked, for example, the various challenges regarding the enzyme-electrode interface can be catered by fabricating a conductive and flexible material having large surface area able to immobilize a large amount of enzyme and enhance the DET while enduring mechanical strains resulting from body movement. This progress will provide promising biocatalytic activity and sufficient electrochemical performance under diverse on-body functions. Besides the mechanical aspects of the material used for developing the flexible wearable EFCs, research should also be aimed at abolishing the use of mediators by synthesizing the new advanced functional materials that support DET. Mediators are generally used in conventional EFCs. Using it in wearable EFCs can reduce the performance, biocompatibility, and stability of wearable EFCs. This challenge can also be mitigated by using a proper strategy that meets the requirements of charge transfer processes and correct enzyme orientation. Additionally, the research on making enzyme's conductive by genetic modification for efficient DET appears as a promising method to address this issue (Tan et al., 2016).

**<Preferred position for Figure 1>**

### **3. 3D-printed structures to fabricate electrodes for EFCs via 3D-printing techniques**

3D printing is also known as additive manufacturing. It is widely utilized in the field of electrochemistry to prepare electrodes and devices for electrochemical energy conversion (water splitting, BFCs) and storage (batteries and supercapacitors). This technique provides

fast prototyping of simple and complex structures from various precursor materials and is relatively inexpensive (Ngo et al., 2018; Tofail et al., 2018). However, to get a particular design and performance compatibility to develop the complex electrode structure and electronic device, there are certain things to take care of the 3D printing techniques. The 3D printing depends on the selection of ink material (well-balanced rheological properties including viscosity, shear-thinning), substrate properties, electrochemical performance, flexibility, and feature size.

The fabrication of electrodes plays an important role in promoting the performance of EFCs. The text regarding electrode preparation is provided in supplementary document under heading S3.

Inkjet printing can fabricate complicated structures in confined spaces. For example, a commercial desktop inkjet printer printed a supercapacitor electrode of different shapes (Sundriyal and Bhattacharya, 2017). Further, along with the activated carbon, graphene oxides (GO),  $\text{MnO}_2$ , and composite of GO- $\text{MnO}_2$  were examined as electrode materials. Further, a research work reported a wearable wristband fabricated from inkjet printing having a self-powered sensor system detecting gas, supercapacitors, and embedded solar cells as energy sources on a piece of the flexible plastic substrate, as shown in Figure 2B (Lin et al., 2019). This wearable system consisted of printed silver (Ag) interconnects, amorphous silicon (a-Si) solar cells array as energy harvesting and conversion module, planar  $\text{MnO}_2$ -based supercapacitors as intermediate energy storage devices, and  $\text{SnO}_2$  gas sensor with a light emitting diode (LED) indicator as active and power consumption units. The whole system was designed into a planar configuration for the sake of monolithic integration. To realize such a system, various types of inks were utilized to prepare printable devices with decent performances. However, the optimized hybrid ink made of ( $\text{MnO}_2$ )/reduced graphene oxide (RGO)/poly(3,4-ethylene dioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) were used successfully. Efforts have also been taken to prepare freestanding paper-like electrode materials for the utilization in flexible and lightweight energy storage devices (Figure 2C) (Chi et al., 2014). The freestanding graphene paper electrode supporting 3D porous graphene hydrogel-polyaniline nanocomposite was synthesised by well-controlled full inkjet printing method. These nanohybrid binder-free paper electrodes were employed to construct flexible and light-weight supercapacitors with high performance. The developed supercapacitor exhibited remarkable mechanical flexibility, high cyclic stability, and an acceptable energy density of  $24.02 \text{ Whkg}^{-1}$  (at a power density of  $400.33 \text{ Wkg}^{-1}$ ).



A research work coated a primer layer of cellulose nanofibre on commercial A4 paper to tune the wetting and non-wetting features, hence aiding high resolution inkjet-printed paper based supercapacitors (K.H. Choi et al., 2016) (Figure 2D). Table S2 mentioned in supplementary document summarizes the various examples of inkjet-printed power sources which could provide ideas for further development. Further text regarding 3D printing is given in supplementary document under heading S3.

However, macro and microscopic 3D porous structures of graphene and its derivatives have been studied for printed electrodes (Fu et al., 2016; Lacey et al., 2018; Zhu et al., 2016). A research on holey graphene oxide was carried out to construct a hierarchical porous structure simplifying ion transport and interfacial electrochemical reactions over the electrodes (Lacey et al., 2018). The hybrids based on graphene-based 2D materials resulted in a mixed-dimensional (2D+nD, where n=0,1,2, and 3) composite materials that have well interconnected electron conducting networks and ion transporting pores. For instance, a research study reported a mixed-dimensional activated carbon (3D)-RGO(2D)-carbon nanotube (1D) 3D printed electrode. These 3D printed mixed-dimensional hybrid material electrodes exhibited hierarchical porous structures ranging from the nanoscale to macroscale and presented good capacitance retention (Gao et al., 2019). The 3D printed graphene-poly(lactic acid) (PLA) electrodes were prepared and considered to be eco-friendly due to the involvement of PLA, one of the most commonly used biodegradable polymers for 3D printing as presented in Figure 2D (Manzanares-Palenzuela et al., 2019). PLA can be partially or completely digested in a controllable fashion by enzymes like proteinase K. This enzyme catalysed the digestion of 3D-printed graphene-PLA electrodes, revealing electroactive graphene-based 3D printed structures for sensing application based on the immobilization of alkaline phosphatase at the electrodes. These electrodes were used for the detection of 1-naphthol.

Further, 3D printed RGO-PLA electrodes were developed for sensing and biosensing applications (Silva et al., 2020). The 3D printed RGO-PLA electrode was used to detect serotonin in synthetic urine and the biosensing application of this electrode involved the immobilization of tyrosinase for the detection of catechol in natural water samples (Figure 2F).

The electrode designing on the mixed-dimensional materials for the electrode preparation of enzymatic BFCs may offer several advantages such as high-power density. However, recently the 3D printing technique is employed for the construction of Li-ion batteries, electrical/electrochemical sensors, various antenna, and titanium based microsupercapacitors

(Foster et al., 2017; Jun et al., 2018; S.B. et al., 2019). Especially, for EFCs, 3D printing was utilized to prepare the mold for the construction of microchannels through lithography (du Toit and Di Lorenzo, 2015). So far, the 3D printing technique is yet to be explored for the whole EFC fabrication.

A research study reported the fabrication of a fully integrated miniature 3D printed microfluidic membraneless EFC (Rewatkar and Goel, 2020, 2019). Polylactic acid/graphene were used to construct the 3D printed electrodes which were further treated by dimethylformamide to enhance the electrochemical performance. The fabricated miniature 3D printed microfluidic membraneless EFC showed excellent performance under laminar flow of fuel for 9 days. The power density obtained from this BFC was found to be  $4.15 \mu\text{Wcm}^{-2}$  for a  $500 \mu\text{Lmin}^{-1}$  optimized flow rate. Further, a glucose/ $\text{O}_2$  BFC was realized by assembling 3D printed multifunctional electrodes having high porosity made of carbon-based conductive filaments. The Fused Deposition Modelling method mostly dealing with polymeric filaments was utilized to fabricate the electrodes. This research work compared the performance of two different bioelectrodes made of carbon filaments having different conducting properties due to some structural variations. It was found that the carbon black filament based bioelectrodes performed better than the amolen filament based bioelectrodes. The carbon black-based BFC generated a power density of  $0.1 \mu\text{Wcm}^{-2}$ . It showed that the modelling of electrodes morphologically has a significant effect on the power density (U.S. and Goel, 2020). A new platform for DET-based third-generation biosensor was developed by 3D-printed graphene/PLA electrode immobilized with horseradish peroxidase (López Marzo et al., 2020). This third-generation biosensor avoided mediators and showed high selectivity and sensitivity for  $\text{H}_2\text{O}_2$  detection. This research work provided a new direction for the construction of third generation biosensors through 3D printing technique.

In a nutshell, 3D printing can facilitate a simple, low cost, time-efficient, fast prototyping fabrication of complex structures for various applications in one step in comparison to expensive, complex, and time-consuming conventional methodologies. However, the 3D printing electrodes required post 3D-printing modification like atomic layer deposition or electrodeposition, potentiostat polishing, solvent activation, enzyme activation, thermal activation, etc, as summarised in Table S3 with their advantages and disadvantages.

**<Preferred position for Figure 2>**

#### 4. Micro and Nano Patterning in fabrication of Bioelectrodes for EFCs

Chen et al. (Chen et al., 2001) reported the first micro-sized glucose/O<sub>2</sub> EFCs in 2001. The developed micro-sized EFCs were comprised of two carbon fibres having a length of 2 cm and diameter of 7 μm functioning in aqueous solution pH 5.0 at ambient temperature. The area of the micro-sized EFCs electrodes was about sixty times smaller than the smallest reported fuel cell and one hundred and eighty times smaller than the formerly developed smallest BFC. The power density obtained from this miniature EFC was 64 μWcm<sup>-2</sup> and 137 μWcm<sup>-2</sup> at 23 °C and 37 °C, respectively, which could provide sufficient power to drive small silicon-based microelectronics. Further, Mano et al. (Mano et al., 2003a) fabricated a miniature EFC comprised of carbon fibre-based bioelectrodes immobilized with redox biocatalysts and redox hydrogel mediators, operating in a physiological buffer. Additionally, they studied the behaviour of this miniature EFC operated in grapes, which generated 2.4 μW at 0.52 V. The further informing text is provided in supplementary document under sub-heading S4.1.

The reason for constructing miniaturized and self-reliant electronic devices is to construct a new generation of wireless, implantable and minimally invasive biomedical devices like sensor transmitters, pacemakers, defibrillators, and drug delivery pumps. The research activities have confirmed that the micro-EFCs are the most promising candidates for the in-built or *in-vivo* supply of micropower. Miniaturization of various functional parts like power supply, electrodes, and signal processing units should be done to construct micro-EFCs. Significant attempts have been made to realize miniature BFCs to provide long-term power to implantable devices where frequent batteries substitution is not possible. The conversion of intrinsic fuels into electrical energy via biocatalyst makes miniature BFCs a promising candidate because of biocatalysts' replenishing ability that ensures the self-sustained and long-term power supply as long as sufficient fuel supply is there.

The most general methods used to fabricate microfluidic systems are polymer molding, photolithography, metal deposition, and etching. All these mentioned methods have been widely employed for the fabrication of miniature BFCs. Back-to-back research activities have been carried out on carbon microelectromechanical systems (CMEMS), carbon nanomechanical systems (CNEMS), carbon micro/nanofibres, carbon whiskers, carbon nanospheres, and 3D graphene for various applications like sensors, biomedical engineering, and electronic applications (Sharma et al., 2009; Wang et al., 2008). Silicon was commonly used for MEMS preparation as the integrated circuit industry exclusively relied on it. Earlier, the use of MEMS was restricted to accelerometers, gyros, and pressure sensors. But with time

MEMS has started to be used in biomedical applications, known as bioMEMS, that can be processed using a much broader range of materials. The text from here is provided in supplementary document under sub-heading S4.2.

The recent efforts to miniaturize EFCs are based on the CMEMS method. This method was used to fabricate the 3D microelectrodes, which is explained further, followed by the functionalization of these microelectrodes for enzyme immobilization.

The power of a cell depends upon the surface area of the electrodes. However, a traditional 2D setup consists of a planar anode and a cathode separated by an electrolyte (solid or liquid). The CMEMS preparation technique has provided the platform to fabricate complex 3D dense microelectrode arrays having a high surface area for employment in carbon-based EFCs. CMEMS are fabricating by baking ultraviolet (UV) sensitive polymer materials at high temperature in an inert surrounding. Based on the application, the positive photoresist and negative photoresist can be altered into carbon via pyrolysis. Various carbon architectures can be made by using the CMEM technique. This versatile strategy also provides the flexibility to involve nanofeatures like carbon nanofibres, suspended carbon nanowires with microelectrodes (Malladi et al., 2006; Song et al., 2011).

The CMEM based 3D microstructures have higher surface area and power output compared to thin-films or two-dimensional planar electrodes for the same foot print area to power miniaturized bioelectronic devices. But there are some significant issues regarding the use of CMEM microstructures which need to be addressed for their proper utilization. Some research groups investigated these issues (mass transport and reaction kinetics) associated with micro-and nano-electrodes by employing a finite element analysis approach (Anandan et al., 2007; Godino et al., 2009). They suggested that the true advantage of employing micro-and nanostructured electrodes in energy storage and conversion or biosensor related applications could be achieved when the geometry of the micro or nanostructured arrays like spacing, height, and width, are optimized to accommodate a particular analyte species. Additionally, they explained the top portion of the nanostructured pillars quickly reacted with the glucose because of the higher reaction rate of enzymes, while the bottom portion of the nanostructured pillars, lacking glucose diffusion, did not enhance the EFCs performance. On the other hand, a study reported the issue of non-uniform current distribution around the 3D microelectrode array affecting electrodes stability because of non-uniform stresses and non-uniform heat dissipation, which lowers the cell efficiency (Long et al., 2004). Hence, it is mandatory to optimise the electrodes geometries in order to get the uniform current

distribution around microelectrodes. The further text is given in supplementary document under sub-heading S4.3.

A research work achieved an efficient and high enzyme loading by integrating the graphene/enzyme onto 3D microstructured arrays, facilitated DET(Figure 3B) (Song et al., 2015). The maximum power density ( $=136.3 \mu\text{Wcm}^{-2}$  at 0.59 V) obtained with this micro-EFC was almost seven times the maximum power density generated by the bare 3D microstructured array-based EFC.

Recently, a closed-loop study around theoretical and experimental results on a micro-EFC was carried out (Song and Wang, 2019). The micro-EFC was based on coating of RGO, CNTs, and biocatalysts on 3D carbon microstructures, as displayed in Figure 3A. The fabrication of the 3D carbon microstructures array was carried out by the top-down CMEMS technique. This 3D carbon microstructures array platform was coated with reduced graphene oxide (RGO), CNT, and biocatalysts through bottom-up electrophoretic deposition approach. The obtained 3D carbon microstructures have high aspect ratios which considerably enhanced the surface area for high amount of enzyme loading. The theoretical study of cell performance and efficiency for the RGO/CNT coated microstructure array-based EFC was done by finite element analysis. The experimental maximum power density ( $196.04 \mu\text{Wcm}^{-2}$  at 0.61 V) was found to be 71.1% of the theoretical value and about double of the maximum power density obtained from RGO-based EFC.

The practical application of EFC is limited by several factors that regulates the lifetime of EFC. Mostly, the stability of the enzyme decides the lifespan of the BFCs. The CMEMS microstructure surfaces were covalently functionalized for the immobilization of enzymes; namely, direct amination, diazonium grafting, and diamine grafting (Bocanegra-Rodríguez et al., 2021; Tsujimura et al., 2021). All of these types of covalent functionalization graft the functional group on the surface of pyrolyzed carbon (Yang et al., 2009). Still, the performance and the stability of EFCs is in need of huge improvements. However, the miniaturization feature and the implantable biological compatibility of EFC open the way for future investigations on *in-vivo* assessment of the stability and cell performance in real implantable systems.

### <Preferred position for Figure 3>

The 3D micro/nano dual-scale carbon array was fabricated by three critical steps (Jiang et al., 2014). The cost-effective nanoimprint lithography was employed to obtain a hemispherical

photoresist micro-array, as shown in Figure 3C. Further, to enhance the surface area, oxygen plasma etching was done to convert the micro-array into hierarchical structures by developing longitudinal nanowires on the microstructure surface. Lastly, carbonization of these hierarchical photoresist structures provided a micro/nano dual scale carbon array. The experimental results confirmed the excellent electrochemical performance of the micro/nano dual scale carbon array compared with planar and hemispherical carbon structures. The developed micro/nano dual-scale carbon array can be used as microelectrodes for a wide variety of applications, including on-chip energy storage devices and microsensors. The proposed method for the fabrication of a micro/nano dual scale carbon array provided a facile way for large scale production.

A research developed polydimethylsiloxane (PDMS) microbial fuel cell (MFC) with embedded micropillar electrodes (Siu and Mu Chiao, 2008). This BFC has flexibility and biocompatibility which is apt for implantable bioMEMS devices (Figure 3D). The biocatalyst *Saccharomyces Cerevisiae* was used to convert chemical energy of glucose from the blood stream to electrical energy. This BFC was a laminated design having a 0.2  $\mu\text{m}$  anode and cathode made of thick gold evaporated PDMS, which was separated by nafion membrane as shown in Figure 3D. The overall size of this BFC was 1.7 cm  $\times$  1.7 cm  $\times$  0.2 cm, carrying a net weight less than 0.5 g. This BFC having a micropillar structure showed 4.9 times more average current density and 40.5 times more average power density compared to the silicon based micromachined BFCs, when functioned at identical operating conditions. The experimental analysis showed electricity generation from a droplet of 15  $\mu\text{L}$  human plasma, which partially confirmed its *in-vivo* application. However, for the practical application, the designed BFC needs improvements regarding blood and carbon dioxide exchange between the BFC interior and exterior. In addition, the leaching of mediator, microbes and oxidant should be prevented from entering the blood stream. A long-term steady state performance should be recorded for *in-vivo* power generation.

However, CMEM based 3D microelectrodes architectures require design optimization of the micropillar quantity and geometry, which may further enhance the electron harvesting capacity. In addition, several other analyses like biocatalytic reaction and diffusion rate, electrode impedances, electron mediator interaction, and oxidant reaction can enhance the coulombic efficiency and longevity of the EFCs.

## **5. Conclusions and future prospects**

This review article outlined the new advancement in the field of flexible EFCs and how these are attractive for various applications. The article also summarised the recent past

advancement with the example of textile, tattoo, and yarn-based flexible EFCs. This explanation ensures the capacity of EFCs technology for wearable energy harvesting. Like conventional EFCs, the electron transfer efficiency between the electrodes and active sites of enzyme and supply of the fuel determines the energy harvesting potential of the wearable EFCs. However, the article also highlights the main challenges that flexible/wearable EFCs are facing today. The major challenges include a. realization of flexible EFCs that can withstand mechanical strains due to body movements, b. maintaining high current at desired voltages, c. development of advanced protein engineering and immobilization techniques that can help to realize long-lasting EFCs, d. aesthetically appealing devices in order to get easier acceptance from market. This article also describes some solutions to resolve these issues. On concentrating the suggested solutions, it is found that the issues that need to be addressed require a collaborative effort by researchers of various fields. Nevertheless, the major advances in the field of EFCs as an alternative energy source and the growing interest they arouse, show that the time is not far away for real applications of powering electronic systems by wearable / flexible EFCs.

Further, in this article the advancement of 3D printed structures to fabricate electrodes for EFCs via 3D-printing techniques was also explained. This perspective review article also explained the employment of advanced materials with consistent physical and electrochemical properties for energy-based applications. 3D printing provides various benefits like rapid prototyping, preparation of complicated structures having high surface area, and quick and low-cost processability. The article explained how the 3D printing techniques is useful and easier to construct complicated structures and designs in contrast to the conventional methods which need huge efforts. On the basis of the application, suitable 3D printing techniques must be used on the basis of the feature size of the final products and processability. The article also explained various 3D printing techniques on the basis of their usage for various applications. For the purpose of 3D printing, the printing ability and the electrochemical performance of the designed ink material should be the two main important factors for electrode preparations. The control on the rheology of inks and drying ability influences the processability of printing along with the electron/ion conduction ability of the printed devices. So, deeper understanding of colloidal chemistry and advanced materials is required to achieve this goal. So far, the 3D printing technique is yet to be explored for the whole enzymatic BFC fabrication.

This review article also focussed on the development of CMEMs and CNEMs based 3D micro- structured electrodes employment for energy storage and conversion applications. The

article summarised the various research work in which techniques and expertise developed in this field can play important roles in the construction of bioMEMS electronics used for implantation. The development of micro-EFCs still has some challenges to overcome because of the miniaturization feature, long-term stability, insufficient power/current output and the implantable biological compatibility.

The actualization of EFCs for various applications requires interdisciplinary understanding and efforts like the knowledge of mechanism of electron transfer from chemistry, the development of advanced biocompatible and conductive materials from material science, understanding of the biomolecules and their engineering from biology, and chemical engineering to design the efficient system.

### **Acknowledgement**

The Authors are grateful to the MANF and French National Research Agency in the framework of the ANR-18-CE09-0022-01.

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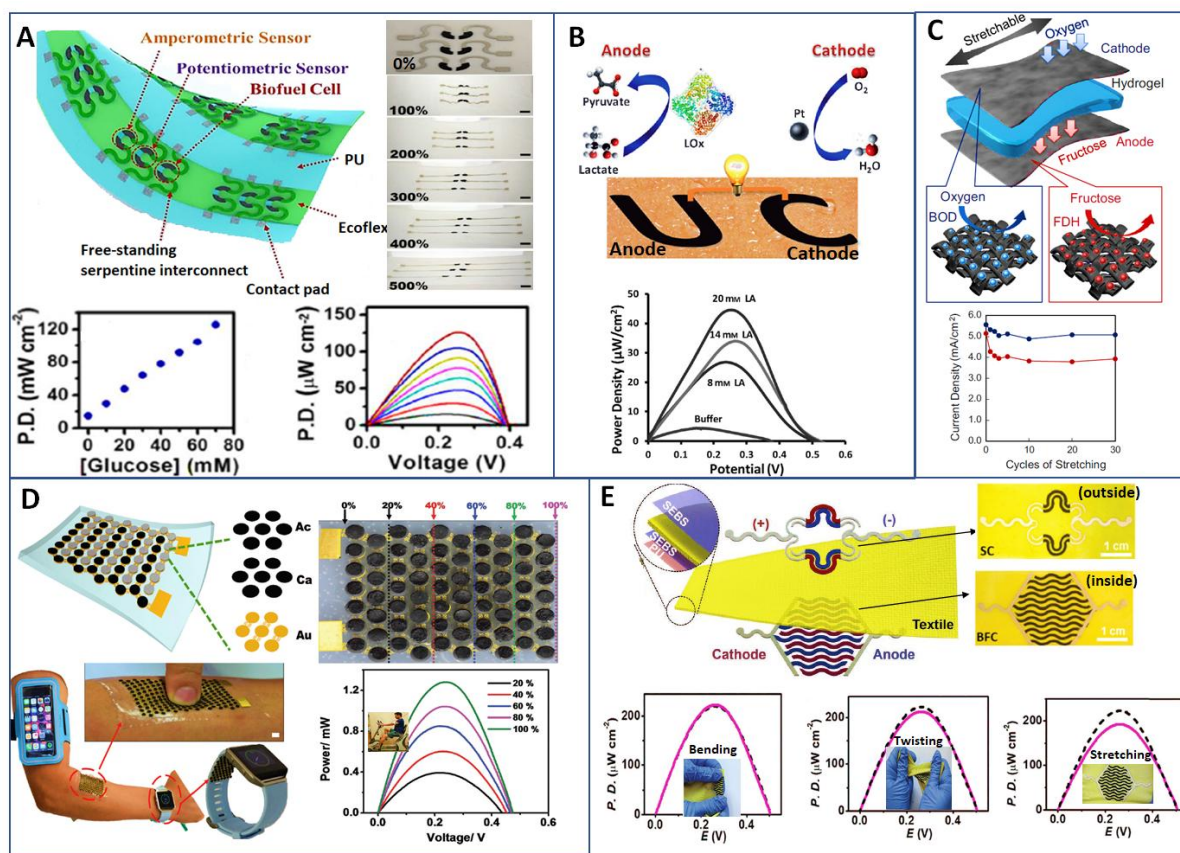
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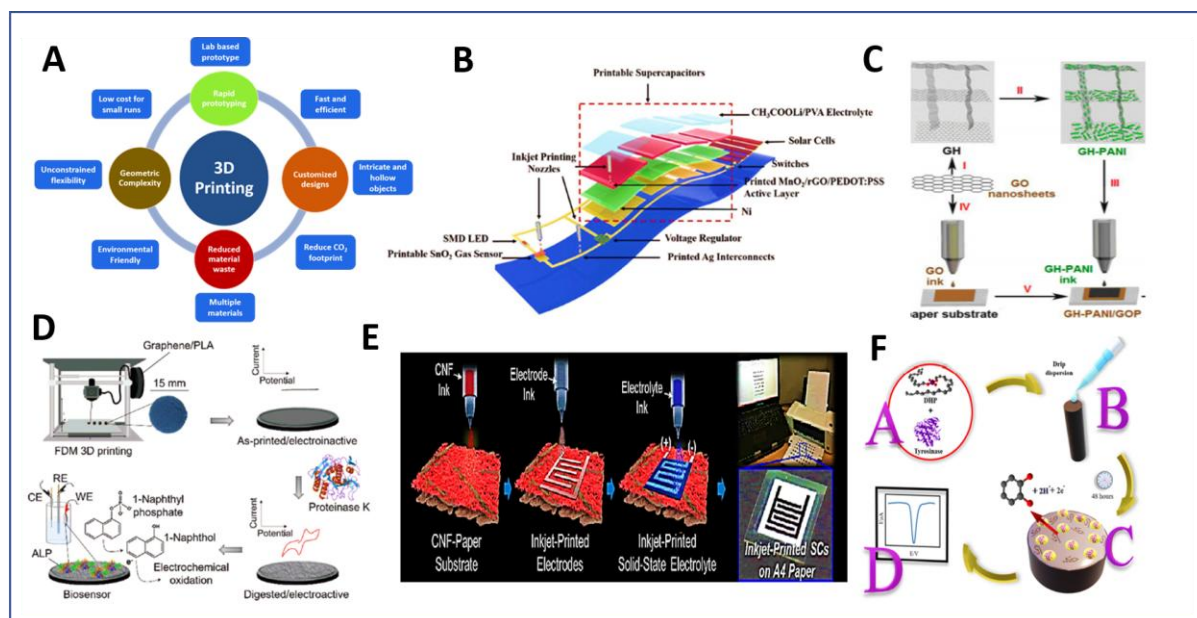
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## Figure Captions



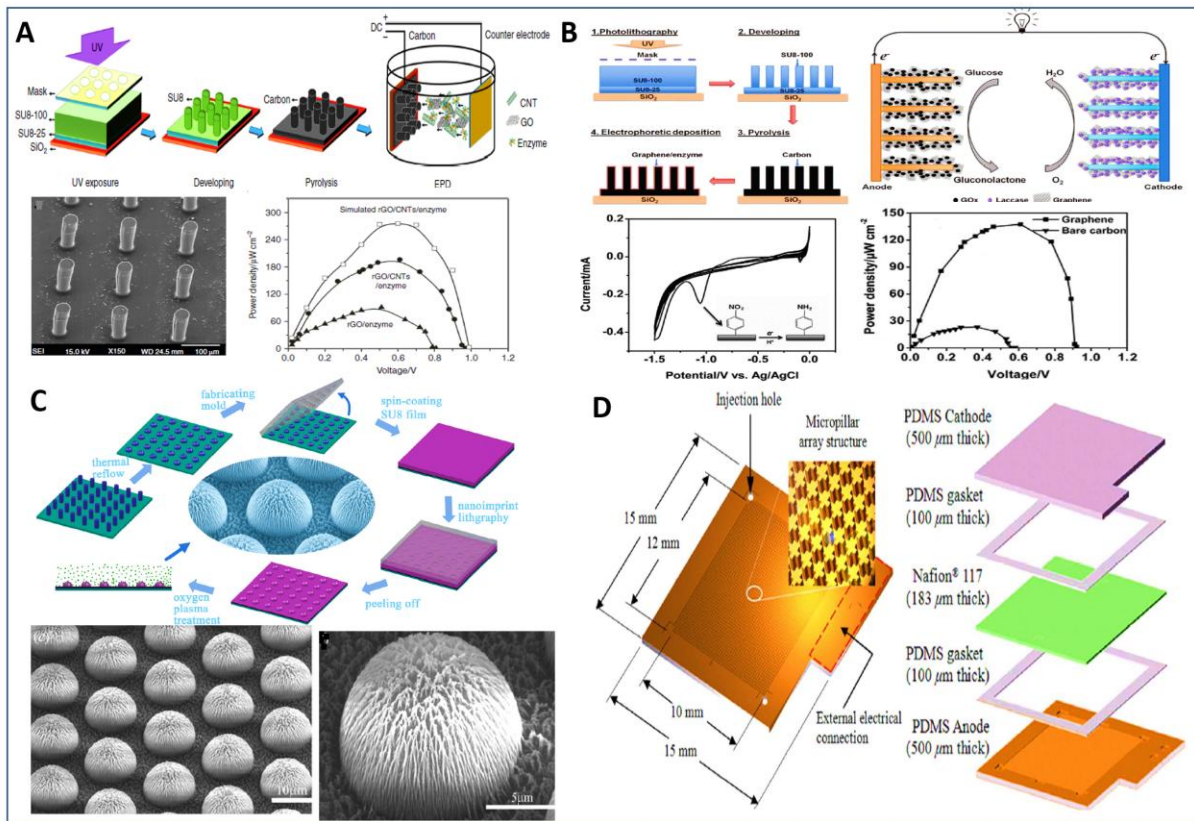
**Figure 1.** Illustration of flexible EFCs harvested biochemical energy: A. Highly stretchable fully printed CNT-based BFC with repeated stretch upto 500%. Adapted with permission (Bandodkar et al., 2016b). Copyright 2016, American Chemical Society B. Schematic showing tattoo-based LOx/Pt BFC and with generating respective power density from human sweat (lactate) at varying lactate concentration during physical activity. Adapted with permission (Jia et al., 2013). Copyright 2013, Wiley-VCH C. Stretchable BFC based on conductive textile and exhibiting the deviation in current density at 0.6 V on stretching 10% (blue) and 50% (red). Adapted with permission (Ogawa et al., 2015). Copyright 2015, Elsevier D. Electronic skin-based lactate/Ag<sub>2</sub>O EFC (Au-gold, Ca-carbon, Ac- active bio-anodic and cathodic components) tested by human for its wearable applications. Adapted with permission (Bandodkar et al., 2017). Copyright 2017, The Royal Society of Chemistry E. Wearable BFC (inside)-supercapacitor (outside) hybrid device harvesting energy from sweat lactate and storing it in supercapacitor and the power curves under mechanical deformation of BFC i.e., bending, twisting, and stretching (black dash line before mechanical

deformation and solid pink line after 100 repeated bending, twisting, and stretching upto 20%). Adapted with permission (Lv et al., 2018a). Copyright 2018, The Royal Society of Chemistry.



**Figure 2.** A. 3D printing advantages over traditional manufacturing methods for energy conversion and storage applications. B. Schematic representation of wearable, flexible, and monolithically integrated self-powered smart sensor printed on plastic substrate. Adapted with permission (Lin et al., 2019). Copyright 2019, Wiley-VCH C. Fabrication steps for free standing flexible inkjet printed supercapacitor. Adapted with permission (Chi et al., 2014). Copyright 2014, American Chemical Society D. FDM 3D printed graphene/PLA electrodes immobilized with alkaline phosphatase (ALP) enzyme catalyzing conversion of 1-naphthyl phosphate to naphthol. Adapted with permission (Manzanares-Palenzuela et al., 2019). Copyright 2019, The Royal Society of Chemistry E. Pictorial presentation of the stepwise procedure for the inkjet printed supercapacitor on A4 paper. Adapted with permission (K.H. Choi et al., 2016b). Copyright 2017, The Royal Society of Chemistry F. Pictorial presentation of 3D printed working electrode modified with a mixture of DHP and tyrosinase for biosensing application. Adapted with permission (Silva et al., 2020). Copyright 2020, Elsevier.





**Figure 3.** A. Top-down and bottom-up fabrication method for the preparation of CMEMS and electrophoretic deposition of RGO/CNTs/Enzyme onto electrode surface, respectively with SEM image of RGO/CNT/GOx-encrusted 3D carbon micropillar arrays and power density performance of the RGO/CNT/EFC, rGO/enzyme-based BFC, and the simulated RGO/CNT/enzyme-based BFC (Song and Wang, 2019). B. Schematic illustration showing EFC based on C-MEMS micropillar arrays and the power density performance of bare carbon-based and graphene-based eBFCs (Song et al., 2015). C. Development of the hemispherical hierarchical structure with SEM images taken after oxygen plasma treatment for 5 min (Jiang et al., 2014). D. Fuel cell electrode fabrication with micropillar structures (Siu and Mu Chiao, 2008).