Perspectives on C-MEMS and C-NEMS biotech applications

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Abstract

Carbon microelectromechanical system (C-MEMS) and carbon nanoelectromechanical system (C-NEMS) have been identified as promising technologies for a range of biotech applications, including electrochemical biosensors, biofuel cells, neural probes, and dielectrophoretic cell trapping. Research teams around the world have devoted more and more time to this field. After almost two decades of efforts on developing C-MEMS and C-NEMS, a review of the relevant progress and addressing future research opportunities and critical issues is in order. This review first introduces C-MEMS and C-NEMS fabrication processes that fall into two categories: photolithography- and non-photolithography-based techniques. Next, a detailed discussion of the state of the art, technical challenges and opportunities associated with C-MEMS and C-NEMS devices used in biotech applications are presented. These devices are discussed in the relevant sub-sections of biosensors, biofuel cells, neural probes, and dielectrophoretic cell trapping. The review concludes with an exposition of future perspectives in C-MEMS and C-NEMS.

1. Introduction

Carbon allotropes have dominated materials science and engineering for several decades due to their unique features making them suitable for a wide variety of applications. The feasibility of carbon-based devices for various biotechnology applications along with various fabrication techniques have been studied and demonstrated extensively. One effective way to synthesize carbon is a top-down manufacturing approach via an organic polymer precursor and pyrolyzing patterning. In the past two decades, a variety of radiation-induced micropatterning techniques have been adopted for polymer-derived carbon patterning. Notable examples include photolithography (Wang et al., 2004a), electron-beam lithography (EBL) (Malladi et al., 2006), nanoimprint lithography (Pennatna et al., 2012a), and X-ray lithography (Maldonado and Peckerar 2016). These techniques usually involve coating a suitable substrate (e.g., silicon wafer), pre- and post-patterning bakes at temperatures above the glass-transition temperatures ($T_g$), and radiation exposure.

One of the carbon fabrication techniques this review paper focuses on is photoresist derived glassy carbon (designated here as GC). SU-8 photoresist has several desirable features: low shrinkage, high adhesion to different substrates, ease of curing and processing, making it a suitable precursor for the top-down fabrication of GC devices (Vilckova et al., 2017). Micro and nanopatterned SU-8 devices are converted to GC using thermochemical decomposition (also known as pyrolysis). During pyrolysis, the patterned SU-8 devices are heated up to high temperatures above 600 °C in an inert atmosphere. The pyrolysis step defines the physico-chemical properties of C-MEMS/C-NEMS, such as microstructure, shrinkage, electrical and thermal conductivity, mechanical stiffness, and chemical reactivity. Therefore, it is crucial to adjust the pyrolysis procedure to achieve the desired properties for the intended application (Pramanick et al., 2018b).

Besides the pyrolysis details, the surface of C-MEMS and C-NEMS devices can also be functionalized and activated for different applications, including for electrochemical biosensors, biofuel cells, and micro-supercapacitors. For example, the surface of C-MEMS devices can be directly functionalized with oxidation techniques such as vacuum ultraviolet (VUV) treatment, electrochemical activation (EA), UV/Ozone...
Integration of various nanomaterials such as reduced graphene oxide (rGO) (Penmatsa et al., 2012b; Yang et al., 2009), carbon nanotubes (CNTs) (Beidaghi and Wang 2012), zinc oxide (ZnO) (Hai et al., 2017), and gold nanoparticles (AuNPs) (Sharma et al., 2017) in C-MEMS and C-NEMS devices have been demonstrated to be feasible for developing new types of microdevices with novel properties and applications. Despite the vast potential for C-MEMS/C-NEMS based devices, there are only a few commercially available applications. Notably, those are non-biotech applications such as Enevate (Patent no. US20140170498A1) and capacitive pressure sensors (Stange et al., 2017). Notably, although commercial GC, such as thermal and electrical conductivity, impermeability, and brittleness, are inadequate in addressing the challenges of this class of biotechnology devices.

2. Fundamentals and fabrication of C-MEMS and C-NEMS

2.1. Fundamentals of SU-8 derived glassy carbon

The name glassy carbon comes from the fact that this material features a smooth, shiny glass-like appearance and a conchoidal fracture. Although the GC structure is amorphous, it cannot be referred to as amorphous carbon since this term is restricted by the International Union of Pure and Applied Physics (IUPAC) to describe carbon materials with localized II-electrons (Fitzer et al., 1995). The microstructure of GC is yet to be completely understood, but the widely accepted GC microstructural models are consist of either of these two models (1) interconnected graphene ribbons with voids or (2) cage-like graphene structures similar to fullerenes (Pesin 2002; Sharma et al., 2018b). These models explain the most experimentally determined characteristics of commercial GC, such as thermal and electrical conductivity, impermeability, and brittleness, but they are inadequate in addressing the microstructural variations in miniaturized GC (Jenkins et al., 1972; Kakinoki 1965; Pesin 2002; Sharma et al., 2018b). In terms of GC micro and nanodevices derived from SU-8 precursor, the exact microstructure is known to be affected by pyrolysis parameters, the chemical composition of the precursor, and the forces applied during polymer-patterning (Ferrer-Argemi et al., 2018; Sharma et al., 2011, 2012).

GC is highly inert and impermeable to gases and comes with high resilience against corrosive agents such as bromine and strong acids like sulfuric and hydrofluoric (Martinez-Duarte 2014). The oxidation rate of GC in carbon dioxide, oxygen, or water vapor is lower than that of any other carbon material, but it can be etched at high temperatures in an oxygen atmosphere. With a wider electrochemical stability window than platinum and gold, GC is ideal for many electrochemistry experiments. With a wider electrochemical stability window than platinum and gold, GC is ideal for many electrochemistry experiments. With a wider electrochemical stability window than platinum and gold, GC is ideal for many electrochemistry experiments. With a wider electrochemical stability window than platinum and gold, GC is ideal for many electrochemistry experiments.

2.2. Photolithography based fabrication

Photolithography of SU-8 photoresist provides a reliable patterning means to fabricate various shapes with an extensive range of dimensions on both hard (e.g., silicon wafer) and flexible substrates (e.g., polyimide). Thus far, various innovative structures such as dense 3D GC micropillars (Amato et al., 2015; Beidaghi et al., 2011), nano-electrodes (Heo et al., 2011; Sharma et al., 2017), nano string resonators (Quang et al., 2018), suspended nanowires (Malladi et al., 2006; Pramanick et al., 2017), and the integration of carbon electrodes on flexible substrates (Vomero et al., 2018b) are some of the milestones achieved. In the following, fabrication of C-MEMS/C-NEMS devices and related technical points are discussed in two major categories based on photolithography or non-photolithography techniques (e.g., X-ray lithography, EBL).

2.2.1. Fabrication of 3D C-MEMS structures

As a deep UV photoresist, SU-8 is a perfect choice to fabricate high aspect ratio structures for various applications, in which it can be used as a mold for patterning (i.e., nanoimprint lithography) or as the primary precursor of GC electrodes. For example, 3D carbon micropillars mounted on thin-film or interdigitated arrays (IDA) can be modified to act as an active electrode for biosensing (detailed in section 3.1) and biofuel cells (detailed in section 3.2), as neural probes (detailed in section 3.3), or as lateral flow arrays for cell trapping (detailed in section 3.4). The schematic illustration of the photolithography process of 2-dimensional (2D) and 3D C-MEMS microelectrodes is represented in Fig. 1. The fabrication process of high aspect ratio C-MEMS structures typically starts with photopatterning of the base layer (e.g., an IDA electrode) with a thickness between 5-25 μm, followed by the patterning of a second layer on top of the first layer. The developed structure is pyrolyzed under an inert atmosphere at high temperatures above 600 °C (Song et al., 2015; Vazquez-Pinon et al., 2019; Wang et al., 2005).

2.2.2. Effect of pyrolysis parameters

The SU-8 pyrolysis process is a sensitive and essential step in the C-MEMS and C-NEMS fabrication process, and several pyrolysis
parameters can directly or indirectly affect the final GC structures. Parameters such as maximum pyrolysis temperature, temperature ramp rate, and type of inert gas (e.g., nitrogen), and flow rate can directly affect the final carbon structures. To achieve high-quality carbon—such as a higher percentage of carbon and a lower percentage of oxygen—the maximum pyrolysis temperature is the most critical parameter. For instance, Pramanick et al. have concluded that 900 °C is the optimized temperature for electrochemical sensing applications (Pramanick et al., 2018b). Furthermore, the maximum pyrolysis temperature can affect the pore size of GC, in which the increase of maximum pyrolysis temperature decreases the pore size of the material.

Heating ramp or temperature ramp rates affect the quality of carbon by directly affecting the chemical reaction rate at which the bi-products generation and their removal from the structure surface should be optimized. The temperature ramp can also affect the pore size of GC in which in lower temperature rates (i.e., below 10 °C min⁻¹), increasing the temperature ramp decreases the pore size while for higher temperature ramps (i.e., above 10 °C min⁻¹), increasing the temperature ramp increases the pore size (Pramanick et al., 2018b). A ramp rate of 10 °C min⁻¹ is typically considered as an optimum temperature ramp for electrochemical performances (Pramanick et al., 2018b; Wang et al., 2005).

The inert gas flow rate (e.g., nitrogen gas) should be high enough to keep the furnace atmosphere inert since even a small amount of oxygen
can destroy the intended C-MEMS device. The flow rate affects the pore size, percentages of carbon, and electrochemical performance of the pyrolyzed carbon and should be adjusted accordingly depending on the tube furnace volume. Pramanick et al. reported the gas flow rate of furnace tube volume in liters per minute (volume/min) as an optimum gas flow rate for pyrolysis in which the highest percentage of carbon and optimized electrochemical performance were achieved (Martínez-Duarte 2014; Pramanick et al., 2018b; Wang et al., 2004a).

Shrinkage of SU-8 devices during pyrolysis is another critical factor that indirectly affects the GC structure, and therefore, should be factored into the process design. The shrinkage of SU-8 structures during pyrolysis strongly depends on pyrolysis parameters and the geometry of the SU-8 precursor. The three controlling parameters for shrinkage are surface area ratio (lateral surface area divided by the top surface area), pyrolysis temperature, and pyrolysis atmosphere (Heikkinen et al., 2020; Natu et al. 2016, 2018). However, the specific geometry does not affect the shrinkage of the SU-8 during pyrolysis. For instance, Natu et al. reported that different 3D geometries (cylinder, triangle, and square) with similar surface area ratios had similar shrinkage percentages (Natu et al., 2018).

### 2.2.3. Fabrication of flexible C-MEMS

Critical parameters for intracorporeal neural probes include biocompatibility, high physico-chemical stability, and small footprint area—all can be satisfied by employing C-MEMS technology (Kassegne et al., 2015). Several studies have demonstrated that polyimide-based C-MEMS provide electrochemically stable and biocompatible neural probes with long-term reliability (Goshi et al., 2018; Kassegne et al., 2015; Vomero et al., 2016). The fabrication process can be summarized into three main steps: (1) standard C-MEMS fabrication process...
including photolithography and pyrolysis, (2) spin-coating the GC with photosensitive polyimide, and (3) customized steps for adding extra layers of metal traces and bump pads (Kassegne et al., 2016; Vomero et al., 2018b).

An example of a flexible C-MEMS neural probe is shown in Fig. 1a. The low adhesion of gold or platinum on polyimide is one of the more significant challenges for the fabrication of durable implants. An innovation implemented here was replacing the chromium adhesion layer—a standard adhesion layer for gold deposition—with non-cytotoxic alternatives such as silicon carbide and diamond-like carbon adhesion promoters. These adhesion promoters enhanced the durability of the electrodes and increased the flexibility of the electrode while also making them less toxic and thus more biocompatible (Vomero et al., 2018b).

2.2.4. Fabrication of suspended C-MEMS and C-NEMS

Suspended carbon structures are another fascinating application of C-MEMS and C-NEMS technology and can be fabricated using photolithography or electrospinning. Electrospinning is a deposition technique that utilizes high electrical potentials to force a liquid to be broken down into charged droplets. These droplets then self-assemble into nanofibers on the collecting substrate. The process is the shrinkage during the pyrolysis. High shrinkage can result in higher residual stress, leading to deformation, delamination, and collapse of the 3D microstructures. Careful design of suspended mesh window size and the radius of the supporting carbon pillars can considerably improve the quality and reproducibility of the intended devices (Hemanth et al., 2017).

Another innovative method for the fabrication of suspended structures is combining electrospinning with C-MEMS technology. This technique minimizes production costs while maximizing throughput. Unfortunately, traditional electrospinning in far-field mode is less controllable for precise positioning and controlling the numbers of nanofibers or nanowires deposited on the electrodes compared to synthesizing carbon nanofibers and nanowires with techniques such as EBL and dip-pen lithography. To overcome this drawback, Bisht et al. adopted electro-mechanical spinning—also known as low-voltage near-field electrospinning—to enhance the positioning accuracy and number control of SU-8 precursor nanofiber deposition (Bisht et al., 2011; Canton et al., 2014). Electro-mechanical spinning provides a more stable and controllable fiber deposition method by: (1) lowering the electrospinning deposition voltage, (2) requiring a smaller distance between the needle and the substrate electrodes, and (3) the use of highly visco-elastic polymer solutions (Canton et al., 2014; George et al., 2020).

The fabrication of C-NEMS devices via photolithography starts with the fabrication of 3D high aspect ratio structures with an extra step of ultraviolet (UV) exposure for crosslinking the suspended sections (Fig. 2b). One of the significant challenges associated with this process is the shrinkage during the pyrolysis. High shrinkage can result in higher residual stress, leading to deformation, delamination, and collapse of the 3D microstructures. Careful design of suspended mesh window size and the radius of the supporting carbon pillars can considerably improve the quality and reproducibility of the intended devices (Hemanth et al., 2017).

2.2.5. Fabrication of C-NEMS nanogap devices

Electrospinning for C-NEMS fabrication has also been used for fabricating carbon-based nanogap devices. Developing carbon-based nanogap devices have been the subject of great interest for researchers in the field of molecular-scale devices because of their numerous advantages over gold or platinum electrodes in terms of resistance to electromigration, better stability at or above room temperature, and easier binding for a greater variety of molecules for biosensing applications.

Initially, carbon-based nanogap electrodes were fabricated using carbon nanotubes (CNTs) (Marquardt et al., 2010). Nanogap devices based on CNTs exhibit high electrical and thermal conductivities. However, this technique fails to provide a desirable level of control on the position and orientation of CNTs on the contacting electrodes, making it challenging to obtain reproducible ohmic contacts (Otsuka et al., 2016; Prins et al., 2011; Vijayaraghavan et al., 2007). Another issue with more traditional carbon-based nanogap electrodes is the high contact resistance at the carbon and metal contact pads (Sharma et al., 2012). Fabrication of carbon nanogap electrodes based on the process explained earlier for the fabrication of suspended carbon nanofibers on C-MEMS electrodes can overcome both drawbacks. The current process provides a highly controllable deposition process while eliminating the contact resistance since the nanofiber is deposited on carbon posts. The studies on the correlation between gap size and the length of fiber have demonstrated that the shorter fibers produced smaller nanogaps, which implies the necessity of fibers shorter than 2 μm for attaining nanogaps with separation of 10 nm or less (Salazar et al., 2017). Fabrication of fibers that are less than 2 μm with conventional photolithography is very challenging. The electrospinning of SU-8 precursor fibers can eliminate the need for using fibers with lengths on the order of 2 μm or less and successfully overcome the problem associated with the limited resolution of photolithography (Salazar et al., 2020).

2.3. Non-photolithography methods

Conventional photolithography is a powerful means for the fabrication of C-MEMS and C-NEMS devices. However, several factors limit the achievable resolution for conventional photolithography tools, including but not limited to projection conditions, mask characteristics, properties and thickness of the photoresist, and depth of focus (Andrews et al., 2019). The resolution limitation of conventional photolithography has encouraged scientists to deploy alternative nanopatterning techniques such as EBL, nanoimprint, and X-ray lithography to fabricate C-NEMS devices. For instance, EBL—as a direct writing method—offers high resolution for nanoscale patterning. Because of the small beam size, the minimum feature size can be reduced to 20–50 nm, and the scanned area is highly localized. This technique can be directly used to pattern the C-NEMS structure or fabricate a nanoscale mold for soft-lithography or nanoimprint processes. In 2006, Wang et al. reported utilizing EBL for the fabrication of GC nanowires mounted on C-MEMS micropillars (Fig. 3a) (Malali et al., 2006).

The EBL is a highly precise technique with high resolution; however, it is impractical for mass-production because of the cost and complexity of the procedure. An effective way to make EBL more feasible is to fabricate nanomold and use the nanomold for patterning the intended photoresist. In 2012, the application of this technique was reported by Penmatsa for the fabrication of C-NEMS with sub-micron resolution in which they used a UV transparent nanoimprint mold followed by ion reactive etching (Penmatsa et al., 2012a). As the SEM image presented in Fig. 3b shows, the nanoimprint has provided a high resolution. Since the emergence of this technology, devices for various applications have been developed, including cell culture (Boxthorpe et al., 2015), optical resonators (Shneidman et al., 2018), flexible electronics (Shao et al., 2019), magnetic devices (Asari et al., 2017). However, a limited number of studies have reported the nanoimprint method for the fabrication of C-MEMS and C-NEMS, which indicates the high potential of this
technique for further investigations.

The application of conventional photolithography and EBL for complex 3D structures fabrication is laborious and time-consuming; thus, making them impracticable for mass production purposes. Two techniques of grayscale lithography and additive manufacturing can efficiently resolve this problem. Photolithography and EBL are binary patterning techniques in which only two areas of crosslinked and non-crosslinked could exist. Such binary feature dictates the fabrication of 3D structures in several steps. In contrast, in grayscale patterning, the gray tone masks allow different amounts of light to be shined to the photoresist resin. The variation in light exposure dose enables mass production of complicated 3D morphologies with a single exposure step and eliminates the need for different masks and laborious alignment procedures (Martinez-Duarte 2014). For instance, the needle-like C-MEMS microstructure presented in Fig. 3c was fabricated using one mask and a single setup (Rammohan et al., 2011). The grayscale lithography has been developed based on UV light (Fallica et al., 2017; Rammohan et al., 2011), X-ray lithography (Mekaru 2015; Mouroulis et al., 2003), and holographic lithography (Liang et al., 2012; Shrauger et al., 1994). Various applications such as C-MEMS microcantilever, bridges, and micromixers have been demonstrated using grayscale lithography (Martinez-Duarte 2014; Rammohan et al., 2011).

Another feasible technique for the fabrication of complex 3D structures is additive manufacturing based on stereolithography printing. This novel technique can fabricate complex freestanding 3D C-MEMS structures with high accuracy that are impossible to pattern via traditional methods. Rezaei et al. have investigated the application of this method for patterning the complex SU-8 precursors (Fig. 3d). Their studies showed that this technique could provide sub-100 μm resolution, which can be used for fabricating devices for a broad spectrum of applications (Rezaei et al., 2020).

2.4. Common fabrication challenges

Several issues, including adhesion, uniformity, and repeatability, challenge the fabrication of C-MEMS and C-NEMS devices (Cardenas-Benitez et al., 2019; Martinez-Duarte 2014; Wang et al., 2004b). Weak adhesion of the SU-8 photoresist to the wafer could result from trapped air bubbles in the photoresist, contamination on the wafer, moisture on the wafer before coating it, and intrinsic stress in SU-8 layers. The adhesion can be improved by thorough wafer cleaning and dehydration bake of the wafer at 150–200 °C for 5–15 min to evaporate the remaining solvent and moisture (Forouzanfar et al., 2020b; Li 2018; Mishra et al., 2018a). Furthermore, the peeling of the SU-8 structures, especially structures with high thickness, can result from insufficient UV dose, inadequate post-exposure bake, or non-uniform coating of the SU-8 photoresist. Several approaches are proposed to resolve this issue, such as conducting the post-exposure bake on hot plates instead of the convection oven, increasing the post bake time, and an extra step of hard baking at 190 °C (Pinion et al., 2017; Vazquez-Pinon et al., 2019).

Applying proper pyrolysis parameters, including but not limited to (1) factoring-in the anticipated shrinkage, (2) optimizing the temperature ramp, and (3) applying multistep heating steps (Beidaghi and Wang 2012; Forouzanfar et al., 2020a; Tang et al., 2010) are known to improve
the durability and adhesion of the final C-MEMS and C-NEMS devices. Along with the inappropriate pyrolysis parameters, the high intrinsic stress in SU-8 layers can lead to cracks, deformation, and low adhesion of SU-8 and consequently C-MEMS and C-NEMS devices. The intrinsic stress can be related to soft and post-exposure bakes (e.g., mismatch of coefficients of thermal expansions) and UV exposure (Anhøj et al., 2006; Keller et al., 2008; Zhang et al., 2004). Several measures can be considered to reduce the intrinsic stress, such as lowering soft and post-exposure bakes temperatures, ramping the soft bake temperature, and factoring-in the type of substrate in calculating the UV dose (e.g., 1.5 times higher for glass substrates). Application of different materials with SU-8 and the incompatibility of design with intended thickness SU-8 can also cause stress. However, systematic studies on the effect of design on intrinsic stress have not been found. Such study can enlighten the roots of intrinsic stress in SU-8 derived C-MEMS and C-NEMS devices.

Uniformity and repeatability of the C-MEMS and C-NEMS process could be challenging, especially for the fabrication of 3D dense structures. The problems of uniformity and low repeatability can be managed by considering several factors in designing the fabrication process, such as changes in ambient conditions, inadequate amount of dispensed photoresist, different waiting times between each lithography steps, changes in viscosity of photoresist because of long storage time, and T-topping of tall pillar-like structures (Li 2018; Martínez-Duarte 2014). T-topping is a well-known phenomenon, which leads to joining the tall structures at their top. These phenomena result from the diffraction of light at the photoresist-mask interface because of existing air gaps (Yang and Wang 2005). One solution could be using hard contact mode in which the photoresist is pushed against the mask can eliminate the air gaps between mask and photoresist. Another way is to do the coating process in several steps for layers above 100 µm thickness. Furthermore, Martínez-Duarte has proposed using a lower UV dose than the recommended dose in the fabrication datasheet for the specific thickness. For instance, Martínez-Duarte has proposed 180 mJ cm⁻² for thick layers up to 200 µm followed by increased post-exposure bake time of 60 min at 90 °C (Martínez-Duarte 2014).

3. Application of C-MEMS and C-NEMS in biotechnology

C-MEMS and C-NEMS devices possess the unique features of carbon such as wide electrochemical window, high physico-chemical stability, high tolerance against biofouling, and highly accessible surface...
functionalization (Beidaghi et al., 2011; Yang et al., 2009). Such features combined with a wide accessible range of resolution and geometry— as discussed in the previous section—make C-MEMS and C-NEMS structures exceptionally versatile for numerous biotechnology-based applications. Thus far, various biotechnology-based applications such as biosensors (Forouzanfar et al. 2020a, 2020b), biofuel cell (Song et al., 2015; Song and Wang 2019), neural probes (Vomero et al., 2017a), and cell trapping (Yildizhan et al., 2017) have been developed using C-MEMS and C-NEMS technology.

In Fig. 4, examples of the recent C-MEMS and C-NEMS based biotechnology devices for the various applications are presented. Fig. 4a shows SEM image, schematics, and the amperometric response of a C-MEMS enzymatic biosensor for cholesterol detection based on IDA electrodes decorated with AuNPs (Sharma et al., 2017). The AuNPs were selectivity electrodeposited on IDA C-NEMS electrodes in which AuNPs facilitated cholesterol oxidase enzyme covalent immobilization via diazonium moity functionalization. The example calibration curve shows the superb performance of the enzymatic biosensor.

Fig. 4c shows a schematic illustration and an SEM image of the enzymatic biofuel cell and the power density of the biofuel cell fabricated with and without CNT, as well as simulated values. The proposed biofuel cell was developed based on decorating C-MEMS micropillars with CNT + rGO nanocomposite. This nanocomposite, along with the high surface area of 3D carbon micropillars, provided large accessible surfaces for enzyme immobilization, which considerably enhanced the biofuel cell’s performance (Song and Wang 2019).

In Fig. 4e, an SEM image and cross-section illustration of the microchannel for analyzing the dielectrophoretic behavior of three salmonella strains proposed in reference (Islam et al., 2020) are presented. As the opto-microscopic image illustrates, the C-MEMS based microchannel was successfully trapped the salmonella strains.

Fig. 4d shows a digital and SEM image of C-MEMS based flexible and implantable neural probe for simultaneous electrical stimulations and recording. In this study, Nimballkar et al. successfully implanted the proposed neural probe in a mouse’s brain. The amperometric calibration plot confirms the high accuracy of the developed neural probe (Nimballkar et al., 2018).

Another interesting biotechnology application of C-MEMS is its integration with optical fibers. Fig. 4e illustrates a leaky opto-electrical neural probe for stimulation and electrochemical detection of dopamine exocytosis. The provided curve represents a good electrochemical sensitivity of this probe to dopamine concentration (Vasudevan et al., 2020). Biocompatibility and good stiffness of C-MEMS micropillars have been successfully harnessed to fabricate microneedle applicable for drug delivery. Fig. 4f shows the illustration and the SEM image of a C-MEMS microneedle developed in (Mishra et al., 2018b). The graph in Fig. 4f shows force vs. displacement of microneedles tests, which confirms the capability of microneedles to stand enough force for skin penetration without breaking.

Thus far, significant progress has been made in developing C-MEMS and C-NEMS biotechnology devices; however, the authors believe that the true potential of this technology is yet to be reached and that there is room for further development of C-MEMS and C-NEMS based biotechnology applications. Hence, a deep understanding of recent accomplishments in this field would be a good starting point for further studies. This section summarizes the C-MEMS and C-NEMS based biotechnology devices in five major groups of (1) biosensors, (2) biofuel cells, (3) intracorporeal neural probes, (4) microfluidics-based cell trapping devices, and (5) cell culture platforms. Such distinguishing would help demonstrate the specific appropriate functionalization and modifications for each application, existing drawback, and possible enhancements associated with each group of devices.

3.1. C-MEMS-based biosensors

The novel application of C-MEMS for biosensing was reported in 2008 for glucose enzymatic biosensors in which glucose oxidase enzymes were immobilized on 3D C-MEMS micropillars (Xu et al., 2008). Since then, various types of biosensors, including enzymatic (Forouzanfar et al., 2020a; Pennmata et al., 2012b; Sharma et al., 2015), optical and electrochemical aptasensors (Forouzanfar et al., 2020b; Pennmata et al., 2013; Thiba et al., 2018; Yang et al., 2009), and immunosensors (Pramanick et al., 2018a; Sharma et al., 2018a) were developed using C-MEMS and C-NEMS technology. The examples of recently developed biosensors are given in Table 1. As shown, the C-MEMS and C-NEMS technology are adaptable for all three types of biorecognizers (i.e., enzymes, antibodies, and aptamers). The developed biosensors exhibited low limits of detection (LoD) and wide linear ranges. Having low background noise and easily accessible functionalization are perhaps the main reasons for excellent biosensing performances of C-MEMS and C-NEMS based biosensors. Herein, the demonstrated functionalizations for C-MEMS and C-MEMS platforms are discussed.

The surface of C-MEMS and C-NEMS can be used directly or with modifications to accommodate the biorecognition agents. The surface of GC has local oxidations, which can be used to directly immobilize the biorecognition agents such as enzymes (Sharma et al., 2015) and antibodies (Pramanick et al., 2018a). As mentioned above, the surface of C-MEMS can be functionalized with carboxyl groups via four techniques of VUV, EA, UV/Ozone, and RIE. Pennmata et al. have reported that carboxyl group concentration was highest in the case of VUV pretreatment (15%) followed by oxygen RIE (12.5%) and EA pretreatments (12.5%), and UV/O3 pretreatment showed a significantly lower carboxyl group percentage of 6% (Pennmata et al., 2014). The carboxyl modified C-MEMS electrodes can be used for covalent immobilization of amino-group modified biorecognizers. Similarly, the surface of C-MEMS electrodes can be functionalized with amino groups using directamination and diazonium grafting, which can be used to immobilize carboxyl-terminated bioreceptors. The easily attainable functionalization of C-MEMS and C-NEMS’s surfaces is highly feasible for developing biosensing and biofuel cells for two reasons: first, they eliminate the need for using self-assembled monolayers, and second, the biorecognizers can be covalently immobilized.

Several examples of the recently developed C-MEMS based biosensors using RIE (i.e., oxygen-plasma) for carboxyl group functionalization are given in Table 1. The RIE technique was used for both enzymatic and aptamer-based biosensors (aptasensors), which implies that this functionalization technique is a simple yet efficient means for stable covalent immobilization of different biorecognizers.

Integration of nanomaterial onto the surface of C-MEMS electrodes is another powerful and efficient functionalization method. Deposition of CNT, CNT based nanocomposites, and metal-based nanoparticles (e.g., AuNPs or ZnO) can simultaneously functionalize and increase the active surface area of C-MEMS microelectrodes. These nanomaterials can be deposited using electrostatic spray deposition (ESD) (Song and Wang 2019), drop-casting (Hemanth et al., 2018), and electrodeposition (Sharma et al., 2017).

In the ESD technique, the nanocomposite is dispersed in a proper solvent and sprayed under high voltage acceleration on a heated substrate. Achieving various and uniform morphologies is the main advantage of this technique, while requiring high voltage and nonselective deposition are the downsides. ESD has been employed to deposit CNT and CNT + rGO nanocomposites on C-MEMS devices envisioned for biosensor and biofuel cell applications (Song et al., 2015; Song and Wang 2019). Drop-casting is a simple and effective way for depositing thin-films on flat substrates. This technique is widely used for developing biosensors and biofuel cells because of its simplicity; however, this method lacks the selective deposition and the coverage of 3D structures (i.e., the side of micropillars). Electrodeposition is a versatile technique for depositing charged nanomaterials. This technique is developed based on the effect of electric fields on charged particles. Electrodeposition is most useful for selective deposition on IDA arrays since the voltage can...
be selectively applied onto the desired arrays. This technique’s main challenge is preventing the deposition between the arrays since this unintended deposition can shortcut the connections. This problem can be solved by increasing the solution’s conductivity or using a sacrificial photoresist to cover the gap between arrays and remove it after the deposition.

The C-MEMS technology has been successfully deployed to develop enzymatic biosensors for detecting various biomolecules, including glucose, lactic acid, and cholesterol. The developed C-MEMS enzymatic biosensors show highly promising performances, including low limits of detection and high stability. For instance, the glucose enzymatic biosensors based on 3D C-MEMS micropillars decorated with rGO showed a wide linear range of 0–10 mM and a low LoD of 1.2 μM. The biosensor exhibited high accuracy for glucose measurements from blood samples (Hemanth et al., 2018). The recently developed cholesterol enzymatic biosensors based on C-NMES IA IDA decorated with AuNPs demonstrated highly promising performance with a low LoD of 1.28 μM and a wide linear range of 0–10 mM (Hemanth et al., 2018). Besides the third generation enzymatic biosensors (i.e., biosensors coupled with immobilized enzymes), C-MEMS platforms are highly promising for non-enzymatic bioanayltic detection such as glucose. Sharma et al. demonstrated the non-enzymatic glucose biosensors based on nanoporous C-MEMS structures decorated with AuNPs in which the C-MEMS structure was etched with O2 microwave plasma to form the nano-pores. The proposed non-enzymatic glucose biosensor exhibited a low LoD of 36 μM and a wider linear range of 0.05–10 mM (Sharma et al., 2019). By proving a stable covalent immobilization of enzymes and low background noises, the developed C-MEMS biosensors are highly promising for wearable or implantable enzymatic point-of-care (POC) biosensors.

The accessible functionalization of C-MEMS and C-NEMS structures make this platforms an excellent choice for aptasensors and immunosensors. The direct functionalization enables covalent immobilization of various aptamers and antibodies without using self-assembled monolayers (SAM layer). Elimination of the SAM layer simplifies the fabrication process while immensely improving the stability of the C-MEMS based aptasensor and immunosensors.

Furthermore, the unique properties of GC, such as low background noise, resistance toward biofouling, and good conductivity, make GC a potent candidate for label-free detection. Label-free detection is significant for POC application because it simplifies the sample preparations and reduces operational costs. For instance, Forouzanfar et al. have proposed label-free PDGF-BB aptasensors in which the affinity aptamers were covalently immobilized on RIE-treated C-MEMS thin film. The proposed aptasensor exhibited a low LoD of 1.9 pM and a wide linear range of 0.005–50 nM, as well as a robust and stable performance in the presence of external interference agents (Forouzanfar et al., 2020b). The application of suspended C-MEMS and C-NEMS structures for biosensing has been demonstrated to improve the performance of the biosensors by providing better access to the redox species and easier sandwich formations (e.g., the sandwich formations in immunosensing). For instance, salmonella aptasensors were developed based on the suspended GC nanowires that demonstrated excellent label-free detection in which a low LoD of 10^3 CFU mL^-1 and a fast reaction time of 5 min was achieved. The affinity aptamers were immobilized on RIE-treated GC nanowires (Thilha et al., 2018). Sharma et al. have proposed the application of C-NEMS suspended mesh structure for immunosensing. In the demonstrated immunosensor, affinity antibodies were immobilized on a suspended mesh structure, and the IDA below the mesh provided vast access of redox species to the formed antibody-target sandwich formation. The proposed immunosensor was highly sensitive and selective toward cardiac myoglobin in mouse serum (Sharma et al., 2018a).

In addition to the prominent properties of C-MEMS and C-NEMS biotechnology devices mentioned in the previous section, the exceptional performance of the electrochemical C-MEMS biosensors can be addressed C-MEMS and C-NEMS features such as low background noise, low ohmic drop at the surface, and tunable active surface area. Furthermore, C-MEMS and C-NEMS biosensing platforms in comparison to gold—as a common contender for biosensors—offer several advantages, including (1) higher stability of immobilized bio-recognotor (e.g., the covalent bond between bio-recognizer and GC) which can not only immobilize the stability and robustness of the biosensor, (2) higher tolerance of GC toward biofouling which is highly crucial for selectivity of the biosensors, (3) higher compatibility of the GC with carbon-based and other organic nanomaterial and elimination of SAM layers, and (4) lower fabrication costs (Kassegne et al., 2016; Pramanick et al., 2016). These features are most probably due to the homogeneity of GC in which lack of crystalline structure eliminates granular surfaces that could act as points of initiation of side chemical reactions or attachment of non-intended (i.e., biofouling) molecules (Bath et al., 2006; Forouzanfar et al., 2020b; Hirabayashi et al., 2013). Although more evidence is required to compare the electrochemical C-MEMS biosensors with optical C-MEMS biosensors, some general facts about electrochemical biosensors could be applied for electrochemical C-MEMS biosensors. Electrochemical biosensors, including C-MEMS and C-NEMS biosensors in which GC is used as the active electrode, suffer from some drawbacks such as (1) lower selectivity than similar optical biosensors, (2) lower signal-to-noise ratio in low-target concentrations which can result in lower sensitivity and necessity of complicated mediator composites to enhance the output signal, and (3) complicated

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### Table 1

Examples of recent C-MEMS based biosensors (selected studies from 2015-2020).

<table>
<thead>
<tr>
<th>Sensor type</th>
<th>Target</th>
<th>Structure</th>
<th>Modification</th>
<th>LoD</th>
<th>Special Features</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzymatic</td>
<td>glucose</td>
<td>IDA</td>
<td>N/A</td>
<td>0.4 μM</td>
<td>reproducible and selective enzyme immobilization</td>
<td>Sharma et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>micropillars</td>
<td>sol-gel Al(OH)₃</td>
<td>0.12 mM</td>
<td>test in human blood</td>
<td>Hai et al. (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>micropillars</td>
<td>rGO</td>
<td>1.2 μM</td>
<td>high selectivity and repeatability</td>
<td>Hemanth et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>lactic acid</td>
<td>IDA</td>
<td>oxygen plasma</td>
<td>1.45 μM</td>
<td>covalent immobilization of enzymes</td>
<td>Forouzanfar et al. (2020a)</td>
</tr>
<tr>
<td></td>
<td>cholesterol</td>
<td>IDA</td>
<td>AuNPs</td>
<td>1.28 μM</td>
<td>nano-sized; wide linear range</td>
<td>Sharma et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>immunosensor</td>
<td>suspended nanowire</td>
<td>oxygen plasma</td>
<td>10 CFU mL⁻¹</td>
<td>rapid, sensitive and selective whole cell detection</td>
<td>Thilha et al. (2018)</td>
</tr>
<tr>
<td>PDGF-BB⁺</td>
<td>thin film</td>
<td>oxygen plasma</td>
<td>1.9 μM</td>
<td>label-free detection; wide linear range</td>
<td>Forouzanfar et al. (2020b)</td>
<td></td>
</tr>
<tr>
<td>cardiac myoglobin</td>
<td>suspended mesh and IDA</td>
<td>EDC/NHS</td>
<td>0.4 pg mL⁻¹</td>
<td>efficient redox cycling; selective detection in human serum</td>
<td>Sharma et al. (2018a)</td>
<td></td>
</tr>
<tr>
<td>Hep-B⁻</td>
<td>thin film</td>
<td>N/A</td>
<td>1 μM</td>
<td>direct immobilization of antibodies</td>
<td>Pramanick et al. (2018a)</td>
<td></td>
</tr>
<tr>
<td>non-enzymatic glucose</td>
<td>thin film</td>
<td>O₂ microwave plasma etch/ AuNPs</td>
<td>36 μM</td>
<td>direct detection without enzyme</td>
<td>Sharma et al. (2019)</td>
<td></td>
</tr>
</tbody>
</table>

* a Platelet-derived growth factor-BB.
* b Hepatitis-B.
reaction pathways and kinetics which could lead to misinterpretation of existing electrochemical processes in the system (Dai and Liu, 2019; Labib et al., 2016). In the light of mentioned facts about C-MEMS and C-NEMS-based biosensors, a systematic comparative study of electrochemical versus optical C-MEMS and C-NEMS biosensors can be an interesting topic for future studies. In general, the good biosensing performance of C-MEMS and C-NEMS-based biosensors and the feasibility of the C-MEMS and C-NEMS fabrication processes illustrate the high potential of this technology for developing various POC biosensors.

3.2. C-MEMS-based biofuel cells

Driven by developed implantable devices and the increasing number of patients with chronic diseases, the demand for implantable medical devices has drastically increased (Song and Wang, 2019). One of the concerns for implantable medical devices is a reliable and biocompatible power source. Enzymatic biofuel cells (EBFCs)—a class of biofuel cells that employ enzymes to convert biological energy sources to electrical power—have been demonstrated as potent candidates for replacing commercially available lithium-ion batteries. EBFCs provide several advantages over conventional batteries such as application of nontoxic renewable biocomponents (e.g., glucose), high abundance of the biofuels in the human body, high reactivity and reaction selectivity of the biocatalysts (e.g., glucose oxidase), and compatibility of the EBFCs performance conditions with human physiological conditions (e.g., body temperature and pH) (Heller, 2004; Palmore and Whitesides, 1994; Vielstich et al., 2003). Since the first demonstration of micro EBFCs in 2001, in which Katz et al. proposed self-powered enzymatic biosensors, a noticeable attention has been devoted to improving the micro EBFCs (Katz et al., 2001; Song and Wang, 2019).

In choosing a suitable contender for developing high-powered micro EBFCs, various parameters should be considered, including having a large surface area, availability of a suitable confined area for enzyme immobilization, the biocompatibility of the EBFCs electrodes, and high-efficiency of electron transfer. The majority of the required parameters can be efficiently fulfilled via functionalizing a suitable C-MEMS or C-NEMS structure with the CNT-based nanocomposites using methods mentioned in subsection 3.1. Such integration has been investigated by Song and Wang in which CNT + rGO nanocomposites were co-deposited with enzymes on 3D C-MEMS micropillars. The experimental performance of the developed EBFCs reached 71.1% of the theoretical value, with a maximum power density of 196.04 μW cm$^{-2}$ at 0.61 V and 64.5% power remaining after 7 days (Song and Wang, 2019).

The performance of recently developed glucose-based EBFC systems in which carbon-based material is used as a promoter is given in Table 2. The performance of the C-MEMS EBFC in comparison with other EBFS systems with CNT shows higher open-circuit voltage and better stability while providing high power density. The excellent performance of the proposed C-MEMS EBFCs can be explained with several facts, including (1) the 3D geometry of the C-MEMS provided more sites for co-deposition of the enzymes + rGO/CNT nanocomposite, (2) the application of CNT prohibited the stacking of graphene and improved the accessible surface area, and (3) GC facilitated the better charge transport which yielded high power density. The achieved power density of the proposed EBFC is sufficient to support wearable and intracorporeal implant devices. Hence, the C-MEMS based EBFCs are highly promising for future wearable POC biosensor and C-MEMS based intracorporeal implants and probes.

3.3. C-MEMS-based intracorporeal neural probes

Several parameters, including architecture, mechanical and electrochemical properties, and durability, should be considered in developing micro and nanoelectrodes for intracorporeal neural probes. C-MEMS technology can satisfy the majority of required features for viable neural probes. As discussed in the previous section, the C-MEMS and C-NEMS devices can be fabricated in various sizes and geometries by deploying proper fabrication techniques. Thus far, thin-film (Grossenbacher et al., 2015; Vomero et al., 2018a), IDA (Goshi et al., 2018; Nimbalkar et al., 2018; Vomero et al., 2017a), micropillars on thin-film (Mishra et al., 2018a), and microneedles (Vasudevan et al., 2020) have been envisioned for neural probes applications.

The application of C-MEMS for intracorporeal neural probes is a relatively novel concept, and the reported studies have revealed the superior performance of GC electrodes over traditional gold or platinum-based probes. The performance of GC intracorporeal neural probes is highly dependent on pyrolysis parameters. Their different properties, such as mechanical properties (e.g., stiffness and hardness), electrical

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Bioanode</th>
<th>Biocathode</th>
<th>$P_{\text{max}}$ (μW cm$^{-2}$)</th>
<th>OCV$^1$ (V)</th>
<th>Stability</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>bioelectrode made of pellet gold electrode</td>
<td>GOX/catalase-compressed MWCNT$^b$</td>
<td>laccase-compressed MWCNT$^b$</td>
<td>190</td>
<td>0.57</td>
<td>N/A</td>
<td>Zebda et al. (2013)</td>
</tr>
<tr>
<td>3D C-MEMS</td>
<td>GOX/rGO</td>
<td>laccase/rGO</td>
<td>136.3</td>
<td>0.59</td>
<td>N/A</td>
<td>Song et al. (2015)</td>
</tr>
<tr>
<td>3D C-MEMS</td>
<td>GOX/rGO</td>
<td>BOD$_2^+$-MWCNT</td>
<td>196</td>
<td>0.61</td>
<td>64.5%</td>
<td>Song and Wang (2019)</td>
</tr>
<tr>
<td>glassy carbon</td>
<td>GOX/PANI/MWCNT</td>
<td>laccase/Ox/BPY/PVII/MWCNTs</td>
<td>430</td>
<td>0.59</td>
<td>56% after 7 days</td>
<td>Zhong et al. (2018)</td>
</tr>
<tr>
<td>glassy carbon</td>
<td>GOX/PANI/MWCNT</td>
<td>laccase/Ox/BPY/PVII/MWCNTs</td>
<td>430</td>
<td>0.59</td>
<td>56% after 7 days</td>
<td>Zhong et al. (2018)</td>
</tr>
</tbody>
</table>

$^a$ glucose oxidase
$^b$ multi-walled carbon nanotube
$^c$ pyrroloquinoneinequinone-dependent glucose dehydrogenase
$^d$ polyaniline
$^e$ bilirubin oxidase
$^f$ 5,5'-dithio-bis(2-nitrobenzoic acid)
$^g$ 1-pyrenobutyric acid
$^h$ NAD-dependent glucose dehydrogenase
$^i$ single wall carbon nanotube
$^j$ flavin adenine dinucleotide-dependent glucose dehydrogenase
$^k$ Os(4,4’-dimethyl-2,2’-bipyridyl)$_2$(poly-vinylimidazole)$_2$Cl$^-$
$^l$ open circuit voltage.
property, and electrochemical property (e.g., double-layer capacitance),
can be tuned to be more compatible with the target tissue. Kassegne et al. studied the effect of pyrolysis parameters on electrical, mechanical, and electrochemical properties of GC neural probes and intracorporeal implants in which they were measured at in vitro conditions. Their study showed that the electrical properties of GC (i.e., impedance (Ω) at 1 kHz) is tailorable between 10-100 kΩ, in which for the same geometry, maximum impedance was recorded for GC pyrolyzed at 600 °C. In contrast, the minimum was recorded for GC pyrolyzed at 1000 °C. The maximum pyrolysis temperature and temperature ramp both had affected the electrochemical property. For instance, to have high double-layer capacitance and low charge transfer resistance, high-temperature ramps (e.g., >8 °C min⁻¹) and a maximum temperature of 1000 °C were suggested. Furthermore, their research showed that the highest hardness could be achieved at middle-range pyrolysis temperatures (800–900 °C) and fast temperature ramps decrease GC’s hardness (Kassegne et al., 2015).

The integration of C-MEMS on polyimide substrate for long-term electrocorticography is one of GC microelectrodes’ unique applications. GC has a wider electrochemical window compare to gold and platinum as it can tolerate the larger voltage excursions without producing irreversible faradic reactions (Nimbalkar et al., 2018; Vomero et al., 2016, 2017a, 2017b). The larger electrochemical window can induce the physico-chemical stability of the electrocorticography electrode. For instance, the comparative case study by Vomero et al. on platinum and GC electrocorticography electrodes showed visible corrosion on platinum electrodes after 5 million stimulation pulses while GC electrodes remained almost intact (Vomero et al., 2017a). This side reaction could result from the adhesion layer’s oxidation (i.e., chromium) that it is typically used for the deposition of platinum. The corrosion resistivity of GC probes can be explained by two facts (1) GC is a homogeneous material with no crystalline structure; hence it lacks the granular surfaces that could act as points of initiation of corrosive chemical reactions, and (2) the absence of metal adhesion layers in GC neural probes (Ranganathan et al., 2000; Schueller et al., 1997).

The comparative case studies on the biocompatibility of GC electrocorticography microelectrodes showed a minimum difference in cell viability on the GC devices compared to the control cells. Furthermore, GC had lower background noise (due to its higher capacitance), enhancing the probe’s signal-to-noise ratio and improving the sensitivity, spatial selectivity, and spatial resolution of GC electrocorticography microelectrodes (Nimbalkar et al., 2018; Vomero et al., 2016, 2017a, 2017b). For instance, the C-MEMS based neural probes developed by Nimbalkar et al. have shown high performance, including (1) high signal-to-noise (>16) recordings, (2) exceptionally high charge storage capacity for the non-coated neural probe with a value of 61.4 ± 6.9 mC cm⁻², (3) highly sensitive dopamine detection (10 nM level), (4) dual recording of both electrical and electrochemical signals, and (5) no failure after 3.5 billion cycles of pulses (Nimbalkar et al., 2018). The achieved high signal-to-noise ratio and high charge storage capacity can result from several factors such as fast electrode polarization, low ohmic drop on the surface of the GC probe, and higher surface charge density (Ranganathan et al., 2000; Schueller et al., 1997). The high sensitivity of the developed GC neural probe can be explained by the fact that GC has high intrinsic hydroxyl groups on its surfaces. The hydroxyl groups are favorable for the adsorption of cationic species such as dopamine, whose amine side chain gets protonated at physiological pH (Bath et al., 2000; Hirahara et al., 2013). Moreover, Vomero et al. have investigated the in vitro stability of GC-based intracorporeal neural probes in which the GC neural probes implanted in rat brains for several weeks showed no significant change in morphology and performances (Vomero et al., 2017b).

Penetrating neural probes based on 3D C-MEMS is another noteworthy application of this technology. The envisioned penetrating neural probes were developed based on 3D GC micropillars (Mishra et al., 2018a), 3D origami-styled GC (Goshi et al., 2018), 3D GC microcones fabricated by additive manufacturing (Chen et al., 2020), and leaky opto-electrical probes (Vasudevan et al., 2020). The 3D structure of the GC neural probes allows simultaneous electrophysiological signals recoding from both the brain surface (electrocorticography) and depth (single neuron). Furthermore, the neural probe can be factionalized to measure the neurotransmitters such as dopamine beside the double signal recoding. The multiplexing of the probe’s function can be tremendously important since it can reduce operating costs and minimize tissue damage (due to lessened penetration sites). To the best of our knowledge, such integration for C-MEMS-based penetrating neural probes has only been investigated by S. Vasudevan et al. They have investigated the proposed neural probe’s electrochemical performance in the presence of various dopamine concentrations (Vasudevan et al., 2020).

3.4. C-MEMS microfluidics based cell trapping

Dielectrophoresis (DEP) is an electrical field-based technique enabling selective manipulation of target particles and cells, using an asymmetrical (non-uniform) electrical field with a sufficient dipole moment of the targeted particles or cells. The main advantage of DEP over other techniques (e.g., magnetic-activated cell sorting) is conducting the separation solely based on the target’s intrinsic physical properties (e.g., surface structure and internal compartmentalization). Tag-free target separation is essential for various applications, especially VOC biosensors, since it eliminates the expensive and time-consuming labeling process and enables fast and real-time operations. DEP has been established for various applications ranging from separation of molecules and proteins to manipulating cells and bacteria (Kim et al., 2018; Martinez-Duarte 2014; Seyed and Matyushov 2018).

C-MEMS based DEP electrode—also known as carbon-electrode DEP—provides several advantages over more traditional DEP devices. Perhaps feasible fabrication of high aspect ratio structures is the main advantage of carbon-electrode DEP. The high aspect ratio structures can improve the efficiency of the DEP devices in two ways of (1) increasing the effective surface area, which can improve the flow rate, and (2) covering the entire height of a flow channel, which reduces the mean distance of any particle to the closest electrode surface. The electrochemical stability and wider electrochemical window of GC compared to other commonly used metal films (e.g., gold or platinum) are other significant advantages of carbon-electrode DEP, allowing higher applied voltage in a given electrolyte without electrolysing it (Kim et al., 2018; Martinez-Duarte 2014; Seyed and Matyushov 2018).

It is worth noting that the photolithography of 3D C-MEMS DEP structures is very similar to the process mentioned in section 2.1, with a minor extra step of hard baking at 190 °C after developing the SU-8 structures. The hard bake is crucial to improve the adhesion of SU-8 to the silicon wafer and prevent the peeling off during the extended time of operations. Several applications have been established using 3D carbon-electrode DEP, including separation of target cells (Islam et al., 2020; Jaramillo et al., 2010; Puri et al., 2018), separation of live and dead monocytes (Yildizhan et al., 2017), decontamination of persisting bacteria from an antibiotic-treated sample (Elitas et al., 2014), lambda-DNA trapping (Martinez-Duarte et al., 2013), and live-cell lysis (Mernier et al., 2012). The proposed applications suggest the high potential of 3D C-MEMS DEP technology for developing lab-on-chip sample preparation platforms for VOC diagnosis devices.

3.5. C-MEMS-based cell culture platform

The development of engineered microenvironments that can provide proper chemical growth factors and mechanical properties (e.g., morphology and texture) is vital to have a more realistic in vitro cell culture model (Benzonì et al., 2016). C-MEMS technology can provide the proper microenvironment for cell growth. For instance, Ferraro et al. investigated the effect of geometry on interaction and orientation of
human iPSCs-derived neural stem cells (Ferraro et al., 2017). Their study showed that the biocompatibility of C-MEMS structures provides a sustainable substrate for the adhesion and proliferation of cells. Moreover, their studies demonstrated that human iPSCs-derived neural stem cells recognize the differences in two configurations and orient their growth according to the structure (Ferraro et al., 2017). This interesting behavior can be the topic of future studies by designing a complex 3D C-MEMS structure (e.g., using additive manufacturing mentioned in section 2.2) to mimic a biological morphology and investigate the human iPSCs-derived neural stem cells’ response to various therapeutic stimulations.

Alongside the geometry manipulations, the integration of nano-materials on C-MEMS microelectrodes allows controlling cell behavior on sub-micron levels. Sub-micron cell growth patterning presents fascinating opportunities in the field of bioelectronics and organ-on-chip technologies. Chauhan et al. have investigated this novel approach by integrating AuNPs within the synthesizing process of GC microelectrodes. Their proposed fabrication approach provided various AuNPs size distributions and interparticle separations on fibronectin functionalized surfaces. The proposed AuNPs/fibronectin-modified GC substrates were highly biocompatible with a cytocompatibility of \( \approx 80\% \) after 3 and 8 days (Chauhan et al., 2020). The compatibility of the metal-salt precursor (i.e., NaAuCl₄) with SU-8 photoresist had better control of fabrication parameters, including the size, distribution, and spacing of AuNPs without disturbing micropatterning and pyrolysis process on metal-loaded SU-8 microstructures. The control over AuNPs properties and the aforementioned unique features of C-MEMS technology make this metal-C-MEMS microfabrication process a promising approach for future organ-on-a-chip systems, bioelectronics, and biosensing applications.

4. Future Outlook

Since the introduction of C-MEMS and C-NEMS technology, various structures for a wide variety of applications have been demonstrated. However, there is still considerable unexplored room for future C-MEMS and C-NEMS biotechnology studies. Herein, several possible new applications and enhancement are presented.

1. Multiplexing the C-MEMS and C-NEMS based biotechnology devices: Multiplexing of biotechnology devices is an important topic in lab-on-chip and point-of-care biodevices studies since they offer cost reduction and expedited operations while minimizing the possible tissue damages (e.g., fewer penetration numbers). Multiplexed biosensing is referred to a class of biosensors that can simultaneously perform or measure several bio-related phenomena. Multiplexing of electrochemical C-MEMS biosensors can be achieved via fabricating several interconnected sensing units and use microplasma writing to functionalize the surface of C-MEMS selectively. Selective functionalization eliminates the need to use complicated masks (Thiha et al., 2019). Multiplexing can also be applied to 3D C-MEMS neural probes (e.g., GC micropillar) via selectively functionalize them to detect neurotransmitters (e.g., dopamine) or any desirable biomarkers from the brain and neural environments.

2. Integration of C-MEMS devices for optofluidic biotechnology devices: Application of polymer-based substrates for surface-enhanced Raman spectroscopy (SERS) provides several advantages over more traditional plasmonic materials such as cheaper production, high-throughput fabrication processes, amenability to integration with microfluidics, and transparency (Reyer et al., 2017; Viebreg et al., 2018; Yan et al., 2017). Recently, SU-8 based SERS platforms have been reported. The developed SU-8 based SERS platforms demonstrated outstanding nanoplasmonic heat resistance, great SERS sensitivity, and a small standard deviation of unmatched SERS signal uniformity (Wu et al., 2019). This novel platform is a promising candidate for optofluidic-based biosensors since it can be integrated with C-MEMS microfluidics on the wafer level. Such integration is highly promising for lab-on-chip sample preparations in which they are applicable to point-of-care biosensors.

3. Exploring synthesis and application of new material phase of SU-8 derived glassy carbon: Pyrolysis is a transient process of making a non-conductive polymer (i.e., SU-8) to a conductive material (i.e., glassy carbon) in which the applied parameters define the properties of the achieved material. Hence, tuning the fabrication and pyrolysis parameters could alter the material properties and consequent device performance. For instance, the pyrolysis parameters can be manipulated to attain semiconductor nanostructures, opening a vast horizon of opportunities for nanoelectronics and nano-biotech devices. An interesting application could be computer chips based on DNA-modified SU-8 derived nanoelectronics. To the best of our knowledge, such an application has not been explored yet.

The suggested future topics are only a limited number of possible research opportunities in C-MEMS and C-NEMS biotechnology. Alongside the promising research-based developments, the successful commercialization of the C-MEMS and C-NEMS-based biotechnology-related devices could be possible by optimizing several factors usually overlooked in the research stage. Improving the factors such as manufacturing cost, robustness under mechanical and environmental stress, fabrication failure rate, ease of production, and integration and packaging flexibility can considerably enhance the commercialization chance of C-MEMS and C-NEMS biotechnology devices. In light of the potentials and challenges, it is clear that more research needs to be devoted before commercial C-MEMS and C-NEMS biotechnology devices become readily available.

5. Conclusion

C-MEMS and C-NEMS technologies are feasible techniques for manufacturing glassy carbon devices in a wide range of structures and sizes. A vast variety of structures can be made by choosing the proper lithography technique and optimizing the pyrolysis parameters. The mechanical and material properties of C-MEMS and C-NEMS devices can be tuned by careful structure design and adjusting the pyrolysis properties to match the envisioned application better. The prominent features of glassy carbon, such as wide electrochemical window, high physico-chemical stability, and biocompatibility, have made C-MEMS and C-NEMS devices highly versatile for various biotechnology-related applications. Highly accessible functionalization, the possibility of covalent immobilizing different biorecognizers, and resistance toward biofouling are unique advantages of C-MEMS and C-NEMS biosensing and biofuel cell over more traditional MEMS devices. Low toxicity, elimination of metal adhesion layers, and lower degradation rate are significant benefits of these devices over traditional metallic-MEMS intracorporeal neural implants. The developed C-MEMS and C-NEMS biotechnology devices have demonstrated that these devices are highly promising for various biotechnology applications. Nevertheless, it should be noted that challenges and issues need to be addressed before C-MEMS and C-NEMS biotechnology devices are commercially available.

CRediT authorship contribution statement

Shahrzad Forouzanfar: Writing – original draft, Writing – review & editing. Nezih Pala: Writing – review & editing, Supervision. Marc Madou: Writing – review & editing, Supervision. Chunlei Wang: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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