Laser induced graphene for biosensors

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A B S T R A C T

Biosensors can sensitively and selectively detect a wide range of compounds and macromolecules strongly relevant to human health diagnosis and environment monitoring. Laser induced graphene (LIG) fabricated from polyimide has recently received intense interest for biosensor application due to its unique properties, such as three-dimensional macroporous structure, good conductivity and superior facile laser fabrication process. This laser direct writing technology demonstrates a great potential for developing graphene-based electronics for its chemical-free and direct patterning of graphene, as well as suitability for roll-to-roll production. In this review, we summarize the recent development of the fabrication of LIG and its modification for meeting the needs of biosensor development. The LIG has been directly employed as electrode, modified with enzyme, aptamer or other catalyst for biosensing. The review also highlights integrated LIG biosensors that can simultaneously measure multiple objectives.

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1. Introduction

Biosensors are analytical devices that convert a biochemical/biological reaction into a measurable physico-chemical signal, which provides a quantitative assessment of the analyte concentration [1]. Biosensors can detect a wide range of chemicals with applications in food security, diseases analyses, and environmental safety [2]. Recently, graphene has attracted significant interests for biosensor applications such as glucose monitoring [3,4], DNA sensing [5,6], and folic acid detecting [7,8], owing to the extraordinary electrical and optical properties of the graphene. Reliable, scalable fabrication and modification methods for developing biosensors are highly on demand to bridge the existing gap between lab research and commercialization [9].

Many approaches have been developed to produce graphene such as mechanical exfoliation [10], chemical vapor deposition (CVD) [11] and the reduction of graphene oxide [12]. In 2014, Tour et al. fabricated macroporous laser induced graphene (LIG) from commercial polyimide (PI) films with a CO2 laser, exhibiting high electrical conductivity and large specific surface area. This laser based reduction method for graphene shows unique advantages of selective and localized reduction, flexibility in patterning and no requirement for chemicals [15]. More importantly, the precursor of PI and the effective laser treatment demonstrate great potential for roll-to-roll manufacturing [16], which is critical for future commercial applications of PI-based devices. Following works have demonstrated the potential PI-derived LIG in various applications, including nitrogen sensor [17], strain sensor [18,19], gas sensor [20,21], and acoustic sensor [22], antibacterial application [23,24], energy storage [25,26], and antennas [27] etc. Compared to graphene fabricated with other method, LIG generates a number of defects, which is not always a disadvantage. Li et al. and Banerjee et al. demonstrated that the laser reduction process induced by laser [42]. Two sub-processes, namely the direct conversion from sp² to sp³ carbon and removal of oxygen functional groups coexist during the laser reduction process [15].

Diverse substrates such as cloth, paper, potato skins, coconut shells, cork [43], wood [44], lignin [45], and phenolic resin [46] can be transformed into graphene directly by laser irradiation, as shown in Fig. 1(d-g). Fig. 1(h) illustrates the scheme for industrial production of LIG film and its modification based on the precursor of commercial polymer and the effective laser reduction process. Compared with other graphene sensor, this LIG sensor shows the great advantage of facile fabrication and the huge potential for commercialization. The LIG sensor can be patterned by direct laser writing without additional chemical or high temperature treatment. The PI precursor is ready for roll-to-roll production and laser process is effective and applicable for large-scale production. The laser direct writing method is also considered to be an effective for graphene fabrication. Kaner et al. reported that more than 100 graphene devices can be produced over large areas in 30 min or less by direct laser writing, demonstrating scalable fabrication of graphene [47]. The combination of commercial precursor such as polyimide, an effective laser direct writing system, and an automated roll-to-roll fabrication process holds the key to making LIG an industrialized production for biosensors.

2. Laser induced graphene

2.1. Fabrication of laser induced graphene

In 2014, Tour’s group [16] reported porous graphene films fabrication from commercial polymer films using a CO2 laser [37]. The LIG exhibits high surface area (≈340 m² g⁻¹), high thermal stability (>900 °C), and excellent conductivity (5–25 S cm⁻¹) [38]. Fig. 1(a-c) shows the laser patterning process on PI, SEM images and Raman spectrum. The 3D porous structure, which renders enhanced-accessible surface areas and facilitates electrolyte penetration into the active materials, can be formed by the release of gas produced during laser treatment. The LIG demonstrates a 2D Raman band (centered at 2700 cm⁻¹), which is typically found in 2D graphite consisting of randomly stacked graphene layers along the c axis [39]. Considering the long wavelength (10.6 μm) of laser, the LIG formation is more likely to be caused by photothermal effects. The extremely high localized temperatures (>2500 °C) induced by laser irradiation could easily break the bonds including C—O, C=O and C—N bonds. Simultaneously, the aromatic compounds are then rearranged to form graphic structures.

Diverse laser with varied wavelength, such as CO2 laser (10.6 μm) [16], semiconductor laser (405 nm) [40], and near-infrared (NIR) laser (1064 nm) [41] were reported to fabricate LIG. The mechanism of laser reduction largely depends on the wavelength of lasers, which determines the photochemical effect and photothermal effect involved in the reduction process induced by laser [42]. Two sub-processes, namely the direct conversion from sp² to sp³ carbon and removal of oxygen functional groups coexist during the laser reduction process [15].

1) Laser parameters adjustment, atmosphere and process.

Various methods have been developed to tune or improve the physical and chemical properties of LIG by varying the laser parameters or controlling the reduction atmosphere, as shown in Fig. 2(a-c). Yang et al. demonstrated the surface modification of LIG, including surface morphologies, carbonization and wettability by adjusting laser powers, scanning speeds and pulse repetition frequencies [16,49]. Controlling the laser reduction atmosphere (O2, air, Ar, H2 and SF6) can modulate the water contact angle of LIG from 0° (O2 or air) to >150° (Ar or H2) or >160° (SF6) [50]. The structure of PI-derived LIG can be modified from its original macroporous foam to an intermediate concave corrugated tile structure and to a final carbon nanotube structure by repeated laser irradiations [51]. The abundant hydrophilic surface of LIG facilitates large amounts of defects and accessible oxygen containing functional groups, which can provide numerous active sites and accelerate electron transfer between the electrode and species in solution [52]. By modified the properties of LIG, the combined effects of the specific surface area, hydrophilic surface, electronic conductivity and available edge plane sites can adjusted be to enhance the electron transfer rate and analytical performance in LIG biosensors [53,54].

2) Doping of LIG.

Tour’s group [16] fabricated boron-doped LIG from H3BO3 mixed poly (amic acid) (PAA) with a CO2 laser (Fig. 2(d)), and the microsupercapacitors based on the boron doped LIG exhibit a high areal capacitance...
of 16.5 mF/cm² (3 times higher than nondoped devices) and an 5–10 times increased energy density [55]. In a similar process, Co₃O₄, MoO₂, or Fe₃O₄ were individually mixed into PAA solution for laser treatment, contributing to the metal nanocrystals embedded LIG, which exhibit excellent oxygen reduction reaction (ORR) catalytic activity [56]. Heteroatom doping in carbon material demonstrate great potential to improve the electrocatalytic activity in biosensor [57,58]. Compared to ordinary graphene materials, the N-doped graphene provides
significantly enhanced oxidation currents for the enzymatic detection of glucose [59]. Metal nanoparticles (Au, Pt, etc.) doped graphene also exhibited excellent electrocatalytical activity for biosensing, resulting from the large surface area and good electrical conductivity of graphene, and the synergistic effect of graphene and metal nanoparticles [60]. Therefore, in-situ doping and metal nanoparticle doping of LIG can be effective strategies to enhance the performance of LIG biosensor.

3) Post-surface modification (Fig. 2(e-g)).

Pseudocapacitive materials such as manganese dioxide (MnO$_2$), ferrocene (FeOOH) or polyaniline (PANI) were simply electrodeposited on the surface of LIG for all-solid-state flexible supercapacitors with improved performance [61]. By using a laser direct writing technique and an electroless Ni plating, the LIG/Ni composite structures were prepared with a low resistance of less than 0.1 Ohm/sq, demonstrating an integrated wireless charging and storage device and a near-field communication (NFC) tag applications [40]. With post-surface processing, such as, deposition, dispersing, polymerization, or binding, single stranded DNA (ssDNA), RNA, antibodies, enzyme molecules, and aptamers can be immobilized onto LIG surface for direct target probing [62,63].

The LIG demonstrates a great potential for developing environmentally friendly electronic devices. In terms of the raw material, besides the commercial PI that shows stable thermal, chemical and mechanical properties. Various natural precursors such as wood, cotton, paper and lignin (an abundant natural polymer in plants) were extended for the fabrication of LIG [43,44,64]. In terms of the laser irradiation process, the fabrication of LIG and device patterning can be simultaneously completed without traditional etching, lithography and deposition process. There is no involvement of strong acid and oxidants, which can cause pollution or high temperature furnaces that requires enormous consumption of energy [15]. The thickness of LIG can be modulated from 20 $\mu$m to 38 $\mu$m on the 125 $\mu$m PI by different laser parameters [65]. The repeated laser irradiations on PI-derived LIG can modify the morphology and structure of LIG [51], indicating the possible recycle of...
the LIG products. In terms of device performance, LIG applications reflect distinguished electrical and mechanical properties and demonstrate excellent stability and cyclability [21,61], which illustrate possible future application in wearable and flexible electronics. Therefore, it is very promising that the further development of natural precursors, the simple and repeatable laser process and the high performance of LIG devices contribute to environmentally friendly and biocompatible electronic applications.

3. Laser induced graphene for biosensor

3.1. LIG-based sensors for ionic species

Li et al. reported a simple one-step and scalable direct-laser-writing method for the LIG in situ decorated with metal sulfide (MS) nanocomposite (CdS and PbS) as an efficient transducer for photovoltaic detection of Cu$^{2+}$ with low detection limit of 2.0 nM and good selectivity shown, Fig. 3(a-c) [66]. The LIG decorated with metal nanocomposite photoelectron were synthesized by laser irradiation on the metal-complex containing polyethersulfone on indium tin oxide (ITO) glass, simultaneously producing the LIG and the crystalization of laser-induced metal sulfide [66]. The LIG with CdS interact with Cu$^{2+}$, generating Cu$_2$S ($x = 1, 2$), which serves as exciton trapping sites on the surface, decreasing the efficiency of charge separation and contributing to a prominent decline of photocurrent output in LIG with CdS photoanode [67].

Glaussen et al. demonstrated the LIG functionalized with a poly(vinyl chloride) (PVC)-based ion-selective membrane for electrochemical sensing of plant available nitrogen (NH$_4^+$ and NO$_3^-$) in soil samples (Fig. 3(d-e)) [17]. This LIG solid contact ion-selective electrodes (SC-ISE) SC-ISEs demonstrates near Nernstian sensitivities ($54.8 \pm 2.5$ mV/dec for NH$_4^+$ and $-54.8 \pm 2.5$ mV/dec for NO$_3^-$), low detection limits (28.2 $\pm$ 25.0 μM for NH$_4^+$ and 20.6 $\pm$ 14.8 μM for NO$_3^-$), and a linear sensing range of 10$^{-3}$–10$^{-1}$ M for both. With a facile low-cost fabrication process and no requirement of metallic nanoparticle decoration, LIG electrochemical sensors are promising for a wide range of in-field or point-of-service applications for soil health management. In a similar way, Kucherenko et al. reported LIG electrodes functionalized with ion-selective membranes for monitoring the concentrations of NH$_4^+$ and K$^+$ in urine samples [68]. This electrochemical LIG sensor exhibit a broad sensing range (0.1–150 mM for NH$_4^+$ and 0.3–150 mM for K$^+$) with high stability across a wide pH range (3.5 to 9.0) in aqueous solutions [68].

3.2. LIG-based sensors for small molecules

3.2.1. Ascorbic acid (AA), dopamine (DA) and uric acid (UA)

Dopamine (DA) is an important neurotransmitter, which plays a significant role in the central nervous, renal, hormonal, and cardiovascular systems [69]. Its detection has gained significant attention. Due to the overlapping voltammetric response between DA and its coexisting ascorbic acid (AA) and uric acid (UA), it is a challenge to distinguish the coexistence of DA, AA, and UA in a biological environment [34]. Xu et al. applied a poly(3,4-ethylenedioxythiophene) (PEDOT) modified LIG for a highly selective electrochemical dopamine (DA) sensor in the presence of ascorbic acid (AA) and uric acid (UA) with high selectivity, sensitivity of 0.22 $\pm$ 0.01 μA/μM and a low detection limit of 0.33 μM [70]. PEDOT, a conducting polymer was electrodeposited on the LIG electrodes to promote electron transfer responses in electrochemical sensors due to its high electrical conductivity, high stability and excellent biocompatibility [71,72]. The PEDOT-LIG electrodes demonstrate great potential for biosensors, as well as other integrated bioelectronic devices. Nayak et al. reported Pt nanoparticles decorated LIG for electrochemical sensors that can simultaneously detect biomarkers including AA, DA and UA with high sensitivity of 250.69 μA mM$^{-1}$ cm$^{-2}$ (AA), 6995.6 μA mM$^{-1}$ cm$^{-2}$ (DA) and 8289 μA mM$^{-1}$ cm$^{-2}$ (UA) and selectivity in a wide concentration range, Fig. 4 [33]. The 3D macro/mesoporous LIG with abundant edge plane sites and large electrochemical surface area, facilitates ion diffusion and leads to efficient electron transfer. The Pt nanoparticles were then simply electrodeposited on the surface of LIG to improve electron transfer and sensitivity of the sensor.

3.2.2. Hydrogen peroxide (H$_2$O$_2$)

Hydrogen peroxide, one of the most common molecules in biological tissue is a general enzymatic product of oxides and a substrate of peroxidases [73,74]. Hydrogen peroxide plays an important role in physiological processes such as diabetes, lung disease, cancer, neurodegeneration and aging [75]. Therefore, it is of great interest to develop an effective, easy, real-time and accurate H$_2$O$_2$ detection technology. Nanoparticles decoration on the surface of graphene has been reported as an effective approach for non-enzymatic H$_2$O$_2$ detection [76]. Zhang et al. reported a simple and low cost non-enzymatic electrochemical detection of hydrogen peroxide (H$_2$O$_2$) with Pt decorated LIG electrode (Pt/LIG), which exhibited an improved electrochemical performance with a limit of detection of 0.1 mM and a sensitivity of 248.4 mA mM$^{-1}$ cm$^{-2}$ [77].

3.2.3. Urea

Urea measurement has been attracting great attention due to its relations with the health hazards such as kidney dysfunction [78], cardiovascular events [79], and environment events [80,81]. Sharma et al. fabricated a pH-based, flexible and catheter–compatible urea sensor based on modified LIG, which can successfully detect urea concentrations as low as 10$^{-4}$ M with a response time of less than one minute, Fig. 5(a) [82]. By electrodepositing the chitosan hydrogel films on the surface of LIG, the quantity of the immobilized urease enzyme can be improved due to the electrostatic and covalent immobilization strategies of chitosan. These urease enzymes can catalyze the hydrolysis of urea into carbon dioxide and ammonia [83], which simply can be detected with a direct pH indicator paper. The low-cost materials and instruments make this technique attractive to a large range of potential users.

3.2.4. Thrombin

Fenzl et al. demonstrated a universal modification route of LIG electrodes for electrochemical biosensor as shown in Fig. 5(b). The sensor exhibits a low thrombin detection limits of 1 pM in buffer and 5 pM in the complex matrix of serum [84]. After fabricating LIG with a CO$_2$ laser on commercial PI, the 1-pyrenebutyric acid (PBA) was immobilized to the surface of LIG via π-stacking and hydrophobic interactions and then amino-functionalized bioreceptors were conjugated to the carboxyl groups of PBA using standard coupling chemistry [84]. Avoiding the existing complex sensors and methods, this modification strategy is an effective approach for developing sensitive, flexible and miniaturized LIG sensors for advanced clinical diagnostics.

3.2.5. Bisphenol A

Bisphenol A (BPA) is a monomer broadly used in diverse consumer products and its concentration in water is significant for evaluating water quality and risk levels of human health and environment [85,86]. Hu et al. presented a facile method to fabricate BPA sensors with a limit of detection of 58.28 aM and a response time of 20 s on the flexible PI by dual-beam direct laser writing, demonstrating an effective approach for low-cost practical sensing for on-site environmental monitoring (Fig. 5b-c) [87]. A 1030-nm femtosecond (fs) laser (400 fs, 120 kHz) and a 532 nm continuous wave (CW) laser were combined for the fabrication of the LIG, integrating both the multiphoton effect and the thermal effect of laser [88], to irradiate a commercial flexible PI sheet under ambient conditions. After plasma treatment, the LIG electrode were functionalized with receptor incubation and
uncovered surface blocking process, which minimized the non-specific binding [89]. Capacitive sensing based on AC electroosmotic (ACEO) was applied to detect the binding of BPA with aptamer on the electrode surface, since the variation of the interface properties can trigger the shift of the interfacial capacitance [87].

3.2.6. Glucose

Tehrani et al. developed a sensitive, enzyme-less glucose sensor based on LIG decorated with copper nanocubes (CuNCs). The sensor shows a linear behavior in the range of 0.25 μM - 4 mM, an excellent sensitivity of 4532.2 μA mM⁻¹ cm⁻² and a low detection limit of 250 nM as well as a rapid amperometric response of less than 3 s [90]. This sensor demonstrates great potential for glucose detection in tears, saliva, sweat, and partial in urine. The porous LIG with an abundance of crystallographic defects and large surface area enhances the electroplating process of the CuNCs (as the catalyst for oxidation of glucose) and increases loading of the highly reactive CuNCs as well as accessibility of glucose molecules. The oxidation of glucose at the CuNCs modified electrode may occur in the following process: (1) the CuNCs were oxidized into CuO in the alkaline media; (2) CuO is electrochemically oxidized to Cu(II) species; and (3) glucose is irreversibly oxidized by the Cu(III) species [91,92].

3.2.7. Biogenic amines

As an indicator of food safety and quality, biogenic amines (BA) is the product of microbial metabolism in foods and its level is affected by temperatures and storage conditions [93,94]. Vanegas et al. reported a low-cost LIG biosensor for BA detection, which exhibits an average histamine sensitivity of 23.3 μA μM⁻¹ with a low detection limit of 11.6 μM and a response time of 7.3 s [95]. Following electroplating of the copper nanocubes, the modified LIG electrodes were then biofunctionalized with diamine oxidase (DAO) to provide selectivity toward biogenic amines since DAO enzyme can catalytically deaminate the primary amines, diamines, and substituted amines to aldehyde, ammonia, and hydrogen peroxide [34], which are then readily decomposed at a working electrode polarized at +500 mV and produce an oxidative current [95]. The decorated LIG biosensors were successfully used to measure the total BA concentration in fish paste samples subjected to fermentation with lactic acid bacteria, demonstrating its great potential for screening food samples, diminishing food waste and reducing the chance of foodborne disease outbreaks [95].

3.3. LIG-based sensors for nucleic acids

MicroRNAs (miRNAs, ~22 nucleotides), small noncoding single-stranded RNA molecules play regulatory roles in cell development and

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**Fig. 3.** (a) Schematic illustration for the laser treatment of the Cd²⁺-containing polyethersulfone (PES) on indium-tin oxide (ITO), (LI-CdS-G@ITO) photoelectrode. (b) The photograph of Cd²⁺-PES on ITO (left) and LI-CdS-G@ITO (right). (c) Principle of the Photoelectrochemical (PEC) Cu²⁺ sensor on LI-CdS-G@ITO photoanode. Reproduced with permission [66]. Copyright 2019, John Wiley and Sons. (d) Photograph of five LIG solid contact ion-selective electrodes (SC-ISEs) on a single polyimide swatch and the illustration of SC-ISE ion sensing. (e) Soil column studies with LIG SC-ISEs compared to commercial ion probes. Representative real time plots of NH₄⁺ and NO₃⁻ ions during soil column flush experiments. Reproduced with permission [17]. Copyright 2018, American Chemical Society.
are significant biomarkers for cancer and other diseases [96, 97]. We recently reported a sensitive LIG biosensor for femtomolar microRNA (miRNA) detection shown in Fig. 6(a-c) [65]. With laser treatment on PI, the LIG with an effective N-doping (2.4% - 4.5%) contributes to the low resistance of LIG and likely improves the affinity with nucleic acids. This self-nitrogen-doped porous LIG was patterned as an electrode for electrochemical sensing. Combined with the miRNA extraction and magnetic isolation process, specific purified miRNAs were directly adsorbed on the surface of LIG electrode and then electrochemically quantified. The detection of miRNA up to a concentration as low as 10 f. with excellent reproducibility can be achieved, indicating the great potential for miRNA detection in biomedical applications.
Fig. 5. (a) Schematic diagram of enzyme adsorption on LIG with and without chitosan layer. Reproduced under the terms of the Creative Commons Attribution 4.0 International license [82]. Copyright 2019, Nature Publishing Group. (b) Schematic of the LIG Electrode fabrication, the functionalization process and the electrochemical thrombin detection mechanism. Reproduced with permission [84]. Copyright 2017, American Chemical Society. (c) Schematic of ultrasensitive LIG capacitive sensors with directed movement of complex sample particles with the applied ACEO effect. (d) Detection response of BPA samples as a function of concentration. Reproduced with permission [87]. Copyright 2016, American Chemical Society.
3.4. LIG-based sensors for immunology

3.4.1. Chloramphenicol

Cardoso et al. reported a LIG electrochemical biosensor with molecularly-imprinted material as biorecognition element for detecting chloramphenicol (CAP), which is an antibiotic effective against many bacteria and a commonly found contaminant in the aquatic environment, Fig. 7(a–c) [98]. The biorecognition elements were assembled on the surface of LIG with molecularly-imprinted polymer (MIP) technology as the following procedures: (1) electrochemically polymerization of the 3,4-ethylenedioxythiophene (EDOT) in the electrode sensing area to render a homogeneous surface; (2) incubation in 0.01 M 4-aminophenol (4-AMP) to ensure the bonding between PEDOT and the MIP layer; (3) electropolymerization of functional monomers eriochrome black T (EBT) in the presence of the template CAP; (4) the removal of CAP template by incubating the sensing layer in acetonitrile (CAN) and performing consecutive cyclic voltammetry (CV) scanning [98]. This LIG biosensor exhibits the linear response from 1 nM to 10 mM, a high sensitivity with a limit of detection of 0.62 nM and good selectivity, representing a promising approach for the future of on-site analysis in different contexts, including environment, industrial and health applications [98].

3.4.2. Acetylcholinesterase inhibitor

A reliable and sensitive detection of the acetylcholinesterase (AChE) inhibitor is highly demanded because the inhibition of AChE contributes to the continuously stimulation of the nerve conduction [99]. Ge et al. report a rapid and stable photoelectrochemical (PEC) enzymatic biosensor based on a TiO2 decorated LIG on ITO glass, demonstrating sensitive detection of an AChE inhibitor (EDOT) shown in Fig. 7(d–e) [99]. Considering that the graphene/TiO2 composites can extend the light absorption range and improve efficiency of electron–hole pair disassociation and electron collection [100,101], the TiO2-decorated LIG photoelectrodes were fabricated by laser treatment of Ti4+-PAA containing poly (amic acid) (Ti4+-PAA) on the surface of an ITO. The photocurrent output can be significantly enhanced by incubating acetylthiocholine (ATCl) with AChE because the AChE can catalyze hydrolysis of ATCl, producing thiocholine as an ideal hole scavenger [102]. Conversely, an obvious photocurrent decrease is noticed when the AChE inhibitor was involved to impaired enzymatic activity of AChE. In this way, the LIG photoelectrode could be used for sensitive photoelectrochemical biosensing, demonstrating a simple approach for free-standing photoelectrodes of various applications.

4. Laser induced graphene for multimodal sensor

The general biosensors that are specifically designed for a certain physical/chemical parameter, can be totally irresponsible to other parameters [103]. In order to provide a more comprehensive perspective, signals from chemical sensors and physical sensor are normally incorporated [104]. However, the incorporation process usually involves multiple complicated integration of diverse materials and layers, which may prevent mass-production and deteriorate the sensing accuracy of the devices [105]. Compared to the biosensor that are specifically designed for one variable while totally irresponsible to other variables, integrated biosensor assays had been reported to measure multiple variables at one time [103,106]. The LIG technology by being based on one main precursor and the laser direct writing process, various multimodal sensors can be fabricated in a straightforward manner, avoiding the potential deterioration on device performance and complicated integration optimization.

Recently, wearable sweat sensors had drawn much attentions [107,108], due to its rapid, continuous and non-invasive health monitoring for a wealth of physiologically relevant information [109]. Gao et al. reported a wearable hybrid sweat sensor, consisting of temperature sensor, strain sensor, and uric acid (UA) and tyrosine (Tyr) sensor that are all based on LIG, Fig. 8(a–h) [105]. For temperature sensing, the conductivity of LIG was enhanced with the increased temperature owing to increased electron–phonon scattering and thermal velocity of electrons in the sandwiched layers [110]. For strain sensing, the porous structure under an external strain will deform and enhance contacts, resulting in a decreased resistance. The rapid and accurate detection of UA and Tyr in human sweat in situ, which are related with diabetes, gout, metabolic disorders and other diseases was achieved due to fast electron mobility, high current density and ultra large surface area of LIG [105]. To continuously measure temperature, respiration rate and low concentrations of UA and Tyr, a microfluidic platform was engraved on PI film with the laser for dynamic sweat
Electrode modification

a) PEDOT deposition, Amination, Electropolymerization, Template removal

b) Graphene, EBT, PEDOT, Amine, CAP

c) $y = 162.5x + 1813.3$, $R^2 = 0.9923$

d) AChE, $e^-$, Photocurrent / $\mu$A

e) Photocurrent / $\mu$A vs. Time / s

d) AChE, $e^-$, Inhibitor, Photocurrent / $\mu$A
sampling [105]. This entirely laser engraved sensor, which facilitate scalable manufacture and flexibility for the wearer's comfort, was validated in physically trained and untrained subjects and demonstrated its potential for monitoring gout.

Park et al. demonstrated stretchable and flexible device with modified LIG electrodes for monitoring the glucose level in sweat, pH sensing, and electrocardiogram (ECG), Fig. 8(i) [106]. The silver nanowire (AgNW) film were prepared between the PDMS and LIG to enhance the electrical conductivity. The platinum and gold nanoparticles (PtAuNP) were deposited on the 3D porous LIG to improve the electrochemical performance by enhancing the electron transfer rate and catalytic activity [111,112]. The various functional layers were deposited on these LIG electrodes for sensing. For glucose sensing, the glucose oxidase enzyme was drop-cast onto the surface, exhibiting a low detection limit (5 μM) and a high linearity (0.99). For pH sensor, the PANI film were electrodeposited as a pH sensing membrane, showing a linear response (66 mV/pH) in the range from 4 to 7. For ECG sensor, the LIG electrodes were utilized to detect the small electric current generated by the contracting muscle for ECG signals, which provide useful information about cardiovascular conditions [106]. The integrated biosensors demonstrate the potential of this novel fabrication technique and stretchable LIG metal nanocomposite for wearable electrochemical-physiological hybrid biosensors [106].

Beside sweat sensor, Hu et al. reported a multiflavor sensing system, which was fabricated on PI using a laser direct writing and functionalized with gold nanoparticles, reduced-graphene oxide, and polyaniline for sensing NaCl, sugar, and vinegar, Fig. 8(j) [103]. In this sensor, the porous LIG as the electrode provides a large surface to volume ratio and achieves a high sensitivity. Gold nanoparticles were deposited to improve the conductivity of the sensor and enhance its sensitivity [113]. Reduced graphene oxide composing a high density of edge-plane-like defect sites was dropped onto the electrode's surface and served as the positive site in electron transfer to chemical and biological species [114], responding differently to Na+, Cl−, H+, CH3COO− ions and sugar molecular. PANI, a conductive polymer, was employed to modify the surface of LIG to detect vinegar solution, NaCl and sugar for its ion selectivity [115–117]. By applying principal component analysis, this sensing system shows a high sensitivity and selectivity with
5. Conclusions and outlook

In this paper, we first discussed the fabrication of LIG, the mechanism and the strategies to engineer the properties of LIG by the regulation of laser parameters, atmosphere, doping and surface modification. Due to the 3D porous structure, the good conductivity of LIG, and the simple fabrication with laser patterning process in laboratory and industrial scale, various state-of-the-art LIG biosensors have been reported in the literature and summarized in this review. The LIG electrodes were employed directly or were modified with ion-selective membranes, catalytic nanoparticles, aptamer or enzyme for sensing (AA, DA, UA, H2O2, Urea, BA, ions, PBA, BPA, CAP and AChE inhibitor). In the end, the integrated LIG biosensors combining several sensing functions (temperature, strain, glucose, microfluids, pH, ECG, multi-flavors) were also demonstrated. Considering that the research in this field is still in infancy and is advancing with a rapid pace, there is plenty of room for future development of LIG biosensors for detecting biomarkers such as DNAs, RNAs, proteins, peptides as well as whole cells. Also, the performance of LIG such as its electrocatalytic activity can be optimized by controlling the porosity, composition, morphology or surface modification. Currently, most LIGs are fabricated by laser ablation directly under ambient air condition. When the power density of incident laser exceeds the threshold ionization of dielectric media, the plasma can be generated on the focal surface, resulting in a density of incident laser exceeding the threshold ionization of dielectric materials [118]. For high-power laser induced plasma, the plasma can be induced by the laser and subsequently focuses on the sample surface, producing the LIG immersed in a dielectric medium such as water [119]. To date, most current measurements rely on electrochemical test. Other methods of analysis such as fluorescence or field-effect transistor-based biosensor should also be explored for LIG biosensing. We expect much more multifunctional LIG biosensors will be developed in the near future because of the beauty of this simple fabrication process of laser direct writing and the maturing functionalization strategies for multiplexing. Finally, a LIG biosensor could be integrated with other flexible LIG devices such as supercapacitor for potential one-step manufactured portable electronics for applications in safety, health care and environment monitoring.

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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