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Review—An Overview on Recent Progress in Screen-Printed Electroanalytical (Bio)Sensors

Sima Singh,[1] Joseph Wang,[2,*,z] and Stefano Cinti[1,3,z]

[1]*Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy*
[2]*Department of NanoEngineering, University of California, San Diego, La Jolla, California 92093, United States of America*
[3]*BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples Federico II, 80055 Naples, Italy*

Portability is one of the essential keys in the development of modern analytical devices. Screen printing technology is an established technology for both chemical and biosensor development. Screen printing technology has been used to generate a variety of electronic sensors that are rapid, cost-effective, on-site, real-time, inexpensive, and practical for use in healthcare, environmental monitoring, industrial monitoring, and agricultural monitoring. This review aims to describe recent research progress related to the development and improvement of screen-printed electrodes (SPEs). We also demonstrate the wide range of applications, also highlighting the market directions and the need for novel devices to be used by non-specialists. Finally, we conclude and provide an overview of the constraints and future opportunities of SPEs in biosensor application.
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During the last decade, electroanalytical technology has paved the way for analysis and continued its path for the advancement of people's lives. It has enormous hidden potential to solve various analytical problems of real-world applications that consistently encompass all phases of people's protection and environmental health, such as clinical analysis, foodstuffs analysis, environmental monitoring, etc., at more rapid and in reliable manner.[1] Yet, despite its fundamental reach, current traditional techniques have been constrained within the blocks of a laboratory because of their instrumental sophistication and overpriced, limiting external application in low-resource settings, making them bot suitable for in situ and point-of-care (POC) solutions.[2] One of the major areas of interest for both centralized and non-centralized analysis is the development of portable systems and the reduction of sample volume. And this interest is fulfilled by combination of electrochemical techniques with screen printed electrodes (SPEs). SPEs have revolutionized the area of decentralised electroanalysis due to their capacity to bridge the gap between lab to hand implementation.[3,4]

Screen printing is a highly competitive method for fabricating scalable and rapid printed microelectronics, owing to its superior advantages of cheap cost, ease of operation, and scalability. They are fabricated by printing different varieties of inks on ceramic, plastic or paper substrates. It offers adaptability in terms of electrode setup, material compatibility, and modifications yet offers economical and profoundly reproducible sensors and biosensors, as reported in pioneering works published by leading groups in the field.[5–13]

The field of screen-printed electrodes and screen-printed sensors is progressively developing and having significant impact into praxis. Globally, SPEs business has incredible growth potential; the expanding market for SPEs is predicted to grow rapidly in the future years, making it one of the fastest-growing industries in the world. Studies predict that the printed electronics industry will develop tremendously over the next decades, with the entire market predicted to rise from USD 41.2 billion in 2020 to USD 74 billion in 2030 (https://www.idtechex.com/en/research-report/flexible-printed-and-organic-electronics-2020–2030-forecasts-technologies-markets/687). The number of patents relating to SPEs has risen at double a rate. The highest number of patents has been issued in the last five years. The number of patents is expected to continue to rise in the future. It is worth noting that with the keyword, screen-printed electrodes in PubMed, the number of articles published in the field of analytical sensors based on SPEs has increased rapidly in recent years. The data on published output are based upon the PubMed-reported number of publications in year 2010 (115), 2015 (224), 2020 (415) and currently for 2022 (till March) (147). More articles have been published on SPEs due to its numerous advantages. The number of papers cited almost doubled in the recent years as shown in Fig. 1.

SPEs are simple, economical, highly sensitive, selective and fast response techniques suitable for onsite analysis as shown in Fig. 2a. Perhaps surprisingly, the SPEs are offered by many companies and the current market is quite competitive. These include companies like Gamry Instrument, PalmSens, Zimmer and Peacock, Metrohm AG, Nano Research Elements, Rusens LTD., BVT Technologies etc.[14] It's worth noting that, several research efforts are being made to produce screen printed biosensors for a variety of applications including clinical pathogen detection, biomarker development, environmental monitoring, medicines, and agro-food sectors (Fig. 2b).[8,15,16] SPEs also provide platform for detecting several biological species DNA, antigen-antibody, hormones, forensic. As a result, there is a considerable wealth of authoritative literature reviews on the application of screen printed electrodes that has been published in recent years.[8,16,17] Further, SPEs hold the potential for on-site analysis; hence SPEs is the main topic of this review. SPEs also commonly integrate the 3-electrode system, compared to classical beaker-based disk electrodes.

Screen-Printed Electrode Fabrication

The fabrication of SPEs relies on thick-film technology that has existed since their beginnings. According to popular belief, it originated in China and left imprints on both the Great Wall of China and ancient Egyptian cloth patterns.[18] In order to meet the growing need for reliable, stable, and disposable electrode devices that are appropriate for large scale production, researchers have turned to the screen-printing approach to construct electrode devices on a variety of substrates. Typically, SPEs contain an inert substrate on three electrode systems, i.e., the namely the working electrode (WE), reference electrode (RE) and counter electrode (CE), system printed on a solid substrate (Fig. 3a). As a result, electrode made through screen printing technique have piqued the curiosity of numerous researchers.[19–21] Screen printing has a long history of being used to fabricate a variety of sensors. The overall fabrication procedure consists of 3–4 steps: fabrication of screen printed electrode, surface design of the screen printed electrode, and its

*Electrochemical Society Member.
zE-mail: josephwang@eng.ucsd.edu; stefano.cinti@unina.it

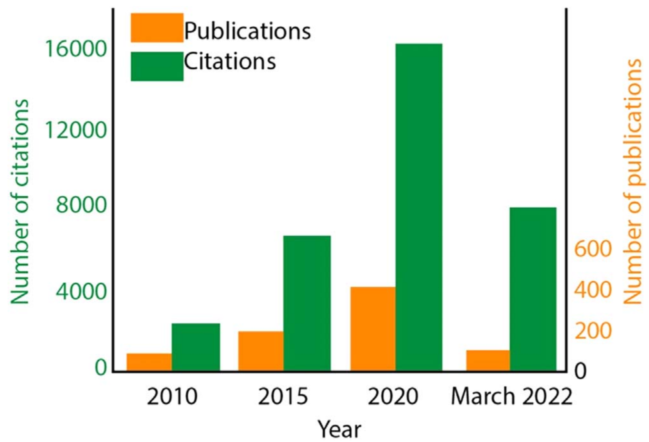

Figure 1. Research reports on screen-printed electrode publications and citation over the past decade, and till date. Publication data is taken from pubmed and citation from scopus (Access date 30th March 2022).

sensing applications.[22] Screen printing is a process in which ink or paste is applied to the substrate by squeezing it through a woven mesh and further printed on to the solid surface.[23] There are several factors that go into designing an effective SPE sensor, including the ink composition, curing temperature, pre-treatment methods and surface roughness, which all contribute to defining the sensor's sensitivity and selectivity, as recently reported for thick-film technology.[24]

Ink or pastes.—The use of a print medium, such as ink or paste, to make a sensor within the screen-printing procedure is crucial to transferring the printed design (Fig. 3b). The composition of paste is typically kept as a trade secret since it is responsible for determining the entire analytical performance and economic value of the sensors that are manufactured. Many times, the chemical formula of these inks is treated as confidential private information by the manufacturer. Conductive inks consist of up three components: (i) the conductor material, (ii) the inorganic binding agent, and (iii) the vehicles (Figs. 3c & 3d). The properties and printing capacity of the conductive paste depend on the solid load, the dispersion of the

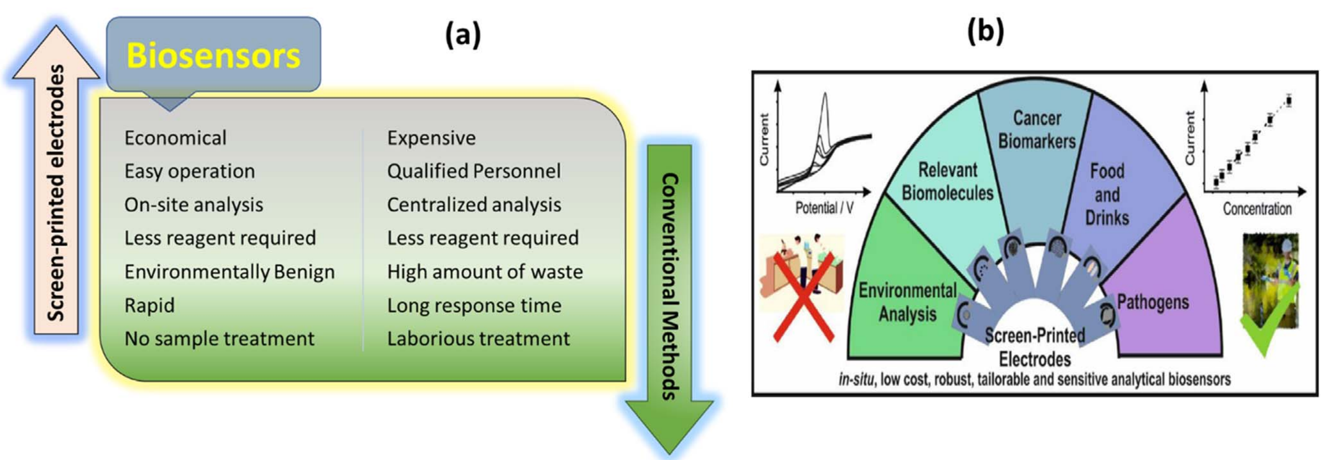

Figure 2. (a) Comparison between screen-printed electrodes and conventional methods; (b) application of screen-printed electrodes.[8]

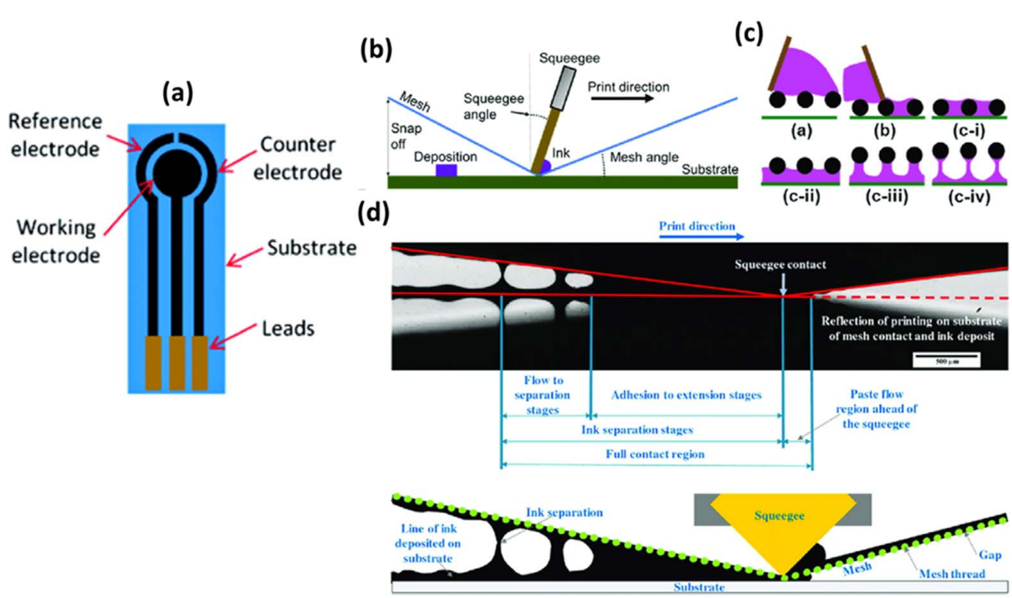

Figure 3. Summary of screen printing techniques: (a) electrode module used for SPEs;[25] (b) depiction of the ink transfer process during screen printing from screen to a substrate; (c) three stages of screen printing process (d) High-speed image and illustration of the final stage involved in ink transfer.[26]

particles, the rheology, the specific surface of the particles and the density. Ink usually contains conducting materials such as carbon, graphene and metals (silver, gold, platinum etc.). The reactivity of electron transport and analytical performance of SPEs are greatly influenced by variations in the composition of screen printed carbon inks.[27] Conductive silver and gold inks are also employed in SPEs. Silver ink is used to print the conductive track, whereas carbon or gold inks are used to print the working electrode.[23]

Printing SPEs has long relied on carbon inks because of their low cost, high conductivity, great mechanical resistance, and favourable electrochemical performance along with lower background currents than most other inks. Carbon inks are made up of graphite particles, a polymeric binder, and dispersion, printing, and adhesion agents. To enhance the post-treatment procedure for printed patterns, Liu and colleagues developed a unique graphene-based screen-printing conductive ink. The new inks are screen printable and have excellent electrical and mechanical properties as printed patterns. Inks using graphene particles with thick flakes and 15% carbon black content is present in the total conductive fillers. It shows a print pattern conductivity of 2.15×10^4 S m^{-1} at 7 μm thickness with magnificant mechanical properties.[28]

In addition to the ink's chemical composition, the electrode's electrochemical activity can be affected by the curing temperature. Choosing the right solvent, employed within the "ink" qualifies for ideal curing times and temperatures to envisage a concern. Dispersion stability and rheological behavior are primarily controlled by single solvents such as cyclohexanone or mixtures of solvents such as cyclohexanone and acetone. The vehicle permits paste to flow through the mesh. It also controls the drying of ink during and after printing.[5] Traditional carbon ink formulations contain organic solvents and must be heat-cured at high temperatures after printing, which limits its further application. Environmentally friendly water-based ink is a better solution to this problem since it is less harmful and more affordable than currently available commercial inks.[29] Hughe et al. developed micron-scale microband glucose biosensors by directly screen printing a water-based carbon ink onto a PVC substrate. Working electrodes were fabricated by in carbon inks containing cobalt phthalocyanine for hydrogen peroxide detection.[30]

SPEs consisting of multiple layers are susceptible to shortening when it is not insulated properly. Most of the time, it happens when current runs via an unanticipated channel with a lower impedance, resulting in Ohmic heating and, as a result, damage to the electrode. To avoid these losses, conductive ink layers are separated correctly by an insulating ink layer. Dielectric materials are insulating in nature and prevent the transmission of electrical current through them, ensuring the safe flow of electricity.[31] Bhatt et al. reported graphene-based screen-printed field-effect transistor with good performance and low leakage current on a paper substrate. The channel and dielectric layer were screen printed on cellulose paper with graphene conductive composite dielectric ink. The constructed device has hole and electron mobility of 135 cm^2 Vs^{-1} and 98 cm^2 Vs^{-1}, respectively. It has an ultra-low leakage current of $\sim$25 nA.[32] It should be noted how easier and low-cost solutions can be also reached by using classical adhesive tape to insulate and define the electrochemical cell.[33]

Supporting substrates for screen-printing ink.—The substrate provides the base for the electrodes of desired design. SPEs have an advantage over solid electrodes due to their simplicity and inexpensive cost of manufacture. There are mostly two types of substrates are preferred i.e., non-flexible (ceramic) and flexible (Paper, polyesters etc.). SPEs can be made on a wide range of materials, including paper sheets, plastic, fabric, and even the skin or a tattoo, as well as other materials.[34] They are characterized by a high versatility in selecting sizes, geometry, dimensions and customization methods that will help in enhancing sensitivity, specificity and stability.[35] Paper has been shown to be an ideal substrate for the building of incredibly novel analytical platforms. The integrated electrochemical detection with paper-based devices offers miniaturization, low cost and simple techniques. Electrodes can be manufactured on various types of paper-based substrates such as Whatman filter paper, cellulose paper, conductive papers, office paper etc.[36] Depending on the porosity, the different type of papers can be adopted to provide the analytical system the optimal features like robustness, loading capacity, flexibility, and even combination of different paper-based substrates can be used simultaneously to improve the applicability towards real settings analysis, i.e. preconcentration and interferences decrease.[37] Cellulose can be obtained from vegetable or microbial sources. Bacterial cellulose is a chemically pure, high water-retention and mechanically stable polymer produced by certain microorganisms, and an extracellular polymer. It was proposed to develop an enzyme-active paper, including lipase. Bacterial cellulose offers better mechanical resistance than vegetable cellulose, even when hydrated.[38,39]

Modification of Printing Ink to Improve the Sensitivity of SPEs

The three primary components of conductive screen-printing inks are solvents, conductive nanoparticles or microparticles, organic binders, and conductive compounds. However, SPE-based sensors have been extensively documented in the literature in order to enhance their analytical behavior. In order to employ them in sensing platforms, numerous SPEs have been shown in Fig. 4. SPEs can be drawn in different shapes, made of different materials,

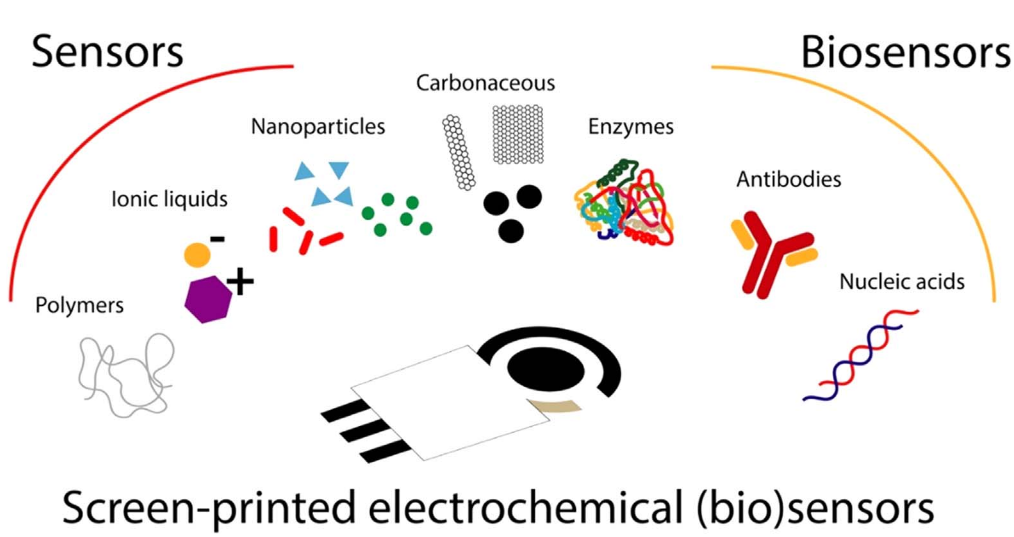

Figure 4. Schematic representation of some of the SPEs for sensors and biosensors.

and changed with different modifiers, so they can be used in a variety of ways.[40]

One of the main advantages of SPEs is the versatility that allows various modified materials to be incorporated into the printing paste to improve the performance of the sensor. As a result, SPEs are becoming one of the fastest-developing topics of interest of the 21st century. Material science has repeatedly overcome uncountable hurdles with the assistance of nanotechnology, meeting both the key criteria of possibility and feasibility. The screen-printed electrodes are modified using different materials and can be more selectively modified by adjusting the sensor elements to respond solely to the target analytes.[41,42] Numerous academics have spent decades researching novel modifying materials that can be used to improve the composition of printing inks. These materials are classified mainly into inorganic. (silver, gold), organic (chitosan and organic conducting polymers), carbon-based (carbon nanotubes, carbon black, graphene), and composite modifiers. A screen-printed electrode modification using nanoparticles is the first application of this technique. It has been successfully modified with a variety of functional nanomaterials and synthetic detection elements.[26]

Inorganic nanomaterials.—Inorganic modification is essentially used in the SPE to improve analytical performance. Due to the simplicity of the process, direct modeling and large fabrication capacity, metal nanowire printing is particularly attractive compared to conventional coating processes. Various metal materials such as silver and gold are usually used in the fabrication.[9] To this end, high-conductivity and long-term silver nanoparticles are crucial to the development of high-quality conductive inks. It is useful for the production of inks with high dispersion stability and good conductivity at low burning temperatures, among other applications.[43] For the fabrication of an Ag@PDMS composite-based strain sensor, Soe et al. synthesized silver nanoparticles (AgNPs) and polydimethylsiloxane (PDMS). The manufacturing technique has a significant influence on the conductivity and sensitivity of the composite strain sensor. It was found that the composite sensors were highly responsive in deformation, very sensitive, and hysteresis-behaving up to 70% strain test.[44]

Polymeric nanomaterials.—Polymer are promising materials for SPEs applications due to lower material cost and easy technologies for structuring. Conducting polymer (CP) nanomaterials have found applications in a variety of fields in recent years due to their superior chemical and physical properties when contrast to conventional metal materials. Additionally, CP nanoparticles offer intrinsic benefits like as ease of surface modification, biocompatibility, and wide surface areas, making them great candidates for application in electrical and optoelectronic sensing devices.[45,46] Electrically conducting polymers are well-known for a variety of properties that make them ideal for immobilizing biomolecules and facilitating electron transfer in the fabrication of effective biosensors. With the use of a macroporous gold screen-printed electrode, Tabrizi and colleagues developed an ultrasensitive molecularly imprinted polymer-based electrochemical sensor for the detection of SARS-CoV-2 RBD. The microporous gold-screen printed electrodes were used to manufacture MIP sensors. The detection limits of MIP sensors were 0.7. pg ml^{-1} (3–4.8 $\times$ 10^2 Virus μl^{-1}).[47]

Carbon-based nanomaterials.—Carbon nanomaterials have been integrated into the design of working electrodes in various sensor platforms. Jiménez-Pérez et al. modified different carbon-based SPEs for electrochemical detection of hydroperoxides. The greatest electrochemical performance was achieved with multi-walled carbon nanotubes based screen printed electrodes (aSPCNTE/PAA:Pt) with a limit of detection in the range of 24–558 nM with no interference among all carbon-based composites tested.[48] Graphene is a two-dimensional monolayer sheet made up of sp^2 bonded carbon atoms organized in a hexagonal lattice. The key advantages of employing graphene as a modifying material to develop SPE are its unique properties of large surface area, excellent electron transfer rate, and high mechanical strength.[49] In this situation, the use of graphene-containing inks for printing is a reasonably easy way for fabricating a graphene-based working electrode using a screen-printing technique. The resulting SPE demonstrated outstanding electrocatalytic activity and resolution of ascorbic acid, dopamine, and uric acid in the DPV tests when ink containing reduced graphene oxide and ionic liquid was used.[50]

Composite modifiers.—It is possible to enhance the performance of electrical sensors even more by mixing various materials and composite design.

With the use of a modified graphite screen printed electrode, Beitollahi et al. developed a graphene oxide/ZnO nano composite that was sensitive and selective in the electrochemical detection of levodopa and tyrosine. The suggested nanosensors were very sensitive, selective, and accurate in real samples analysis. It is possible to increase electrochemical signals by combining graphene oxide/ZnO nanorods nano composite with screen-printed electrode. Under optimized conditions square wave voltammetry (SWV) exhibited linear dynamic ranges from 1.0×10^{-6} to 1.0×10^{-3} M and 1.0×10^{-6} to 8.0×10^{-4} M with detection limits of 4.5×10^{-7} M and 3.4×10^{-7} M for levodopa and tyrosine respectively.[51] Using the SWASV approach, Hwang et al. coupled the biocompatibility of chitosan and Fe to design a composite modified sensor to detect As(III) in mine wastewater and soil leachate. SPCE without chitosan-Fe modification had a surface area of 0.0105 cm^2 and SPCE with chitosan-Fe modification had a surface area of 0.17769 cm^2, respectively. The result shows that the electrode surface can be increased by the composite modification process. The chitosan-Fe modified sensor demonstrated high sensitivity to As(III), with the LOD of in mine wastewater being 1.12 ppb and soil leachate being 1.01 ppb.[52]

Advancement and Commercialization Efforts in SPEs Platforms

Significant research and commercialization efforts are being made to develop new wearable electrochemical biosensors that can evaluate biochemical markers in body fluids non-invasively and continuously for the prediction, diagnosis and management of diseases, as well as monitoring physical conditioning. The evolution is also graphically depicted in Fig. 5a.[53] IDTechEx's reported annual revenue from wearable sensors as shown in Figs. 5b & 5c.[54] The number of electronic devices that are being sold annually is already enormous. It provides a thorough description and view of the types of sensors used in current and future wearable products.

Eliminating bulk materials and equipment from the analytical protocol is an important step for analytical chemists. In light of rising healthcare costs and the enormous success of paper-based (glucose) biosensors, consumers are now clamouring for inexpensive, integrated, and less-invasive sensors that can be made and sold for use in wearable biosensors to support the maintenance of wellbeing and pocket-sized portable POC systems for regular health monitoring. Biosensors have great promise in wearable applications because they have high precision, speed, portability, low cost and low energy consumption. Innovative biosensor systems for non-invasive chemical analysis of biofluids, such as sweat, tears, saliva, or interstitial fluid (ISF), have already been widely deployed to a range of head-to-toe application locations in proof-of-concept demonstrations, targeting a number of critical analytes.[55,56]

In recent years, advances in screen printing technology have provided unprecedented opportunities for electrochemical and biochemical sensors. SPEs have been very successful in both commercial and academic fields, and the need for new, easy-to-use, home and decentralized diagnostics is now more than ever before. It is quickly recognized that these sensors can significantly reduce health care costs and improve the quality of life of citizens. We can take a good example of temporary tattoos have become an attractive platform for the fabricating of skin-wearing devices. Incorporating

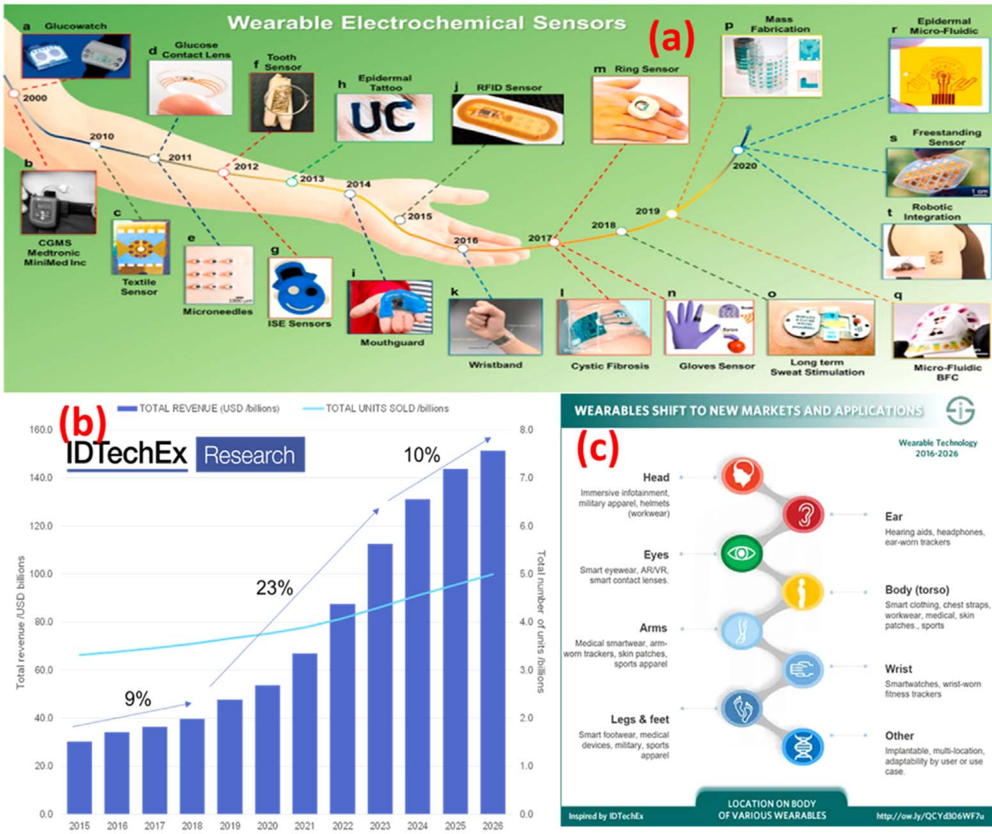

Figure 5. (a) Time-line on the progress of wearable sensor;[53] (b) wearable market growth forecast; (c) wearables shift to new markets and applications across several locations on the body. (I-SCOOP, Wearables Market Outlook 2020: drivers and New Markets, 2016, [Online] https://www.i-scoop.eu/wearables-market-outlook-2020-drivers-newmarkets/ (Accessed March 3, 2021).

screen-printed flexible electrodes into tattoos provides a platform for skin-worn biosensors since they allow for direct and continuous skin contact. Tattoo-based skin-like sensors are used to analyze key electrolytes and metabolites in real time without interference, and their sensing capabilities are reported to be remarkable.[57] Later, Bandodkar et al. published a proof-of-concept demonstration of an all-printed temporary tattoo-based glucose sensor for non-invasive glycaemic monitoring. The sensor is the first example of a flexible tattoo-based epidermal diagnostic device that combines reverse iontophoretic glucose extraction with an enzyme-based amperometric biosensor. In-vitro tests show that the tattoo sensor responds linearly to physiologically appropriate glucose levels with minimal interference from other electroactive species. This concept test demonstrated the possibility of portable glucose sensing devices based on tattoos to use reverse iontophoresis for ISF sampling but needed electronic integration and confirmation of long-term operation in the direction of continuous monitoring applications.[58]

Additionally, the commercialization of wearable glucose sensors based on microneedle-based minimally invasive wearable ISF glucose sensors is presently being pursued for fitness, military, and industrial workforce applications. Wearable sweat sensors that can detect sweat sodium and potassium levels have been in the works for a long time. Companies like General Electric Global Research have been working on them for years: they can use electrochemical systems to detect sweat sodium and potassium.[59]

The illustrative instances described in the above section demonstrate the rapid advancement of epidermal wearable platforms for noninvasive sweat or ISF monitoring, as well as the future possibilities of such wearable epidermal biosensors. Recent innovations in device integration, sensing accuracy, sweat/ISF generation and replacement, signal transduction, data transport, and multiplexed sensing, as well as related flexible and self-healing materials, have resulted in substantial advances. Thus, screen printing is a promising technique for numerous printed microelectronics applications in industry.

Challenges and Concluding Remarks

In 1962, the electronic chemical biosensors made great progress.[60] There are several advantages to electroanalytical procedures, including their mobility, low detection limit low cost and wide future improvement. Many challenges remain, one of which is application at the required/used location. To conduct an effective analysis on site, it is necessary to replace the analysis system with small and portable tools. Due to advantages in high-throughput fabrication and customizability in terms of material support and system process, SPEs will play an increasingly significant role in the electronics sector. SPE sensors are unable to obtain adequate data to receive clinical validation and market acceptance. The forefront of SPE biosensor research is focused on the development of thick-film electrodes and the utilization of nanomaterials to enhance electron transmission. The development of 4D printing in recent years has enabled screen-printing to be printed directly on smart materials substrates in the future.

In order to advance this field, many aspects of screen printed electrode recognition need to be developed. Many wireless sensor applications include attachment to the body surface or installation in the body. Consequently, further research is needed into the interference and reliability of signals in biological systems by wireless technologies. A full validation of clinical trials through large-scale clinical trial investigations, together with coordination and collaboration with medical practitioners, is therefore essential. It will help to clarify the relationship between analytic concentrations and clinical acceptability, which is now unclear. Given their versatility, these printed electrochemical devices require significant work to advance beyond prototypes and into commercial use. The entire potential of SPEs has yet to be grasped, and there is plenty of room on the electronic screen to do so. In addition, the growing field of chemometrics, machine learning and artificial intelligence (AI), through multivariate approaches, could represent a crucial advance toward the development of both optimized platforms and the application towards multiplexing.[61–64]

In summary, SPE technology is a crucial component of future research. Because of the many unresolved issues related to data collection and processing, communications, security and privacy, hardware limits and user acceptance, this technology is still in its infancy. This paper emphasizes these elements and provides readers with a general summary of possible solutions to overcome the current literature.

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ORCID

Stefano Cinti https://orcid.org/0000-0002-8274-7452

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