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A holistic pathway to biosensor translation

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Point-of-care (POC) biosensors have enormous potential to help guide and inform clinical decisions at a patient's location. They are particularly relevant to underserved populations, and people living in remote locations where healthcare infrastructure and resources are often limited. The translation of effective POC biosensors into commercial products is rapidly growing across many research fields. A significant quantity of scientific articles focused on the fundamental, applied, and proof-of-concept aspects of biosensing are reported each year. However, this extensive body of work is not reflected in the comparatively small number of commercial biosensors available on the market. Here, we discuss key aspects of the biosensor translation process including the selection of analytical biomarkers in various body fluids, clinical trials, regulatory approval, consumer engagement, manufacturing and scale-up strategies, health economics, and legal and ethical considerations.

Introduction

Sensors and biosensors applied to biomedical diagnosis are a quickly evolving multidisciplinary field. The development of biosensors for the detection of clinically relevant biomolecules requires a multi-disciplinary approach that includes chemistry, biology, physics, engineering, medicine, computer science, information technology, and data analytics. The ubiquitous personal electrochemical glucose sensor has revolutionized the management of diabetes, with the ability to self-monitor blood glucose levels daily to slow the progression of the disease and improve the quality of life of millions of patients.[1] These sensors owe their success to the use of enzymes, such as glucose oxidase or glucose dehydrogenase, which can generate amplified signals through high catalytic turnover.[2] Unfortunately, such a sensing format is not easily attainable for the detection of other biomolecules such as small molecules, proteins, and nucleic-acid analytes; effective point-of-care biosensors for detecting clinically relevant biomolecules (*e.g.*, cancer biomarkers) require sensing platforms with sensitivities and specificities considerably higher than those of glucose sensors. Therefore, achieving the same practicality of glucose sensors for the diagnosis and monitoring of other diseases requires continuous exploration, development, and optimization of different sensing platforms.

There is ample research focused on creating innovative, sophisticated sensing mechanisms for detecting specific biomolecules or other biologically relevant materials whilst improving analytical sensitivity. We have also seen considerable advances in sensor portability and miniaturization (which is essential for point-of-care diagnostics), signal amplification, and improved limits of detection in analyte targeting, as well as cost reduction. However, it remains challenging to attain the practical and scientific simplicity and performance of enzymatic glucose sensors in platforms that detect other analytes. Consequently, despite the number of potentially promising sensors developed in research laboratories, very few end up being translated into commercialized products.

The translation of point-of-care (POC) sensing technologies to end-users, which will eventually allow monitoring of biomarkers and inform clinical decision-making, requires a holistic design-led process. It is necessary to establish a workflow that covers aspects beyond biosensor design and

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initial proof-of-concept validation in academic settings. We direct readers to excellent review articles in the literature that discuss the design of biosensor devices for health monitoring and disease diagnostics. These reviews cover a range of topics, including approaches for developing innovative biorecognition elements, signal amplification, and selectivity improvement, as well as device integration into wearable, portable, and implantable systems.[2–10] Here, we will discuss other important practical aspects that need to be considered for the successful translation of POC biosensors into medical diagnostics. Fig. 1 summarizes the key aspects of sensor translation, which include the selection of the right analytical biomarkers, working with the different biological fluids, preparing for clinical trials, obtaining regulatory approval, strategies for manufacturing, legal and ethical considerations, health economics, and the involvement of consumers throughout the process.

Biomarkers selection

The selection of the optimal biomarker(s) for a POC sensor is key to its success. There are multitudes of cataloged biomarkers described in the literature and used in diagnostic devices. Given this range of available information and the complexity of developing and evaluating sensing architecture prototypes, biomarker selection is one of the most challenging aspects when developing new POC diagnostic systems.[11]

It is common to see in the literature many different types of biomarkers (such as nucleic acids, proteins, and others) being reported for the same disease, or a biomarker that is being used but is not disease-specific. For example, the blood concentration of prostate-specific antigen (PSA) is often elevated in people with prostate cancer; however, in addition to prostate cancer, various non-cancerous conditions including prostate inflammation or

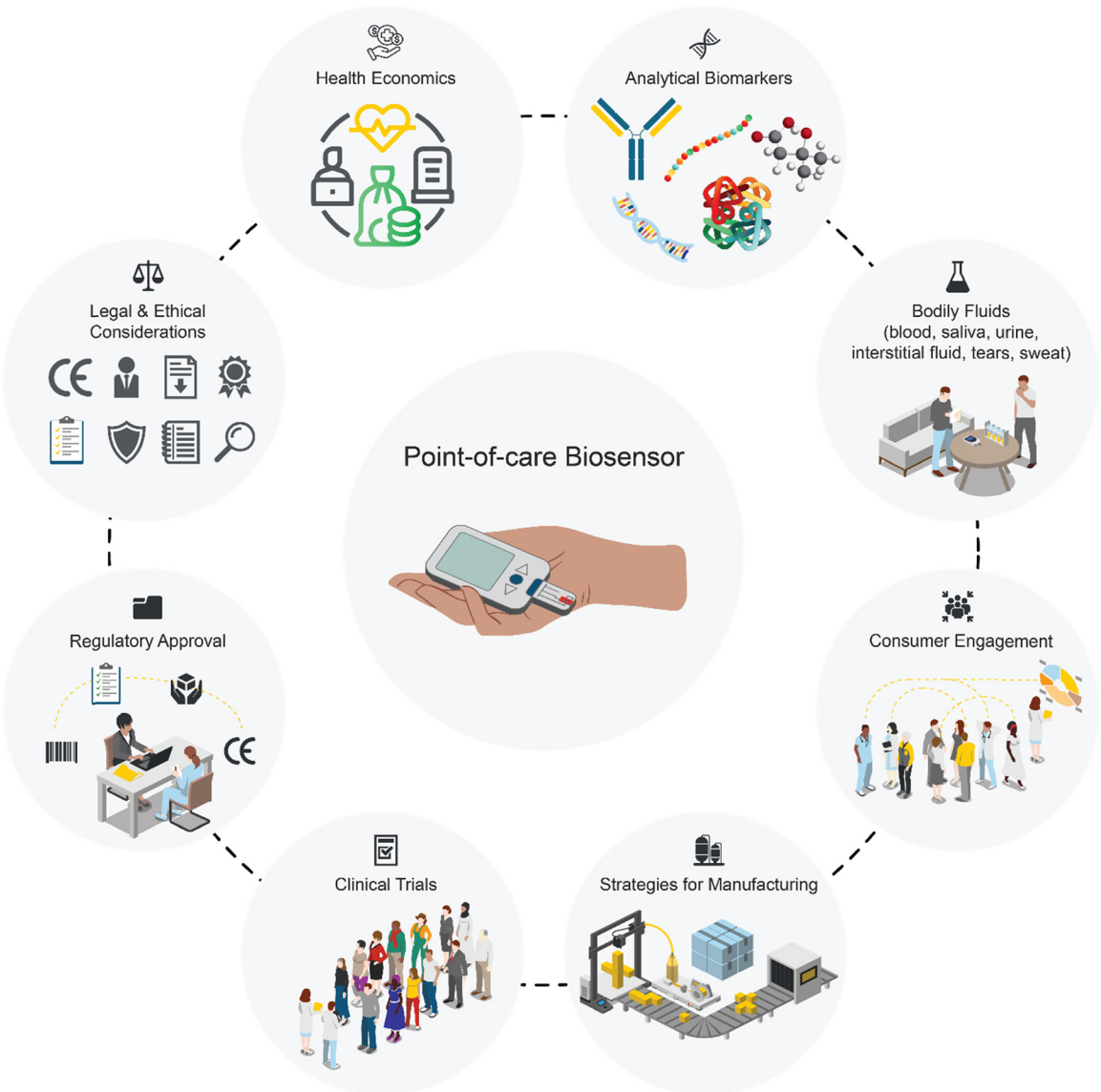

Fig. 1 Scheme of the design-led process for translation and commercialization of biosensors.

enlargement may result in elevated levels of PSA. Thus, it is recommended that the PSA test be used in conjunction with other medical exams to avoid false positive results, which limits the usefulness of PSA testing in POC settings. Another promising approach to avoid false positives or false negatives in molecular medical diagnostics is the detection of multiple biomarkers simultaneously in a single assay or multiplex analysis. Beyond enhanced diagnostic precision, multiplex biomarker detection also offers improved diagnostic efficiency because using multiple biomarkers provides insights into the holistic progression of diseases, reduces diagnostic costs, and alleviates pain in patients as it may require only a single collection of biofluids to analyze many different biomarkers.[12]

Due to the importance of biomarkers in medical diagnostics and in understanding a disease's development and progression, an entire field of research is dedicated to biomarker discovery and validation. Ultimately, selecting the right biomarker(s) also impacts aspects of the final diagnostic test such as sensitivity, selectivity, cost, usability, and logistics for biosensor storage and handling.

In the search for biomarkers, people may seek to engage with clinicians, or scientists working in genomics, proteomics, metabolomics, lipidomics, and glycomics.

The following questions can be used as a framework for selecting biomarkers that are most likely to provide useful information about a patient's disease state in POC settings:

- Has the candidate biomarker(s) been extensively evaluated to establish their utility in a clinical setting?
- What is the relevant clinical concentration range of the biomarker(s) in different body fluids?
- Does the relevant concentration range of the biomarker(s) vary across the different fluids?
- Is the concentration range of the biomarker(s) affected by disease progression or genetic factors?
- Is the concentration range of the biomarker(s), or reference intervals, affected by people's age, gender, and ethnicity differences?
- What are the biomarker concentrations in a healthy control group and what are the real-time dynamics of this biomarker?
- Is the biomarker unbound in the sample or does it require sample processing for biomarker release?
- What sample volume is required for collection? Will the collected volume contain an adequate quantity of biomarker(s) to detect using the desired assay?
- Does the analytical limit of detection of the intended detection method meet the concentration range of the relevant biomarker(s) and matrix effects?
- How stable is the biomarker in biological fluids before it is measured?
- Can the biomarker(s) be directly measured in the targeted setting given resource and personnel constraints?

It is important to mention that the Food and Drug Administration (FDA) has developed a guide to define biomarkers, the BEST (Biomarkers, EndpointS, and other Tools) Resource.[13]

Working with body fluids

Directly linked to the selection of the target biomarker(s) is the decision about what type of sample to analyze. Patient samples including different body fluids (*e.g.*, blood, urine, saliva, sweat, tears) can be utilized for clinical investigations. Once the biomarker levels and its role across different body fluids is understood, it is important to focus on the final intended application for the biosensor technology and the end-users. Since the ultimate goal of POC biosensors is to perform the diagnostic or prognostic test at the location of the patient so that the result can be immediately acted upon, the samples collected from patients need to be easily, quickly, and non-invasively accessed. Additionally, biosensors deployed in remote locations need to be usable by personnel with minimum training (including sample collection, test performance, and data interpretation). This reinforces the need for using body fluids or specimens that can be easily and safely collected and require minimal or no processing. For working with different biological fluids, pathology/medical laboratories and physiologists may be able to provide advice and input.

Blood-based biomarkers

Blood-based biomarkers are historically the most researched for biosensing applications. Blood is a complex fluid constituting red and white blood cells; cell fragments called platelets; and plasma, the liquid portion of blood.[2] Blood plasma is comprised of 91% water and proteins (*e.g.*, antibodies, hormones), nutrients, ions, lipids, dissolved gases, and other metabolites. One of the major proteins present in plasma is human serum albumin (HSA), which accounts for 60% of the total plasma proteins, and is usually present in the concentration range of 35–60 mg mL^{-1} with a half-life of 21 days.[14,15] The other two major proteins present in blood plasma are immunoglobulin G (IgG) and fibrinogen, which are commonly found in concentrations of 6–16 mg mL^{-1} and 2 mg mL^{-1}, respectively.[14,15] These three proteins together can be responsible for decreasing the sensitivity and functionality of biosensors through the natural phenomenon of nonspecific adsorption of proteins on solid surfaces.

Human saliva

Human saliva is a multi-component oral fluid that has shown great potential as an alternative medium for the surveillance of general health and disease.[16,17] Some of the saliva's constituents include nucleic acids, proteins, electrolytes, and hormones that originate from local and systemic sources.[16,18] It is estimated that saliva contains approximately 30% of the biomolecules that are found in blood. It also carries viral microorganisms, which can be practical for detecting infectious diseases such as COVID-19.[19] One of the advantages of using saliva for POC testing is that it is less invasive than blood collection, enabling home sampling.[17] Another advantage is that saliva can be used in scenarios where obtaining blood could be difficult, such as

acquiring samples from young children or more anxious patients with underlying co-occurring conditions. Furthermore, saliva samples can be stored in stabilizing solutions for several days at ambient temperature.[16,17]

Salivary biomarkers have been demonstrated to be useful for diagnosing cardiovascular diseases,[20] bacterial or viral infections,[16] cancers,[21–23] diabetes,[24] and Alzheimer's disease.[25] Despite the great advantages of using saliva for POC testing, its clinical use is still in its infancy in comparison to blood tests. For many listed disease biomarkers, there is no clear correlation between the levels of biomarkers in saliva and blood. Additionally, the concentration levels of salivary biomarkers are lower when compared to blood. For example, protein biomarkers are present at significantly low concentrations in saliva, which requires biosensors with extremely low limits of detection.[4] Other issues include diurnal variation of biomolecules, matrix effects, interferences, and differences in saliva pH or viscosity from patient to patient (or even differences for the same patient caused by food intake).[26]

Interstitial fluid (ISF)

Interstitial fluid (ISF) is the medium *via* which cells receive nutrients, secrete waste, and transmit molecular signals. In terms of volume, the human body is more abundant in ISF than blood.[27] Dermal ISF, which is the ISF within the skin, is rich in diverse analytes including proteins, amino acids, fatty acids, electrolytes, glucose, and other nutrients.[28,29] An early study has indicated that around 83% of proteins found in blood can be similarly found in ISF; however, some proteins present in ISF have not been found in blood.[30] Proteomic studies also suggest that the ISF proteome corresponds to plasma and serum proteome.[31] There have been great efforts in understanding biomarkers in ISF and the development of new biosensing strategies for these biomarkers. Despite this, the physiological concentrations of many clinically relevant biomarkers in ISF compared to their concentration in blood continue to be poorly characterized.

One advantage of dermal ISF for diagnostics is that it is presented near the skin's surface, allowing easier access to biomarkers without the pain or clotting related to blood draw. Although access to dermal ISF is easy, the accurate collection and low volume available of such fluid has proven to be challenging.[32] For example, constituents of the extracellular matrix within the interstitial space (specifically, glycosaminoglycans and collagen) can bind water, rendering ISF a hydrogel-like consistency, which makes it difficult to collect.[33] To circumvent this issue, microneedle patches have been developed that can simultaneously perform the ISF collection and analyze biomarkers directly on the microneedles and *in vivo*.[32,34,35] The use of microneedle patch-based biosensors for the detection and quantification of biomarkers in ISFs is interesting because it allows for real-time, longitudinal, and continuous monitoring. The detection of glucose in ISF using microneedle patch-based biosensors has been already demonstrated,[36] where these biosensors can detect glucose in ISF with excellent correlation to blood.[36] The question now is: can we extend this to the detection of other biomarkers?

Urine

Urine is a particularly attractive biofluid for POC biosensing as it offers an easy and non-invasive sample collection procedure with no discomfort for the patient. Urine provides an overview of the whole metabolic status of the human organism as it contains more than 3000 metabolic species which can be used for diagnostics.[37] Some of the most abundant organic metabolites present in urine are, on average, creatinine (~10 mM), urea (~22 mM per mM of creatinine), hippuric acid (~298 mM per mM creatinine), and citric acid (~280 mM per mM creatinine).[37] There is also a considerable amount of inorganic ions and gases such as chlorine, potassium, ammonia, and sodium (in the mM per mM creatinine range).[37]

Urine has been very well established as a biofluid for at-home hormone testing in the form of pregnancy tests that can be easily purchased in any pharmacy or even supermarkets. The at-home pregnancy tests detect gonadotrophin (hCG), a glycoprotein hormone secreted by the trophoblast cells of the placenta during pregnancy.[38] Urine is also commonly used for the detection of drugs and their metabolites. Injected or inhaled drugs rapidly appear in high concentrations in plasma, and their urine excretion commences almost concurrently. In chronic users – especially cocaine and cannabis users – illicit drugs can be detected even for several weeks after the last use.[39]

Some of the benefits of using urine as a biofluid for POC sensing include easy collection and analysis; it is usually accessible in sufficient quantities for retesting if necessary; it carries metabolites or drugs in higher concentrations than other biological specimens; and well-established point-of-need tests are already available which makes rapid screening possible. On the other hand, urine presents a relatively shorter detection window compared to hair and sweat; samples can be easily adulterated; and patients might be unable to provide urine samples immediately when requested, *e.g.*, when access to toilet facilities is limited. Other disadvantages of urine as biofluid for analysis, regarding its impact on biosensor performance, include variations in pH and ionic strength (which can impact electrochemical sensors negatively) as well as variations from person to person in urine color and turbidity (which can impact the response of optical sensors).

Tears

Tears have recently been demonstrated to be a very attractive fluid for bioanalysis.[40] They are extracellular fluids secreted by the lacrimal gland and consist of an outer lipid layer, a middle aqueous layer, and an epithelium-covering mucoid layer. Tears are referred to as a hypotonic purified form of blood plasma and comprise a mixture of small molecule

metabolites, proteins, mucins, and lipids. Proteomics studies show that tears contain 500–1500 proteins involved in multiple signaling pathways as well as over 400 microRNA sequences.[40–42] The collection of tears for bioanalysis is already established in ophthalmology clinics, where tools such as polyester/polyvinyl wicks, microcapillary tubes, and Schirmer strips are employed for collection.[43] One limitation of using tears as a biofluid in POC settings is that they are not always readily available for patients, and the sample volume is small.

Sweat

Sweat as a diagnostic biofluid is very attractive for use within a POC setting: it can be collected non-invasively and avoids many issues that occur with other non-invasive biofluids, including irritation and inconvenience.[44] Sweat also presents a wealth of biochemical information including micronutrients (electrolytes), hormones, metabolites, nucleic acids, proteins, and exogenous agents.[45] Therefore, sweat diagnostics can provide information about physiological health, psychological stress, nutritional balance, and exposure to foreign substances.

Traditional sweat assays rely on collection into absorbent pads or tubes followed by laboratory analysis. Some examples of well-established uses of sweat as a biofluid include the measurement of chloride concentration for cystic fibrosis diagnosis;[46] determination of water and electrolyte loss for sports performance evaluation;[47] and trace analysis of prohibited substances for drug screening.[45] The use of sweat for bioanalysis is a rapidly evolving field: skin-interfaced wearable platforms exploit recent advances in soft flexible/stretchable electronics, electrochemical sensing, and microfluidic technologies to support continuous or intermittent assessment of sweat composition in a variety of settings or conditions.[45,48] Despite being a promising biofluid, and extensive literature demonstrating the feasibility of wearable sensors, a validation of the clinical value of several biomarkers in sweat is still missing. A robust correlation between the concentration levels of biomarkers in blood and sweat is challenging since biomarkers are transported into sweat from capillaries with distinctive partitioning profiles.[49] Additionally, biomarkers can reach the sweat by passive (*e.g.*, diffusion) or active mechanisms, as well as be produced within the sweat duct itself.[49]

Other potential issues related to sweat sample collection should also be considered since sweat is prone to evaporation, external contamination, and interference from the environment.[49] Additionally, it can produce inconsistent and/or low sample volumes, and its composition (including electrolytes and metabolites) may vary at different excretion times.[49] Therefore, establishing a suitable sampling protocol is imperative to performing an effective and reproducible biosensing analysis.

After selection of the appropriate biomarker and body fluid, the next step is the development and prototyping of a biosensing interface. The sensing interfaces are generally first tested in controlled environments (*e.g.*, buffered solutions), the analytical performance is determined, and then the newly developed sensors are challenged in a scenario as close as possible to the final intended application. Researchers in the field of sensing and biosensing typically validate the functionality of developed sensors by testing them with a limited number of actual patient body fluids. This process serves to demonstrate the sensor's efficacy for the envisioned application. This is generally sufficient for academic validation and scientific publication and is typically performed simply by a collaborative multidisciplinary research team. However, the next steps in the sensor translational process require clinical validation of sensors with a much larger number of patients' biospecimens to truly demonstrate their clinical potential and usefulness. This is where the clinical trial happens.

Clinical trials

Typically, sensor clinical validation begins with testing body fluids that have been collected retrospectively: biofluid samples that already exist and have been previously stored or archived. Commonly, retrospective biospecimens – *e.g.*, plasma, serum, tissues, cells, and DNA samples – are stored, long-term, frozen. Body fluids may have been collected and stored for various reasons, such as diagnostic remnant samples or bio-banked samples. Diagnostic remnant samples are from samples which were collected in routine clinical care, but not completely used up during clinical testing. Therefore, the remainder of the sample can be stored for subsequent analysis if required. If such samples are intended to be used for biosensor clinical trials, ethical approvals need to be obtained to use them.

In contrast, bio-banked samples refer to samples that already have been collected by research institutes, hospitals, or even commercial companies for the purpose of future use in research and development. In this instance, the body fluids would have been collected and stored with ethical approval for subsequent research use. Thus, accessing body fluids that were collected retrospectively allows initial clinical studies to be performed comparatively quicker and easier as the samples are already available when they are requested. This is a useful way to prove that the sensor mechanism is working in complex biological media and that it has the required analytical sensitivities and specificities. At this step of biosensor translation, cross-validation of the retrospective clinical samples with established technologies, such as enzyme-linked immunosorbent assays (ELISAs), is very important to gain confidence in the sensor output. Additionally, multisite validation (same samples analyzed by different laboratories) using retrospective body fluid samples can also build confidence in the translation potential of the sensor.

The next step in biosensor clinical validation involves testing using prospective biospecimens, which are body fluids (*e.g.*, whole blood) collected freshly at the time in which testing is happening. This step allows the biosensor to be validated

beyond the analytical realm and is where the clinical relevance and accuracy of the biosensor are truly demonstrated. Prospective clinical studies are synonymous with longitudinal studies where individuals/patients are observed over extended periods, occasionally spanning several years. Prospective studies offer the ability to infer causality with greater confidence and allow the determination of risk factors, as well as establish relationships between possible variables more precisely. This is particularly important for biosensors that are designed for monitoring diseases progression or treatment efficacies, thus requiring testing of a given patient's sample(s) at different time points (*e.g.*, a cancer patient pre- and post-surgery).

While they present more accurate information *versus* retrospective studies, prospective studies can require significant resources as they often span several years. The clinical study size (number of patients to be recruited) is correlated to the study phase. Early-phase trials may involve few patients (approximately 30 subjects) while more advanced trials may require hundreds of subjects or even thousands: this results in very high financial costs. The high costs associated with clinical trials can be a significant barrier for researchers working on translational biosensors. The cost of a clinical trial depends on various aspects, including study size (quantity of patient samples tested), locations (number of countries used for testing different ethnicity samples and exploring different potential markets), number of clinical sites, and the particular tests and procedures needed to benchmark the new biosensors against, amongst other factors. Therefore, planning for a clinical trial can be a complex task, and preparing a detailed budget and securing large funds for this step of sensor translation is vital. Another difficulty encountered with prospective clinical studies is the attrition of participants over time, which can occur for a variety of reasons, and which can create bias or decrease the reliability of results.

Altogether, implementing sensor testing for large cohorts of patient biospecimens, and ensuring the chosen sample cohorts are suitable for and representative of the intended POC application, is complex. Therefore, it can require the involvement of partners and additional infrastructure. It is crucial to work with local hospitals, clinicians, and/or pathology laboratories to design a clinical cohort to validate a POC sensor. These stakeholders, along with experienced researchers and private companies, can also assist with clinical trial planning and development.

Pathway for regulatory approvals

Regulatory approvals are essential for POC biosensors used in biomedical analysis to guarantee their safety for the end-user in terms of electrical, biological, physical, and chemical aspects. Because of the potential risks to patients associated with POC tests, it is illegal to market or sell a POC test without it undergoing the appropriate regulatory process. It is important to mention that each country has its medical regulatory authorities, which can make it challenging to decide which regulatory authority to follow. For example, in the USA, the FDA is the responsible agency for ensuring safety of medical devices. In Europe, the European Union (EU) oversees such regulations, while the Therapeutic Goods Administration (TGA) is Australia's regulatory authority for therapeutic goods. Of course, the decision of which authority to seek approval from will be dependent on the target market for the translated POC biosensor. The degree of regulation required depends on the level of risk associated with the device. For example, according to the TGA, an HIV self-test (unsupervised and at home using a POC test) demands more stringent regulation than non-invasive urine-based pregnancy self-test kits.

A common consideration across the different regulatory bodies includes a clear definition of the intended use of the POC biosensor. The claimed performance characteristics of a POC biosensor will be evaluated by the regulatory agencies in the context of the intended use as well as the public health risk or personal risk that may occur from an inaccurate result. Based on this, the newly created biosensors will need to be designed and manufactured in a manner that the claimed analytical and clinical characteristics permit the intended use. Another important consideration for POC biosensors by the regulatory agencies is whether it can be properly validated by the intended users. An example could be the control line on a COVID-19 rapid antigen test: if the control line does not appear, the information obtained with the device is not valid, and the user should re-test with a new device. Finally, significant clinical considerations include the anticipated literacy of the intended user; the validity of performance claims that are being presented to the consumer; and the level of contact that the user has with a health practitioner who may be able to assist in case of an error, or an unexpected result.

To obtain approval for new POC biosensors applied to medical diagnosis, the manufacturer must demonstrate clinical efficacy by collating data and information that shows the performance and validation of the biosensors when it is used as intended. Clinical evidence can be demonstrated by a combination of scientific validity, analytical performance, clinical performance, and clinical utility (Fig. 2). In addition, any clinical validation should be performed on the final device design taking into account any differences in production or materials used at small- and large-scale volume manufacture.

The scientific validity

The scientific validity of a biosensor can be defined as the correlation between biomarker concentrations in the human body and a clinical or physiological condition. It can be initially identified from academic literature research describing previous uses of a given biomarker or the potential of using a given biomarker for the intended clinical applications. These applications may be corroborated by feasibility studies using the new biosensor design for the detection of that same biomarker reported in the literature. For several well explored

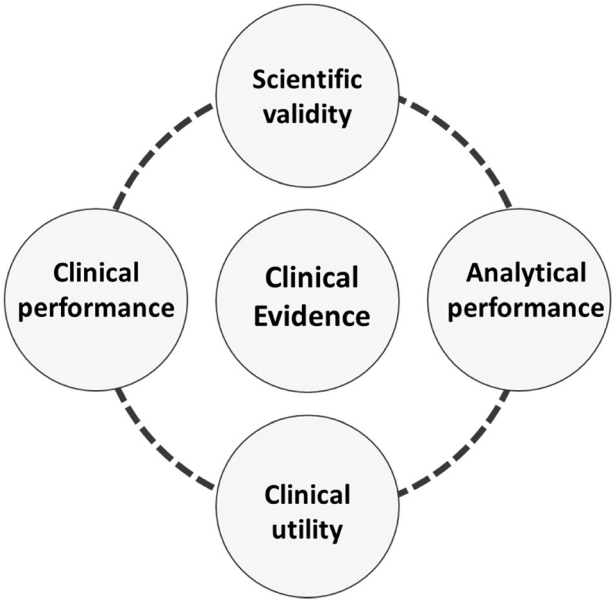

Fig. 2 All the required information that needs to be provided to regulatory agencies as part of the clinical evidence due diligence.

biomarkers, the scientific validity is very well established: for example, calcium in serum for diagnosis of parathyroid diseases. In such cases proving the scientific validity for a new biosensor can be easier. However, for emerging biomarkers, such as newly characterized biomarkers that could potentially be used in a biosensor to monitor the progression and treatment efficacy of patients with cancer, it would require extensive scientific validation.

Analytical performance

Analytical performance is the ability of the POC biosensor to precisely detect a particular analyte/biomarker in the target biological fluid. It can be demonstrated by the collection of experimental data designed to determine the analytical performance (*e.g.*, concentration linear range, limit of detection, and limit of quantification), analytical selectivity (*e.g.*, interference and cross-reactivity), accuracy (precision to obtain a true result), reproducibility of the obtained results, and biosensor stability.

Clinical performance

Clinical performance of a POC biosensor can be broadly defined as its ability to generate analytical results that are directly associated with a physiological state or clinical condition of a target population or intended user. Clinical performance is crucial in establishing the intended use of the POC biosensor. It demonstrates that the POC biosensor can identify a change in a patient's state. The data used to determine clinical performance should include expected values, diagnostic specificity and sensitivity based on the known clinical state of the patient, and negative and positive predictive values based on the prevalence of the disease. This data can be obtained through clinical trials for the POC biosensors, and it is important to ensure representative and varied populations.

Clinical utility

Clinical utility essentially describes the usefulness of the obtained results using a POC biosensor and the value of the information to the patient being tested, as well as to the broader community. It can be informed by a combination of the data that support scientific validity, analytical performance, and clinical performance. It should also consider current clinical practices in place as well as proposed diagnostic algorithms.

Accordingly, before approving a new POC biosensor, the regulatory agencies will evaluate the data and information related to all the above elements (scientific validity through to clinical utility) and hence determine the benefits/risks profile associated with the POC biosensor. It can be complex process, but nowadays there are plenty of consulting companies that can assist with concept development through the FDA/TGA/EU, and are specialists in all aspects of medical device and diagnostic regulation throughout the product lifecycle.

Consumer engagement

Consumer engagement is increasingly recognized as essential to introducing new and effective medical technologies to the market, including new POC biosensors. Many institutions and international bodies now require it as part of the research and funding process, and its importance has led to a push for academic research to happen in direct conjunction with industry. The term 'consumer' generally refers to stakeholders who have lived experience using similar medical technology to the one being developed. It includes patients or potential patients, carers, health practitioners, and the wider community.[50,51] Ideally the consumers engaged with will be diverse to enable universal design principles to be followed, including people of different ages, sexes, ethnicities, and socioeconomic cohorts; disabled and/or neurodivergent people; people whose first language is not the dominant language in the country of research; people who are indigenous, or experience racial discrimination; people who identify as part of the LGBTIQA+ community; and those who live in non-metropolitan areas.

Consumer engagement in the context of biosensor creation and development refers to the authentic involvement of these stakeholders at all stages of the research and translation process. The goal of consumer engagement in biosensor design is to take consumers beyond the role of passive end-user and to place them in a position of active contributor and co-creator. It should happen at different stages of the process, from early to late, including identification of target biosensor application; seeking funding, planning and execution of research steps; the performance of analytical or clinical tests; product design; the test process; data interpretation; implementation of the findings; and avenues for feedback and continued optimization

post-commercialization. For example, a biosensor that is most relevant to older people should consider that visual and/or fine motor impairment is common, so tests should avoid small writing and buttons, and fine, precise movements.

Consumer engagement provides numerous benefits for the biosensors industry. Firstly, from an ethical perspective, consumers should be involved in research decisions that may impact their health and well-being, or that of people they care for. Secondly, the quality and relevance of the biosensor in development will be improved because of the unique perspectives and knowledge that consumers can offer. This can result in a product that is more effective and positively contributes to health equity, and more potential consumers. Finally, the adoption of the POC biosensor by consumers, once it is available on the market, might be easier as they have more confidence in the final product, given they participated in the research and translation process.

Strategies for large-scale manufacturing

Another common practice in the POC biosensing academic field is to perform experiments using prototyped sensors or sensors produced using small-scale pilot production lines. This is partially because researchers are often focused on the development of an innovative surface chemistry or transducer paradigm used for the sensing approach, or only in demonstrating a proof-of-concept study of new sensor architectures. Taking POC biosensors out of the laboratory and translating it into manufacturable sensor products is challenging, and the absence of functional manufacturing integration and high manufacturing costs are major challenges for commercialization. Therefore, understanding the different potential scalable manufacturing processes for biosensors is crucial for successful translation. Traditionally, large-scale manufacturing of biosensors for health monitoring aims to generate significant quantities of single-use test strips (paper-based or flexible materials/substrates), microfluidic devices, and screen-printed electrodes. However, the POC sensing field is constantly evolving and new fabrication technologies continue to emerge.

Roll-to-roll (R2R)

Roll-to-roll (R2R) manufacturing consists of a collection of foil-based processes in which additive and subtractive techniques are implemented to create functional structures coatings on a continuous substrate called a web. In R2R, the web goes through a multitude of processing stations. A R2R process usually involves the following steps: a flexible web roll is unwound on the unwinder by means of a web transport system; foreign substances are removed from the manufacturing system itself; pre-processing of the web's surface treatment (or pre-coating); the main process which is surface coating (which could be the dispensing of chemicals or biological solutions); a controlled heat treatment process such as drying and sintering; and finally, winding of the web roll to the winder for further processing (*e.g.*, individualization of single test strip sensors).[52]

A great example of a worldwide commercialized biosensor manufactured using R2R processes is the POC glucose test strip. The glucose test strips used for diabetes monitoring are usually fabricated using webs consisting of a thin film of gold deposited on flexible polymeric substrates like polyimide or polyethylene terephthalate (PET) on which the chemistries (*e.g.*, enzymes plus redox moieties) are deposited. This example highlights the potential of R2R fabrications as a technology for supporting production in large scales at a low cost. One downside of R2R processes is that involves large and expensive machinery, which presents a financial barrier to producing prototype sensors for initial validation studies. Another disadvantage is that it can be difficult to customize sensor configurations using R2R processes.

Additive manufacturing (AM)

Additive manufacturing (AM), also called 3D-printing, has been widely investigated in recent years for the fabrication of the next generation of biosensors. This fabrication process constructs a 3D structure using numerically-controlled 2D stacking: therefore, AM is a manufacturing process that uses layer-upon-layer stacking.[53] Such a methodology has experienced an exponential evolution regarding deposition mechanisms and diversity of printable materials. High-resolution AM has allowed the microfabrication of diverse architectures of electrodes and sensing layers for improved sensing. The possibility of using different printable materials, such as biocompatible materials, polymers, gels, and metals, has vastly expanded the horizons in biosensors and diagnostic devices for both academic research as well as commercial purposes.

The AM process usually begins with a 3D modeling stage by using a computer-aided design (CAD) software. Once the 3D geometry is created using CAD, it is then imported into a slicing software in which the number and thickness of layers is determined. This will generate the tool paths required to start the printing. The next step is the actual printing of the designed structure, which can take place using diverse AM processes. Based on the forming mechanisms and working materials, AM processes can be classified into powder binding processes (*e.g.*, high energy binding and additive biding), lamination processes, photopolymerization (*e.g.*, stereolithography), and extrusion deposition processes (*e.g.*, fused deposition modeling or direct ink writing). The advantages of the AM process include limitless geometry possibilities, short lead time (*i.e.*, hours) for the fabrication of small batches of prototyped biosensors, and customizable biosensing designs. AM is often used for the fabrication of microfluidic biosensors and soft wearable sensors.[54]

Inkjet printing

Inkjet printing, a simpler version of AM, is a promising fabrication process that can be used to directly manufacture

biosensors and electronics on a range of different substrates such as flexible plastics and soft silicones. Inkjet printing consists of precisely depositing ink microdroplets onto the chosen substrate. The controlled formation of microdroplets is achieved *via* piezoelectric and thermal technologies. Thermal technologies use a heating resistor inside the nozzle which creates a vapor bubble, leading to an increase in the pressure and dispensing of the ink.[55] One drawback with thermal printers is that they can reach high temperatures inside the nozzles (~300 °C), which is unsuitable for printing biological components and some synthetic compounds of the conductive inks. Therefore, depending on the printing materials, thermal printing may not be possible. In piezoelectric technologies, an electrical field is applied to crystalline materials contained in the nozzles of piezoelectric printheads.[55] The applied electrical field creates mechanical stress on the crystals, hence reducing the available space for the ink, which raises the pressure and dispenses the ink droplet.[55]

Advantages to inkjet printing include its simplicity, maskless patterning of materials, relatively fast production, compatibility with diverse substrates, and cost-effectiveness. The properties of the inks such as viscosity, particle size, and surface tension, are crucial parameters that need to be well controlled. Generally, inadequate control of these parameters can cause clogging of the printheads, which is one of the main issues faced by inkjet printing.

A great possibility for a next-generation approach for large-scale manufacturing of biosensors is the integration of AM processes with a R2R systems, which permits the biosensor production to become a continuous process. AM provides greater control over the manufacturing process, enabling reproducible and consistent biosensor production; R2R systems allow for high-volume and continuous production, hence lowering manufacturing costs and increasing productivity. This combination could enable the production of high-performance biosensor strips with improved efficacy and low manufacturing costs.

Legal and ethical considerations

Understanding the intricacies involved in obtaining a strong intellectual property (IP) is a vital aspect for biosensor translation. Having a strong IP position is one of the main factors considered by investors when determining whether to fund a biosensing technology: it can serve as a source of potential revenue through licensing agreements, can help in alleviating expensive legal settlements, and of course, it can assist in preventing potential competition from similar biosensing technology.[56] To attain a successful IP position, academic researchers or innovators are required to obtain a robust understanding of the patenting process; work with an experienced IP attorney; pursue patents with all necessary claims; and most crucially, understand that patenting is a continual process that necessitates ongoing monitoring of the IP landscape, and that possible modifications to the patent portfolio might be required.[56]

When it comes to patentability there are a few things that should be considered. The first aspect is deciding if the best approach is to file a provisional application or advance directly to a full utility patent. Usually, thoughtful preparation of a provisional patent can provide the benefit of securing one extra year of protection. The utility patent application has a twenty-year lifespan from its filing date and can be filed within a year of the provisional filing date. Thus, the provisional patent determines the priority date, which is the earliest filing date in a family of patent applications. Once the biosensor is on the market – which can take up to several years after provisional filing – then the security offered by the utility patent will be one extra year *versus* the case where the utility patent was filed directly.

Once the provisional is filed, the second aspect to be considered is if it is necessary to strengthen the IP portfolio. Commonly, scientists or innovators make substantial discoveries or include new features to the biosensor technology during the translation process, even after filing a provisional patent. In this case, there is the option of filing a new provisional application or initiating the utility filing that includes both the original application and the new discoveries.[56] Another consideration is to whether combine all aspects of the biosensing technology into one patent only or split it up into multiple patents. In the case of provisional applications this is not very crucial, but it could be very interesting in the utility application stage, especially if a particular aspect of the sensing technology is undoubtedly original and not as obvious (or more innovatively) as other aspects of the technology. Pursuing separate patent filings for different aspects of the technology, if done correctly, could help companies who are commercializing the technology to maintain a strong position in the market. One drawback of multiple patent filings is the expense. Notably, the patent filling costs can be much higher if it is done across multiple jurisdictions. Thus, securing large budgets for the IP protection journey is essential. Altogether, the IP protection trajectory can be a complex one, and that is why developing a deep knowledge of the process and working together with a very experienced IP attorney is so crucial.

Once the patent is granted, the next step is to investigate if the sensing technology can be commercialized through a freedom-to-operate (FTO) search. That a sensing device, or features of the technology, is patentable does not necessarily mean that it is cleared to be commercialized, as some of the claims of technology could be infringing on claims of other deposited patents that are active. An FTO search is essentially a systematic review of prior art in the same area as the invention and which exists as an active patent, granted in the last two decades. Thus, this review should be searching for claims in other active patents in the same field of the new technology. Again, this is a very crucial step in the translation process and should be carefully considered and advised by an expert IP attorney in order to avoid any FTO problems.

Apart from the IP regulations, ethical aspects should also be considered. The number of potential ethical issues is expansive and challenging to cover in a single article. However, during the translation process, researchers/innovators should consider the

following ethical issues when introducing new biosensors to the market: i) accessible, equitable, and non-discriminatory health care opportunities, ii) medical research with human beings or animal testing, iii) autonomy and best interest of the human being including children, iv) citizen's right to information, v) informed consent, and vi) privacy and personal data protection. It is important to mention that some of these issues are regulated through legal instruments, which should also be considered.

Other ethical aspects that need to be considered during the biosensor commercialization journey are the issues regarding the sourcing of materials/components and fabrication processes, the so-called supply chain. As society is gaining more awareness about issues associated with the environment, sustainability, and forced labor, the supply chains for new POC diagnostic products are required to meet ethical standards related to environmental stewardship; sustainable sourcing; reducing waste, or adopting a circular economy approach; and better work conditions. Adopting an ethical supply chain means that startups and companies will incorporate social and human rights and environmental considerations into their business models.

The adoption of sustainable practices for the POC diagnostic industry encompasses a holistic approach to reducing environmental impact throughout the entire life cycle of its products. It begins with eco-conscious product design and material selection, opting for materials that are sustainable and have minimal environmental footprints. During manufacturing, energy-efficient processes should be adopted to reduce carbon emissions and resource consumption. Waste management practices should also be prioritized, aiming to minimize waste generation and promote recycling and reuse of materials wherever possible. Throughout distribution and logistics, sustainable transportation methods should be employed to lower the carbon footprint during POC product delivery. Packaging materials should also be chosen with sustainability in mind, focusing on materials that are recyclable or biodegradable.

The following questions can be used by researchers/innovators to establish the standard operational procedures for the manufacturing of new ethical and sustainable biosensing technologies:

- Is the sourcing of materials achieved from sources with renewable or low-impact extraction/fabrication methods? Are the providers certified?
- Are the suppliers and supply chain partners trustworthy?
- Do the suppliers and supply chain partners uphold their ethical and moral commitments consistently, even when it may involve extra expenses or challenges?
- Does each partner of the supply chain ensure fair wages, manageable workloads, and ethical behavior towards its workers?
- If unethical conduct is identified, what steps will be taken to address it? Will every partner in the supply chain collaborate to ensure it is promptly rectified?

Health economics

Health economics analysis is an important process for the introduction of new POC diagnostic tests. It offers evidence-based observations into the economic implications and value of adopting these biomedical tests in clinical practice, helping decision-makers allocate resources efficiently and improve healthcare delivery.[57–59] A thorough health economics analysis should include multiple components:

Cost-effectiveness analysis: this should assess whether the new diagnostic biosensor provides value for money compared to existing pathology tests. It considers the costs of fabricating the biosensor itself, including equipment, consumables, and personnel time, against the health outcomes it can achieve.

Patient outcomes: this should evaluate the impact of the new POC diagnostic test on patient outcomes including quality of life, rates of mortality, and morbidity. This is where the determination of the clinical benefits or improvements in health outcomes related to using the newly introduced diagnostic test occurs.

Effect on clinical decision-making: this should be designed to estimate how the new POC biosensors will impact clinical decisions and patient management compared to standard laboratory-based tests. This includes assessing whether the test will provide more accurate and timely diagnoses, reduce unnecessary treatments or referrals, or improve patient outcomes.

Budget impact analysis: this is designed to predict the financial consequences of adopting the new diagnostic tests within a healthcare system or institution. A budget impact analysis should consider costs related to POC diagnostic test implementation, alterations in treatment pathways, prospective savings from improved patient outcomes, and overall budget inferences.

Cost–benefit analysis: estimates whether the benefits resulting from the new diagnostic test compensate for the costs incurred. It should calculate the monetary and non-monetary benefits and assess them compared to the costs to determine if the investment can be justified from a wider societal prospect.

Sensitivity analysis: this should be designed to predict uncertainties in the data and assumptions used in the economic analysis. This encompasses testing how modifications in key variables (*e.g.*, test accuracy or cost of treatment) impact the findings and conclusions of the economic assessment.

Final considerations

The biosensor industry is blossoming, with market predictions of continued growth in the next few years. There are many opportunities for translating biosensor technologies into medical diagnostic devices for numerous human conditions and health monitoring. Academic research in biosensing plays a pivotal role in the translation ecosystem: it sits on one side of the valley of death (the gap between proof-of-concept or

preclinical research and commercialization of biosensors for clinical diagnostics). If a university academic has a new POC diagnostic test with commercial potential, they should discuss it early on with the university or research institution's technology transfer or business and development office, which will assist researchers in pursuing IP protection and deciding the best commercialization route. Spinouts and startups are a progressively popular pathway for commercializing academic research. However, pursuing such a pathway requires knowledge in different fields such as business, development, manufacturing, quality control, marketing, and leadership which is not part of most science Ph.D. training. Of course, it also necessitates securing funding and resources. For those navigating the entrepreneurial world for the first time, it can be difficult to imagine what the process entails – who should be participating? What funding sources are available? Or where it begins? Deciding whether a venture should or should not become a spinout is a decisive and frequently irreversible decision. Beyond the crucial translational points discussed in this perspective, the following considerations can help academics in making an informed choice:

Market research: analyse the market potential of the new POC diagnostic biosensor. Is there a clear demand for the biosensors? Is the market of the newly created spinout big enough to sustain a stand-alone company?

Business model: assess whether the newly formed spinout's business model can sustain itself independently of its parent institution, or if it will falter once it loses its initial support.

Resources allocation: evaluate the resources needed to develop and expand the new concept. This encompasses financial needs, qualified staff, infrastructure, and time. Decide whether these resources can be adequately provided by the university or home institution internally, or if it would be preferable to source them from third parties.

Securing funds: spinouts and startups coming out of academia can benefit from a range of federal funding that specifically targets the development and commercialization of basic research. Thus, working alongside the academic business and development unit to investigate all possible funding opportunities is vital for early-stage startups.

Entrepreneurial team: evaluate the team responsible for the spinout once it leaves the parent institution. Do they possess the entrepreneurial mindset, expertise, and practical experience to lead a startup? A competent and driven team is essential for the success of a spinout.

Risks and challenges: identify and outline potential risks and obstacles related to the new venture. This encompasses technical difficulties, regulatory and legal complexities, market acceptance uncertainties, and competitive pressures (it is important to know the competitors well to develop specific strategies). Establish risk tolerance procedures and create the capability to mitigate such challenges.

Financial feasibility: perform a comprehensive financial assessment. Calculate the anticipated expenses for research and development, market entry, and continuous operations. Evaluate these against projected revenues and potential profitability, considering scenarios where the new venture operates independently as a spinout/startup or remains integrated within the parent institution.

Taking a POC diagnostic sensor from the lab bench into the hands of the end-users is certainly not an easy and quick process, and it requires a lot of effort and investment. Thus, the POC biosensor translational process needs to be thought out carefully, and all of the necessary considerations discussed here should be reflected upon. Delivering POC biosensors to the market certainly presents great potential to create societal, health, and economic impacts. Glucose test strips and meters provide a solid example of how POC biosensors positively impact the lives of many people around the globe. The question now remains, can we replicate this success to many other biomedical applications? The future looks very encouraging for the POC diagnostic field.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

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