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Comparative analysis of electrochemical and optical sensors for detection of chronic wounds biomarkers: A review

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ABSTRACT

Chronic wounds (CW) present a significant healthcare challenge due to their prolonged healing time and associated complications. To effectively treat these wounds and prevent further deterioration, monitoring their healing progress is crucial. Traditional wound assessment methods relying on visual inspection and subjective evaluation are prone to inter-observer variability. Biomarkers play a critical role in objectively evaluating wound status and predicting healing outcomes, providing quantitative measures of wound healing progress, inflammation, infection, and tissue regeneration. Recent attention has been devoted to identifying and validating CW biomarkers. Various studies have investigated potential biomarkers, including growth factors, cytokines, proteases, and extracellular matrix components, shedding light on the complex molecular and cellular processes within CW. This knowledge enables a more targeted and personalized approach to wound management. Accurate and sensitive techniques are necessary for detecting CW biomarkers. Thus, this review compares and discusses the use of electrochemical and optical sensors for biomarker determination. The advantages and disadvantages of these sensors are highlighted. Differences in detection capabilities and characteristics such as non-invasiveness, portability, high sensitivity, specificity, simplicity, cost-effectiveness, compatibility with point-of-care applications, and real-time monitoring of wound biomarkers will be pointed out and compared. In summary, this work provides an overview of CW, explores the emerging field of CW biomarkers, and discusses methods for detecting these biomarkers, with a specific focus on optical and electrochemical sensors. The potential of further research and development in this field for advancing wound care and improving patient outcomes will also be noted.

1. Introduction

Chronic wounds (CW) represent a growing global health challenge, affecting currently about 1.5–2 million people across Europe, and in the United States about 5–6.5 million people, which leads to thousands of euros in medical costs and imposing substantial burdens on health systems. Between hospitalization, nursing, dressings, and the difficulty of effectively treating non-healing wounds, the costs are around 85% of the total value for treating wounds. The number of people suffering from this problem will continue to increase without control since the means of diagnosis and treatment for this type of situation have not yet been fully defined (Advances in Wound Care 8, 2019; Phillips et al., 2016; Sen, 2019).

CW are characterized by the inability to complete the healing process in an orderly and sequential manner to fully recover the functional and structural integrity of the damaged barrier. Characterized by their prolonged healing time of more than six weeks, these sores often stem from conditions such as diabetes, poor circulation, immune system disorders, and pressure injuries. The repercussions include not only physical pain and decreased quality of life, but also increased health expenses and imminent risks of serious complications such as infections and amputations (Falcone et al., 2021; Li et al., 2020; Nussbaum et al., 2018).

Effective management of CW is imperative to improve patient outcomes, alleviating healthcare costs and pressure on medical systems. Currently, several techniques, predominantly visual inspection, are used to detect and evaluate CW. Although widely employed due to their ease and non-invasiveness, these methods can be less accurate than other analytical methods, since they are subject to the interpretation of the

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healthcare professional performing them. The results may be subjective and vary considering the health professional who observes. Furthermore, with this method it is difficult to visualize small changes in the wound, especially in the initial stages, which leads to a lack of essential information for targeted diagnosis and treatment (Bandodkar et al., 2016; Li et al., 2021; Wang et al., 2022).

A promising way in treating CW revolves around biomarkers, that according to the National Institute of Health (NIH), is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Atkinson et al., 2001). These biomarkers encompass a variety of substances, including proteins, enzymes, cytokines, and other molecules intrinsically involved in processes such as inflammation, angiogenesis and tissue regeneration. By meticulously monitoring these biomarkers, clinicians can gain valuable insight into fundamental mechanisms that govern the healing of CW (Pereira et al., 2021). To this end, innovations in wound treatment are materialized in the development of wearable sensors capable of detecting these biomarkers associated with CW. These wearable sensors are designed to provide real-time information on biomarker levels in wounds, allowing healthcare professionals to quickly identify changes in wound status and adapt treatments immediately. Many proof-of-concept examples of these wearable sensors have been published recently, however, although there are some commercialized sensors (Yang et al., 2023b), to date none of them have been clinically implemented on a large scale and used routinely, due to certain limitations (Ciani et al., 2012; Rajeev et al. 2018, 2020; Simoska et al., 2020; Youssef et al., 2023). The development of wearable sensors faces several challenges about different characteristics that must be considered when using them, such as the materials used, energy sources, data acquisition, appropriate and safe forms of wireless communication between the sensor and devices such as computers or cell phones and the miniaturization of certain elements that must be incorporated for the sensor to function effectively. Among the various types of wearable sensors available, electrochemical, and optical sensors stand out for their potential in detecting relevant biomarkers. They are characterized by their minimally invasive nature, ease of use and cost-effectiveness - ideal attributes for deployment in clinical settings (Pereira et al., 2021).

Electrochemical sensors operate by measuring electrical signals resulting from chemical interactions between the biomarker and the sensor surface. Its merits include high sensitivity and ability to detect multiple biomarkers simultaneously. For example, glucose biosensors track glucose levels, which can serve as predictive biomarkers for wound healing outcomes (Chen et al., 2013). In contrast, optical sensors depend on the assessment of absorption, scattering or emission of light by the biomarker. Its strengths include high specificity and the ability to identify biomarkers in complex biological environments. One example is the use of fluorescent sensors to detect matrix metalloproteinases (MMPs), enzymes crucial for the breakdown of extracellular matrix during wound healing (Chen et al., 2013).

The progression of biomarkers and sensors targeting CW marks a transformative step in wound care. The detection of these biomarkers is the key to the diagnosis, prognosis, and effective treatment of CW. This field promises to be fertile ground for future research and innovation (Dargaville et al., 2013a).

The main objective and novelty of this review is to discuss on electrochemical and optical sensors aimed at detecting CW biomarkers that are described in the literature, and to present the latest contributions in this area. First, the most important biomarkers related to CW will be presented, and an extensive compilation of analytical methods for their detection using sensors will be discussed, focusing on electrochemical (potentiometric, voltametric, amperometric and impedimetric) and optical (UV/Vis spectrometry, fluorometric, colorimetric) methods. Specific examples of sensors for each type of biomarker will be explained, compared, and critically discussed.

2. Chronic wounds

A skin lesion can disrupt the skin's layers and microcirculation, resulting in a wound that affects the structure and sometimes function of the affected organ (Murphree, 2017; Velnar et al., 2009). Depending on their origin, wounds are categorized as acute wounds (caused by chemical or physical injuries or surgical procedures) or CW (resulting from conditions like diabetes, infections, vascular diseases, or cancer) (Eming et al., 2014; Mast and Schultz, 1996; Sun et al., 2021). Acute wounds generally follow a structured healing process (Table S1), while CW do not fully recover their functional and structural integrity (Mast and Schultz, 1996).

For a detailed molecular and cellular level comparison between chronic and acute wounds, refer to Table 1 (Mast and Schultz, 1996; Morton and Phillips, 2016; Sun et al., 2021).

CW have certain characteristics that allow them to be identified as such, for example high levels of cytokines and proteases, high amounts of reactive oxygen species (ROS), senescent cells, as well as persistent infections (Table 1). In this type of wound, microorganisms and platelet-derived factors successively and constantly stimulate the production of immune cells and, in this way, the cascade of proinflammatory cytokines is amplified and prolonged, leading to increased levels of proteases. What normally happens (AW) is that the levels of proteases are regulated by the respective inhibitors, which does not happen in CW, where the level of proteases exceeds the levels of their inhibitors. This leads to the destruction of the extracellular matrix (ECM) which prevents the wound from progressing to the next stages of the healing process and attracts more and more inflammatory cells. Immune cells can produce ROS, which are beneficial at low concentrations for wound defense against microorganisms. However, in CW the predominance of a hypoxic environment increases ROS production to levels that will destroy the ECM

Table 1
Different characteristics of chronic and acute wounds. ↑ Increased concentration at the wound site; ↓ decreased concentration at the wound site.

	Acute wounds	Chronic wounds
Bacteria levels	↓	↑
Inflammatory cytokines levels	↓	↑
Proteases concentration	↓	↑
Reactive oxygen species levels	↓	↑
Matrix	Intact and functional	Degraded and nonfunctional
Mitogenic activity	↑	↓
Cells	Mitotically competent	Senescent

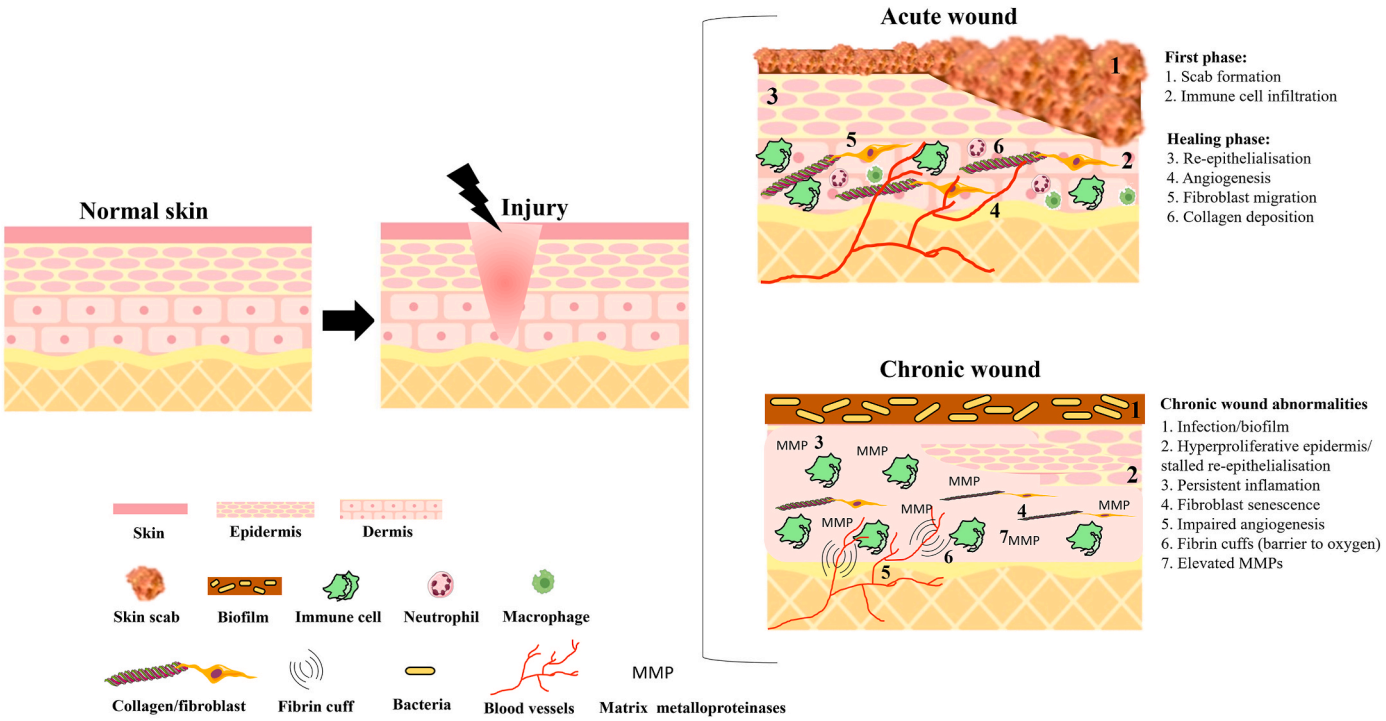

Fig. 1. Differences between chronic (non-healing) and acute (healing) wounds at the cellular and molecular level.

and cause cellular damage. The predisposition of CW to bacterial colonization is greater than in AW, for the reasons mentioned above. In this way, CW are prone to bacterial infections from the low-oxygen, high-protein environments, creating a repetitive cycle (Fig. 1) (McCarty and Percival, 2013; Menke et al., 2007; Mota et al., 2021; Schreml et al., 2010).

CW are generally perceived as a co-morbidity of other conditions (age, illnesses, etc.), which limits the efforts dedicated to their treatment and the growing obstacle they represent in society (Frykberg and Banks, 2015). The distinction between CW and AW is crucial in the clinical context, as it directly influences treatment strategies and patient management. This distinction is of great importance for some reasons that must be taken into consideration (Table S2).

3. Chronic wounds biomarkers

Addressing CW is an immediate and critical concern. These wounds display a disruption in their biological environment, accompanied by alterations in specific biomarkers that deviate from their normal ranges. Biomarkers, quantifiable indicators of biological processes, serve as versatile tools in the medical field, encompassing roles in disease diagnosis, prognosis, risk assessment, and prediction of therapeutic outcomes. These markers are classified, by U.S. Food and Drug Administration and National Institutes of Health (Califf, 2018), into distinct categories based on the type of information they offer, including safety, diagnostic, risk, prognostic, predictive, response, and monitoring biomarkers (Fig. 2) (Table S3) (Atkinson et al., 2001; Hahm et al., 2011; Yang et al., 2023a).

The evaluation and utilization of biomarkers extend to efficacy and

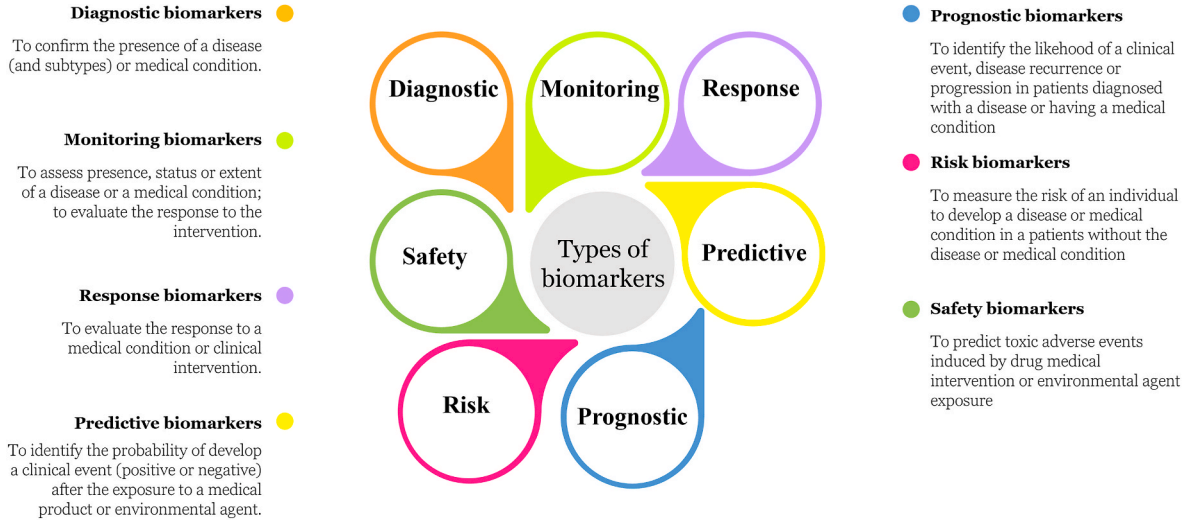

Fig. 2. Different types of biomarkers based on their main clinical application.

safety assessments, involving studies in animals, tissue samples, and early-stage clinical trials. The process of discovering biomarkers starts with identifying molecules linked to the regulation, stimulation, or inhibition of the healing process. Subsequent validation involves statistical analysis and clinical assessments to confirm their relevance and significance. The evaluation of biomaterials through molecular and cellular biology techniques provides a means to track biomarker presence and activity throughout the wound healing process. The main human sources of these biomarkers include wound fluids, tissues, swabs, and serum samples from patients, each offering unique insights into the healing process. Biomarkers found in these sources can be both biochemical and physical, with changes in their levels carrying crucial physiological implications. For instance, wound fluids offer insights into proteins and lipids present, while tissue samples reveal pathological histology and diagnostic biomarkers. The patient's blood, plasma, and serum samples, either individually or in conjunction with other sources, provide a comprehensive assessment of systemic markers associated with wound healing (Lindley et al., 2016; Mota et al., 2021; Mouës et al., 2008; Shah et al., 2012). The presence of altered biomarker levels in CW signals disruptions in the healing process. Incorporating biomarkers into wound detection, diagnosis, and ongoing monitoring enhances precision and accuracy in assessing wound status. The concentrations of key biomarkers associated with CW are highly relevant clinically, potentially providing critical insights into the wound's progression and response to treatment (Fig. S1).

A detailed overview of CW biomarkers, encompassing their types, functions, and applications, can be found in Table 2. The integration of biomarkers into wound care practices holds immense promise for advancing our understanding of CW and improving patient outcomes (Hahm et al., 2011; Kandhwal et al., 2022; Lindley et al., 2016; Shah et al., 2012; Stojadinovic et al., 2005; Sun et al., 2021).

3.1. Meaning of biomarkers derived from chronic wound fluids

3.1.1. Uric acid (UA)

Studies show that uric acid (UA) accumulates in CW (CW), particularly in their fluids, serving as a biomarker for wound severity. This marker is linked to oxidative stress and bacterial infections. Elevated UA levels in CW fluids, ranging from 220 to 750 mM/L, correlate with greater wound severity. The skin damage caused by the wound and accompanying cellular damage trigger abnormal release of adenosine triphosphate (ATP), which is then converted into UA by specific enzymes. As wound severity increases, more skin and cellular damage occurs, leading to higher ATP release and subsequently greater UA production. Recent research also connects UA presence in wounds with bacterial infection, notably *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Microbial uricase (UOx) catalyzes UA breakdown in the presence of bacteria, causing UA concentrations to fluctuate and potentially indicating bacterial infection. UA is a biomarker that can be measured by electrochemical or optical sensors. Electrochemical sensors, when applied to UA, employ electrodes modified to have high selectivity to UA. These electrodes can react specifically with UA, providing a measurable electrochemical response. For example, an amperometric sensor was developed based on modifying the surface of an electrode with uricase. This enzyme could catalyze the reaction of UA, converting it into detectable products. The sample collected from the wound containing UA is placed in direct contact with the sensor, in this case, the electrode, and electrochemically detectable products, such as hydrogen peroxide, are measured by the sensor. Based on the magnitude of the electrical response, it is possible to estimate the concentration of UA in the wound sample. In the case of optical sensors for the detection of UA, several spectroscopic methods such as UV/Vis, fluorescence, or colorimetry, use the absorption of UV/Vis light, or

Table 2
Several types of chronic wound biomarkers. ↑ Increased concentration at the wound site; ↓ decreased concentration at the wound site.

Type of biomarker	Biomarterial		Biomarker		Quantified Measure of Non-healing
Biochemical	**Wound Fluid**		Uric Acid		↑
			Nitric oxide		↓
			Cytokines		↑
			Reactive oxygen species		↑
			Gene expression		↑ ↓
			MMP/TIMPS		↓
			Growth factors	PDGF	↓
				EGF	↓
				VEGF	↓
			Bacteria	*Staphylococcus*	***Positive***
				Pseudomonas	***Positive***
				Corynebacterium	***Positive***
			Enzymes	MMP	↑
				MPO	↑
				CAT G	↑
				HNE	↑
			pH		↑
	Wound Tissues		β- catenin		↑
			C-myc		↑
			Growth factors	PDGF	↓
				EGF	↓
				VEGF	↓
	Systemic	Serum and plasma	MMP		↑
			Cytokines		↑
			Procalcitonin		↑
Physical			Temperature		↑
			Humidity		↑
			Impedance		↑

emission of fluorescent light after sample excitation, to identify and quantify UA. In the case of fluorimetry, specific fluorophores (such as DAPI-type dyes (4′,6-Diamidino-2-Phenylindole) or fluorescein and reagents such as 2,3-Naphthalenediol acid) can be used to interact with the UA and generate fluorescent signals. In the case of colorimetry, a colorimetric test strip was developed based on the immobilization of specific reagents (peroxidase) that react with UA. Peroxidase reacts with hydrogen peroxide generated by the action of uricase. This reaction leads to a change in color on the strip, which with a specific reader for more precise measurement or visually correlates the amount of UA with the color, based on its intensity (Fernandez et al. 2012, 2013; Trengove et al., 1996).

3.1.2. Nitric oxide (NO)

The NO is a biomarker produced by macrophages in early stages of the healing process. This marker induces angiogenesis, and intensifies collagen deposition and wound resistance, and in addition, its antimicrobial properties act to fight infection. For this reason, it has been indicated as a therapeutic agent for CW. The reduced concentrations of NO in CW impair re-epithelialization and collagen deposition, thus causing failures in the healing process. However, when it is found in excessive concentrations, associated with infected wounds, it causes more tissue damage. Thus, it is important that there is a balance and modulation in the levels of NO in wounds, as a solution for wounds with impaired healing. In terms of sensor detection, NO is more easily measured by electrochemical sensors than by optical sensors. The ease of modifying the electrode surface in an electrochemical sensor improves NO selectivity. However, optical spectroscopy such as absorption spectroscopy can be applied to analyze spectral features associated with nitric oxide (Brown et al., 2018; Malone-Povolny et al., 2019; Nathan and Hibbs, 1991).

3.1.3. Cytokines

Cytokines are chemotactic for fibroblasts and manipulate the inflammatory phase of wound healing. CW fibroblasts do not respond to healing as they have lost their receptors which now respond to inflammatory cytokines. Inflammatory cytokines, specifically enhancing interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF-α) cytokines, are found in greater concentrations in impaired healing wounds than in normally healing wounds. In CW, the concentrations of these cytokines are unregulated, leading to a detrimental effect on the healing process. IL-1 is present at persistently high levels and contributes to chronic inflammation, preventing the transition to later phases of healing. Furthermore, it stimulates the production of enzymes such as matrix metalloproteinases (MMPs), which can lead to the degradation of the surrounding tissue if not controlled properly, which can result in wounds that do not heal properly. Similarly high concentrations of IL-6 are found in CW, suggesting a possible contribution to persistent inflammation and healing difficulties, interfering with normal tissue repair processes, contributing to wound chronicity. High levels of TNF- α can inhibit the synthesis of collagen, a crucial protein in the formation of scar tissue. Furthermore, this cytokine is also related to the production of enzymes, which degrade the extracellular matrix. This can lead to degradation of the surrounding tissue, making healing difficult. Studies indicate (Trengove et al., 2000) that IL-1 concentrations in healing wounds is normally 2700 U/mL compared to non-healing wounds which is 9200 U/mL. The same study indicates that this value dropped when healing began to occur. The detection of cytokines in CW using electrochemical and optical sensors is an important area of research, as they may be crucial to understanding the wound environment. Functionalized electrodes for cytokines, with specific materials to selectively interact with them, allow their direct or indirect detection in the wound. Furthermore, techniques such as electrochemical immunoassay, which uses specific antibodies or aptamers for cytokines, can be used to improve the selectivity and sensitivity of the sensor. To detect the cytokine IL-6, a gold electrode was modified on its surface with monoclonal antibodies specific for IL-6. The cytokine, when present in the sample, bound to the molecular recognition sites on the electrode surface. The binding of IL-6 to the electrode generates a quantifiable electrochemical response. Another type of specific molecular recognition layers that can be used for the detection of cytokines, such as Peptides and Functionalized Nanoparticles. In the case of optical sensors, optical spectroscopy techniques, such as absorption or scattering spectroscopy, can be applied to analyze spectral features associated with the presence of cytokines (Gohel et al., 2008; Harris et al., 1995; Trengove et al., 2000).

3.1.4. Reactive oxygen species

CW are also characterized by elevated levels of ROS which results from impaired antioxidant defense, prolonged inflammation, mitochondrial dysfunction, ischemia, bacterial presence, and an altered wound microenvironment. These species are released by neutrophils and represent a key step in the pathogenesis of CW. They motivate the persistence of inflammation, increased tissue degradation and lipid peroxidation, sustaining an increasingly hostile microenvironment. The detection of ROS in CW allows us to understand oxidative stress and the healing process, and in this sense, electrochemical sensors, such as amperometry, and optical sensors such as fluorescence, are mostly used. Amperometric techniques are employed to record the electrical response generated by the interaction between the ROS and the electrode. Modifying the surface of the electrodes with catalytic materials such as platinum or metal oxide facilitates the ROS reduction reaction. Hydrogen peroxide is oxidized on the surface of electrodes with this constitution, generating electrons. The electrical current resulting from the ROS oxidation reaction is proportional to its concentration present in the sample. The use of fluorescent probes as sensors allows the study of the interaction between the species to be detected and the probe, resulting in measurable changes. The use of probes such as DCFH-DA (2′,7′-Dichlorofluorescein diacetate), react with ROS (such as hydrogen peroxide) present in the sample, resulting in the formation of 2′,7′-Dichlorofluorescein (DCF), which is highly fluorescent. Fluorescence intensity correlates with the concentration of hydrogen peroxide in the sample, indicating a measure of oxidative stress (Diegelmann and Evans, 2004; Dunnill et al., 2017; Wenk et al., 2001).

3.1.5. Gene expression

Gene expression is a recently studied biomarker, whose study is still under development, however it is expected to be a very viable option in the future and that can provide a lot of information. One study demonstrates a change in gene expression of certain bacterial genes. This change is fluctuating, since depending on the bacteria, its gene expression can increase or decrease. In this study, they realized that a certain bacterial virulence factor gene was more likely to be expressed in the presence of invasive infection (non-healing wounds) than in a wound with controlled colonization. On the other hand, several host maintenance genes, which are genes expressed to carry out common cell functions, were expressed more in healing wounds than in infected, non-healing wounds. In this way, gene expression analysis can be a valuable tool to establish the state of the wound (Asada et al., 2012; Butte et al., 2001; Lindley et al., 2016).

3.1.6. Matrix metalloproteinases/tissue inhibitors of metalloproteinases (MMPs/TIMPs)

MMPs are enzymes of the protease group and tissue inhibitors of matrix metalloproteinases are the regulators of these enzymes, inhibiting them in a ratio of 1:1 (inhibitor: enzyme), through the interaction of the N-terminal domain of TIMP molecule with the active site of MMP. This MMPs/TIMPs complex interferes with and regulates most phases of the wound healing process. However, MMPs are only favorable for the wound to heal correctly, at adequate levels, in the correct places and for an exact period. Therefore, chronically elevated levels of these enzymes, and reduced levels of TIMPs, or aberrations in their proportions, are

related to non-healing of CW. In this type of wound, the levels of TIMPs tend to be reduced, and these changes lead to an exaggerated increase in the levels of MMPs and, consequently, an inadequate deposition of matrix proteins (Gill and Parks, 2008; Ladwig et al., 2002; Ra and Parks, 2007; Wysocki et al., 1993; Yager et al., 2007).

3.1.7. Growth factors (GF)

GF are released by macrophages, as are the cytokines mentioned above. Among these GF are mainly platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). PDGF is one of the main growth factors involved in regulating cell migration and proliferation, stimulating the formation of new blood vessels (angiogenesis) and collagen synthesis. It plays a key role in the formation of granulation tissue, an essential step in healing that sets the stage for wound closure. EGF stimulates the proliferation and differentiation of cells, especially epithelial cells and keratinocytes, which are essential for the healing of the epidermis and promotes the migration of epithelial cells to the wound site, facilitating wound coverage and closure. In turn, VEGF also promotes the formation of new blood vessels and plays a role in the formation of granulation tissue, a structure rich in blood vessels that is essential for the healing process. The presence, production and release of GF are essential in mediating key cellular activities throughout the healing process, and therefore a deficiency in the concentrations of these factors causes impaired healing in CW. In this sense, CW fibroblasts themselves show poorer responses to GFs than acute wound fibroblasts (Bao et al., 2009; Cowin et al., 2001; Rumalla and Borah, 2001). Studies report that CW administered with GFs, after one week of treatment, show improved healing compared to CW without GF treatment (Blume et al., 2011).

3.1.8. Bacteria

The study of biofilms in CW has been increasingly studied and detailed. CW usually harbor a high polymicrobial load susceptible to the formation of biofilms, since these wounds are the ideal environment for this formation. Debris and necrotic tissue allow for better attachment of bacteria and due to compromised host immune system wounds are more susceptible to infection. Biofilms can have a varied composition depending on the etiology, location, extension and clinical setting of the wound. However, the most common bacterial species in CW are Staphylococcus (*S. aureus*), Pseudomonas (*P. aeruginosa*) and Corynebacterium. About 60% of CW samples showed bacterial biofilm, with only 6% of acute wounds containing biofilm. Bacteria secrete a variety of biochemical byproducts to attend to their physiological activities, which easily react with cellular metabolism to generate ROS that cause damage to host tissues (as is the case of pyocyanin produced by *P. aeruginosa*, which can be used as an indicator to reflect the severity of the infection). Bacterial infections in CW lead to poor wound healing, prolonging the inflammatory phase and delaying epithelialization. The persistence and proliferation of bacteria in wounds depend on several factors such as wound treatment, virulence, pathogen characteristics and the ability of the host's immune system to control or even eliminate the infection. Detection of bacteria in CW is an essential aspect of assessing wound health and implementing appropriate therapeutic strategies. Electrochemical and optical sensors offer distinct approaches for this purpose. Voltammetric techniques include electrodes functionalized with antibodies or aptamers specific to the surface of pathogenic bacteria. The connection between the target bacteria and the recognition sites on the electrode generates changes in the electrochemical characteristics. Bacteria-specific electrochemical biosensors use biological elements, such as enzymes or other cellular components, to generate specific electrochemical responses when in contact with bacteria. In relation to optical sensors, light scattering spectroscopy can be applied to analyze characteristic patterns of light scattering caused by the presence of bacteria in the wound, as well as the use of fluorimetric sensors (Robson, 1997; Robson et al., 1990; Zhao et al., 2013).

3.1.9. Enzymes

Enzymatic activity, specifically and mostly of proteolytic enzymes, which should decrease as fibroblasts deposit collagen, is substantially higher in CW than in AW. Some studies have shown that concentrations of human neutrophil elastase (HNE), MMPs, and cathepsin-G (Cat G), remain elevated in CW, and the concentrations of their inhibitors remain low. The activity of proteases is essential for the healing process and therefore the levels of these enzymes are more reliable to be used as biomarkers of poor wound healing. The failure between the production and activation of proteases and their inhibitors leads to a wound becoming chronic since the balance between degradation and deposition of ECM is disrupted. In this way, CW tend to remain in a state of persistent inflammation, characterized by exaggerated concentrations of proteases and neutrophils (cells that secrete proteases) and which increases the degradation of connective tissue. MMPs are considered a key element in tissue repair by ECM degradation and remodeling, and by their role in leukocyte influx, angiogenesis, and re-epithelialization. By degrading ECM components, MMPs contribute to the several ways of regenerating injured tissues such as elimination of damaged proteins, destruction of provisional matrix and migration of new cells to the wound area. However, these enzymes are only favorable for healing when their expression is controlled, if their expression is high and prolonged, it disrupts the balance between tissue degradation and repair, resulting in exaggerated degradation of the ECM associated with impaired healing. There are several cells that lead to the elevated proteolytic environment in CW, such as keratinocytes that inhabit the wound edge, fibroblasts and endothelial cells, neutrophils, and macrophages, which lead to the production of several classes of proteases. The high activity of proteases used as a detection tool can be considered the best biochemical marker available in the CW. Regarding enzymatic detection, optical sensors are mostly used for this purpose. Techniques such as Surface Plasmon Resonance (SPR) are used to detect biomolecular interactions, including the binding of enzymes to their substrates. Changes in the SPR spectrum indicate the presence and activity of the enzyme. This technique can be coupled to optical immunosensors, for example for the detection of the MMP enzyme using an SPR-based immunosensor. The surface of the SPR sensor is functionalized with molecules that can selectively bind to the target enzyme, in this case, antibodies (e.g. monoclonal or polyclonal antibodies) specific to the MMP to be detected. Antibodies present on the sensor surface selectively interact with MMPs in the sample. Binding of MMPs to antibodies results in a change in the surface plasmon resonance angle, which can be monitored in real time. Other types of molecules, such as aptamers and MIPs (Molecularly Imprinted Polymers), are often described. MIPs are synthetic polymers that are designed to mimic the three-dimensional structure of target molecules. Specific MIPs can be developed for MMPs (Martin and Nunan, 2015; McCarty and Percival, 2013; Snyder et al., 2011a; Yager et al., 1996).

3.1.10. pH

The concentration of hydrogen levels is a critical biochemical marker in the determination of healthy or pathological states. The pH of a wound with a normal healing is between 4 and 7, since it is a pH that favors tissue repair by decreasing abnormal collagen formation, increasing fibroblast activity, slowing MMP degradation rates and produce a severe environmental condition to reduce bacterial viability and on -site infection. However, CW exhibit a more basic pH between 7 and 8, which signals abnormal healing conditions and can suggest alkalosis or pathogenic infection. When this occurs, it may reflect two aspects. On the one hand, the alkaline pH of the CW cause's changes in enzymatic concentrations, for example in MMPs, which are essential in healing. The presence of MMP inhibitors can limit their overexpression, that is, the alkaline environment will break this balance, which leads to overexpression of MMPs and makes healing difficult. On the other hand, alkaline pH provides bacteria proliferation and expression of bacterial secretions toxicity. Several studies have reported that pH has a direct or

indirect influence on the biochemical processes, physiology, immunology and microbiology of the healing process (Martin and Nunan, 2015). Thus, it is assumed that low pH, as found under normal conditions, is more favorable for wound healing. Furthermore, it is only recently known that pH has been considered an explanation for many therapeutic strategies failing in the treatment of CW. In conclusion, pH is a valuable tool that can be used to assess whether wounds have healed. To detect the pH of a wound, pH electrodes are usually used. pH electrodes rely on a combination of electrochemical reactions and an ion-selective salt bridge to produce a measurable voltage potential between solutions. However, more sensors can be applied to this purpose, such as electrochemical biosensors that incorporate specific pH-sensitive enzymes. The activity of these enzymes can be correlated to local pH, or optical pH indicators, such as pH-sensitive dyes. pH-sensitive dyes, such as phenolphthalein or bromothymol blue, are substances that exhibit different colors or fluorescence depending on the pH of the environment in which they are found. The color change usually occurs due to protonation or deprotonation of the dye molecule in response to changes in hydrogen ion concentration (pH). This change in color can be detected by optical techniques (Greener et al., 2005; Jones et al., 2015; Kuo et al., 2020; Martin and Nunan, 2015; Schneider et al., 2007).

4. Detection methods for chronic wound biomarkers

The time between the occurrence of a wound and the start of its diagnosis and treatment is generally lengthy, and follow-up monitoring of wound healing after treatment remains a challenge. As has been mentioned, CW are considered an epidemic with an increasing rate of growth and alarming diagnosis, leading to huge expenses in the financial structure of the health economy. Due to the lack of specific guidelines that allow a rigorous diagnosis and treatment, the burden of this type of pathology is increasing (Darvishi et al., 2022; Dreifke et al., 2015). Currently, there are several approaches practiced by traditional and conventional clinics, as shown in Fig. 3 (scheme 1 and 2) (Darvishi et al., 2022; Sun et al., 2021). However, there are so many more modern, practical, fast, and up-to-date approaches, which are mostly just developed but not applied in the clinic on a large scale (Fig. 3 scheme - 3) (Darvishi et al., 2022).

Current measures on the management of CW are based on certain guidelines regarding visual and physical examination, the type and location of the wound, the patient's medical history as well as family and social background and in terms of medication. In relation to these traditional methods, that is, in relation to a qualitative assessment of CW, the visual and physical examination is very subjective and susceptible to the professional's experience. Some types of wounds are not visible to the naked eye, and others are not likely to be identified as CW in the early stages of formation (Frykberg and Banks, 2015; Morton and Phillips, 2016). Therefore, this has not been a very effective strategy, as this type of wound is the main cause of limb amputation (Derakhshandeh et al., 2018). This approach is based on regular analysis of the wound surface and mainly the skin around it, following its healing kinetics. However, this requires a lot of experience on the part of the professional that takes many years to acquire, and in addition these approaches are unable to provide information about the dead or infected tissues under the skin, which are the most predominant parameters for the stagnation of wound healing. Although there are some clinical symptoms related to the formation of pathogenic biofilms, such as yellowish wound exudate, pale wound bed, high tissue fluid, redness, burning, bacterial aggregates in wound biofilms are not distinguishable with the naked eye, as they are smaller than 100 μm in diameter (Darvishi et al., 2022; Pal et al., 2018).

Various conventional methods have been developed and employed to detect early CW, with a particular focus on identifying microorganisms at the wound site. The presence of microorganisms is a highly important biomarker in the diagnosis of CW, as it plays a crucial role in wound infection and biofilm formation (Fig. 3 scheme - 2). In clinical practice, microbiological assays, molecular assays, and imaging assays are commonly used for CW biofilm detection. Microbiological culture, a widely used method, aims to identify culturable and viable bacteria in the wound. However, this method has its limitations, including its inherent fallibility, lack of precision, and accuracy when it comes to confirming the existence of bacteria in the wound. One significant drawback is the fact that many bacteria do not form colonies under standard culture conditions. Moreover, when they do, it often occurs in

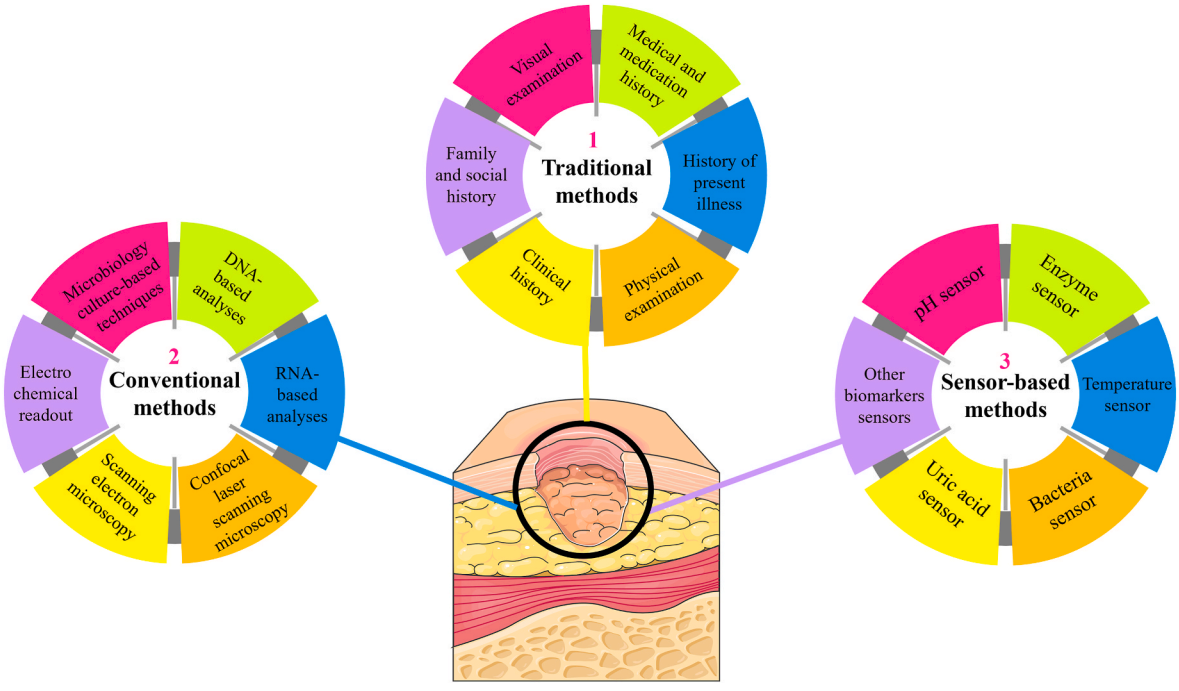

Fig. 3. Methods for detection of chronic wound biomarkers. This image is composed of three schematics separated into: 1. Traditional methods; 2. Conventional methods; 3. Methods based on sensors. All methods are based on the literature, essentially methods based on sensors that are recent, current, and under studied.

the advanced stages of CW, hindering early diagnosis. We can also add the fact that it is an unsuitable method for non-culturable bacteria, as it cannot identify non-culturable or viable but non-cultured bacteria, which are increasingly recognized as significant contributors to CW infection. Consequently, several studies based on this method have been disregarded due to insufficient data and inconclusive results. On the other hand, methods based on bacterial DNA and RNA are considered more accurate and offer the advantage of detecting both cultivable and non-culturable cells, as well as mixed species of bacteria, including aerobic and anaerobic varieties. Molecular sequencing methods, such as denaturing gradient gel electrophoresis (DGGE), ribosomal RNA-polymerase chain reaction (rRNA-PCR), and various ribosomal amplification techniques, have been implemented in this context. Nonetheless, these methods also have their limitations, such as the risk of sample contamination with environmental DNA, inability to provide information about cell viability, difficulty in distinguishing between biofilms and planktonic microbes, difficulty in distinguishing an active infection from colonization, the inability to detect certain mycobacteria, and the potential unavailability of sequencing results in existing databases. Despite these drawbacks, it's worth noting that these methods offer the advantages of rapid quantification, analysis, and detection of specific bacterial populations in CW. Various microscopy techniques are also employed for CW analysis, though they have certain limitations. Microscopy often produces false-negative results, primarily due to the irregular and scattered distribution of bacteria in the samples described in this work. Electrochemical bioimaging is another conventional method used to record CW biofilm surface activity. However, these imaging techniques frequently require specialized equipment and facilities, which may not be readily available in all clinical settings. While conventional methods have been valuable in diagnosing CW and detecting biofilms, each method has its limitations. In general, they are limited in providing comprehensive biomarker information because they generally focus on identifying bacterial pathogens, but CW involve multiple biomarkers, including assessment of host immune response, growth factors, cytokines, and others molecular indicators. These methods may not provide a holistic view of the wound microenvironment. These limitations underscore the need for continued research and innovation in the field to develop more accurate and accessible diagnostic techniques for CW (Darvishi et al., 2022; Davies et al., 2004; Fisher et al., 2017; Lawrence et al., 1991; Vyas and Wong, 2016).

Although the more traditional and conventional methods described above (Fig. 3 scheme - 1 and 2) have brought evolution and advances regarding the diagnosis of CW biomarkers, there is still great urgency in the development of detection methods, accurate and reproducible, and still easy to handle, fast and low cost. This is because the ineffective diagnosis of CW leads to a delay and worsening of the healing process, leading to greater complications. That said, it is imperative to find new methods of high efficiency and low cost and solve the problem of detection and recovery of CW using technological innovation (Barros Almeida et al., 2021; Sun et al., 2021).

Currently, several studies have been carried out in the area of sensors (Fig. 3 scheme - 3) as potential means of diagnosing CW biomarkers, and their provision of reliable and accurate information on the state of the wound. In a CW, its biological environment is constantly changing, this includes changes in the concentrations and levels of the biomarkers mentioned above. For example, only in CW or those with infection (since most infected wounds are CW), there is an increase in the concentration of certain enzymes, as well as with the pH, whose sudden increase is often indicative of bacterial infection, which increases the likelihood of a CW appearing. However, the biological environment of a wound is very complicated and constantly changing. Although the previously presented biomarkers are related to the non-healing process of CW, there are a large number of them that are only present in trace amounts, and for this reason they are difficult to detect. For this reason, it is increasingly important to develop sensor methods with low detection limits, high precision, easy handling, and low cost. In addition, the development of these detection sensors will lessen the discomfort for the patient in the traumatic process of removing the dressing for the clinical analysis of the state of the wound. In a futuristic and ideal perspective, these wearable sensors are thought to be included in dressings for monitoring biomarkers. There are already described in the literature numerous proofs of concept of these types of sensors recently published (Fuchs et al., 2023; Gao et al., 2021; He et al., 2021; Macovei et al., 2023; Pal et al., 2018; Pusta et al., 2022; Simoska et al., 2020; Simoska and Stevenson, 2022), however, none of them has been implemented in the clinic on a large scale due to some limitations that still exist. The sensors developed for the detection of biomarkers have been essentially represented by electrochemical sensors and optical sensors, this being the main focus of this review. The main advantages and disadvantages of each type of sensor are summarized in Table 3 (Ashraf et al., 2022;

Table 3
Comparison in terms of advantages and disadvantages of electrochemical and optical sensors for the detection of biomarkers of CW.

Type of sensor	Sensor	Advantages	Disadvantages
Electrochemical	Amperometric Voltammetric Impedimetric Potentiometric	✓ Low cost ✓ Large scale production ✓ Versatility ✓ High sensitivity (higher than optical) ✓ Good repeatability ✓ High reproducibility ✓ High accuracy ✓ Possibility of using different materials (nanomaterials or polymers) ✓ Portable and compact ✓ Miniaturization capacity (more easy than optical) ✓ Real-time measurement ✓ Online monitoring ✓ Multiparameter sensing	x External energy requirement x Short or limited shelf life x Cross-sensitivity x Incrustation of the electrode x Complex calibration process x Longer detection time x Difficulty in complex matrices
Optical	Colorimetric Fluorimetric UV/Vis Spectrometric	✓ Good resolution (higher than electrochemical) ✓ Good repeatability ✓ Possibility of estimating with the naked eye ✓ No need for specially designed probes ✓ Real-time measurement ✓ Faster detection ✓ Label-free detection ✓ Highly specificity ✓ Good sensitivity ✓ Miniaturization capacity ✓ Range of light dissemination configurations ✓ Multiparameter sensing ✓ Online monitoring ✓ Aptitude for remote sensing ✓ Ability to improve contrast for images ✓ Complex matrices	x Compatibility issues regarding the dyes used x Need for measuring and/or quantification devices x Low reproducibility x Depth of light penetration x Complex calibration process

Bandodkar et al., 2016; Khan et al., 2015; Nejadmansouri et al., 2021b; Pirzada and Altintas, 2020a; Sazonov and Daoud, 2021).

Electrochemical sensors have emerged as a promising tool for CW monitoring, as they offer some more optimized advantages. Numerous applications have been tested for electrochemical sensors in the most diverse areas, such as environmental (Du et al., 2021; Hart et al., 2007; Williams, 2020), industrial (Hart et al., 2007) and biomedical applications (Hart et al., 2007; Maduraiveeran et al., 2018; Ozoemena and Carrara, 2017). These sensors have advantages that do not necessarily mean that optical sensors do not, however, they may be more optimized in one type of sensors than in the others, for example, both have high sensitivity, however, if we compare the two types of sensors, the electrochemical sensors can almost always exhibit higher sensitivity and lower limit of detection (LOD) values. For example, the specific case of some biomarkers such as UA which is a proof of concept of an electrochemical wearable sensor based on differential pulse voltammetry where the detection limit was 3.11 μM, compared to a proof of concept of an optical fluorescent sensor based on fluorescence resonance energy transfer (FRET) technique where the detection limit recorded was 125 μM. The concentration of UA in healthy skin conditions is 52 μM and in fluids from CW it can vary between 220 and 700 M, the electrochemical sensor can more easily quantify UA levels in any of the situations mentioned above, and even in another type of sample, such as plasma or urine where the concentration is lower between 0.13 and 0.46 mM and between 1.49 and 4.46 mM, respectively (Hirt et al., 2019; Nawrot et al., 2018; Sun et al., 2021).

The same goes for using these sensors in a non-invasive and continuous way, providing real-time information about the wound environment. The great advantage and difference of electrochemical sensors in relation to optical ones is their cheap, easily reproducible manufacturing, with reduced production of waste and on a large scale, which justifies the greater use of these sensors for this purpose. Another big, and more recent, difference that may justify the high application of these sensors is their miniaturization capacity. Optical sensors are also likely to be miniaturized, however, the process is more easily performed with electrochemical sensors, making them lighter, portable, and compact sensors. The disadvantages to be overcome with this type of sensors persist, such as the lack of external energy sources like batteries, the short half-life, and a longer detection time (Bandodkar et al., 2016; Broekaert, 2015; Han and Ceilley, 2017; Macovei et al., 2023; Sharma et al., 2021). Recently, an article on "battery free" sensors was published (Xiong et al., 2021; Xu et al., 2021), which solved the problem of using batteries in sensors. However, proof of concept regarding this type of sensors is still limited, especially when the objective is biological and point-of-care application. Xu et al., (2021), described a battery-free sensor embedded in a wound dressing, with wireless, controlled with a smartphone that is used to wirelessly power the device, wireless data transmission, signal processing, and control wound biomarker detections (Fig. 4) (Xu et al., 2021).

Electrochemical sensors work by measuring and interpreting the electrical signals generated by the sensor after interacting with biomarkers present in the wound. For example, changes in the concentration of glucose, oxygen, and other key wound healing indicators can be measured using electrochemical sensors. This information can then be used to adjust the wound care regimen and monitor healing progress. In addition to measuring the wound environment, electrochemical sensors can also be used to detect the presence of harmful bacteria, such as MRSA, in the wound. This is particularly important in CW, as bacterial infections can significantly delay healing and increase the risk of amputation (Ashley et al., 2019; Ghoneim et al., 2019; Han and Ceilley, 2017). In Fig. 5, an example of an electrochemical sensor for the detection of biomarkers is schematically represented in relation to its operating principle, the answer to which can be given by the 4 types of sensors that we have discussed throughout this review.

Among electrochemical sensors, amperometric, potentiometric, voltammetric and impedimetric sensors stand out. Amperometric sensors measure the current generated by an electrochemical reaction and are used in a wide range of applications, including the detection of chemicals and biomolecules, such as UA, NO, and hydrogen peroxide. However, amperometric sensors also have some limitations. They require a small electrical current to operate, which can cause tissue damage if the sensor is not properly designed or positioned. Additionally, the sensors may be affected by the presence of other substances in the wound tissue, such as blood or other body fluids (Punter-Villagrasa et al., 2013). Potentiometric sensors are devices that measure the potential difference between two electrodes in a solution and are used in a wide range of applications, including the measurement of pH, ion concentration, and redox potential (Ding and Qin, 2020). Voltammetric sensors measure changes in the electrical current generated by the oxidation and reduction of certain chemical species in solution (Farghaly et al., 2014). They can be used to detect and quantify various chemical species, including biomolecules, in a sample. This can be used to monitor pH (Sharifuzzaman et al., 2020), UA (Jarošová et al., 2019; Simoska et al., 2020), pyocyanin (Jarošová et al., 2019), NO, ROS (Simoska et al., 2020), cytokines and *S. aureus* (Gao et al., 2021). Impedance sensors measure the electrical impedance of a sample and are used in a wide range of applications, which is the resistance of the tissue to the flow of electrical current. In the context of CW, impedance sensors have been studied as a tool for monitoring the healing process including the measurement of cell viability, the detection of bacteria and other

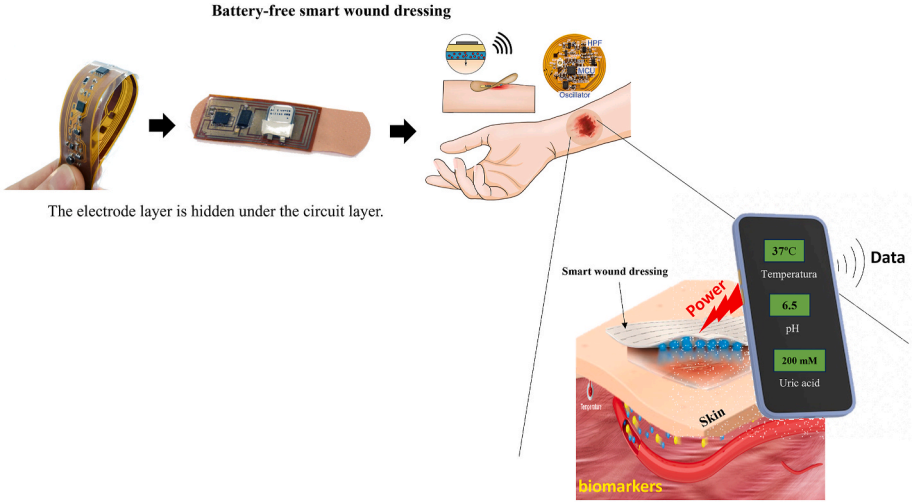

Fig. 4. Schematic representation of a battery-free sensor inserted into a wound dressing for the detection of biomarkers.

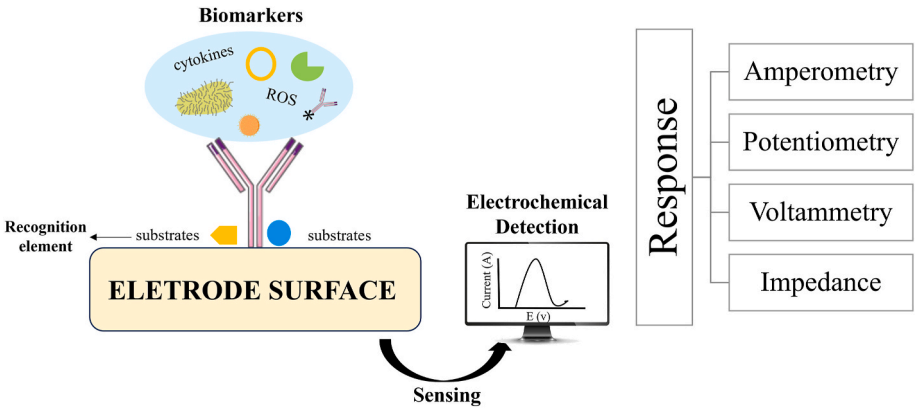

Fig. 5. Schematic representation of an electrochemical sensor for the detection of biomarkers.

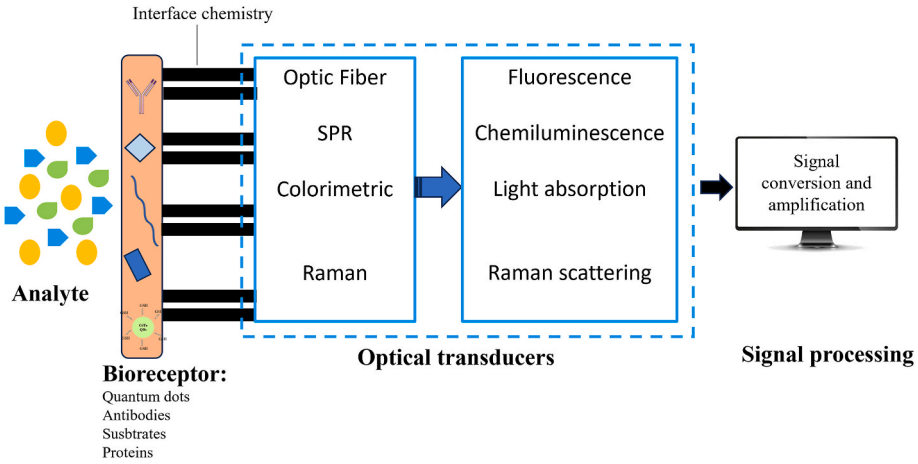

Fig. 6. Schematic representation of an optical sensor for the detection of biomarkers.

microorganisms, and the monitoring of wound healing (Kekonen et al., 2017; Manjakkal et al., 2018; Pal et al., 2018). The measurement of these species can provide important information about the state of the wound, including its progression and healing status. In addition to the electrochemical sensors mentioned above, there are also other types of sensors such as Quartz Crystal microbalance (QCM), Micro-electromechanical systems (MEMS). Currently, there are no proofs of concept for this type of sensors applied to wounds, however, it is a modern and promising concept, especially if coupled. The combination of both technologies can offer synergistic advantages. For example, a system that integrates a QCM for detection of specific biomarkers with MEMS devices for continuous monitoring of wound conditions could provide a comprehensive and sensitive approach to the assessment of CW (Akgönüllü et al., 2022).

Relative to optical sensors they are devices that are designed to detect and quantify optical changes that occur in the wound, taking advantage of the properties of light and the interaction of light with tissues and biological fluids present in the wound (Dargaville et al., 2013b). Like the electrochemical ones, they also allow a non-invasive and continuous monitoring of other parameters of wound healing such as temperature, oxygen saturation, and blood flow (Salvo et al., 2017). This information can be used to assess the overall health of the wound, detect any potential infections, and determine the best course of treatment. Furthermore, this type of sensors are also often used to evaluate the effectiveness of different treatment options. They can provide real-time information on the healing process and help detect potential problems early on. These sensors have the advantage of fast detection and a longer half-life compared to electrochemical sensors. By using optical sensors, healthcare providers can make informed decisions about the best course of action for their patients and improve the chances of successful wound healing (Nejadmansouri et al., 2021b; Pirzada and Altintas, 2020a; Tran et al., 2022). In Fig. 6, an example of an optical sensor for the detection of biomarkers is schematically represented by its operating principle, the answer which can be given by the different types of optical techniques covered in this review.

Colorimetric sensors have shown potential in CW monitoring. These sensors use chemical reactions to detect changes in wound pH, temperature, and oxygen levels, and can produce a color change that is visible to the naked eye. This makes them useful for patients to monitor their own wounds and for healthcare providers to quickly assess wound healing progress. However, further research is needed to optimize the design and performance of colorimetric sensors for use in CW management. One advantage of colorimetric sensors is that they can be easily integrated into wound dressings, making them easy to use. They can also provide real-time feedback on wound conditions, allowing for early detection of potential complications such as infection. However, there are also some limitations to consider. Colorimetric sensors may not be suitable for all types of wounds, and there is a risk of false positives or false negatives depending on the specific wound conditions and the design of the sensor. In addition, further research is needed to optimize the performance and reliability of colorimetric sensors for use in CW management (Nejadmansouri et al., 2021b; Pusta et al., 2022; Salvo et al., 2017; Sharma et al., 2021). Fluorescent sensors are another type of sensing technology that has shown potential for monitoring CW. These sensors use light-emitting molecules to detect changes in wound conditions. They emit light of a different wavelength in response to changes

in the environment, providing a highly sensitive and quantitative method of wound monitoring. Within the optical sensors, these are among the most sensitive, meaning they can detect small changes in wound conditions with high accuracy. Like the previous ones, they also allow real-time, continuous monitoring of wound healing, which can be useful for detecting early signs of infection or other complications. A special highlight of this type of sensor over others is its ability to be combined with other sensing technologies, to provide complementary information on wound healing. For example, the signal amplification through integration with other sensing technologies to improve detection limits FRET can be employed to enhance the sensitivity of fluorescence-based sensors. These sensors that is excellent spatial and temporal resolution, which can be advantageous when combined with other sensor modalities. As with all sensors, these also have some limitations such as the technology is still relatively new and may require further optimization for use in clinical settings. Additionally, the cost and complexity of the equipment required for fluorescent sensing may limit its accessibility for some healthcare providers (Cheng et al., 2018; Pusta et al., 2022; Salvo et al., 2017). UV/Vis spectrometric sensors are a type of sensing technology that can be used for monitoring CW. These sensors use light to detect changes in the chemical composition of wound tissue, providing information on wound healing progress, inflammation, and infection. They can provide quantitative information on the chemical composition of wound tissue and can be used to detect subtle changes in the tissue that may not be visible to the naked eye. Furthermore, they can also be relatively quick to perform, making them a convenient and practical method for wound monitoring. Relative to the limitations, the equipment required for spectroscopy sensing can be expensive and may require specialized training to operate. The sensors may also be sensitive to external factors, such as the presence of water or other substances, which can interfere with the accuracy of the measurements. There are several types of optical sensors based on absorption, fluorescence, Raman spectroscopy, SERS (Surface-Enhanced Raman Spectroscopy), infrared, SPR (Surface Plasmon Resonance)-LSPR (Localized Surface Plasmon Resonance), nuclear magnetic resonance (NMR) and X-ray (Dargaville et al., 2013b; Tran et al., 2022). Some techniques mentioned above, such as SERS and SPR, have had continuous development over the last few decades, with advances in nanotechnology, materials, and experimental techniques. Although sensors based on these types of techniques are not the focus of this review, it is important that a brief review of the area is made as several applications of this type have been made in the detection of wound biomarkers. However, mainly areas such as environmental (pollutant detection (Tang et al., 2021)) and biomedicals (respiratory diseases (Sun et al., 2024; Wu et al., 2014), neurodegenerative diseases (Eremina et al., 2024; Jiang et al., 2021), also in wounds (Perumal et al., 2021; Tanaka et al., 2021)) have been reported to use the SERS technique. Some proof-of-concept SERS sensors for biomarker detection have been described, but only for a limited number of biomarkers such as MMP, cytokines, and bacterial metabolites. (Perumal et al., 2021), developed a SERS sensor for the detection of wound biomarkers (TNF-α, IL1-α, IL1-β, MMP-2 and MMP-9), as this technique exploits Raman scattering by absorbed molecules in a physical or chemical form on a substrate. This technique is highly sensitive, specific, and targeted for chemical and biomolecular detection, as it leads to the production of unique vibrational spectra ("fingerprints") for individual molecular species. The sensitivity of this sensor has been demonstrated at concentrations of 10–5000 ng/mL for MMP-9 concentrations, and a range of 5–100 ng/mL for TNF-α concentrations. The levels of TNF-α reported for healing wounds are approximately 1 ng/mL and in CW these levels reach 15 ng/mL, that is, the sensor was able to detect concentrations of CW more easily. Still in the area of wounds, another SERS sensor was reported for the detection of pyocyanin, where different substrates were tested to understand which was most compatible with the desired detection. AgNP and AgAuNP sensors (detection limit of 1.1 μM (in a linear range of 0.1–25 μM; 10.9 μM (in a linear range of 5–100 μM, respectively) together cover the sensitivity requirements and dynamic range for the clinical detection of wound infection, where pyocyanin is present at a concentration of 1–50 μM (Tanaka et al., 2021). SPR sensors have also shown significant developments in recent decades, due to their ability to respond to various criteria when developing a sensor. This is an optical detection technique where the target molecule is detected through the change in the refractive index (RI) that occurs near the detection layer because of the presence of a new substance, that is, analyte molecules. Few wound-related applications exist using this type of sensors, but a proof of concept on the development of an SPR fiber optic system for the detection of biologically relevant levels of IL-1, IL-6 and TNF-α has been reported (Battaglia et al., 2005b). Fiber-optic SPR sensors are coated with an antibody binding layer and antibodies specific to the cytokine of interest are covalently attached to this layer. It was revealed that the developed sensor was not able to obtain detection limits low enough for the detection of TNF-α but was capable of detecting other cytokines. The main obstacle of these sensors is, currently, inhibition by the nonspecific binding (NSB) signal. For example, in blood, plasma, serum or fluid samples, which are the most promising applications in the field of biomarkers, has not yet been achieved due to the NSB of blood borne proteins on the sensor surface. Currently, the only SPR-based measurements in blood samples are whole blood and blood plasma coagulation (Janith et al., 2023).

Table S4 will review most of the optical and electrochemical sensors mentioned above described in the literature for biomarkers of CW. Several papers have been published but on an overview of monitoring just 2 or 3 CW biomarkers (e.g. pH and temperature or UA and pH) by wearable sensors through different detection mechanisms (Sani et al., 2021; Tang et al., 2021). Additionally, there are published reviews on wound biomarkers, as well as their detection focused on different types of sensors (Pusta et al., 2022; Sun et al., 2021). However only an overview of the main sensors described for wound infection biomarkers are being considered, and only a few examples shown in relation to electrochemical sensors, and fluorimetrics. The main insight of the current review is going further to the specific targeting of CW biomarkers and not infection biomarkers in general, as well as a compilation of all optical and electrochemical sensors described for each type of biomarker, (Pusta et al., 2022), is carried out. More information is, also given about each referred sensor, and a comparison is made in terms of sensitivity, linear response range and their advantages.

Considering Table S4, which contains a review of most of electrochemical and optical sensors described in the literature and applied in the detection of biomarkers of CW, can be observed that there are a total of 144 sensors described between electrochemical and optical. Among these, 23 described for UA detection (20 electrochemical and 3 optical), 13 described for NO detection (10 electrochemical and 3 optical), 20 described for cytokine detection (9 electrochemical and 13 optical), 17 reported for ROS detection (8 electrochemical and 11 optical), 24 cited for bacteria detection (12 electrochemical and 12 optical), 13 cited for enzyme detection (7 electrochemical and 6 optical) and, finally, 29 described for pH (19 electrochemical and 10 optical). For most of the mentioned biomarkers (except for cytokines and ROS), there are more electrochemical sensors studied than optical sensors. This can be justified by the fact that electrochemical sensors can detect very low concentrations of biomarkers, even at parts per billion (ppb) levels, presenting in most cases lower LOD values. This is because electrochemical signals are based on electron transfer reactions, which can produce a strong signal. Another justification is that its production is designed to be highly selective for a specific biomarker using specific recognition elements such as antibodies, aptamers, or enzymes. This ensures that the sensor only responds to the target biomarker and not other interfering substances. In addition, the production of these sensors is cheaper, easier and on a large scale when compared to optical ones (Pusta et al., 2021; Simoska et al., 2020; Sun et al., 2021).

In the case of ROS and cytokines, the report of a greater number of optical sensors can be justified by the multiplexing capacity that allows

multiple detection of cytokines and ROS, simultaneously (within each biomarker). Different optical sensors (especially fluorescent) can be used, each designed to interact specifically with a different cytokine or ROS, since by being designed for different emission spectra, several species can be measured in a single assay, providing comprehensive and efficient profiling of these species. Electrochemical sensors, in contrast, may require different sensors for different ROS and cytokines. Another justification may be the selectivity of optical sensors that can be designed to exhibit high selectivity for specific cytokines and ROS. By using specific recognition elements with fluorescent probes, optical sensors can minimize interference from other components present in the wound environment, ensuring accurate and reliable cytokine measurements. Additionally, these sensors specifically have good imaging capabilities. Optical sensors, coupled with fluorescence microscopy or imaging systems, enable spatial mapping and visualization of cytokine and ROS distribution within the wound bed. This imaging capability provides insights into the localization and heterogeneity of cytokine expression, contributing to a better understanding of the wound healing process and the development of targeted treatment strategies. Lastly, these sensors are more stable and have a longer lifetime than electrochemical sensors, which can be affected by factors such as pH and temperature changes in the wound environment (Cooper et al., 1994; Gao et al., 2021; Hajnsek et al., 2013; Li et al. 2017, 2020; Lu et al., 2022; Xie et al., 2021).

In Table S4 there is a column (LOD) which contains the detection limit ranges, of the referenced articles, for each method. This range contains the lowest LOD (minimum) and highest LOD (maximum) fixed within all articles referenced for this method. That is, between this range of LODs (minimum and maximum) are the other detection limits of the remaining referenced articles. Considering then the LOD values presented, a discussion will be made comparing the different types of sensors and biomarkers with each other. Although a low limit of detection may indicate that the sensor is capable of detecting very low concentrations of an analyte, it does not necessarily mean that the sensor is highly sensitive. The sensitivity of a sensor refers to the response it produces to a change in analyte concentration. Therefore, a sensor with high sensitivity would be able to detect a small change in analyte concentration. A sensor with a very low LOD may not necessarily have a linear or accurate response over a wider range of concentrations. In addition, other factors such as sensor selectivity and background noise can influence the sensor's ability to detect specific analyte concentrations. So, while a low LOD is an important indicator of a good sensor, it is not the only consideration when evaluating sensitivity and overall sensor quality. A low detection limit can improve the accuracy and precision of measurements, reduce false negatives, and enable earlier detection of a problem (Horwitz, 2020). The comparison that will be made below concerns exclusively the electrochemical and optical sensors described in Table S4 for each biomarker and is not a comparison at a more generalized level of sensors.

Regarding the UA biomarker, it is important to highlight that the impedimetric method has the lowest LOD (0.20–9.91 μM). Although voltammetry and amperometry also show the same minimum LOD, and the maximum LOD is considerably higher (50 and 100 μM) than impedimetric methods (9.91 μM). Both the impedimetric sensor and the voltammetric sensor have been applied to wound samples, and are non-invasive, stable under physiological conditions, and allow continuous measurement (Bhushan et al., 2019; Ping et al., 2012; RoyChoudhury et al., 2018). The big difference is that the voltammetric sensor allows real-time measurement at the point-of-care. (Bhushan et al., 2019), reported a bienzymatic voltammetric sensor for monitoring UA in biofluids extracted from wounds, wound skin, and healthy skin. The biosensor is composed of uricase (UOx) as a biocatalyst for UA oxidation, horseradish peroxidase (HRP) for electron transfer and a nanocomposite of multi-walled carbon nanotubes (MWCNTs) and Au nanoparticles (AuNPs) as substrate. Using voltammetry, an effective response was already expected, but the bienzymatic approach provided a twofold increase in current response, while the nanocomposite facilitated increased enzyme loading and rapid electron transfer, allowing a 2-fold increase in current response. The biosensor was able to measure UA levels in human wound exudate and in biofluids extracted from wound and healthy skin. The biosensor exhibited a lowest detectable concentration of 9.91 μM. UA levels in wound exudate and biofluid extract from wound skin samples were 3.7 and 1.2 times higher than in healthy skin. It is also possible to compare them in terms of the linear response range, which in the impedimetric method (0.8–2500 μM) (Ping et al., 2012) has a lower quantification limit than in the voltammetric method (50–650 μM) (Bhushan et al., 2019) referred to above. In the context of sensors, the linear response range is the range of input values over which the sensor produces a response proportional to the quantity or physical property being measured. Outside this range, sensor response may no longer be linear and saturation, distortion, or other unwanted effects may occur. It is important to know the linear response range of a device as it indicates the range in which the response is most reliable and accurate. Outside this range, it may be necessary to apply calibration or compensation techniques to obtain accurate results. Therefore, impedimetric methods present a wider linear response range, and in addition allow quantification to begin from lower concentrations. This is, without a doubt, an added value of the sensor as it allows the detection of any type of wound, considering the concentration of the analyte, whereas in the acute wounds the concentration is lower (52 μM) and in fluids from CW (can vary between 220 and 700 μM). Furthermore, it is also capable of quantifying in other types of samples, such as plasma or urine, where the concentration is lower between 0.13 and 0.46 mM and between 1.49 and 4.46 mM, respectively. When comparing electrochemical and optical methods in terms of LOD, after impedimetric, UV/Vis spectrometry is the method that presents the lowest LOD. According to the article described for the spectrometric sensor (RoyChoudhury et al., 2018), this sensor has an advantage compared to electrochemical methods, in terms of its specificity. As these sensors were developed to be applied to very complex matrices with several analytes, such as wound fluids, the high specificity of spectrometry sensors allow the identification and quantification of specific analytes in a sample such as UA (RoyChoudhury et al., 2018).

Concerning NO, we can see that both electrochemical and optical methods have an equally low LOD (0.0005 μM). The amperometric methods present in their range of LODs very low values, being the maximum LOD 0.5 μM. Equivalent to this are the UV/Vis spectrometric methods, since they are, after the amperometric ones, the ones that present the lowest maximum LOD (5 μM) (Hunter et al., 2013a; Xu et al., 2014; Xu and Ceylan Koydemir, 2022). NO has a very short half-life (6–50 s) and its diffusion length is very low (PintoR et al., 2020). This means that it is preferable to measure NO directly at the point-of-care in order to obtain more realistic concentration values and better understand its physiological role. In this sense, this can be an obstacle for the use of sensors, since, for example, with electrochemical sensors, despite their low LOD, the measured NO is critically low in oxygenated and complex media, giving transient signals, which prevents its quantification in the long term. This issue leads to a frequent need for sensor calibration (eg. after analyzing 1–2 samples) and performance testing to maintain data integrity (Snyder et al., 2011b). It is at this point that UV/Vis spectrometric sensors are advantageous for measuring this analyte, as they allow its quantification in very complex matrices with very varied concentrations of other interfering biomolecules. Hassan et al., reported a compact lab-on-chip optical detection sensor for real-time in vivo detection of NO. They demonstrated for the first time in 2022 that photonic microring resonators (MRRs) can provide real-time, direct, in vivo detection of NO in a mouse wound model. This encodes the NO concentration transfer function information in the form of a resonance wavelength shift. We show that these functionalized MRRs, fabricated using complementary metal oxide semiconductor (CMOS) compatible processes, can achieve sensitive detection of NO (sub-μM) with excellent specificity and without apparent performance degradation for over 24 h

of operation in biological media. With different functionalizations, these platforms can allow the in vivo detection of a multitude of biomarkers (Hassan et al., 2022). With these sensors, continuous and specific monitoring is still possible, with a very short response time of between 1 and 2 s (Hassan et al., 2022; Hunter et al., 2013b). Therefore, in the case of NO detection, despite the amperometry presenting a lower LOD, the UV/Vis spectrometry sensors represent a better option considering the disadvantage-benefit trade-off.

As for cytokines, it is difficult to group the methods by their LOD values, since they are quite dispersed, and alternate in large intervals between LOD minimum and LOD maximum. All the sensors described in the articles cited in Table S4 were applied directly to wound samples in real time, are non-invasive and performed at the point-of-care, showing no significant differences in terms of the characteristics of the sensors themselves (Battaglia et al., 2005a; Gao et al., 2021; Perumal et al., 2021). Regarding the LODs, differences can be verified since it is in the optical methods, UV/Vis spectrometry, that the sensor with the lowest LOD (0.047 ng/mL) is found. The LODs of the electrochemical sensors show higher values (0.1 and 0.25 ng/mL). The cited articles point out that the only specific advantage of electrochemical sensors compared to optical sensors is the miniaturization capacity, because it is a characteristic that is more easily executed in electrochemical sensors than in optical ones. This feature allows electrochemical sensors to be easily transported and used in point-of-care locations (Gao et al., 2021). However, it is important to mention that optical sensors have other characteristics that can make them the preferred choice in many cases. (Beidler et al., 2009), developed an optical fluorescent sensor, based on specific fluorescent antibodies for the detection of several cytokines (TGFβ1; IL-1α, IL-1β, IFN-γ, IL-12p40 and GM-CS). A multiplexed protein assay was used to measure multiple cytokines in a single sample. Levels of these cytokines were determined in untreated CVI ulcer tissue before and after 4 weeks of high-force compression therapy. Most pro-inflammatory cytokine protein levels were elevated in ulcer tissue compared with healthy tissue, and compression therapy significantly reduced these cytokines. The sensors described allow the simultaneous detection of several cytokines, providing a multiplexed analysis with a single measurement (Battaglia et al., 2005a), in addition, these sensors can allow the unlabeled detection of cytokines, avoiding the need for complex labeling steps (unlike the electrochemical sensors that still need tagging (Gao et al., 2021)).

About reactive oxygen species, the situation is the same as for cytokines, that is, it is in optical methods that the method (UV/Vis spectrometry) with the lowest LOD (0.015 μM) is found, compared to electrochemical methods. Regarding the linear response range, it is also possible to verify that the widest range is also found in optical sensors (5–5000 μM) (Safaee et al., 2021), while in electrochemical ones the ranges are 1–25 μM (Hajnsek et al., 2015) and 0.5–500 mM (Li et al., 2017). That said, optical sensors allow the detection and quantification of a wider range of ROS concentrations, since it is a group that includes different types of species with different concentrations, and therefore it is advantageous to have a sensor that is comprehensive of all these concentrations. Also, for this reason, it is advantageous that the sensor used for the detection of ROS allows multiplexing, that is, measurement of different species of ROS without the others interfering, which happens in the articles described. Since cytokines and ROS are the exceptions to biomarkers that have more optical than electrochemical methods described, it makes sense that the lowest LOD would be found in optical methods as they are the most studied and developed (Hajnsek et al., 2013; Li et al., 2017; Ye et al., 2019).

For bacteria, bacterial units are often expressed in CFU/ml, which stands for "colony forming units per milliliter". This is because bacteria cannot be counted individually and need to cluster in colonies to be seen and counted. Colonies are formed from a single bacterial cell that divides several times and forms a visible colony. Counting the number of colonies on a bacterial culture plate and expressing this count in CFU/ml is a way to estimate the density of bacteria in a liquid or solid sample. When detecting bacteria, it is important to take into account some characteristics related to them, such as:

- Target Species and Strains: The sensor must be able to detect the relevant bacterial species or strains for the analysis.
- Bacterial Load: CW can have different levels of bacterial load, ranging from low to high. The sensor must be sensitive enough to detect bacteria even at low concentrations in order to properly assess the bacterial load in the wound.
- Bacterial Diversity: CW can harbor diverse bacterial species or polymicrobial infections. The sensor must be capable of detecting multiple bacterial strains or species simultaneously, providing a comprehensive assessment of the bacterial composition in the wound.
- Biofilm Formation: Bacterial biofilms are commonly found in CW and may contribute to treatment resistance. The sensor must be capable of detecting bacteria within biofilms or have specific biofilm detection capabilities.
- Virulence Factors: Some bacteria produce virulence factors that contribute to wound chronicity and severity. The sensor may need to detect or assess the presence of these virulence factors to provide valuable information for wound care (Sheybani and Shukla, 2017).

Since the previously mentioned characteristics would be the ideal characteristics for choosing a detection sensor, it is almost impossible for a sensor to fulfill all of them. In the articles described, it is the electrochemical, impedimetric sensors that present the lowest LOD (3.0×10^{-6} – 2.2×10^{3} CFU/mL), followed by the voltammetric ones (10–100 CFU/mL). Despite this, not even these sensors meet all the "essential" requirements for detecting bacteria. The described impedimetry articles were developed for the detection of 3 and 4 bacteria, respectively. Since in a CW there are many more strains, they will not be able to detect any existing bacteria (Hannah et al., 2021; Sheybani and Shukla, 2017). However, in relation to voltammetric sensors, which are the ones with the lowest LODs, they were developed to detect only one bacterium (*Pseudomonas aeruginosa*), and for this reason the sensitivity of these sensors is greater since it is easier to detect one bacteria than multiple. The bacteria for which the sensors were not designed work as interferences. In addition, a miniaturization of these sensors has already been tested (Burkitt and Sharp, 2017; Sismaet et al., 2016; Webster et al., 2014). Optical methods present, in this case, much higher LODs, both colorimetric, fluorometric and also UV/Vis spectrometry, 2.5×10^{5} CFU/mL, 2.8×10^{2} - 1×10^{8} CFU/mL, 1×10^{4} – 2.5×10^{5} CFU/mL, respectively. All sensors mentioned here were developed to also detect only one specific bacteria (Fig. 7).

The great advantage of optical sensors is their ability to improve contrast for images, making them valuable tools for visualizing bacteria in CW. (Raizman et al., 2021), described a fluorescent sensor that allows obtaining images of the bacteria *Pseudomonas aeruginosa* in the wound. Point-of-care bacterial fluorescence imaging illuminates wounds with safe, violet light, triggering the production of cyan fluorescence from *P. aeruginosa*. A prospective single blind clinical study was conducted to determine the positive predictive value (PPV) of cyan fluorescence for the detection of *P. aeruginosa* in wounds. Bacterial fluorescence using the MolecuLight *i:X* imaging device revealed cyan fluorescence signal in 28 CW, including venous leg ulcers, surgical wounds, diabetic foot ulcers and other wound types. To correlate the cyan signal to the presence of *P. aeruginosa*, wound regions positive for cyan fluorescence were sampled via curettage. A semi quantitative culture analysis of curettage samples confirmed the presence of *P. aeruginosa* in 26/28 wounds. These findings suggest that cyan detected on fluorescence images can be used to reliably predict bacteria, specifically *P. aeruginosa* at the point-of-care. By leveraging these contrast-enhancing mechanisms, optical sensors can provide clearer and more distinct images of bacteria in CW. This improved contrast aids in the accurate identification, localization, and characterization of bacterial colonies, ultimately assisting

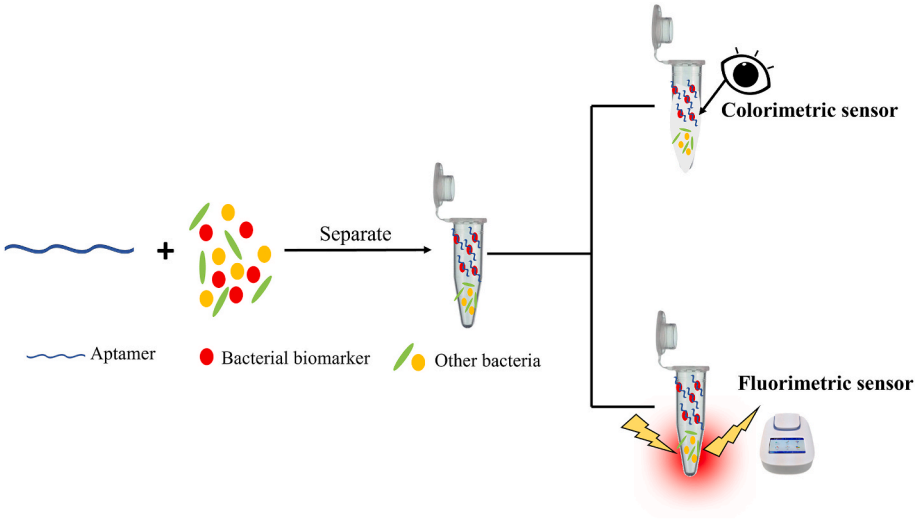

Fig. 7. Schematic representation of an optical sensor developed for the detection of bacteria.

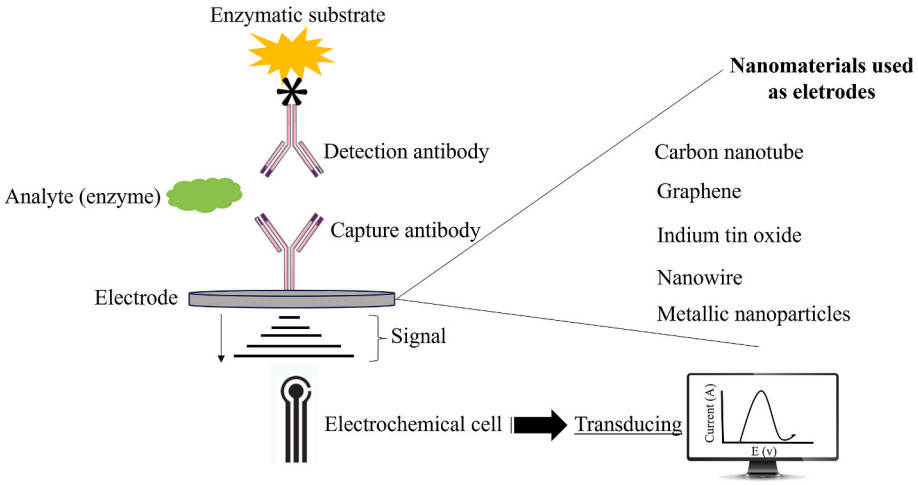

Fig. 8. Schematic representation of an electrochemical sensor developed for the detection of enzyme.

healthcare professionals in diagnosing and managing bacterial infections in wound care (Currie et al., 2020; Raizman et al., 2021; Ye et al., 2023).

Regarding enzymatic biomarkers, are the voltametric (7×10^{-6}μM) and impedimetric (0.001μM) methods that allow the detection of lower concentrations of enzyme levels, although there are few references described for both. The impedimetric sensors cited in Table S4 offer high sensitivity and selectivity, as they have been functionalized on the surface of the electrode with an enzyme-specific recognition antigen, which allows it to detect biologically relevant concentrations. (Ciani et al., 2012), demonstrated a method for immunodetection through label-free electrochemical impedance for the detection and quantification, in simulated wound fluids, of MMP-9, an enzymatic biomarker of CW. Detection is performed with gold screen-printed electrodes modified with a thiolate antibody specific to MMP-9. Detection was performed without the need for prior treatment or sample preparation, in less than 1 h directly from the simulated wound fluid. The 1.1 nM detection limits for MMP-9 are close to or below the threshold necessary to dictate the presence or absence of CW. This electrochemical immunosensor demonstrated the sensitivity required for rapid detection of CW directly from a clinically relevant sample (Fig. 8) (Ciani et al., 2012).

While electrochemical sensors can provide high sensitivity by amplifying and measuring the electrochemical signals associated with the enzymatic process, optical sensors can provide equally high sensitivity by using specific dyes or markers that generate strong signals in the enzymatic reaction. This is what we can observe in the fluorimetric sensors, which also have a very low minimum LOD (0.01 μM). All sensors have their limitations, in the case of electrochemical applied to enzymes, a major problem is the limitation of enzymatic compatibility, that is, these sensors are not compatible with all existing enzymes in CW. Some enzymes may require specific redox mediators or cofactors that are not easily incorporated into the electrochemical detection setup, limiting the applicability of electrochemical sensors to certain enzymes. In addition, another limitation mentioned in some articles is the incrustation of the electrode. Electrochemical sensors are prone to electrode fouling, where the electrode surface becomes coated or blocked by biomolecules or reaction by-products. This fouling can affect the sensitivity, stability, and accuracy of the sensor over time, requiring regular maintenance or electrode replacement. In the case of optical sensors, signal attenuation can be a limitation. The depth of light penetration into tissues may be limited, especially in CW with deep layers of tissue. This limitation can reduce the signal strength or distort it, making it difficult to accurately detect and quantify enzyme activity or concentration. (Ciani et al., 2012; Hasmann et al. 2011, 2013; Lee

et al., 2017; Windmiller et al., 2010).

Regarding the pH, the most important thing will not be to understand which of the sensors can detect the lowest pH, since the objective is that the sensor is calibrated to detect the specific pH of CW. Thus, it is important to note that an alkaline pH (pH 7.0–9.0) has been observed as a predictive indicator of infection and is present in CW, while a slightly acidic pH (pH 3.0–7.0) presents faster healing processes and has a greater chance of healing. Taking this information into account, it is important to know which of the sensors described will be more able to specifically detect the pH of a CW. Based on this, we can conclude that in the different methods, both electrochemical and optical there are sensors capable and developed for this detection. We can verify this through sensors that have a pH range more directed towards alkaline pH, such as the potentiometric sensor referred to in (McLister et al., 2016) (pH 6.0–9.0) and the UV/Vis spectrometic sensors referred to in (Kekonen et al., 2019; Van der Schueren and Clerck, 2010) (pH 5.2–12 and pH 5.0–9.0, respectively). On the other hand, and from another perspective, the wider the pH range that a given sensor allows to detect, the wider the different types of wounds to which it can be applied. Once again, some limitations are associated with optical and electrochemical sensors when determining the pH of a given wound. In optical sensors, the biggest problem mentioned in the cited articles is the influence of luminosity and the need for frequent calibration. The presence of ambient light can affect optical measurements, requiring a controlled environment or compensation techniques to reduce interference. Regarding calibration, optical sensors may require frequent calibration to ensure the accuracy of pH measurements. On the other hand, there are advantages in using these sensors instead of electrochemical ones, such as the possibility of selecting indicators, and the possibility of obtaining images. The sensors described in the articles include specific indicators for the pH range of interest in the wound. There is a wide variety of optical indicators available, allowing to choose specific indicators for the wound pH and range of interest. Additionally, some optical sensors allow obtaining spatial images of the pH, providing information on the pH distribution in the wound. The same happens in relation to electrochemical sensors, where the biggest limitations described are the need to use electrodes, which need to be properly calibrated and maintained to guarantee the accuracy of the measurements. And yet, the possible interference of electrolytes that interfere with the measurements. However, these sensors have a longer service life compared to optical sensors, and have a lower cost both in terms of the sensor itself and the equipment needed for the measurements (Guinovart et al., 2014; Kassal et al., 2015; Liu et al., 2017; Louisa et al., 2017; Panzarasa et al., 2017; Phair et al., 2011; Qin et al., 2019; Rahimi et al., 2017; Van der Schueren and De Clerck, 2012; Yang and Choy, 2021).

In general, in most biomarkers, impedimetric methods are predominant, regarding lower LODs (UA, bacteria, enzymes), followed by amperometric methods (NO, ROS). As for optical methods, UV/Vis spectrometric methods are predominant, after electrochemical ones, in most biomarkers (UA, NO, cytokines, ROS). Therefore, although the number of optical sensors described in the area is lower than the number of electrochemical sensors, these have become increasingly important for the detection of biomarkers mainly due to their particular characteristics that cannot be found in electrochemical sensors and therefore make them unique. Characteristics such as the ability to obtain images with high resolution and high contrast. Also, an important feature of these sensors is their ability to detect biomarkers in complex biological samples, such as blood, WF and urine, without the need for complex sample preparation. These can also detect multiple biomarkers simultaneously, which is essential for the diagnosis of complex diseases where multiple biomarkers are involved, as is the case with CW. Although the miniaturization of electrochemical sensors is more studied and implemented due to its greater ease in the process, this does not mean that this is a feature that does not cover optical sensors. These sensors can also be miniaturized, despite the process being more complex as mentioned above, however, once achieved, it is possible to maximize the individual characteristics of these sensors, through integration into portable devices, making them ideal for point-of-care diagnostics. They can be easily adapted for monitoring and remote sensing, which is particularly relevant for chronic disease monitoring. In the context of CW, there is still a lot of work to be done with regard to optical sensors, however, as technology continues to improve, it is likely that these will become even more versatile, allowing diagnoses and treatments of diseases faster and more accurate (Nejadmansouri et al., 2021a; Pirzada and Altintas, 2020b; Sun et al., 2021).

The trend is the increase and development of sensors aimed at wounds, this being the next generation of devices to be used in the future of diagnosis and faster and more accurate monitoring.

5. Conclusion and future perspectives

CW is a major health problem, affecting millions of people around the world and can lead to serious complications and low quality of life for patients. In this paper, we review the specific application of sensors based on optical and electrochemical methods in the detection of CW biomarkers. It is easy to identify that there is greater progress in the development and research for the detection of the main wound biomarkers, such as pH and temperature. This can be proven by the current commercialization of some sensors of this type. In 2022, at least 3 different types of sensors, Biohealth, VeCare and Smartheal, were commercialized, and in practice, they are capable of accurately identifying these parameters, as is the case with (George et al., 2023; Yang et al., 2023b). However, these devices, despite being currently marketed and tested in the clinic, are still not routinely used there, due to their production cost and large-scale use. In a futuristic and ideal vision, the objective is for them to be quickly implemented on a routine basis, so that increasingly better results are achieved, playing a crucial role in the future of personalized medicine. For these wearable sensors to be successfully implemented, it is essential to ensure certain parameters such as high sensitivity, biocompatibility, stability, as well as autonomous operation and wireless data transmission. Furthermore, if we consider that the use of these wearable sensors is for application in fragile wounds, such as CW, it is essential that substrate materials are increasingly studied that are compatible, soft, non-aggressive, and comfortable for the patient, as well as ways to improve the mechanical and electrical conductivity of the sensor. Integration of sensors with wearable devices can provide continuous monitoring of biomarkers in CW, enabling early detection of complications and timely interventions. Wearable sensors can be designed to adapt to the contours of the wound site, providing real-time monitoring of wound biomarkers (Bandodkar et al., 2016). However, there are limitations that need to be overcome in relation to the use of these sensors. For example, the existence of inadequate markers for detecting the wound, that is, the relationship between the marker and the condition of the wound is not clear, and the relationship between the different markers is not clear, and there is no explicit information on the subject. Therefore, further investigation into this relationship between wound physiology and biomarkers needs to be carried out. Another problem related to biomarkers is the fact that, for example, when using enzymes as biomarkers, they are affected by other factors. Although enzyme-based sensors demonstrate greater specificity and sensitivity, the activity of these proteins is easily affected, leading to the development of wearable sensors with poor stability and repeatability. We believe that the solution to this problem can be linked to the advancement of science in replacing enzymes with new materials, such as nanozymes. A limitation associated with the development of sensors is also the fact that most studies stop at the moment of detection in wound simulation fluid. This reveals a major problem in the practical application of these sensors, due to the inability to find problems that exist in the actual application of these same smart dressings, and not thinking about ways to resolve these situations. We consider that wearable sensors should be used in real biological models that exactly mimic what happens in a wound, and their performance should be

adequately evaluated.

From a future perspective, we believe that research should focus on the development of autonomous sensors, which allow wireless data transfer, and can operate without the need for non-portable devices. Regarding the practicality of using these wearable sensors, there are promising ideas of combining near-field communication (NFC) technologies with these sensors to allow them to be used passively wirelessly. Also, the use of smartphones with applications for the use of sensors allows the results to be more intuitive and easier to read and interpret. Another area that we believe is promising is the development of multiplex sensors that can detect multiple biomarkers simultaneously. Current sensor technologies typically measure one or a few biomarkers at a time. However, the development of multiplex sensors could provide a more comprehensive picture of the biological processes involved in wound healing, leading to more effective management of wound care. The combination between these sensors and the release of medicines will certainly encourage the development of smart bandages. These dressings can contain drug delivery systems that can release therapeutic agents in response to changes in wound biomarkers, providing personalized management of wound care.

As a critical and global analysis, it is important to review all the information described on a topic as fundamental and relevant as this one, considering all the material that exists on the subject. The development of sensors to detect CW biomarkers is expected to be of great importance in the future. As technology advances, sensors can become smaller, cheaper, and more accessible, becoming an essential tool in the fight against CW, and routinely applied in clinical practice.

Author contributions

Fátima A. R. Mota: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. M. Lúcia M.F.S. Marques Ferreira de Sousa Saraiva: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. Marieta L. C. Passos: Supervision. Professor João L. M. Santos: Supervision

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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