chemosensors

Review

Biodetection Techniques for Quantification of Chemokines

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Abstract: Chemokines are a class of cytokine whose special properties, together with their involvement and relevant role in various diseases, make them a restricted group of biomarkers suitable for diagnosis and monitoring. Despite their importance, biodetection techniques dedicated to the selective determination of one or more chemokines are very scarce. For some years now, the critical diagnosis of inflammatory diseases by detecting both cytokine and chemokine biomarkers, has had a strong impact on the development of multiple detection platforms. However, it would be desirable to implement methodologies with a higher degree of selectivity for chemokines, in order to provide more precise information. In addition, better development of biosensor technology applied to this specific field would make it possible to address the main challenges of detection methods for several diseases with a high incidence in the population, avoiding high costs and low sensitivity. Taking this into account, this review aims to present the state of the art of chemokine biodetection techniques and emphasize the role of these systems in the prevention, monitoring and treatment of various diseases associated with chemokines as a starting point for future developments that are also analyzed throughout the article.

Keywords: cytokines; chemokines; inflammation; autoimmune diseases; cancer; biosensor

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1. Introduction

Chemokines or chemotactic cytokines constitute a large group of low-molecular-weight proteins (8–12 kDa) with 70–90 amino acids which play decisive roles in angiogenesis and viral infection processes by inducing the migration of immune cells in specific tissues and organs through chemotaxis [1] with cell differentiation and development, and/or cancer metastasis [2]. These proinflammatory proteins attract leukocytes and other cell types via binding with their corresponding receptors [3,4]. The first chemokine was identified in 1977, when platelet factor 4 (PF4/CXCL4) was purified [5]. Later, chemotactic activity for neutrophils by interleukin-8 (IL-8/CXCL8) was shown [6] and, more recently, it was discovered certain chemokines contribute to the HIV suppressive effect in vitro [7,8]. Currently, it has become apparent that chemokines are involved in virtually all pathologies that present an inflammatory component including neurodegenerative diseases [9].

Although they belong to the cytokine family (Figure 1A), chemokines are considered as a specific group due to their importance. Nowadays, these proteins constitute a separate category of selective biomarkers for the diagnosis and monitoring of disorders derived from inflammatory autoimmune and neurological processes. It is known that chemotactic cytokines are produced in inflamed tissue by a wide variety of cell types in response to exogenous or endogenous factors such as lipopolysaccharides, viruses, autoantigens, and inflammatory cytokines [10–12]. Once secreted by cells they bind and activate G protein–coupled receptors; these proteins act as inflammatory mediators and immunological modulators by creating an extracellular concentration gradient that is specifically recognized by cells and promotes their movement. Thus, chemokines regulate leukocyte

migration in response to extracellular gradients of diffusible chemicals through chemokine receptors [13,14]. This enables the response to inflammation of the immune system by movement of cells responsible for surveillance and antigen uptake. In a second step, subsequent altered expression of chemokine receptors in the cells enables them to exit towards lymphoid organs where specific antigen immunity is initiated. In this way, chemokines fulfil functions such as directing the traffic of various cells to mediate the immune response (i.e., in the case of an infection), as well as promoting homeostasis and development at the cellular level [15].

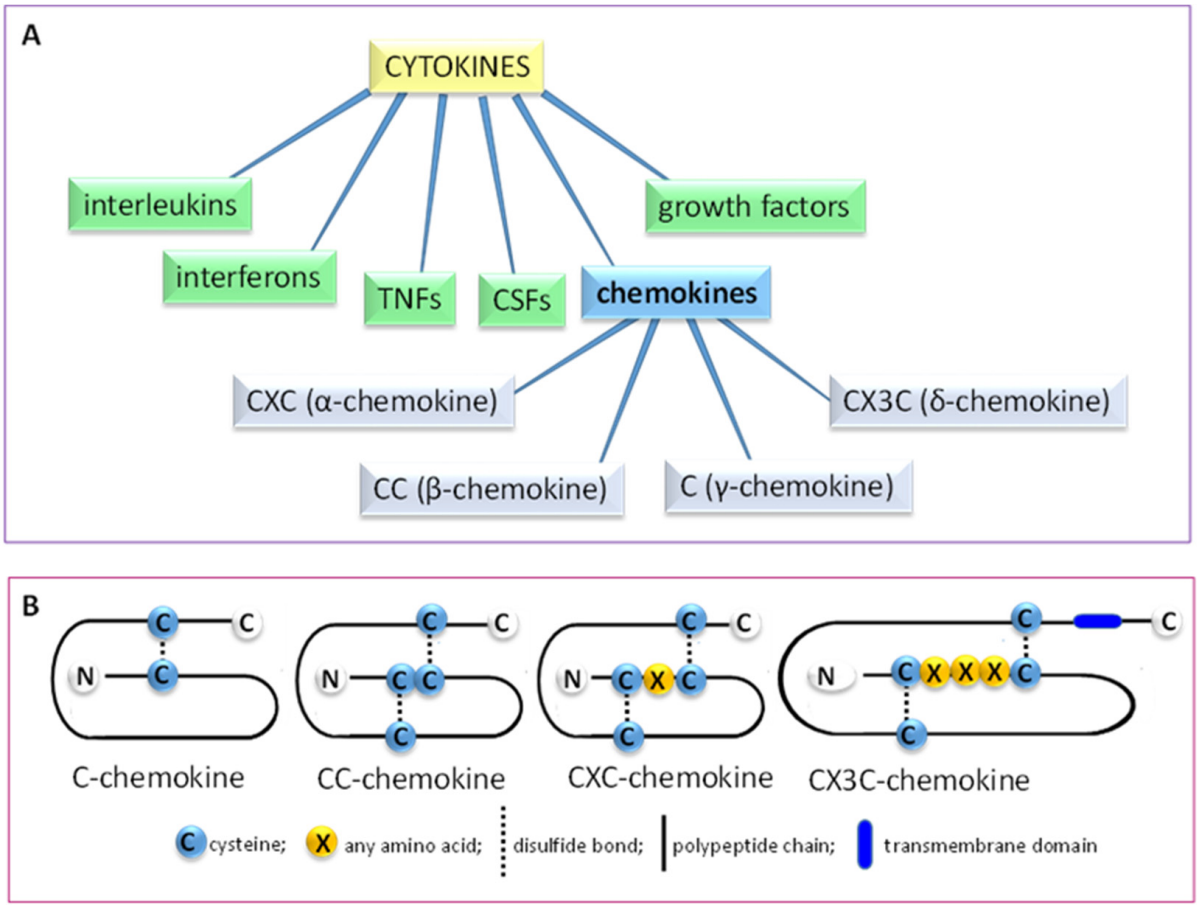

Figure 1. (**A**) Cytokines and chemokines. (**B**) Structures of the chemokine family. Drawn from a schematic of [16].

The expression and production of chemotactic cytokines are induced by a variety of factors in vivo in order to recruit immune cells to inflamed tissue, while the secreted chemokines bind to membrane-bound proteoglycans creating chemotactic gradients that guide the migration of immune cells [17,18]. Quantification of the levels of chemokine secretion by cells, in parallel with the study of their interactions with receptors, are of great interest to understand the regulation and function of chemokines. These objectives justify the creation of new biosensing systems for these proteins in complex samples.

2. Classification of Chemokines

Chemotactic cytokines are characterized by the presence of three or four cysteine residues whose relative position in the amino acid backbone may be used for classifying them into four families: CC, CXC, C, CX3C, where C is a cysteine and X stands for any amino acid residue (Figure 1B) [3,19,20]. More than 50 chemokines and approximately 20 chemokine receptors have been described, with CC (CCL1 through CCL28) and CXC (CXCL1 to CXCL17) forming the two major groups [1,3,10–12,20]. The CXC

chemokine branch can be further subdivided by structure and function into proteins containing or lacking the amino acid motif ELR (Glu-Leu-Arg) terminal to CXC. Particularly, the C–X–C family is characterized by the separation of the two N-terminal cysteines by one variable amino acid residue while in the C–C subfamily the cysteine residues are adjacent to each other [21]. More than forty different chemokines have been identified in humans [22]. Importantly, structural distinctions of the different branches of chemokines have been shown to parallel differences in their biological activities with some few exceptions [23]. As examples, the CC group is chemotactic primarily for T lymphocytes, monocytes and macrophages, natural killer (NK) cells, basophils, and eosinophils, whereas CXC chemokines are chemotactic mainly for neutrophils, whose adherence to endothelial cells is also promoted [1,4,10,19,24].

3. Chemokine Biomarkers and Related Diseases

The critical role that chemokine–chemokine receptor interactions play in the development, function and homeostasis of the immune system, as well as in the pathogenesis of numerous diseases including viral infection [25], cancer [26], neurological disorders [27] and autoimmune diseases [28] has already been discussed. Table 1 summarizes various chemokines whose expression in biological matrices are useful as biomarkers for detection and monitoring of respective diseases [29–43].

Table 1. Some chemokines and chemokine receptors associated with various diseases.

Chemokines, Receptors	Disease	Sample	Observations	Ref.
CXCL12, CXCL14, CXCR4	HIV-1	Peripheral blood mononuclear cells (PBMCs)	CXCL14 synergizes with CXCL12 via allosteric modulation of CXCR4; CXCL14 bound to CXCR4 with high affinity, induced redistribution of cell-surface CXCR4, and enhanced HIV-1 infection by >3-fold	[29]
CXCL8, CXCL10 (and pro-inflammatory cytokines)	Oral squamous cell carcinoma	Saliva, plasma	The levels of CXCL8 and CXCL10 were higher in the OSCC patients than in the controls	[30]
CCL3, MIP-1α	Cystic fibrosis (CF)	Tears	Patients with severe CF have significantly increased levels of MIP-1 α which correlate negatively with clinical status	[31]
CXCL8 (and IL-6, TNF-α)	Dental caries	Saliva	CXCL8 levels significantly higher in dental caries patients	[32]
CXCL8, CCL5/ RANTES (IL-1β, IL-6, IL-13, IL-17A, TNF-α)	Graves' orbitopathy (GO)	Tears	Higher release of CCL5 (RANTES) and cytokines in GO patients	[33]
MCP-1, CXCL8 (and IL-6, IL-1β, TNF-α, γ-INF)	CKD (chronic kidney disease)	Saliva	MCP-1 and CXCL8 levels decrease in patients with CKD	[34]
CXCL8 (and various cytokines)	Thyroid-associated orbitopathy (TAO)	Tears	CXCL8 levels increase in patients of TAO	[35]
CCL2, CCL3, CCL8, CXCL10 (and IL-2, IL-6, IL-15, TNF-α, γ-INF)	Latent tuberculosis infection (LTBI)	Peripheral blood mononuclear cells (PBMCs)	Relevant role of CCL2 relevant for revealing subjects at higher risk of reactivation LTBI	[36]
CXCL8	Glioblastomas	TDECs and UVECs	CXCL8 plays an important role in the process of glioma stem-like cell vascularization	[37]
CCL5/RANTES, MDC (and TGF-β1)	HSC expansion	Stem cell cultures	CCL5, MDC, TGF-β1: secreted factors deleterious to HSC expansion. Significant modulators in stem cell cultures.	[38]
CXCL13	Sjögren's syndrome (SS)	Serum, saliva	Elevated serum or salivary CXCL13 levels in patients with primary SS or SS	[39]

Table 1. *Cont.*

Chemokines, Receptors	Disease	Sample	Observations	Ref.
CXCL8 (and IL1α, IL 1β)	MERS-CoV	Sputum, tracheal aspirate	High expression of CXCL8 in the lower respiratory tract of MERS-CoV infected patients	[40]
CXCL8 (and IL-6, IL-10, TNF-α)	infection/ inflammation	Eccrine sweat	CXCL8 levels in sweat can be correlated to that in serum	[41]
CXCL13, CXCL10, CCL2, CCL3, CXCL12, CCL5	Sjögren's syndrome (SS)	Saliva	Higher levels of CXCL10 and CCL2 in primary SS	[42]
28 cytokines, eight chemokines, and nine growth factors	Sepsis	Serum	Increased levels of CCL2, CCL3, CCL4, CCL5/RANTES, CCL11, CXCL10, CXCL12 in sepsis patients	[43]

In the following, the two most important groups of diseases in which these proteins are involved, cancer and autoimmune diseases, and the role of several chemokines for diagnosis and monitoring of related disorders, are discussed.

3.1. Cancer

Chemokines, which play a crucial role in inflammation and immunity, are also key cancer mediators. They are present at the tumor site in pre-existing chronic inflammatory conditions and are also targeted by oncogenic pathways. Their altered expression in malignant neoplasms drives leukocyte recruitment and activation, as well as angiogenesis, cancer cell proliferation and metastasis at all stages of disease [44]. The amount of evidence and research in this field describing pro-cancer properties of chemokines and their receptors, as well as the possibilities for their use in anti-cancer therapies is enormous, and has been reviewed in various articles [45–47]. Therefore, in this section, representative examples have been selected to illustrate the role of chemokines in cancer, with the criterion of choosing the most recent articles in which methods dedicated to the determination of specific chemokines involving different techniques were developed.

Due to its tumorigenic and proangiogenic properties, interleukin-8 (IL-8, CXCL8) is one of the most investigated chemokines in relation to cancer [37,48]. It plays an important role in the process of glioma stem-like cell vascularization, being expressed and secreted in human gliomas at levels associated with their histological grade [49]. Upregulated CXCL8 is also found in breast cancer and its involvement is reported in progression and metastases in HER2-positive cancers [50]. This chemokine has also been used as a biomarker of bladder cancer (BCa) where urothelial carcinomas constitute the majority of all cases [51]. It has been seen that the determination of CXCL8 (and several cytokines) in urine can provide adequate tools for the diagnosis and therapeutic planning of patients with BCa, since this body fluid maintains close contact with tumor cells and the adjacent inflamed urothelium. [52]. Indeed, urinary concentrations of this chemokine appear significantly elevated in BCa patients with both muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC), compared to healthy individuals and those with hematuria.

Secreted chemokines act as mediators of cell communication by binding to membrane-bound receptors and triggering intracellular signaling. Among them, CXCL12 is a prototype chemokine. Also known as a stromal-derived factor, it binds to the CXCR4 receptor, resulting in a very useful biomarker for monitoring various types of cancer [53]. The role of the CXCL12–CXCR4 axis in tumour growth and metastasis, and in cancer cell-tumour microenvironment interaction and angiogenesis was reviewed [54]. The tumor promoting activity of CXCL12–CXCR4 in various cancers affecting organs such as kidney, lung or pancreas was established [55]. Increased hepatocyte CXCL12 expression is also associated with melanoma [56] and with the process of tumorigenesis and metastasis in colorectal cancer (CRC) [57].

Specifically taking into account chemokine receptors, the recent studies demonstrating their role in processes of metastasis, including those related to squamous cell carcinoma

of the head and neck (HNSCC), should be highlighted [58]. Particular expressions of the paired chemokine–receptor are developed by metastatic tumor cells from cancers affecting breast, liver, prostate or stomach, which significantly correlate with the produced chemokines within distant organs and lymphatic sites to which such cells metastasize. It is known that a wide variety of cell types, mainly leukocytes, express chemokines binding to transmembrane G protein–coupled receptors (GPCRs). This provokes conformational changes in trimeric G proteins that trigger the intracellular signaling pathways required for cell movement and activation. Furthermore, it has been observed that chemokine GPCRs are involved in the initiation and progression of cancers affecting multiple organs [59], suggesting that expression of chemokine GPCRs detecting ligands as growth signals may be the driving force that allows invasion and metastasis in many cancer cells. Among them, one of the most frequently identified chemokine GPCRs in cancer is CXCR4, which has been implicated in more than 23 human cancers.

One of the most common malignancies worldwide is lung cancer, with non-small cell lung cancer (NSCLC) being the most frequent [60]. Importantly, levels of the CXCL10/IP10 chemokine detected in NSCLC tumors are significantly higher than those in adjacent normal lung tissue, which evidences the role of this chemokine in the regulation of NSCLC-induced angiogenesis, as well as in tumor growth and metastasis. In addition to CXCL10, the CXCR4/CXCR7/CXCL12 chemokine axis actively participates in the migration of tumor cells during cancer development by modulating metastasis to specific distant sites including lymph nodes. In this context, it is important to note that high expression of CXCR4 in primary sites and CXCL12 in metastatic lymph nodes have been associated with poor overall survival [61]. Moreover, another chemokine, CCL17/TARC, has been also considered as a highly promising blood biomarker for classic Hodgkin lymphoma (cHL), for its use in early diagnosis, monitoring of treatment response, and relapse detection [62].

3.2. Autoimmune Diseases

Frequently, autoimmune diseases manifest clinically as the consequence of a strong immune response to a particular self-antigen, which leads to significant activation/accumulation of leukocytes in the tissue of the target organ and the subsequent pathology [28]. Chemokines are involved in these processes, since they provide the population of leukocytes necessary to participate in the inflammatory response. Particularly, several chemokines and chemokine receptors, whose role in rheumatoid arthritis (RA) has been recently reviewed [63], are abundant in the peripheral blood and in the local inflamed joints of RA. Expression of CXCL4 and CXCL7 chemokines in patients with early RA may be used to predict progression of early-stage synovitis [64]. Indeed, increased levels of both chemokines were observed in the synovium of these patients compared to those with a resolving disease course. In RA, cells of the immune system including neutrophils, macrophages, B cells, natural killer (NK) cells and T cells migrate to the synovial membrane and, after accumulation in the synovial fluid, they lead to the release of chemokines. Among them, CCL2 (monocyte chemoattractant protein-1, MCP-1) plays an important role in various events related to RA pathogenesis, including migration of effector T cells to the RA synovium tissue and angiogenesis [65]. A significant increase in CCL2 in the synovium of patients with RA is observed, the disease activity score being associated with CCL2 levels, which suggest this chemokine be considered a suitable biomarker of RA [66]. CX3C Ligand 1 chemokine (CX3CL1), also named fractalkine, also participates in monocyte chemotaxis and angiogenesis in RA disease. Increased MMP-2 production from synovial fibroblasts upon CX3CL1 stimulation in vitro was observed, which suggests a pro-inflammatory activity [67].

Multiple sclerosis (MS) is a chronic autoimmune and demyelinating neurodegenerative disorder of the central nervous system (CNS) [68]. This disease is characterized by the attack of abnormally infiltrated immune cells, mainly T-cells, B-cells, and macrophages, that provoke damage to the myelin sheath and oligodendrocytes of the CNS. This action results in demyelination and axonal degeneration to varying degrees, that give rise to

dense scar-like tissue, a hallmark of MS. The clinical manifestations of MS can range from relatively mild neurological symptoms to highly debilitating disease. CXCL13 is a validated biomarker for MS [69], playing a role in B-cell recruitment to the CNS during inflammation. It is also considered as a disease activity biomarker present at increased concentrations in patients with active disease which suffer relapsing–remitting and progressive disease courses. Cui et al. recently reviewed the role of chemokine and chemokine receptors in MS [70]. Several CCL-type chemokines are involved in MS disease. For example, CCL5 (RANTES, regulated on activation of normal T cell expressed and secreted) induce the recruitment of immune cells to the CNS via interaction with its receptor CCR5. Thus, it could in principle also regulate synaptic transmission and brain plasticity, [71] in addition to acting in inflammatory regulation. Indeed, an increase in the central levels of CCL5 is associated with the inflammatory process typical of MS which in turn causes significant changes in synaptic excitability of central neurons. Other chemokines proposed as MS biomarkers are CCL11, associated with disease duration and progression, and CCL20 which is related to disease severity. Immunoassay proteomic research has also allowed identification of up-regulated CCL3 (MIP-1a) and CXCL10 in cerebrospinal fluid (CSF) of patients with MS, and a higher expression of both chemokines in active lesions [72].

The correlation existing between chemokines and thyroid autoimmunity has been demonstrated in various studies. This is evidenced by the results obtained in the investigation of patients with Graves' disease (GD), where the presence of CXCL10 in thyroid follicular cells [73] or the high level of CCL2 (MCP-1) in cultured human thyrocytes [74], and the increased expression of CXCL12 (SDF-1) and CXCL1 (Gro1) in thyroid tissues [75] were reported. Circulating levels of these chemokines are associated with the active phase of GD [76], autoimmune thyroiditis, and other diseases. For example, patients with Hashimoto's thyroiditis exhibited increased expression of CXCR3 and of CCR2 and CCR5 receptors for CCL5 and CCL2, respectively [77]. Importantly, the wide variability in chemokine expression was related to disease duration. Thus, increased CXCL10 expression was found in the thyroid of recent-onset patients compared with long-standing patients [78]. These results agree with those of Armengol et al., showing increased expression of CXCL12, CXCL13, CCL21, and CCL22 in HT thyroid glands [79,80]. Furthermore, Kemp et al. detected CXCL10, as well as CCL2, CCL3, CCL4, and CCL5 in thyroid tissue from patients with HT [81].

4. Biodetection Methods for Determination of Chemokines

Methods to determine chemokine levels are mostly based on immunoassays such as ELISA. At present, commercially available ELISA kits make it possible to measure several cytokines and chemokines in biological samples and cell culture supernatants [82]. Western blotting analysis has also been used to investigate the expression of various chemokines in biological fluids [83]. However, methods involving such techniques have limited sensitivity for detecting very small amounts of the target compounds such as concentrations below pg/mL that appear as cut-off levels for individuals suspected of suffering from some diseases, or to determine mis-expressed proteins [84]. In the case of immunoassays, most require long assay times, taking up to five hours, and do not allow investigation of the secretion of chemokines in real time [85], also relying on the availability of specific and sensitive immuno-reagents. Nevertheless, there are a lot of kits for the determination of individual chemokines which are commonly used as reference methods for validation of results obtained in sample analysis using other methodologies, mainly biosensors.

As will be seen in what follows, the development of biosensors for the determination of chemokines is still scarce. However, it should be emphasized that these devices have ideal analytical characteristics for the diagnosis, prognosis and monitoring of diseases such as cancer or autoimmune disorders, since they allow real-time measurements and can detect minimal amounts of biomarkers (in some cases at the fg/mL level) in very small volumes—below microliter units—of physiological samples, based on antibodies, peptides, nucleic acids, receptors or other types of biomolecules capable of interacting with

the chemokine of interest. Table 2 summarizes the methods and analytical characteristics of some recent biosensors using different detection techniques, which have been applied to clinical samples [37,38,62,85–109]. In the following sections we discuss the relevant aspects of some selected designs. For better understanding, several sub-sections for the different instrumental techniques used were also considered.

Table 2. Some recent biosensors for chemokines and chemokine receptors.

Type of Biosensor	Technique	Target(s)	Biodetection Principle	Dynamic Range	LOD	Sample	Ref.
Chemokine tagging with nanoluciferase fragment HiBiT and to Alexa-Fluor488-tagged CXCR4	Luminescence (BRET)	CXCL12	CRISPR/Cas9 genome editing used to tag the chemokine. CXCL12 secretion monitored in live cells	1 fM–1 nM	-	live cells	[85]
Lentiviral particles with CXCR4 immobilized on MUA SAM onto Au chip	SPR	CXCL12	Specific CXCL12 binding to LVPX4-coated chip in the biosensor	5–50 nM	-	urine of RA patients	[86]
CXCR2-AuNPs/2,2′:5′,2″-tert-thiophene-3′(p-benzoic acid)	Amperometry	CXCL5	Selection of the ligand for CXCR2 receptor by EIS	0.1–10 ng/mL	0.078 ng/mL	human serum CRC cells	[87]
Chip-based gold nanostructured micro-electrode with immobilized thiolated DNA	SWV	CCL5/RANTES CCL22/MDC	Amplified steric hindrance hybridization combining DNA and antibodies signaled by methylene blue	10 pg/mL–10 ng/mL	10 pg/mL	stem cell culture	[38]
Electrochemical sandwich-type immunosensor with immobilized anti-CXCL7 on IgG-MWCNTs/SPCE	Amperometry	CXCL7	Electrode modification by click chemistry	0.5–600 pg/mL	0.1 pg/mL	human serum form patients with RA	[88]
Sandwich-type electrochemical dual immunosensing platform	Amperometry	CXCL7	Simultaneous determination of CXCL7 and MMP3 with COOH-MBs on SPdCE	1–75 ng/mL CXCL7 2.0–2000 ng/mL MMP3	0.3 ng/mL 0.8 pg/mL	+/− RA human serum	[89]
Sandwich-type with cAb immobilized on diatom biosilica/AuNPs/DTNB	SERS	CXCL8/IL-8	cAb/AuNPs-DTNB as the Raman reporter	up to 30 ng/mL	6.2 pg/mL	plasma	[90]
Dual-function microfluidic chip. Sandwich-type with Abs and aptamers	Fluorescence	CXCL8/IL-8	Rolling circle amplification	7.5–120 pg/mL	0.84 pg/mL	TDEC and HUVEC	[37]
Label-free immunosensor with anti-CXCL8 immobilized on 6-phospho-hexanoic acid/ITO	EIS (charge transfer resistance, ΔRct)	CXCL8/IL-8	Phosphonic acid covalently bound to hydroxylated ITO. EDC/NHS activation of carboxyl groups for Ab immobilization	0.02–3 pg/mL	6 fg/mL	human serum and saliva	[91]
Label-free immunosensor with anti-CXCL8 immobilized on SuperP© carbon black/Star polymer/ITO	EIS (charge transfer resistance, ΔRct)	CXCL8/IL-8	The high conductivity of carbonaceous material enhances electron transfer	0.01–3 pg/mL	3.3 fg/mL	human serum and saliva	[92]
Sandwich-type fluorometric immunoassay with fluorophore-Avidin-Biotin-CXCL8-cAb-MBs	Fluorescence	CXCL8/IL-8	Laser excitation of sample fluorophores	up to 5000 ng/L	0.19 ng/mL	spiked human plasma	[93]
SiNMW FET biosensor with Abs immobilized onto APTES and GA modified surface	ΔR in I/V curves	CXCL8/IL-8	Multiplexed determination of CXCL8 and TNF-α. Current/Resistance increase or decrease depending on pI of proteins	10 fg/mL–1 ng/mL	10 fg/mL	saliva	[94]

Table 2. *Cont.*

Type of Biosensor	Technique	Target(s)	Biodetection Principle	Dynamic Range	LOD	Sample	Ref.
Optical modulation biosensing (OMB)	Fluorescence	CXCL8/IL-8	SA-PE-Biotin-Ab2-CXCL8-Ab1-MBs	0.05–10,000 ng/mL	0.02 ng/L	-	[95]
Fluorometric immunosensor with H_2O_2 generating Au/TiO2 photocatalytic film and Cy5/cAb/GNR MEF probes	Fluorescence	CXCL8/IL-8	One-pot, wash-free immunoassay. H_2O_2 induced chloro-1-naphthol precipitation quench Cy5 fluorescence via FRET	1–1000 pg/mL	0.612 pg/mL	serum	[96]
Label-free electrochemical immunosensor	EIS (Rct)	CXCL8/IL-8	Anti-CXCL8 immobilized onto FTO-OH/IPTES platform.	0.04–2 pg/mL	11.9 fg/mL	human serum, saliva	[97]
Label-free synthetic Ab-mimetic protein-monothiol-alkane-PEG SAM-AuE	EIS (change in phase, $\Delta\theta(f)$)	CXCL8/IL-8	Ab-mimetic protein selected via phage display. Capture protein coding region subcloned in top ET11	900 fg/mL–900 ng/mL	90 fg/mL	spiked serum	[98]
Label-free immunosensor with cAb immobilized onto pCBMA/AuE	EIS (Rct)	CXCL8/IL-8	AuE modified by cysteamine adsorption, covalent linking of CB and photo-polymerization.	55 fM–55 nM	10 fM	nasal epithelial lining fluid	[99]
Electrochemical dual biosensing platform with anti-CXCL8 antibody and specific hairpin DNA sequence	Amperometry	CXCL8/IL-8 CXCL8 mRNA	Simultaneous determination of CXCL8 and CXCL8 mRNA involving COOH-MBs, Strept-MBs and dual SPCEs	87.9–5000 pg/mL CXCL8; 0.32–7.5 nM CXCL8 mRNA	72.4 pg/mL 0.21 nM	spiked human saliva	[100]
Array of nanofilm ZnO/metal electrodes functionalized with specific Abs	EIS	CXCL8/IL-8 CXCL10/IP-10	Simultaneous determination of CXCL8 CXCL10, IL-6, IL-10 and TRAIL	0.1–5000 pg/mL CXCL8 1–2000 pg/mL CXCL10	~1 pg/mL	sepsis patients' serum	[101]
Electrochemical DNA biosensor. Immobilized DNA strand with a distal methylene blue reporter on SAM-AuE.	Amperometry	CXCL10 /IP-10	Hybridization to recognition strand with target binding peptide. Current decrease as increase target concentration	1–2000 nM	~60 pM	serum	[102]
DTSSP/Abs solutions immobilized onto ZnO electrodes	EIS	CXCL10/IP-10	Simultaneous determination of CXCL10, TRAIL and CRP	up to 500 pg/mL	<2 pg/mL	human sweat	[103]
Sulfo-LC-SPDP/Ab solution immobilized onto AuE. Sandwich-type electrochemical immunoassay.	Amperometry	CCL17/TARC	HRP-Strept-Biotin-Ab2 conjugates for TMB/H_2O_2 detection	387–50,000 pg/mL	194 pg/mL	serum from patients with cHL	[62]
Sandwich-type electrochemical immunosensor with cAb immobilized on rGO-(rGO-TEPAThi-Au)/GCE	Amperometry	CCL2/MCP1	Signal amplification with Ab2-RuPdPt	20 fg/mL–1000 pg/mL	8.9 fg/mL	spiked serum	[104]
Label-free electrochemical immuno-sensor with cAb immobilized on Au @Pt-CA-AuE	DPV	CCL2/MCP1	Decrease in peak current of $Fe(CN)_6^{3-/4-}$ as increased CCL2 concentration	0.09–360 pg/mL	0.03 pg/mL	spiked serum	[105]
Sandwich-type immunosensor with cAb immobilized onto a silicon photonic micro ring resonator.	Res λ shift	CCL2/MCP1	Shifts in resonance wavelength are related to the target concentration	84.3–1582.1 pg/mL	0.5 pg/mL	spiked serum	[106]
Label-free electrochemical immunosensor with cAb immobilized on PtNPs/SWCNHs	Amperometry	CCL2/MCP1	Reduction current of H_2O_2 decrease as increased CCL2 concentration	0.06–450 pg/mL	0.02 pg/mL	serum	[107]

Table 2. *Cont.*

Type of Biosensor	Technique	Target(s)	Biodetection Principle	Dynamic Range	LOD	Sample	Ref.
Label-free anti-CCR4-PPyr-CSsg-ITO immunosensor	EIS	CCR4	Amino groups of anti-CCR4 antibodies bound covalently to succinimide groups of PPyr-CSsg polymer	0.024–12 pg/mL	7.3 fg/mL	human serum	[108]
Label-free anti-CCR4-P(Pyr-Pac)-ITO	EIS	CCR4	Amino groups of anti-CCR4 antibodies bound covalently to carboxyl groups of P(Pyr-Pac) polymer	0.02–8 pg/mL	6.4 fg/mL	human serum	[109]

4.1. Electrochemical Techniques

A variety of electrochemical biosensors have been proposed for the determination of CXCL8 (IL-8) alone or together with various cytokines. This is probably due to the implication of this chemokine in important inflammatory diseases and cancer [20]. Torrente et al. [100] prepared a dual biosensing platform for the simultaneous determination of CXCL8 and its associated messenger RNA oligonucleotide, which involved the use of carboxyl- and streptavidin-functionalized magnetic microparticles (MBs), respectively, as supports for immobilization of the capture anti-CXCL8 antibody to implement a sandwich-type immunoassay, as well as binding of BHCpCXCL8 for subsequent hybridization with the biotinylated target DNA, which were finally labelled with Strept-HRP. The resulting method, involving amperometric detection at disposable SPCEs, allowed limits of detection of 0.21 nM and 72.4 pg/mL for the respective targets, and it was successfully applied to the determination of CXCL8 in undiluted human saliva. More recently, a label-free impedimetric immunosensor for CXCL8 was also prepared using anti-fouling zwitterionic hydrogel polycarboxybetaine methacrylate (pCBMA) to immobilize polyclonal anti-CXCL8 antibody. The charge transfer resistance (Rct) changes were used as the analytical signal to determine CXCL8 in a wide range of detection, from 55 fM to 55 nM, with a limit of detection (LOD) value of 10 fM. Interestingly, the immunosensor was successfully applied to determine CXCL8 in samples of nasal epithelial lining fluid (NELF) of asthma patients and healthy individuals [99].

Because of the low levels of chemokines existing in biological fluids, ranging between pg/mL to low ng/mL, one of the main objectives in the construction of an electrochemical biosensor for these biomarkers is obtaining high sensitivity. To achieve this, widely used strategies are those involving the employment of nanomaterials capable of amplifying the response due to their high conductivity while enabling stable immobilization of bioreagents. In this context, an interesting article of Aydin et al. [92] reported the preparation of a composite consisting of Super P© carbon black, polyvinylidene fluoride (PVDF) and star shaped poly(glycidylmethacrylate) (PGMA) as a useful modifier of a disposable ITO electrode for the construction of a label-free impedimetric CXCL8/IL-8 biosensor. Figure 2 illustrates the steps of composite preparation and fabrication of the electrode by a spin coating process. As can also be seen, specific anti-CXCL8/IL-8 capture antibodies were bound covalently to epoxy groups of the star polymer. Under optimum conditions, measurements of the charge transfer resistance (ΔRct) provided a wide linear 0.01–3 pg/mL range and a low detection limit of 3.3 fg/mL. The applicability of the proposed immunosensor to determine CXCL8 in saliva and serum samples was also investigated.

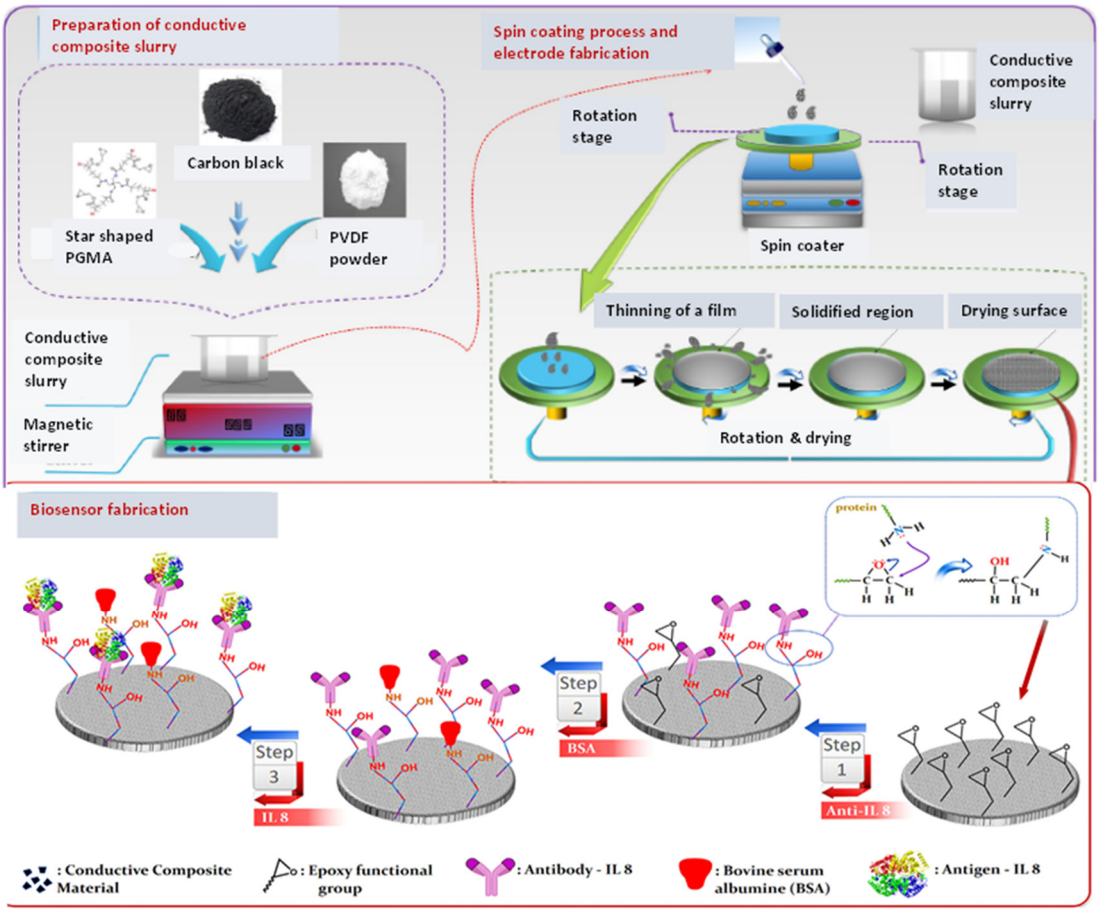

Figure 2. Schematic illustration of the composite preparation procedure and the fabrication process of the label-free CXCL8/IL-8 immunosensor. Adapted from Ref. [92] with permission.

Another label-free biosensor for CXCL8/IL-8 using electrochemical impedance spectroscopy (EIS) with a detection limit of 90 fg/mL in full serum, was developed by Sharma et al. [98]. A self-assembled monolayer (SAM) was prepared using monothiol-alkane-PEG-acid which has anti-fouling properties and also prevents non-specific adsorption onto gold electrodes. Synthetic antibody mimetic proteins with high affinity to the human chemokine were selected via phage display and covalently immobilized on the carboxyl groups. On the resulting electrode surface, binding of biomarkers to the capture reagents resulted in a variation in the local environment of the SAM leading to changes in monolayer defects and in the electrochemical impedance, which can be measured as a change in the phase $\Delta\theta(f)$. Hence, such electrodes are sensitive to the variation of CXCL8 concentrations in the range of 9 fg/mL to 900 ng/mL.

It has been found that CXCL5 also plays important roles in tumorigenesis and cancer progression; studies suggest that this chemokine promotes cell metastasis through tumor angiogenesis in colorectal cancer (CRC). Therefore, the detection of this chemokine could serve as a potential prognostic biomarker for cancer patients [110] However, despite its importance, the number of methods developed for biodetection of CXCL5 are very scarce [111]. As an example, a biosensor for detection of CXCL5 in the CRC cell line was developed by Chung et al. [87]. It was based on the immobilization of CXCR2 onto a glassy carbon electrode modified with gold nanoparticles (AuNPs) and an electro-polymerized 2,2′:5′,2″-terthiophene-3′ (p-benzoic acid) (TBA) nanocomposite film. Interestingly, a chemokine receptor, CXCR2, for which the target has natural affinity, was used for biosensor development. Amperometric detection provided a method with a calibration range between 0.1 and 10 ng/mL CXCL5. The LOD value was 0.078 ± 0.004 ng/mL. The proposed biosensor was successfully applied to determine clinically relevant concentrations

of this chemokine in human serum and CRC cells, with high sensitivity and selectivity. Regarding the latter samples, HT-29 cells which are known to secrete CXCL5 extracellularly, were analyzed for recovery studies after spiking with the chemokine in autoclaved PBS solution containing 105 cells/mL.

Also related to cancer detection, it is worth highlighting the development of an electrochemical affinity biosensor for CCL17/TARC (thymus- and activation-regulated chemokine) as a tool for rapid triage and monitoring of classical Hodgkin lymphoma (cHL) [62]. Indeed, this chemokine is secreted by cHL tumor cells and has recently emerged as blood biomarker for this disease [112]. The amperometric immunosensor proposed by Rinaldi et al., involved a thiolated heterobifunctional cross-linker, sulfo-LC-SPDP, chemisorbed onto gold electrodes for immobilization of the specific capture antibody and preparation of a sandwich design with biotinylated CCL17 secondary antibody conjugated with streptavidin-HRP. The resulting bioelectrode showed excellent analytical performance using 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide for the electrochemical measurements, covering a linear range of 387 to 50,000 pg/mL CCL17 with a LOD value of 194 pg/mL. An important achievement in this work was to demonstrate the ability of the immunosensor to differentiate healthy individuals from 42 diagnosed cHL patients by analyzing blood serum, and to discriminate between samples of patients collected prior to treatment and during chemotherapy on the basis of the decrease in chemokine level.

Multiplexed biosensors for the simultaneous determination of chemokines have also been described. These configurations are of great interest for the diagnosis and monitoring of diseases in which quantification of chemokines, often present in biological samples together with various other cytokines, are essential for a better understanding of the disorder's evolution. As an example, Tanak et al. [101], prepared a multiplexed biosensor involving host immune response for rapid sepsis stratification and endotyping. Sepsis is an intricate condition characterized by a dysfunctional immune response which often is misdiagnosed with serious health consequences [113]. For the purpose of being used as a near-patient point-of-care sensor to detect sepsis, the same team [114] developed the first multi-biosensing platform consisting of five functionalized working electrodes to measure individual biomarkers, with only one chemokine, CXCL10/IP-10, among them. It should be noted that the detection of this chemokine has high importance since it allows identification of the source of the pathogen-triggered immune response. Indeed, viral infection seems to be reflected by an increase in this chemokine [103]. More recently, in this new configuration, CXCL8/IL-8 and CXCL10/IP-10 chemokines were determined, together with three cytokines (IL-6, IL-10 and TRAIL) and two well-established inflammatory biomarkers (PCT and CRP). The multi-assay device, named Direct Electrochemical Technique Targeting (DETecT), consisted of an array of seven electrodes for simultaneous detection of the biomarkers by EIS using a palm-sized, form-factor electronic reader. The sensor surface was modified with dithiobis (succinimidyl propionate) (DSP) to immobilize the specific capture antibodies. It detected all biomarkers in a small plasma sample volume (<40 µL) and the results correlated well with the reference standard method, Luminex, during clinical evaluation of 124 sepsis patient samples. Among other advantages, the method allows stratification of patient samples by measuring a panel of seven host immune response biomarkers on a single platform, providing results in less than 5 min.

Related to the multiplexed electrochemical device described above, fabrication of a wearable biosensor for noninvasive tracking of host immune response biomarkers in sweat was also reported by Jagannah et al. [41,103]. The developed design, named Sweatsenser, allowed the simultaneous and continuous detection of CXCL10/IP-10 and/or CXCL8/IL-8 chemokines together with inflammatory cytokines and C-reactive protein (CRP) for infection monitoring, with limits of detection of 1 pg/mL (IP-10 and TRAIL), and 0.2 ng/mL (CRP) with wide dynamic ranges. The platform (Figure 3) is based on an electrochemical biosensor strip functionalized with capture antibodies via a cross-linker on zinc oxide nanofilm. It allows measurements to be performed with real-time transduction and continuous reporting of targets in sweat. As can also be seen, the sensor strip is comprised of

multiple fluid transport sites that have been carefully designed by means of an absorbent layer interfacing with the skin, to ensure effective capture of sweat, and sweat diffusion through the porous sieve.

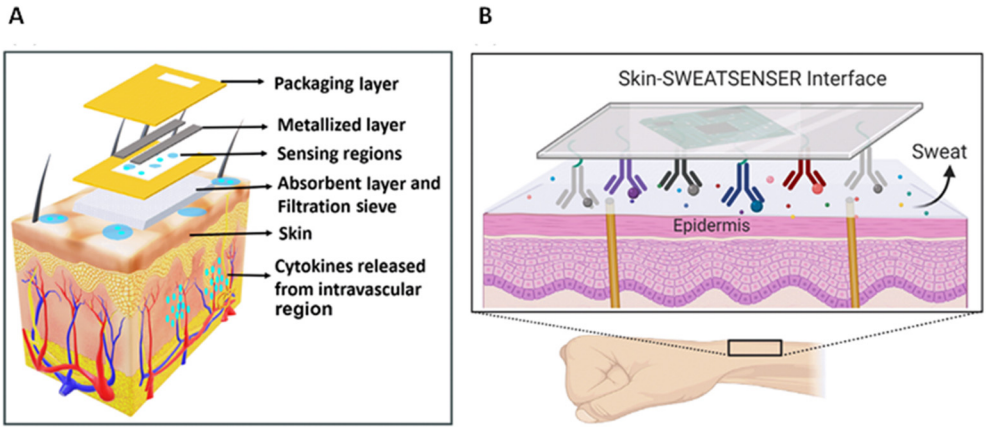

Figure 3. (**A**) Schematics of the layers of the SWEATSENSER strip on contact with skin and (**B**) Skin–device interface where SWEATSENSER is functionalized with specific antibodies to capture the biomarkers. Adapted from Ref. [103] with permission.

Elevated expression of the CXCL7/NAP-2 chemokine has been shown in synovial fluid and serum of patients with rheumatoid arthritis (RA) during the first weeks of the disease but appearing at lower levels in RA with a longer duration. This difference in chemokine concentration is very useful to reflect local pathological changes in the clinical status of patients [115]. Two electrochemical immunosensors were developed by our group for application in the determination of CXCL7 in serum of healthy individuals and patients diagnosed with RA. In a first approach, an original method for immobilization of anti-CXCL7 capture antibodies was developed. It involved a copper (I) catalyzed azide–alkyne cycloaddition reaction (click chemistry) to prepare an integrated disposable immune-platform involving screen printed carbon electrodes modified with azide-modified-multi-walled carbon nanotubes which react with alkynylated IgG by the Cu(I)-catalyzed click reaction followed by the efficient conjugation with antibodies (Figure 4A). A sandwich-type immunoassay was then implemented for the electrochemical detection by differential pulse voltammetry (DPV) using alkaline phosphatase (AP) and 1-naphthyl phosphate (1-NPP) as the enzyme substrate. The immunosensor was successfully employed to determine CXCL7 in human sera with no need for sample treatment except dilution [88].

The second approach [89] (Figure 4B), was designed with the goal of achieving a better assessment of the disease state and treatment success by the simultaneous determination of the CXCL7 chemokine and matrix metalloproteinase-3 (MMP3), an enzyme involved in the degradation of various extracellular matrix components and in the destruction of cartilage and bone in RA [116]. In this configuration, an immunoplatform was prepared using carboxylated magnetic microbeads as substrates for the preparation of the immunoconjugates. The resulting methods, involving amperometric detection with the HRP/H_2O_2/hydroquinone (HQ) system, provided good results in the determination of biomarkers when applied to serum samples of RA patients and healthy individuals. When comparing the methodologies implemented for CXCL7 (Table 2), it can be seen that the one based on electrode modification by click chemistry provided the highest sensitivity, with a LOD value of 0.1 pg/mL vs. 0.8 ng/mL obtained with the magnetic assisted immunoplatform. This may be due to the different configurations prepared for the bioassays and also to the different enzymes, redox probes and electrochemical technique used for detection. However, both configurations were suitable for detecting chemokine overexpression with respect to normal levels of CXCL7 in human serum samples of healthy individuals, which are around 850 ng/mL [117].

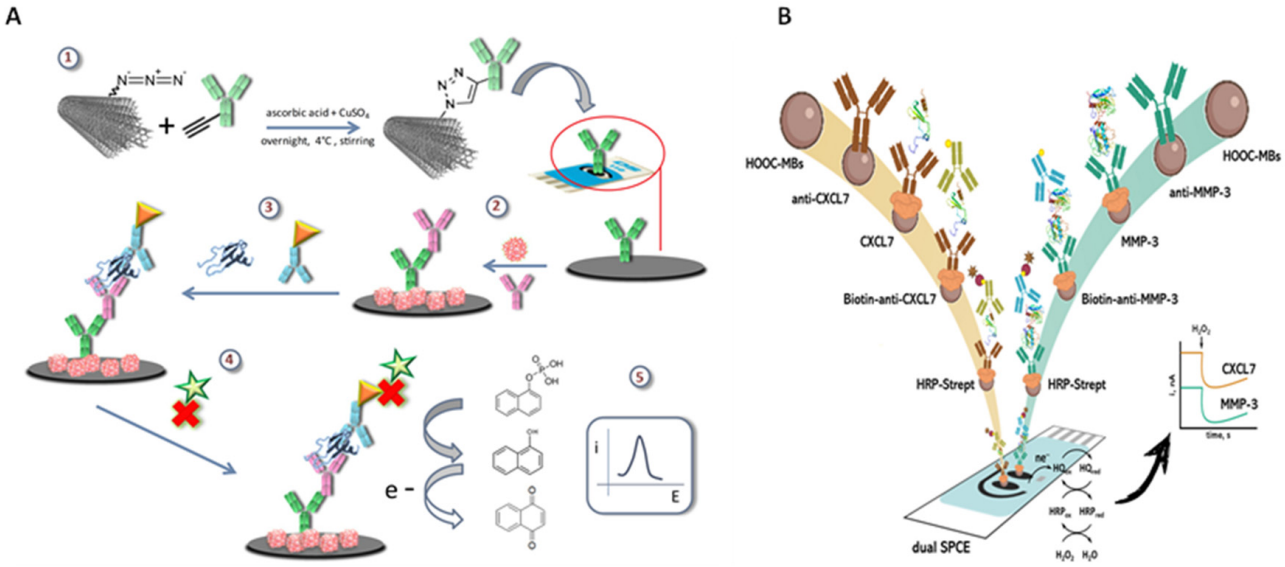

Figure 4. Schematic displays of the different steps involved in the preparation of (**A**) the immunosensor for CXCL7 by click chemistry assisted synthesis of IgG−alkyne−azide−MWCNTs and modification of SPCE (1); blocking step with casein and immobilization of anti-CXCL7 (2); sandwich assay with CXCL7 and biotin-anti−CXCL7 (3); incubation with AP−Strept (4); addition of 1-NPP and DP voltammetric monitoring of the 1-NP oxidation (5). (**B**) the dual CXCL7 and MMP3 immunoplatform involving carboxylated magnetic microbeads as substrates for preparation of the immunoconjugates. Reproduced from Refs. [88,89] with permission.

CCL2 chemokine, also named monocyte chemotactic protein-1 (MCP-1), has relevant roles in pathogeneses of several diseases related to heart and circulatory system, and actively participates in progression of autoimmune diseases. It is considered an initiating factor and biomarker of atherosclerosis and is also involved in several events related to rheumatoid arthritis. Therefore, a there is great interest in the development of sensitive biosensors for detecting CCL2 in different types of clinical samples. Among the electrochemical biosensors proposed for this chemokine, a sensitive electrochemical design based on conductive wires orderly oriented was reported by Li et al. [105]. Such wires were prepared with gold nanoparticles joined to Au@Pt core–shell microspheres via a cysteamine (CA) crosslinker and grafting to the gold electrode to immobilize anti-CCL2 antibodies. After incubation in the presence of the chemokine using only 6 µL of sample, differential pulse voltammetry with $Fe(CN)_6^{3-/4-}$ as the redox probe, provided decreasing changes in the signal response related to the increase in antigen concentration, which reduces the peak current by hindering the spread of redox probe to the electrode surface. A high sensitivity with a calibration interval ranging between 0.09 and 360 pg/mL CCL2, and a LOD value of 0.03 pg/mL was achieved.

CXCL10/IP-10 is an interferon (IFN) inducible chemokine associated with poor prognosis in many types of cancer, showing greater affinity for the CXCR3 receptor. The CXCR3/IFN chemokine axis plays a significant role in cancer progression. To detect this chemokine, Bonham et al. [102] reported an electrochemical DNA biosensor which was prepared by immobilizing a DNA strand with a distal methylene blue redox reporter attached via SAM-AuE. The method involved the grafting of a 21-residue polypeptide binding element derived from CXCR3 receptor onto a peptide–nucleic-acid strand, and hybridization to the AuE-attached methylene blue-modified DNA strand via a terminal thiol group. The binding of CXCL10 to this recognition element reduces current from methylene blue related to the target concentration, which can be measured by square wave voltammetry (SWV). Figure 5 shows the fundamentals of this biosensor, that allows determination of the chemokine in a concentration range between 1 nM to 2 µM.

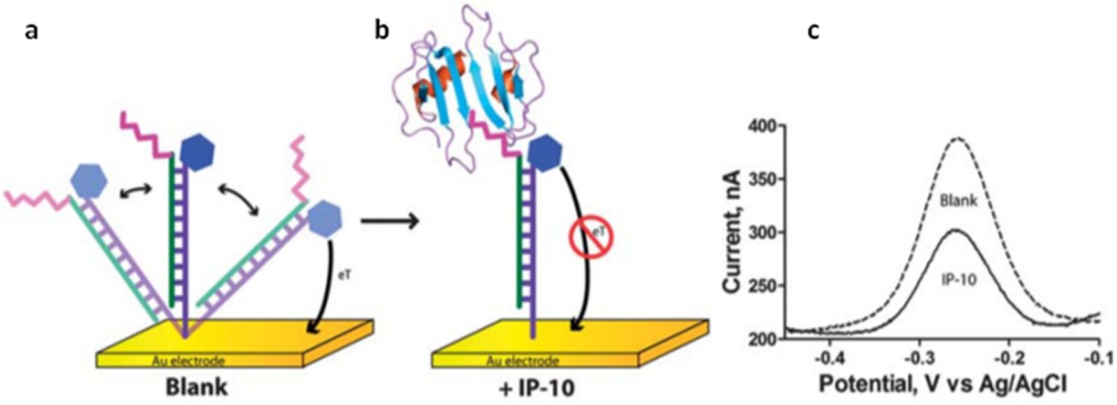

Figure 5. Scheme of the fundamentals of the electrochemical DNA biosensor for the determination of CXCL10/IP-10. (**a**) DNA anchor strand (purple) with a distal methylene blue reporter (blue hexagon) attached via thiol to AuE; (**b**) hybridization to a DNA recognition strand (green) linked to a CXCL10/IP−10 binding peptide; (**c**) SWV current reduction upon chemokine binding. Reproduced from Ref. [102] with permission.

Regarding chemokine receptors, CCR4 has aroused considerable interest as a biomarker of prostate cancer. For its quantitation, an impedimetric immunosensor was prepared using an ITO electrode modified with an acid-substituted polypyrrole (p(Pyr-Pac) containing several carboxyl groups for covalent immobilization of the specific capture anti-CCR4 antibody [109]. As in other research works from Aydin´s group, the implementation of a label-free immunosensor achieves a high sensitivity, with a detection limit of 6.4 fg/mL and a wide linear range extending from 0.02 to 8 pg/mL, which is suitable for application of the developed method to analyze clinical samples.

4.2. Optical Techniques

Although electrochemical techniques comprise the majority for biosensor applications, other forms of transduction such as fluorimetry, have also been used. Figure 6 shows a schematic of the method developed by Verbarg et al. [93] for the detection of CXCL8 using a magnetic modulation biosensing system (MMB) which combines fluorescence measurements with the use of superparamagnetic beads on which capture anti-CXCL8 antibodies were immobilized. As can be seen, a sandwich-type immunoassay was implemented with biotinylated detection antibodies and streptavidin phyco-erythrin (SA-PE) fluorescent dye. In the laser system developed (Figure 6b), the fluorophores in the sample were excited and the emitted response was collected and detected by a photomultiplier tube (PMT). The resulting method allowed determination of CXCL8 through a 6-log dynamic range, being capable of detecting 0.08 ng/L of the chemokine in blood plasma. Important advantages of the MMB system are the ability to measure very low concentrations of analyte such as those from the calibration plot obtained in this method (up to 50 ng/L CXCL8) in a short time (after a 15-min incubation time) and the discrimination between the signal and the background noise due to unbound fluorescent molecules without requiring washing or separation steps.

Using a very different methodology but also involving fluorescence measurements, Zhang et al. [37] developed a microfluidic biosensor with dual function and based on rolling circle amplification (RCA) for on-line detection of CXCL8/IL-8. The analytical concept of on-line detection in this method derives from the configuration depicted in Figure 7. As can be seen, a microfluidic chip was constructed with two passages connected by the vertical channels, where the specific capture antibody was immobilized onto one channel, and the other was used for investigating cell culture. Affinity bioassays to determine CXCL8 were performed by a sandwich-type configuration involving antibodies and aptamers. As Figure 7A shows, once the target is sandwiched, the biotinylated RCA primer template complexes are linked to aptamers followed by isothermal amplification with Phi29 DNA

polymerase. Finally, addition of DNA probes complementary to the amplified sequence provides multiple fluorescent probes to generate enhanced signals observed by a fluorescent microscope. This assay allowed quantitative determination of the chemokine to be performed within a linear range between 7.5 and 120 pg/mL CXCL8. Furthermore, the system was also applied to study the expression of CXCL8 tumor-derived and human umbilical vein endothelial cells (TDEC and HUVEC, respectively) under hypoxic conditions.

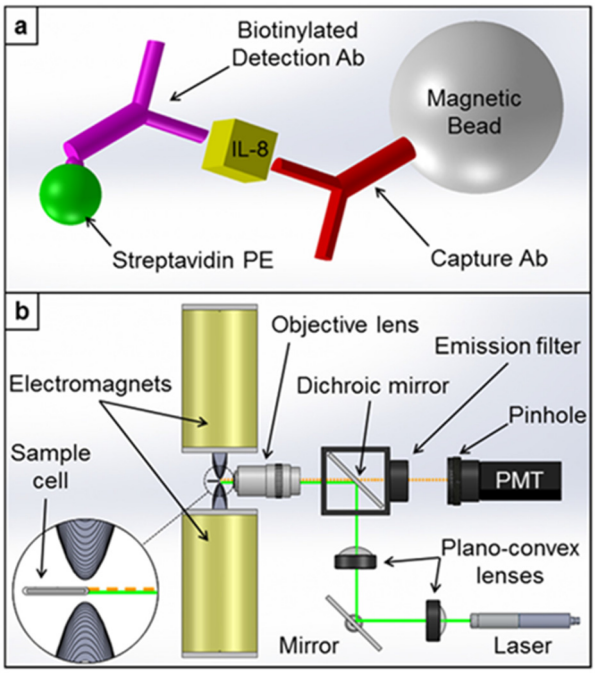

Figure 6. Schemes of (**a**) the magnetic immunoconjugate for the determination of CXCL8/IL8 with a superparamagnetic bead coated with the capture antibody, the antigen, the biotinylated detection antibody, and a fluorophore conjugated to avidin; (**b**) the MMB system (see the text for more information). Reprinted from Ref. [93] with permission.

A surface-enhanced Raman scattering (SERS) immunoassay for the determination of CXCL8/IL-8 was also reported [90], which involves diatom biosilica with immobilized antibodies and integrated gold nanoparticles (AuNPs) using 5,5′-dithiobis(2-nitrobenzoic acid (DTNB) as a Raman reporter molecule. As Figure 8 shows, the immune reaction was performed in a sandwich-type configuration using silica-rich frustules from diatom species (*Pseudostaurosira trainorii*) where anti-CXCL8 capture antibodies were covalently bound. In the presence of the target chemokine, an intense SERS spectrum of DTNB bonded with the detection antibody was obtained, which was suitable for the development of an analytical method for quantitative determination by measuring the peak intensity of the average SERS response. A linear increase in these signals with the CXCL8 concentration in human blood plasma up to 30 ng/mL, and a limit of detection of 6.2 pg/mL were reported.

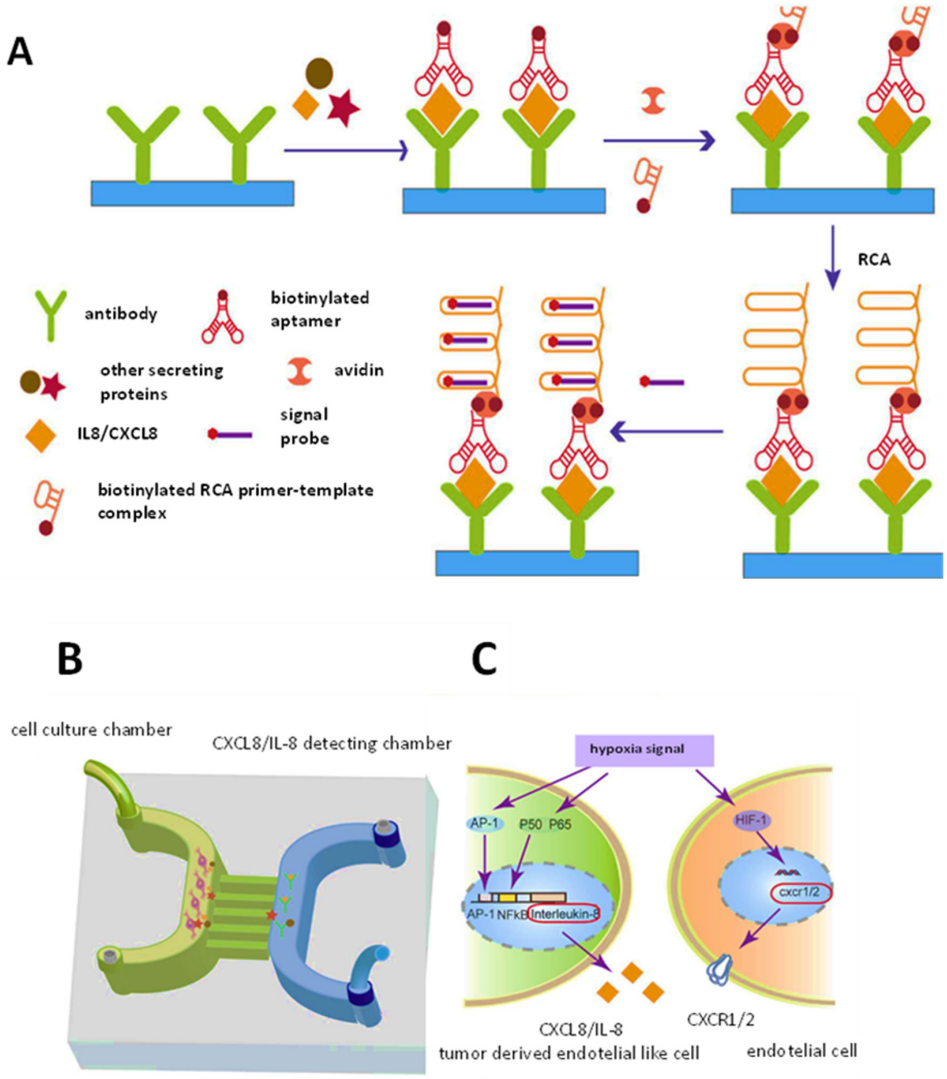

Figure 7. (**A**) Scheme of the steps for CXCL8/IL-8 determination by RCA and a fluorescent probe. (**B**) The design of a dual function microfluidic chip. (**C**) Hypoxia signalling pathway in TDECs and HUVECs. Adapted from Ref. [37] with permission.

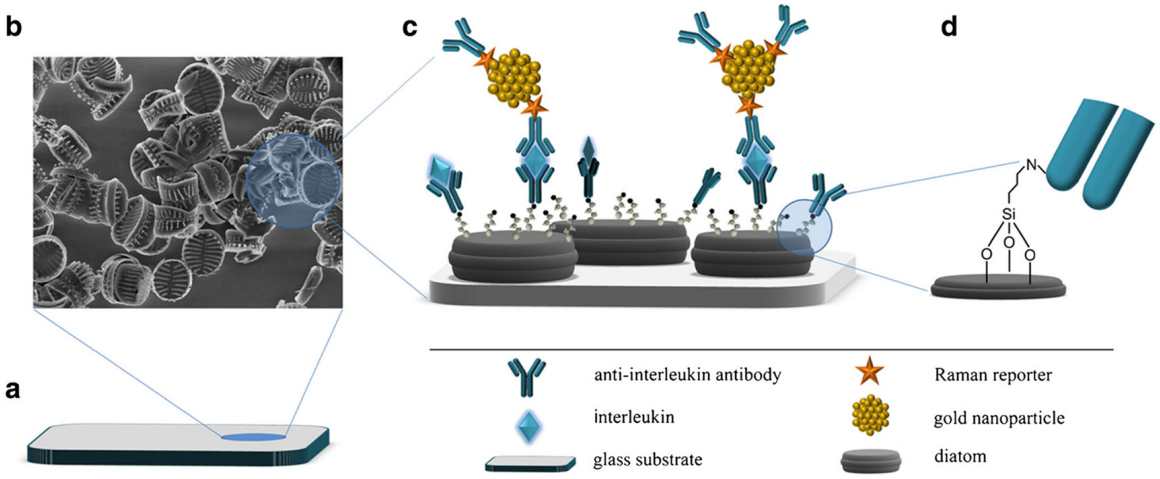

Figure 8. Scheme of the SERS immunosensor for CXCL8 detection (**a**) the glass slide modified with diatom frustules; (**b**) SEM image of a portion of the modified slide; (**c**) the sandwich-type configuration; (**d**) the bonding of the antibody. Reprinted from Ref. [90] with permission.

A sensitive real-time biosensing approach to quantify secretion and receptor binding of native chemokines in live cells was developed by White et al. [85]. CRISPR/Cas9 genome editing was used to tag the chemokine CXCL12 with the nanoluciferase fragment HiBiT and subsequent monitoring and determination by luminescence output. Binding of CXCL12-HiBiT to Alexa-Fluor488-tagged CXCR4 chemokine receptors was analyzed using BRET (bioluminescence resonance energy transfer) in a range between 1 fM and 1 nM. This live cell biomonitoring combines the sensitivity of nanoluciferase with CRISPR/Cas9 genome editing, to detect and quantify binding of low levels of native secreted proteins in real time.

Surface plasmon resonance (SPR) measurements can be used to study interactions between biomolecules immobilized onto metal surfaces and those present in liquid samples. Indeed, SPR biosensors have demonstrated their usefulness as diagnostic tools in a wide variety of biomedical applications [118]. As an example, an SPR biosensor was fabricated by Vega et al. [86] for real-time detection of CXCL12 chemokine in urine samples of RA patients. The developed configuration involved the use of lentiviral particles which bear the chemokine receptor CXCR4 in its native plasma membrane. In fact, CXCL12 chemokine, also named stromal cell-derived factor 1 (SDF-1), is one of the most relevant biomarkers for RA [119]. It is expressed by synovial endothelial cells binding to proteoglycans and participates in bone erosion and progression of the disease. The SPR method showed linear responses to CXCL12 concentration in the 5–40 nM range with good reproducibility and stability of the sensor responses for more than 150 measurements. Furthermore, its application to analyze urine samples demonstrated the high expression of this chemokine in RA patients with respect to healthy individuals, suggesting value of this biomarker for diagnosis of the autoimmune disease.

5. Advantages and Disadvantages of Electrochemical and Optical Biosensors

As discussed above, electrochemical and optical biosensors have been applied to the determination of chemokines. Although all of them are highly sensitive and selective, both have pros and cons. Regarding electrochemical biosensors, they show advantageous properties that, in general, can be summarized as providing wide linear dynamic ranges and low LODs, as well as the ability to be miniaturized and having lower experimental cost compared to optical biosensors. Moreover, they are not affected by interference of the light source and can be used for analyzing turbid samples. However, the electrode surface easily deteriorates, thus explaining why disposable electrodes are more frequently used, with the subsequent increase in the cost of analysis. In addition, and also in general, optical biosensors involving luminescence or absorbance measurements, require more complex instrumentation than that used in electrochemical transduction, and are more susceptible to physical damage and environmental interferences.

Furthermore, amperometric detection is easy to implement and use. The involvement of nanomaterials as electrode surface modifiers to improve the electrochemical responses [88] or as carrier tags for current amplification [104] allow for a high sensitivity. As has been pointed out, an important advantage of these biosensors is the possibility of carrying out the simultaneous detection of several biomarkers, as has been demonstrated with the bioplatforms magnetically assisted with MBs, for the immobilization of the bioreagents [89,100]. In addition, label-free detection involving the EIS technique allows the highly sensitive determination of CXCL8 [94,97] and the CCR4 receptor [108,109]. Regarding optical biosensors, the high sensitivity achieved with sensors using luminescent detection [85] and their suitability for detection in cells [37], as well as the wide dynamic range of calibrations [95] should be highlighted. Also noteworthy is the use of fluorometric detection in microfluidic systems [37] and the one-pot approach using Förster resonance energy transfer (FRET) detection [96]. In this area, the advantages of nanomaterials have been exploited to build SPR-based biosensors that allow real-time monitoring without the need for labels [86], or SERS immunoassay using Raman-reporter-labelled gold nanoparticles for signal enhancement [90].

6. Conclusions and Future Perspectives

In this review, the importance of the determination of different chemokines in clinical samples due to their involvement in diseases of high severity and incidence in the population has been highlighted. The selected examples have demonstrated the need for highly sensitive detection techniques to reach the low concentration levels in which these proteins are usually expressed, but also selective enough to be used directly in the analysis of complex samples without the need for previous treatment. In this context, electrochemical biosensors can offer a wide range of possibilities using different bioassay configurations, by detection with different techniques, and employing nanomaterials and signal amplification strategies.

Assuming that biosensors improve many of the characteristics of conventional methodologies for chemokine determination, it is necessary to pose new challenges that make more efficient detection in the early stages of the disease possible, when the probability of cure is higher, but also during its course by applying accurate methodologies for monitoring capable of detecting selected biomarkers from biological samples obtained by non-invasive collection.

As has been seen, some biosensors are validated by applying them to spiked samples instead of real clinical samples from patients, this being one of the many challenges that still lie ahead when evaluating the clinical utility of biosensors for chemokine detection. On the other hand, there are still only a few examples of multiple biosensing that enable the simultaneous detection of several chemokines and their receptors, to provide more information on the course of the disease. In this context, the use of label-free detection devices should be emphasized, which in the field of electrochemical biosensors has a predominant role in the impedance spectroscopy (EIS) technique.

Additionally, the dissemination of the utility of these devices must be carried out intensively so that the implemented methodologies reach users in clinical laboratories and patients, for point-of-care and self-application. This objective will be easier to achieve if the biosensors are prepared with the additional criteria of ease-of-use and low cost using simple protocols and cheap instrumentation, as well as working with miniaturized systems that allow the patient to monitor parameters of medical interest in spaces that do not necessarily qualify as laboratories or hospitals.

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