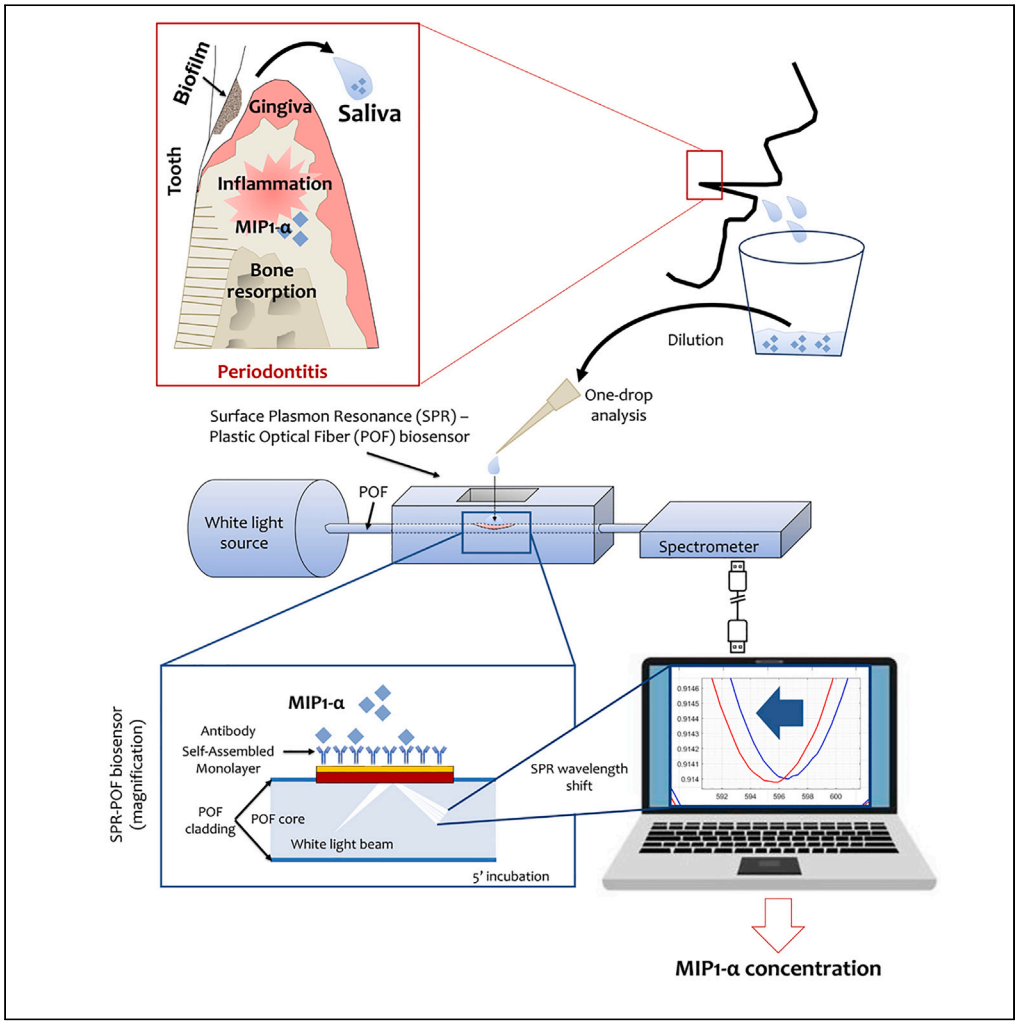
iScience

CellPress
OPEN ACCESS

Article

A novel plasmonic optical-fiber-based point-of-care test for periodontal MIP-1α detection



Marco Annunziata, Francesco Arcadio, Adriana Borriello, ..., Nunzio Cennamo, Fulvio Della Ragione, Luigi Guida

nunzio.cennamo@unicampania.it (N.C.)
fulvio.dellaragione@unicampania.it (F.D.R.)
luigi.guida@unicampania.it (L.G.)

Highlights

MIP-1α is a chemokine involved in periodontitis-related bone resorption

A plasmonic optical-biosensor-based POCT for salivary MIP-1α was described

High selectivity, low LOD (346 fM), and rapid response (5 min) were demonstrated

The present POCT could help in the early and accurate diagnosis of periodontitis

Annunziata et al., iScience *26*, 108539
December 15, 2023
https://doi.org/10.1016/j.isci.2023.108539

iScience

CellPress
OPEN ACCESS

Article

A novel plasmonic optical-fiber-based point-of-care test for periodontal MIP-1α detection

Marco Annunziata,[1,4] Francesco Arcadio,[2,4] Adriana Borriello,[3] Debora Bencivenga,[3] Angelantonio Piccirillo,[1] Emanuela Stampone,[3] Luigi Zeni,[2] Nunzio Cennamo,[2,*] Fulvio Della Ragione,[3,*] and Luigi Guida[1,5,*]

SUMMARY

The analysis of salivary biomarkers as expression of periodontal health conditions has been proposed as a useful aid to conventional diagnostic approaches. In this study, we present a point-of-care test (POCT) exploiting a surface plasmon resonance (SPR)-based optical biosensor to detect salivary macrophage inflammatory protein (MIP)-1α, a promising marker of periodontitis. A plastic optical fiber (POF) was suitably modified and functionalized by an antibody self-assembled monolayer against MIP-1α for plasmonic detection. The proposed SPR-POF biosensor showed high selectivity and very low limit of detection for MIP-1α of 129 fM (1.0 pg/mL) in phosphate-buffered saline and 346 fM (2.7 pg/mL) in saliva. As a proof of concept, this POCT was also able to discriminate between a periodontitis patient and a healthy subject. The obtained results support the future application of this technology for an on-site detection and real-time monitoring of periodontal health conditions for diagnostic and therapeutic purposes.

INTRODUCTION

Periodontitis is a dysbiotic bacterial-biofilm-initiated, multifactorial, chronic, inflammatory disease, causing the progressive resorption of tooth-supporting tissues and, finally, tooth loss, with potential significant implications for systemic health, masticatory function, and aesthetics. Individual susceptibility, strictly tied to a dysregulated host immune and inflammatory response, plays an important role in the development of this pathology. Periodontitis is the most frequent cause of tooth loss in adults in industrialized countries and induces significant adverse social and economic consequences.[1,2] Periodontitis has been moreover linked with several systemic comorbidities such as cardiovascular and neurodegenerative diseases, type-2 diabetes, obesity, rheumatoid arthritis, osteoporosis, respiratory infections, chronic kidney and liver diseases, adverse pregnancy outcomes, and certain cancers.[3] Regarding the causes of these links, periodontitis has been correlated with a systemic status of chronic sub-clinical production of pro-inflammatory agents, defined low-grade inflammation (LGI), a common denominator of many chronic conditions. Periodontitis may contribute to induce and/or maintain a systemic LGI status, and, in turn, LGI may be associated with the onset and progression of periodontitis.[4,5] The onset or exacerbation of periodontitis has been related to the presence in salivary and gingival crevicular fluid (GCF) of biomolecules typical of an inflammatory status.[6,7] Accordingly, their qualitative and quantitative detection in oral fluids could represent a useful tool supporting the conventional diagnostic approaches for this pathology.[8] Nowadays, the health of the periodontium is defined predominantly by clinical and radiographical parameters; this implies that the disease is generally diagnosed after biological damage has already occurred. The use of biomarkers, released into oral fluids from periodontal tissues affected by periodontitis, can facilitate the diagnosis and can even allow to identify the stage at which the biological damage of periodontal tissues is in progress. Such molecules are released in relation to the inflammatory response, including cytokines, particularly interleukin-1β and -6 (IL-1β and IL-6); oxidative stress, like malondialdehyde (MDA); tissue damage, such as matrix metalloproteases (MMPs), in particular MMP-8; and bone remodeling, such as the macrophage inflammatory protein (MIP)-1α.[6,7,9,10]

MIP-1α/CCL3 (hereinafter MIP-1α) is a chemotactic inflammatory chemokine of the cysteine-cysteine subgroup produced by several cells, such as neutrophils, monocytes, lymphocytes, and epithelial cells, at the site of inflammation.[11] When secreted in both extracellular matrix and bodily fluids, its main and well-characterized role is recruiting mononuclear cells and modulating cytokine production. It plays regulatory roles during cell-mediated immune responses, causing the selective migration of human monocytes and lymphocytes (in particular, the $CD8^+$ T-cytotoxic cell subset).[12] Furthermore, it also stimulates osteoclast recruitment and differentiation, eliciting bone resorption primarily through the chemokine receptors CCR1 and CCR5, which are predominantly expressed by osteoclast cells.[13–15] Increased concentrations of MIP-1α have been implicated in the pathogenesis of various inflammatory diseases such as sarcoidosis, Papillon-Lefèvre syndrome, and rheumatoid arthritis.[16] A higher number of MIP-1α-positive and CCR-positive cells have been also reported in inflamed periodontal tissues.[17]

[1]Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania *Luigi Vanvitelli*, Via L. De Crecchio, 6, 80138 Naples, Italy
[2]Department of Engineering, University of Campania *Luigi Vanvitelli*, Via Roma 29, 81031 Aversa, Italy
[3]Department of Precision Medicine, University of Campania *Luigi Vanvitelli*, Via L. De Crecchio 7, 80138 Naples, Italy
[4]These authors contributed equally
[5]Lead contact
*Correspondence: nunzio.cennamo@unicampania.it (N.C.), fulvio.dellaragione@unicampania.it (F.D.R.), luigi.guida@unicampania.it (L.G.)
https://doi.org/10.1016/j.isci.2023.108539

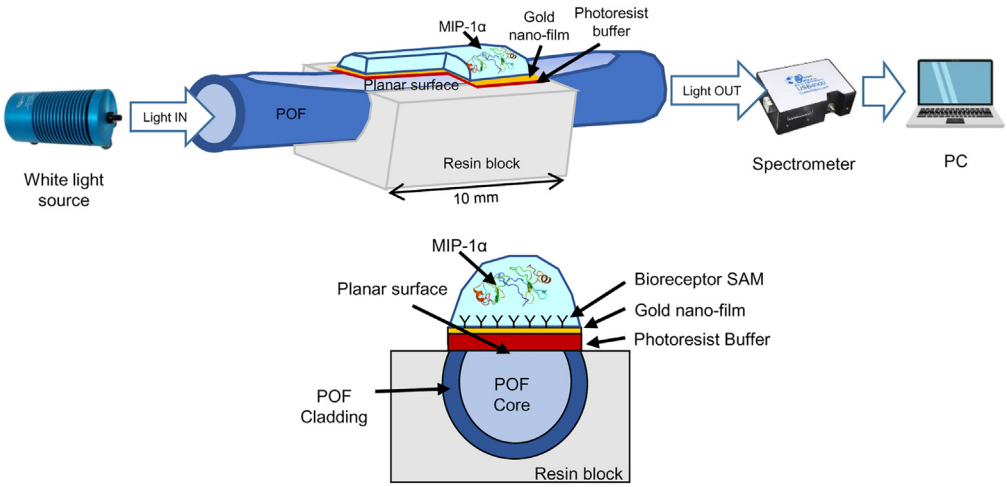


Figure 1. Surface plasmon resonance (SPR) plastic optical fiber (POF) biosensor system

Outline and cross-section view of the SPR-POF biosensor system functionalized by an anti-MIP-1α antibody self-assembled monolayer (SAM).

Higher production of MIP1-α has been reported for gingival fibroblasts when stimulated *in vitro* with lipopolysaccharide from *Porphyromonas gingivalis*.[18] This chemokine has been suggested to play an important role in periodontitis-related bone resorption by inducing the activation of osteoclasts and acting as a potent chemoattractant for T lymphocytes, which, in turn, produce Th-1 cytokines, potent pro-inflammatory mediators promoting tissue destruction.[19,20] MIP-1α salivary levels were found to be significantly higher in periodontitis subjects compared with healthy individuals[21–25] and to decrease following periodontal therapy.[25–28] For this reason, MIP-1α has been proposed as a promising biological marker of periodontitis and as a screening tool being able to discriminate between periodontitis and healthy patients with sensitivity and specificity values up to 95% and 93%,[10,22] respectively.

Currently, the most common techniques for the detection of MIP-1α, as well as of other biomarkers from the oral fluids, are the enzyme-linked immunosorbent assay (ELISA)[24,25] and fluorescent-bead-based immunoassays.[21–23,27,28] Although very specific and sensitive, these laboratory methods are expensive and time-consuming. They generally do not provide a rapid response and require a bulky and not portable equipment. Alternatively, small-size biosensor monitoring systems, directly used at the patient care site (point-of-care tests [POCTs]), appear well suited to complement standard analytical methods, allowing to save considerable amounts of time, reagents, and samples. The development of POCTs, capable of realizing low-cost and rapid analysis, could contribute to the immediate formulation of the correct diagnosis and treatment plan. In this sense, POCTs also show great potential in the public healthcare system for constant monitoring of chronic diseases, with a significant improvement in terms of "secondary prevention" and reduction of public healthcare treatments costs. Furthermore, thanks to the characteristics mentioned earlier, the role of POCTs in collecting data in population-based studies deserves to be emphasized.

Among the different technologies used for this purpose, the surface plasmon resonance (SPR) has gained attention in the recent years. This optical phenomenon has been exploited to detect the binding between an analyte and its specific receptor layer, usually over a planar metal surface (typically silver or gold), by measuring the refractive index changes in response to biomolecular interactions. SPR is the detection principle underlying different biosensors, in which specific analyte-biorecognition elements (i.e., aptamers, recombinant antibodies, etc.) are firstly immobilized on the metallic surface (functionalization procedure), producing a highly sensitive self-assembled monolayer (SAM). Recently, plastic optical fibers (POFs), suitably modified and functionalized with a gold nano-film covered by specific SAMs, have been proposed to produce highly sensitive SPR-based biosensors for a heterogeneous panel of analytes for different chemical and biochemical sensing applications.[29] Because biomarkers of periodontitis are present in very low concentrations in GCF and even more in saliva, the availability of highly sensitive portable biosensors for their detection and quantification is particularly attractive. We have recently developed and described SPR-based POCTs for salivary IL-6, MDA, and MMP-8,[30–32] with promising results in terms of very low limits of detection and selectivity. The aim of this proof-of-concept study was to demonstrate the applicability of this novel POCT exploiting an SPR-based POF biosensor for detecting and quantify salivary MIP-1α, as valuable biomarker of bone resorption in course of periodontitis.

RESULTS

SPR-POF biosensor system

The experimental setup used to monitor the SPR-POF biosensor is based on a white light source and a spectrometer, both directly connected to the SPR-POF chip, exploiting removable SMA connectors.[29] In particular, the white light source connected in input to the SPR-POF sensor presents a wavelength emission range from 360 to 1,700 nm. In contrast, the spectrometer's detection wavelength range connects to the output of the SPR-POF sensor, varying from about 300 to 1,000 nm. The spectrometer is connected to a computer via USB ports. A schematic outline of the SPR-POF biosensor system is shown in Figure 1.

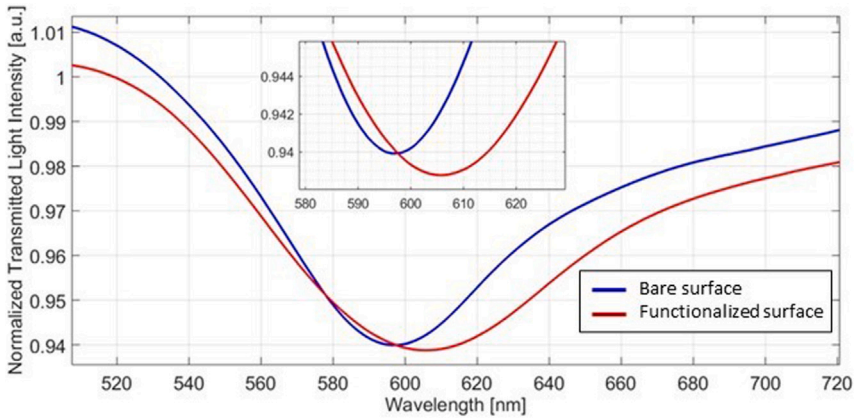


Figure 2. Functionalization of the POF surface for MIP-1α

SPR spectra obtained in PBS, before (bare surface) and after functionalization (functionalized surface). The inset highlights the resonance wavelength shift to the right for the functionalized compared with the bare surface.

Functionalization of the POF surface for MIP-1α

To produce a sensitive SPR-POF for MIP-1α detection, commercially validated anti-MIP-1α antibodies were used for realizing an SAM of the specific bioreceptor. The MIP-1α-specific SAM was tested and characterized acquiring the spectra in phosphate-buffered saline (PBS) before and after the functionalization procedure (Figure 2). In particular, the functionalization steps (lipoic acid, NHS/EDC, and antibody) caused a total resonance wavelength shift toward higher values (red-shift) of ~10 nm. This shift is related to increments of the detected refractive index due to the SAM of the antibody at the gold sensing surface. In fact, when the measured refractive index increases at the interface between the gold surface and the dielectric medium, the resonance wavelength increases, accordingly.

Binding experiments in PBS

The experimental spectra obtained by the developed MIP-1α SPR-POF biosensor at different analyte concentrations in PBS are shown in Figure 3A.

These spectra were obtained by the normalization of all the spectra acquired at different analyte solutions to the reference spectrum (acquired in the air). When MIP-1α concentration increased, the resonance wavelength decreased and shifted to the left (blue-shift), compared with the blank. This SPR shift indicated that when the binding occurred, the refractive index of the receptor monolayer in contact with the gold surface decreased.

The binding kinetics between the bioreceptor and the analyte was also investigated. More specifically, a fixed low MIP-1α concentration (0.5 pM) was followed over time, acquiring SPR spectra every minute. As shown in Figure 3B, the resonance variation relative to the binding occurred in the first 4 min. Therefore, the incubation time was fixed at 5 min for all the following binding experiments.

Dose-response curves in PBS and saliva

A dose-response curve of SPR wavelength variation, compared with the blank, versus the MIP-1α concentration, in PBS (Figure 4A) was calculated (MIP-1α binding isotherm). The tests performed in buffer were also repeated on the saliva collected from a periodontally healthy subject (Figure 4B).

Table 1 reports the limit of detection (LOD), affinity constant (Kaff), and sensitivity at low concentration calculated, as described in method details of the STAR Methods section, from Langmuir parameters of the fitting reported in Figures 4A and 4B.

Selectivity test

Selectivity test showed that both interferents, MIP-3β and bovine serum albumin (BSA), at a concentration of 20 pM, produced only a slight resonance wavelength shift of 0.1 and 0.2 nm, respectively. In contrast, the marker of interest (MIP-1α), at concentration 10-fold less than that of interferents (2 pM versus 20 pM), generated a significantly higher resonance wavelength shift of −2.4. More specifically, these resonance wavelength variations are calculated with respect to the blank and shown in Figure 5. When the not-specific binding occurs, a slight resonance wavelength red-shift is present, in contrast to the binding effect (blue-shift) produced by the specific bioreceptor. Overall, these results demonstrate the efficacy of our developed biosensor in selectively determining the concentration of MIP-1α.

MIP-1α detection in a real clinical scenario

In addition to the main experiments, adjunctive SPR spectra were acquired, as a proof of concept, in a real clinical scenario. A negative salivary sample, collected from a periodontally healthy patient, and a positive salivary sample, collected from a patient with periodontitis, as assessed

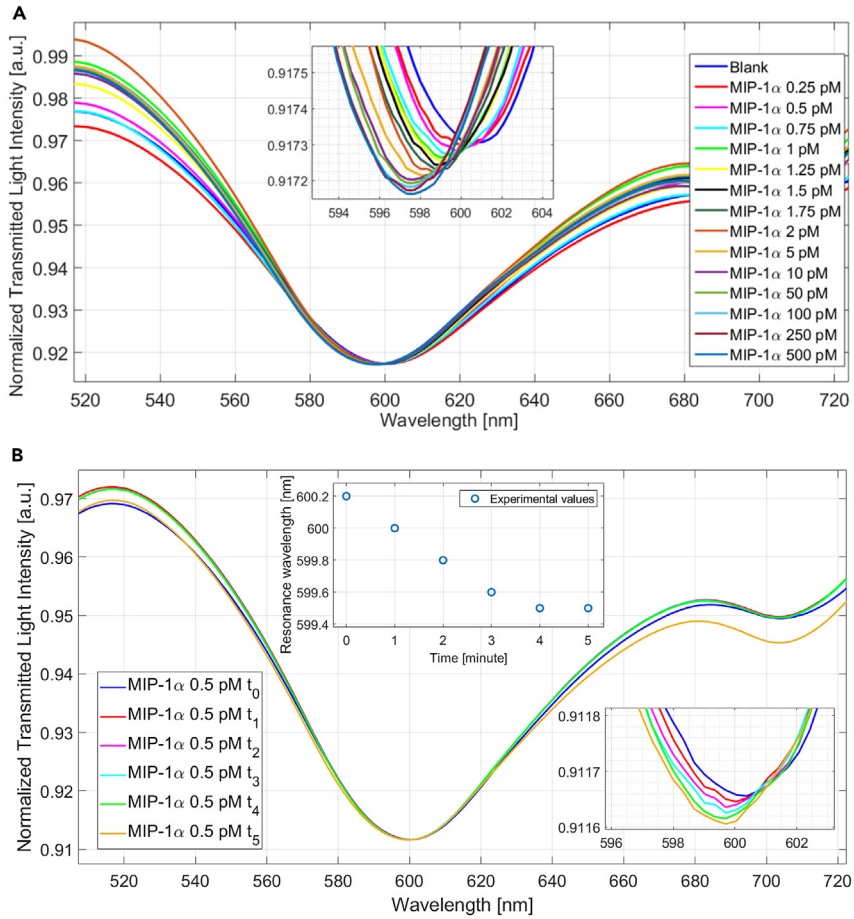


Figure 3. Binding experiments in PBS

(A) SPR spectra obtained by the developed SPR-POF biosensor at different MIP-1α concentrations in PBS. The inset highlights the resonance wavelength shift to the left, compared with the blank, at the MIP-1α concentration increase.

(B) SPR spectra as a function of time at 0.5 pM MIP-1α concentration. The lower inset shows a magnification of the resonance wavelength shift. The upper inset shows the resonance wavelength versus the incubation time.

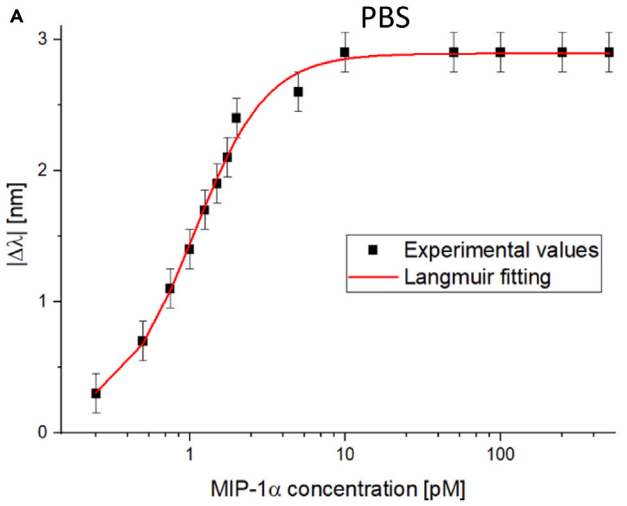
by clinical and radiographic evaluation according to the case definitions provided by the classification of periodontal diseases,[33] were analyzed and compared. When the negative sample was incubated, the resonance wavelength increased (red-shift), whereas when the positive sample was incubated, the resonance wavelength decreased (blue-shift), indicating non-detectable or detectable MIP-1α specifically binding with the bioreceptor surface (Figure 6). On the basis of the obtained SPR spectra (the wavelength shift) and the previous calculated dose-response curve (fitting), the concentration of MIP-1α in the saliva of the periodontitis patient was estimated to be about 15 pM.

DISCUSSION

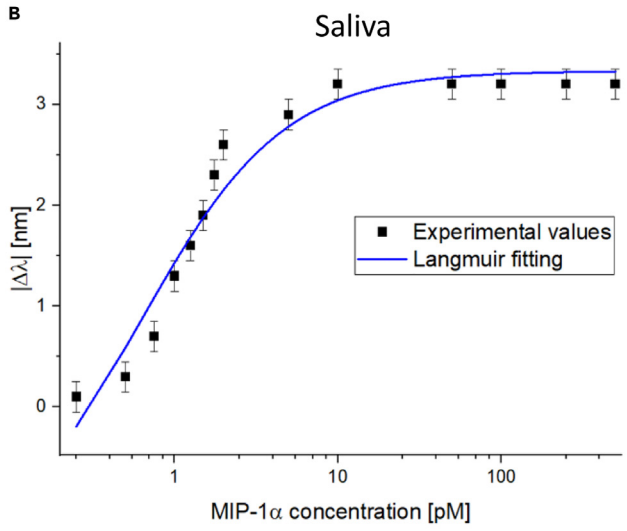
In the present study, we describe a novel plasmonic optical-fiber-based POCT for salivary MIP-1α detection and quantification, demonstrating high selectivity and very low limit of detection in both buffer solution and saliva. Furthermore we have showed that, as a proof of concept, the reported POCT was also able to discriminate between a periodontitis patient and a periodontally healthy subject.

The clinical use of POCTs based on tissue biomarkers may represent a useful aid to conventional diagnostic approaches to real-time monitor the pathophysiological changes caused by several diseases, including periodontitis. In this sense, for the currently used classification of periodontal diseases and conditions, the evaluation of biomarkers levels from oral fluids has been indicated as a future potential grade modifier of periodontitis.[34]

To the best of our knowledge, this is the first example of a POCT for the detection of MIP-1α in oral fluids, so that no comparison can be made with any similar existing device reported in literature. We have recently described the high performance of this SPR-based POCT when applied for the detection of different analytes, such as malondialdehyde (a marker of oxidative stress), demonstrating the high selectivity, thanks to the specific antigen-antibody interaction, and the significantly lowest LOD value that such a technology allows with respect to



λ0 [nm]		$\Delta\lambda_{max}$ [nm]		K [nM]		Statistics	
Value	SE	Value	SE	Value	SE	χ^2	R^2
0.13	0.12	2.98	0.03	1.05	0.06	0.23	0.98



λ0 [nm]		$\Delta\lambda_{max}$ [nm]		K [nM]		Statistics	
Value	SE	Value	SE	Value	SE	χ^2	R^2
0.02	0.25	2.93	0.11	1.32	0.17	2.82	0.95

Figure 4. Dose-response curves in PBS and saliva

Absolute values of SPR wavelength variation (Δλ), compared with the blank, versus the MIP-1α concentration, together with the Langmuir fitting and the error bars (maximum standard deviation) of the experimental values obtained in PBS (A) and saliva (B). Langmuir parameters of the fitting are also reported below the graphs. λ0, resonance value at the blank solution; Δλmax, maximum value of Δλ, calculated by the resonance saturation value minus the blank resonance value; K, dissociation constant; SE, standard error; X^2, chi-square; R^2, R-square.

any other available POCT.[31] Currently, MIP-1α monitoring procedures are primarily carried out via ELISA and fluorescent-bead-based immunoassays. Despite their high sensitivities and broad operating ranges, these methodologies have a very lengthy wait time for results. They are also quite expensive, requiring specialized personnel and complex and costly laboratory equipment, which is bulky and not portable. In contrast to these laboratory methods, the POCT biosensor developed and investigated in the present proof-of-concept study is a portable device, with a short response time (5 min), a high selectivity, and a considerably low detection limit of 129 fM (1.0 pg/mL) in PBS and 346 fM (2.7 pg/mL) in saliva. These values are close to those reported in the literature with the currently available laboratory enzyme immunoassay technologies. The minimum detection limit reported for MIP-1α, indeed, is 23.44 pg/mL using commercial ELISA kits[25] and 0.3 pg/mL using a fluorescent-bead-based immunoassay.[28] The clinical relevance of having a low LOD for a POCT device is easily understood. It may contribute

Table 1. Binding parameters

	LOD (fM)	K_{aff} (pM^{-1})	Sensitivity at low concentration (nm/pM)
PBS	129	0.94	2.82
Saliva	346	0.75	2.21

Limit of detection (LOD), affinity constant (K_{aff}), and sensitivity at low concentration calculated for the detection of MIP-1α in PBS and saliva.

to perform, directly at the dental chair, an early diagnosis and a targeted therapy. Periodontitis biomarkers, indeed, are present in low concentrations in GCF and even more in saliva. Moreover, their levels may be further lowered in the presence of a reduced number of teeth, as in case of advanced periodontitis, or a reduced number of periodontal pockets, as in case of incipient periodontitis, localized periodontitis, or after periodontal treatment. Furthermore, oral biomarkers could also contribute to detect a condition of systemic LGI that, as mentioned, may link periodontitis to systemic diseases such as diabetes, cardiovascular, cerebrovascular, and neurodegenerative diseases.[5]

Previous studies have shown that MIP-1α concentrations in periodontitis patients were significantly increased compared with both healthy[21,22,24,25] and gingivitis subjects.[23] Al-Sabbagh et al. (2012)[22] found that the mean level of MIP-1α in periodontitis subjects was 18-fold higher than in healthy subjects, demonstrating a high sensitivity (94%) and specificity (92.7%).[22] Fine et al. (2009)[21] showed for MIP-1α 50-fold higher salivary levels in a group of young patients who were positive for *Aggregatibacter actinomycetemcomitans* and were longitudinally followed-up developing a form of localized aggressive periodontitis compared with a control group that remained healthy.[35] The authors, indeed, demonstrated how in those patients MIP-1α levels correlated very well with pathological probing depth and the onset of bone loss with a very high specificity (96.8%) and sensitivity (100%). Based on the available studies in the scientific literature, MIP-1α has been described as the key salivary biomarker with highest diagnostic accuracy in a recent systematic review[10] on this topic.

The salivary concentrations of MIP-1α in periodontitis patients, as reported in literature, widely range from picomolar levels[20,24,26,28] to nanomolar levels.[24] In our pilot experiment, in the positive sample we found an MIP-1α concentration of 15 pM (about 117 pg/mL), which is in line with the main part of the abovementioned studies. All the available data, however, are based on heterogeneous study protocols, and further well-designed trials are needed to corroborate these findings and reliably correlate such values with the periodontal conditions of the examined patients.

The POCT described in the present work exploits an SPR-based optical biosensor technology, which has been recently applied to the biomedical field to detect target molecules from different fluids of the human body[36–38] and, recently, to identify IL-6, MDA, and MMP8 from the saliva of periodontitis patients.[30–32] It is a very versatile device in which a highly sensitive SAM can be functionalized for the molecule of interest, in this case the MIP-1α. The resonance shift observed in the present test after functionalization (~10 nm) was in line with the abovementioned studies on similar SPR-based POF biosensors, where a typical resonance wavelength shift ranging from 5 to 20 nm after functionalization was observed for other analytes.[30–32,36–38]

The used SPR platform exploits modified multimode plastic optical fibers. When multimode waveguides are used to excite the SPR phenomenon, the SPR spectra are the result of the convolution of different resonance peaks. More specifically, each peak is obtained for a specific resonance condition defined by a given angle-wavelength couple, a single transverse magnetic (TM) mode. Therefore, the SPR sensors based on multimode waveguides present a better sensitivity with respect to mono-mode waveguides due to the higher modes. On the contrary, the full width at half maximum (FWHM) increases when multimode waveguides are used instead of mono-mode waveguides. In the SPR D-shaped POF sensors used here, a good trade-off between the sensitivity and the FWHM values can be obtained.[29]

The good results obtained with the present POCT in terms of selectivity and accuracy of detection of MIP-1 α as well as other analytes in saliva samples, as mentioned earlier, could open interesting perspectives for its clinical use as a screening tool for periodontitis by multiple simultaneous biomarkers analysis. It has been shown, indeed, that the specificity and sensitivity of this diagnostic approach is considerably increased by the simultaneous detection of multiple periodontitis biomarkers rather than single analytes.[10,39]

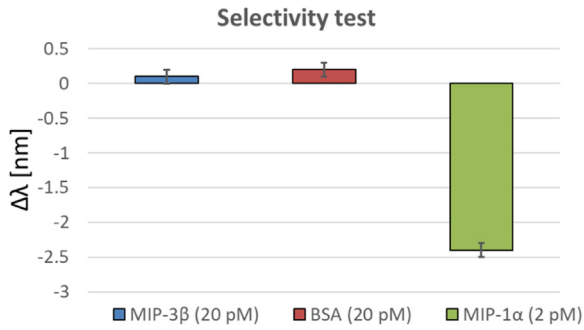


Figure 5. Selectivity test

Resonance wavelength variation (Δλ) obtained by different substances in PBS: MIP-3β (20 pM), BSA (20 pM), and MIP-1α (2 pM). The Δλ is calculated with respect to the blank. Means with error bars (maximum standard deviation).

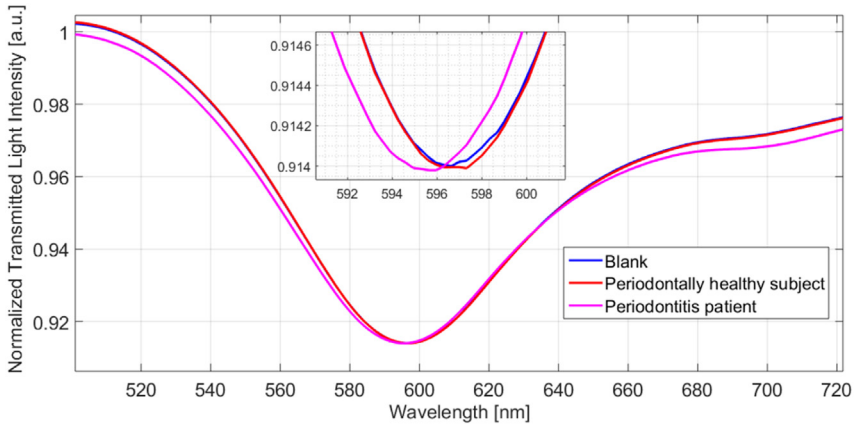


Figure 6. MIP-1α detection in a real clinical scenario

Biosensor response to a negative salivary sample (periodontally healthy subject) and a positive salivary sample (periodontitis patient). The inset highlights the shift of the resonance wavelength to the right, for the periodontally healthy subject, and to the left, for the periodontitis patient, compared with the blank.

In this sense, the use of biomarkers, in addition to increasing the information provided by standard clinical parameters, may contribute not only to improving diagnostic accuracy in the early diagnosis of periodontitis but also to better assess the stage and grade of periodontitis.[34] The current classification of periodontal diseases and conditions, indeed, is an open system in which some grading modifiers, i.e., smoke habit and glycemic status, already exist but where other parameters might be adopted in the near future if reliably sustained by clinical evidence, and saliva biomarkers have been considered promising candidates in this sense.[34]

In conclusion, the POCT described in the present study was able, exploiting an SPR-based optical biosensor technology, to detect MIP-1α from buffer solution and saliva in short time, with high selectivity and very low limit of detection. Furthermore, as a proof of concept, it was also able to discriminate between saliva samples from a periodontitis patient and a healthy subject. These preliminary findings, although needing to be clinically validated, support the hypothesis of a useful diagnostic and therapeutic application of such a device in the periodontal field, as well as for all the other systemic conditions linked to higher levels of this inflammatory chemokine, in the direction of an even more personalized and precision medicine. If confirmed, indeed, the obtained results could support the further development and application of the proposed POCT as an adjunctive low-cost diagnostic tool to real-time monitor, directly at the dental chair, the periodontal health conditions, providing a contribution to perform a targeted and timely therapy, and furthermore, to obtain an important feedback in terms of the efficacy of the periodontal treatment and, consequently, to prevent local and systemic complications.

Limitations of the study

Although the ability of the proposed biosensor to detect and quantify MIP-1α in buffer and saliva has been hereby demonstrated and a pilot test in a periodontally healthy patient and a periodontitis patient has been carried out, the reliability of the measurements effected on patients' saliva needs to be broadly confirmed by comparison with reference laboratory methods (e.g., by ELISA). Such a clinical validation has been scheduled by our research group shortly.

Furthermore, it must be recognized that several systemic and local factors may affect the salivary levels of inflammatory biomarkers, so that some steps are still ahead to reach a breakthrough in the field of diagnostic biomarkers for periodontitis and other systemic diseases. In this perspective, the simultaneous analysis of multiple analytes could be a promising path to take. Currently, we are working to implement our technology for a multiplexed assay that will allow for the simultaneous detection and measurement of different biomarkers, with the aim to increase the diagnostic reliability of the device.

Finally, the hardware and software interfaces of the proposed device need to be improved and kept more user-friendly to simplify both the analysis process and the reading of the results.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- METHOD DETAILS

- Chemicals and reagents
- SPR-POF biosensor system
- Functionalization of the POF surface for MIP-1α
- Binding experiments in PBS
- Binding experiments in saliva
- Binding parameters calculation
- MIP-1α detection in a real clinical scenario

ACKNOWLEDGMENTS

This work was supported by grants from MUR - PRIN 2022 "BOHEMIAN" (CUP: B53D23002680006) and University of Campania *Luigi Vanvitelli* "Kip2PTMCaTR" (CUP: B63C22001470005).

AUTHOR CONTRIBUTIONS

Conceptualization: M.A., F.A., A.B., D.B., L.Z., N.C., F.D.R., and L.G.; data curation & formal analysis: M.A., F.A., L.Z., and N.C.; investigation: M.A., F.A., D.B., A.P., E.S., and N.C.; methodology: A.B., D.B., L.Z., N.C., and L.G.; supervision: A.B., L.Z., N.C., and L.G.; validation: L.Z. and N.C.; writing—original draft: M.A., F.A., A.B., D.B., A.P., E.S., L.Z., N.C., F.D.R., and L.G.; writing—review & editing: M.A., F.A., A.B., L.Z., N.C., F.D.R., and L.G.

DECLARATION OF INTERESTS

The authors declare no competing interests with respect to the authorship and/or publication of this article.

Received: June 14, 2023
Revised: July 19, 2023
Accepted: November 20, 2023
Published: November 23, 2023

REFERENCES

1. Pihlstrom, B.L., Michalowicz, B.S., and Johnson, N.W. (2005). Periodontal diseases. Lancet *366*, 1809–1820.
2. Deng, Z.-L., Szafrański, S.P., Jarek, M., Bhuju, S., and Wagner-Döbler, I. (2017). Dysbiosis in chronic periodontitis: Key microbial players and interactions with the human host. Sci. Rep. *7*, 3703.
3. Hajishengallis, G. (2022). Interconnection of periodontal disease and comorbidities: Evidence, mechanisms, and implications. Periodontol *89*, 9–18.
4. van der Waal, S.V., Lappin, D.F., and Crielaard, W. (2015). Does apical periodontitis have systemic consequences? The need for well-planned and carefully conducted clinical studies. Br. Dent. J. *218*, 513–516.
5. Cecoro, G., Annunziata, M., Iuorio, M.T., Nastri, L., and Guida, L. (2020). Periodontitis, Low-Grade Inflammation and Systemic Health: A Scoping Review. Medicina *56*, E272.
6. Arias-Bujanda, N., Regueira-Iglesias, A., Balsa-Castro, C., Nibali, L., Donos, N., and Tomás, I. (2019). Accuracy of single molecular biomarkers in gingival crevicular fluid for the diagnosis of periodontitis: A systematic review and meta-analysis. J. Clin. Periodontol. *46*, 1166–1182.
7. Chen, M., Cai, W., Zhao, S., Shi, L., Chen, Y., Li, X., Sun, X., Mao, Y., He, B., Hou, Y., et al. (2019). Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. J. Clin. Periodontol. *46*, 608–622.
8. Giannobile, W.V., Beikler, T., Kinney, J.S., Ramseier, C.A., Morelli, T., and Wong, D.T. (2009). Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol *50*, 52–64.
9. de Lima, C.L., Acevedo, A.C., Grisi, D.C., Taba, M., Guerra, E., and De Luca Canto, G. (2016). Host-derived salivary biomarkers in diagnosing periodontal disease: systematic review and meta-analysis. J. Clin. Periodontol. *43*, 492–502.
10. Kc, S., Wang, X.Z., and Gallagher, J.E. (2020). Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: Systematic review. J. Clin. Periodontol. *47*, 289–308.
11. Baggiolini, M. (2001). Chemokines in pathology and medicine. J. Intern. Med. *250*, 91–104.
12. Siveke, J.T., and Hamann, A. (1998). T helper 1 and T helper 2 cells respond differentially to chemokines. J. Immunol. *160*, 550–554.
13. Kukita, T., Nakao, J., Hamada, F., Kukita, A., Inai, T., Kurisu, K., and Nomiyama, H. (1992). Recombinant LD78 protein, a member of the small cytokine family, enhances osteoclast differentiation in rat bone marrow culture system. Bone Miner. *19*, 215–223.
14. Kukita, T., Nomiyama, H., Ohmoto, Y., Kukita, A., Shuto, T., Hotokebuchi, T., Sugioka, Y., Miura, R., and Iijima, T. (1997). Macrophage inflammatory protein-1 alpha (LD78) expressed in human bone marrow: its role in regulation of hematopoiesis and osteoclast recruitment. Lab. Invest. *76*, 399–406.
15. Lean, J.M., Murphy, C., Fuller, K., and Chambers, T.J. (2002). CCL9/MIP-1gamma and its receptor CCR1 are the major chemokine ligand/receptor species expressed by osteoclasts. J. Cell. Biochem. *87*, 386–393.
16. Bhavsar, I., Miller, C.S., and Al-Sabbagh, M. (2015). Macrophage Inflammatory Protein-1 Alpha (MIP-1 alpha)/CCL3: As a Biomarker. General Methods in Biomarker Research and their Applications, 223–249.
17. Kabashima, H., Yoneda, M., Nagata, K., Hirofuji, T., and Maeda, K. (2002). The presence of chemokine (MCP-1, MIP-1alpha, MIP-1beta, IP-10, RANTES)-positive cells and chemokine receptor (CCR5, CXCR3)-positive cells in inflamed human gingival tissues. Cytokine *20*, 70–77.
18. Morandini, A.C.F., Sipert, C.R., Gasparoto, T.H., Greghi, S.L.A., Passanezi, E., Rezende, M.L.R., Sant'ana, A.P., Campanelli, A.P., Garlet, G.P., and Santos, C.F. (2010). Differential production of macrophage inflammatory protein-1alpha, stromal-derived factor-1, and IL-6 by human cultured periodontal ligament and gingival fibroblasts challenged with lipopolysaccharide from P. gingivalis. J. Periodontol. *81*, 310–317.
19. Gemmell, E., Carter, C.L., and Seymour, G.J. (2001). Chemokines in human periodontal disease tissues. Clin. Exp. Immunol. *125*, 134–141.
20. Ryu, O.H., Choi, S.J., Linares, A.M.G., Song, I.S., Kim, Y.J., Jang, K.T., and Hart, T.C. (2007). Gingival epithelial cell expression of macrophage inflammatory protein-1alpha induced by interleukin-1beta and lipopolysaccharide. J. Periodontol. *78*, 1627–1634.
21. Fine, D.H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., McKiernan, M., Donnelly, R., and Gunsolley, J. (2009). Macrophage inflammatory protein-1alpha: a salivary biomarker of bone loss in a longitudinal cohort study of children at risk

for aggressive periodontal disease? J. Periodontol. *80*, 106–113.
22. Al-Sabbagh, M., Alladah, A., Lin, Y., Kryscio, R.J., Thomas, M.V., Ebersole, J.L., and Miller, C.S. (2012). Bone remodeling-associated salivary biomarker MIP-1α distinguishes periodontal disease from health. J. Periodontal. Res. *47*, 389–395.
23. Ebersole, J.L., Nagarajan, R., Akers, D., and Miller, C.S. (2015). Targeted salivary biomarkers for discrimination of periodontal health and disease(s). Front. Cell. Infect. Microbiol. *5*, 62.
24. Kluknavská, J., Rabajdová, M., Urban, P., Špaková, I., Klepcová, Z., Kalinová, K., and Vašková, J. (2022). Expression of selected inflammatory proteins and metalloproteinases in periodontitis. Eur. Rev. Med. Pharmacol. Sci. *26*, 1825–1831.
25. Görgülü, N.G., and Doğan, B. (2022). Effect of non-surgical periodontal treatment on salivary and serum biomarkers in Stage III Grade B and C periodontitis. J. Periodontol. *93*, 1191–1205.
26. Thunell, D.H., Tymkiw, K.D., Johnson, G.K., Joly, S., Burnell, K.K., Cavanaugh, J.E., Brogden, K.A., and Guthmiller, J.M. (2010). A multiplex immunoassay demonstrates reductions in gingival crevicular fluid cytokines following initial periodontal therapy. J. Periodontal. Res. *45*, 148–152.
27. Sexton, W.M., Lin, Y., Kryscio, R.J., Dawson, D.R., Ebersole, J.L., and Miller, C.S. (2011). Salivary biomarkers of periodontal disease in response to treatment. J. Clin. Periodontol. *38*, 434–441.
28. Grande, M.A., Belstrøm, D., Damgaard, C., Holmstrup, P., Könönen, E., Gursoy, M., and Gursoy, U.K. (2020). Salivary concentrations of macrophage activation-related chemokines are influenced by non-surgical periodontal treatment: a 12-week follow-up study. J. Oral Microbiol. *12*, 1694383.
29. Cennamo, N., Pesavento, M., and Zeni, L. (2021). A review on simple and highly sensitive plastic optical fiber probes for bio-chemical sensing. Sensor. Actuator. B Chem. *331*, 129393.
30. Cennamo, N., Piccirillo, A., Bencivenga, D., Arcadio, F., Annunziata, M., Della Ragione, F., Guida, L., Zeni, L., and Borriello, A. (2023). Towards a point-of-care test to cover atto-femto and pico-nano molar concentration ranges in interleukin 6 detection exploiting PMMA-based plasmonic biosensor chips. Talanta *256*, 124284.
31. Bencivenga, D., Arcadio, F., Piccirillo, A., Annunziata, M., Della Ragione, F., Cennamo, N., Borriello, A., Zeni, L., and Guida, L. (2023). Plasmonic optical fiber biosensor development for point-of-care detection of malondialdehyde as a biomarker of oxidative stress. Free Radic. Biol. Med. *199*, 177–188.
32. Guida, L., Bencivenga, D., Annunziata, M., Arcadio, F., Borriello, A., Della Ragione, F., Formisano, A., Piccirillo, A., Zeni, L., and Cennamo, N. (2023). An optical fiber-based point-of-care test for periodontal MMP-8 detection: A proof of concept. J. Dent. *134*, 104553.
33. Caton, J.G., Armitage, G., Berglundh, T., Chapple, I.L.C., Jepsen, S., Kornman, K.S., Mealey, B.L., Papapanou, P.N., Sanz, M., and Tonetti, M.S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J. Clin. Periodontol. *45* (*Suppl 20*), S1.
34. Tonetti, M.S., Greenwell, H., and Kornman, K.S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J. Periodontol. *89*, S159–S172.
35. Fine, D.H., Markowitz, K., Fairlie, K., Tischio-Bereski, D., Ferrandiz, J., Godboley, D., Furgang, D., Gunsolley, J., and Best, A. (2014). Macrophage inflammatory protein-1α shows predictive value as a risk marker for subjects and sites vulnerable to bone loss in a longitudinal model of aggressive periodontitis. PLoS One *9*, e98541.
36. Cennamo, N., Pesavento, M., Lunelli, L., Vanzetti, L., Pederzolli, C., Zeni, L., and Pasquardini, L. (2015). An easy way to realize SPR aptasensor: A multimode plastic optical fiber platform for cancer biomarkers detection. Talanta *140*, 88–95.
37. Cennamo, N., Pasquardini, L., Arcadio, F., Vanzetti, L.E., Bossi, A.M., and Zeni, L. (2019). D-shaped plastic optical fibre aptasensor for fast thrombin detection in nanomolar range. Sci. Rep. *9*, 18740.
38. Zeni, L., Perri, C., Cennamo, N., Arcadio, F., D'Agostino, G., Salmona, M., Beeg, M., and Gobbi, M. (2020). A portable optical-fibre-based surface plasmon resonance biosensor for the detection of therapeutic antibodies in human serum. Sci. Rep. *10*, 11154.
39. Bostanci, N., Mitsakakis, K., Afacan, B., Bao, K., Johannsen, B., Baumgartner, D., Müller, L., Kotolová, H., Emingil, G., and Karpíšek, M. (2021). Validation and verification of predictive salivary biomarkers for oral health. Sci. Rep. *11*, 6406.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Recombinant Anti-Macrophage Inflammatory Protein 1 alpha/CCL3 antibody [EPR16618-90]	Abcam	Cat# ab179638, Rabbit monoclonal; RRID:AB_2927558
Biological samples		
Healthy human saliva	periodontally healthy subject (aged 43)	N/A
Pathological human saliva	periodontitis patient (aged 62)	N/A
Chemicals, peptides, and recombinant proteins		
Plastic Optical Fiber	Edmund Optics	Cat# 02-534
Microposit S1813	Chimie Tech Services	Cat# 021838
Gold	8853	Cat# SP-AU54X0.1
(±)-α-Lipoic acid	Merck KGaA	Cat# T5625
N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride	Merck KGaA	Cat# 03449
N-Hydroxysuccinimide	Merck KGaA	Cat# 130672
Ethanolamine	Merck KGaA	Cat# E9508
Phosphate buffer saline	Merck KGaA	Cat# 524650
Ethanol	Merck KGaA	Cat# 34852
Tween-20	Merck KGaA	Cat# P1379
Bovine serum albumin	Merck KGaA	Cat# A3059
Recombinant Human MIP-1 alpha/CCL3 protein (Active)	Abcam	Cat# ab283442
Recombinant human MIP-3 beta/CCL19 protein (Active)	Abcam	Cat# ab285571
Software and algorithms		
Matlab 2022	MathWorks	https://it.mathworks.com/products/matlab.html
OriginPro 9 (demo version)	Origin Lab. Corp	https://www.originlab.com/try
Spectra Suite	Ocean insight	https://www.oceaninsight.com/support/software-downloads/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Luigi Guida (luigi.guida@unicampania.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Chemicals and reagents

N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), Ethanolamine, (±)-α-Lipoic acid, PBS, BSA, Tween 20, were purchased from Merck KGaA (Darmstadt, Germany). The rabbit monoclonal antibody against MIP-1α/CCL3, the human recombinant active MIP-1α/CCL3 and MIP-3β/CCL19 proteins were from ABCAM (Cambridge, CB2 0AX, UK). All the reagents were at the maximum degree of purity and all the used solutions, prepared just before the use, were obtained by using ultrapure Milli-Q water (Milli-Q® IQ 7000, Merk) and were filtered with 0.2 μm pore size filters before the use.

SPR-POF biosensor system

MIP-1α detection is performed by the plasmonic probe based on a modified POF with a 980 μm poly(methyl methacrylate) (PMMA) core (1 mm of total diameter), monitored by a simple experimental setup. In particular, the used POF-based plasmonic probe has been obtained by three simple manufacturing steps, in order to excite the SPR phenomenon in a planar sensing region 1 cm long, as described in a recent study.[29] The planar sensing surface can be used to carry out the measurements by dropping the solutions under test without a microfluidic system. So, a polishing process, obtained from two different polishing papers, is used to realize a D-shaped POF sensing area. Exploiting this polishing process, the cladding and part of the core of the POF are removed. On the exposed core a multilayer is realized to obtain the SPR phenomenon. More specifically, a polymer layer (Microposit S1813 photoresist) is realized on the core by spinning and heat polymerization steps (1 μm thick). This optical buffer layer presents a refractive index major than that of the PMMA core of the POF to improve the SPR performances.[29] Finally, a sputtering process covers the polymer buffer layer with a gold nanofilm (60 nm thick).

Functionalization of the POF surface for MIP-1α

The POF platform surface was functionalised as previously reported,[30,31,38] with some modifications. Briefly, the POF gold surfaces were sequentially cleaned with Milli-Q water (3 times, 5 minutes each) and 8% ethanol in Milli-Q water (5 min incubation repeated 3 times). Then, the gold surfaces were treated for 18 h at room temperature with lipoic acid (0.3 mM in 8% ethanol solution) to produce surfaces exposing carboxyl groups; then, activation was performed through incubation with 200 mM NHS/ 50 mM EDC mixture in PBS pH 7.4 for 20 minutes at room temperature and washed three times with PBS. After these steps, each surface was incubated 2 h at room temperature with 10 μl anti-MIP-1α antibody (mg/ml) for covalent immobilization. After 3 washes with PBS for antibody excess removal, the remaining free activated carboxyl groups were blocked by treatment with 1 M ethanolamine pH 8.0, for 30 min at room temperature. For washes (3 times, 5 minutes each), 0.005% Tween 20/PBS was used. All the employed solutions were prepared with filtered (0.2 μm pore diameter) MilliQ water and after each step the surfaces were dried under a gentle nitrogen stream. Finally, the functionalised platforms were stored in PBS overnight at 4°C before use. The plasmonic spectra of unfunctionalized (bare) and functionalized SPR-POF chip surfaces were recorded with the same bulk solution (PBS) to monitor antibody cross-linking.

Binding experiments in PBS

The detection of MIP-1α by the SPR-POF biosensor was assessed as follows: 50 μL of recombinant MIP-1α dilutions (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 5, 10, 50, 100, 250 and 500 pM in PBS) were dropped over the sensing region of the SPR-POF biosensor and incubated at room temperature for 5 minutes, to allow the interaction with the immobilized antibody. At the end of each incubation, after a washing step by PBS, the spectrum was recorded and compared to a blank (PBS without analyte). By adopting this protocol, only the shift of the resonance wavelength determined by the specific antigen-antibody binding was measured, eliminating shifts due to bulk changes or non-specific interactions. The Limit of Detection (LOD), the affinity constant (K_{aff}), and sensitivity al low concentrations were calculated by using the Langmuir model, as described below. To test the selectivity of the biosensor, dilutions of MIP-1α (2 pM), human recombinant MIP-3β (20 pM) and BSA (20 pM) were prepared and tested.

Binding experiments in saliva

The measurements were performed also in real matrix (human saliva). The saliva sample, harvested from a 43 years old periodontally healthy volunteer (see details and inclusion criteria below), were collected in 50 ml sterile tubes just before the analyses, centrifuged for cells and debris removal, diluted in a 1:50 ratio with sterile and filtered PBS, and processed. Recombinant MIP-1α was diluted in saliva at the same concentration as those in buffer. The measurements with biosensor were performed by dropping 50 μl of diluted samples on functionalised surfaces. After 5 minutes of incubation and a washing step with PBS, the plasmonic spectra were acquired and saved.

Also in this case, LOD, Kaff, and sensitivity al low concentrations were calculated by using the Langmuir model.

Binding parameters calculation

Data were managed as previously published.[32] Specifically, experimental measurements were taken in triplicate, and data were expressed as mean values and maximum standard deviation (SD). Dose-response curves were realized by plotting absolute values of the resonance

wavelength variation (Δλ), calculated with respect to the blank, versus the MIP-1α concentration. The experimental values were fitted by the Langmuir equation, recalled in Equation 1.

$$|\Delta\lambda| = |\lambda_c - \lambda_0| = |\Delta\lambda_{max}| \cdot \left(\frac{c}{K+c}\right) \quad \text{(Equation 1)}$$

Where λ_c is the resonance wavelength relative to the concentration c of the MIP-1α, λ_0 is the resonance value at the blank solution (zero concentration of MIP-1α), $\Delta\lambda_{max}$ is the maximum value of Δλ, calculated by the resonance saturation value minus the blank resonance value, and K is a dissociation constant.

At low analyte concentration, when c is much lower than K, Equation 1 can be considered linear, and its slope can be approximated as the biosensor sensitivity at low concentration, as described in Equation 2. In fact, by exploiting the Langmuir model (reported in Equation 1), the biosensor parameters can be obtained. Hence, the LOD can be calculated as reported in Equation 3 being approximated as the ratio between three times the SD of the blank, and the sensitivity at low concentration calculated as indicated in Equation 2. Equation 4 reports how the Kaff can be calculated by the Langmuir model parameters. OriginPro software (Origin Lab. Corp, Northampton, MA, United States) was used to obtain the Langmuir fitting of the experimental values.

$$\text{Sensitivity at low c} = \frac{|\Delta\lambda_{max}|}{K} \quad \text{(Equation 2)}$$

$$\text{LOD} = \frac{3 * \text{standard deviation of blank } (\lambda_0)}{\text{Sensitivity at low c}} \quad \text{(Equation 3)}$$

$$K_{aff} = \frac{1}{K} \quad \text{(Equation 4)}$$

MIP-1α detection in a real clinical scenario

The collection of saliva was performed at the Periodontology Unit of the integrated activity care Department of "Oral Surgery and Stomatology, Maxillo-Facial Surgery and Rehabilitation" of the University Hospital *Luigi Vanvitelli* following the protocol approved by the Institutional Review Board (Prot. 0016318/i of 26/05/2022). The two adult donors, both caucasian males, aged 43 (periodontally healthy subject) and 62 (periodontitis patient) were recruited on the basis of the following selection criteria: good systemic health, no medications for any acute or chronic pathological conditions, presence of at least ten couples of teeth, no oral mucosal diseases, no instrumental periodontal therapy, no topical antibiotic or antiseptic periodontal therapy, and nor taking of systemic antibiotics, steroid and nonsteroidal anti-inflammatory drugs in the previous three months. The saliva samples were provided by the two subjects after being warned not to eat or drink for two hours before the collection and immediately diluted (1:20 in PBS) and addressed to the analysis.