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Surface plasmon resonance-based immunoassay for procalcitonin

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HIGHLIGHTS

- We developed a surface plasmon resonance based procalcitonin (PCT) immunoassay.
- It detects PCT in the range of 4–324 ng mL^{-1} with a LOD of 4.2 ng mL^{-1}.
- It is rapid, simplified and analytically-superior to the conventional procedures.
- It determines PCT in diluted serum and EDTA plasma of patients.
- It has high precision similar to that of commercial ELISA.

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ABSTRACT

A surface plasmon resonance (SPR) biosensor has been developed for rapid immunoassay of procalcitonin (PCT) with high detection sensitivity and reproducibility. The 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)-activated protein A (PrA), diluted in 1% (v/v) 3-aminopropyltriethoxysilane (APTES) was dispensed on a KOH-treated Au-coated SPR chip, resulting in the covalent binding of PrA in 30 min. This "single-step" PrA immobilization strategy led to the oriented binding of the anti-PCT antibody (Ab) on a PrA-functionalized gold (Au) chip. The leach-proof immobilization procedure is five-fold faster than conventional counterparts, enabling high detection specificity and reproducibility. The IA detects 4–324 ng mL^{-1} of PCT with a limit of detection (LOD) and a limit of quantification (LOQ) of 4.2 ng mL^{-1} and 9.2 ng mL^{-1}, respectively. It was capable of detecting PCT in real sample matrices and patient samples with high precision. The Ab-bound SPR chips were stable for more than five weeks.

1. Introduction

PCT, a propeptide of calcitonin, has a molecular weight of 13 KDa and 116 amino acids. It comprises an N-terminal region, a C-terminal region of katacalcin, and a middle region of calcitonin consisting of 57, 21 and 32 amino acids, respectively [1]. As first described by Bohoun in 1992, PCT was a critical parameter for inflammation and is now well recognized for acute and severe bacterial as well as viral infection, similarly to C-reactive protein (CRP). Its concentration might be up to 300 ng mL^{-1} in patients with most severe cases in septic shock (unpublished observations). Procalcitonin is the natural precursor of calcitonin produced in the thyroid gland by C-cells and proteolysis, however, multiple tissues secrete procalcitonin during the early stages of inflammation. Considering its stability [2] with a half-life of ~24 h, hyperprocalcitoninemia in systemic inflammation or infection occurs within 2–4 h and often reaches peak concentrations in 8–24 h. It is very stable [3], and persists for as long as the inflammatory process continues [4]. Numerous assays for PCT for *in vitro* diagnostic (IVD) have been developed. Indeed, it is a promising biomarker for bacterial infections [5–7] and the early diagnosis of sepsis in critically ill patients [8,9]. PCT is also a useful biomarker in discriminating infectious fever from non-infectious one in febrile diseases [10] apart from identifying low-risk febrile infants [11], even though

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PCT is not a good infection marker in newborns [12]. With the exception of newborns, PCT serves as an important biomarker in pediatrics as its assessment is of importance in neonatal sepsis, pyelonephritis, pneumonia, cancer, bacterial infections, bone and joint infections, meningitis, and systemic inflammatory response syndrome after cardiac surgery [13].

When compared to generalized infection and sepsis, pulmonary infections do not lead to a very fulminant rise in PCT but still provides important information concerning the risk of ventilator-associated pneumonia [14] and hospital-acquired pneumonia [15]. The serial quantification of PCT can predict clinical outcomes in patients with community-acquired pneumonia (CAP) [16] apart from assessing the treatment response in such patients [17]. PCT can also differentiate between severe and very severe CAP [18] and between CAP and acute decompensated heart failure [19]. Additionally, PCT is a prognostic biomarker of early graft failure after heart transplantation [20]. It has also been associated with the prognosis of acute heart failure [21,22] and might be used as a predictor of long-term mortality in ischemic stroke patients [23]. The measurement of PCT can exclude bacterial infection and guide antibiotic treatment in congestive heart failure patients in the emergency department [24]. Longitudinal PCT measurements support the timely reduction of antibiotic treatment in intensive care units' (ICUs) patients, who are treated for suspected bacterial infections [25]. The serum PCT level is a marker of lower respiratory tract infections, which can be used for the diagnosis, prognosis and follow-up of antibiotic therapy [26]. Even in the absence of other predictors, a plasma PCT concentration of $\geq$10 ng mL^{-1} at the time of admission to ICU is a strong predictor of short-term mortality [27]. Along the same lines, elevated PCT levels have also been shown to predict mortality in critically ill neurological patients [28].

Despite a wide range of IA formats for the detection of PCT, the clinically-accredited IAs are still based on enzyme-linked immunosorbent assays (ELISA) [29,30], electrochemiluminescent IA [31] and immunoturbidimetric assay [32]. This adoption is mainly due to the high precision and high sensitivity of these formats, and the prominent role that it plays in the clinical diagnosis and decision-making in the healthcare settings. However, most of these conventional IA formats can take a few hours, which substantiate the critical need for rapid IA formats that enable PCT detection in minutes. Of importance is the development of a label-free SPR [33] has emerged as a potential rapid IA format, which enables label-free and real-time detection of numerous analytes. A wide range of SPR IA formats has been developed and used in bioanalytical settings. However, most of these formats employ expensive commercial SPR chips that are functionalized with carboxymethyl dextran, streptavidin, nitriloacetic acid, long chain alkanethiol molecules or lipophilic groups to facilitate antibody bioconjugation. Various immobilization chemistries [34–41] have also been developed for the binding of antibodies to the SPR Au chip, which directly impacts the bioanalytical performance of the assay [34,39]. In general, the selection of an optimal Ab immobilization strategy plays a critical role in assay development.

This paper advocates the use of a rapid Ab immobilization strategy, which involves the initial covalent binding of an intermediate Fc binding protein (PrA), followed by the oriented immobilization of Ab. The EDC-crosslinked PrA, prepared in APTES, was dispensed onto KOH-treated SPR Au chip and left incubated for 30 min at room temperature (RT). This strategy provides the covalent crosslinking of free amino groups of APTES to the carboxyl groups on capture Ab, and the simultaneous binding of the alkoxy groups of APTES to the hydroxyl groups present on the KOH-treated chip. The analytical performance of the developed PCT IA was compared with that of a conventional SPR IA procedure using a commercial carboxymethyl (CM5) dextran chip. The assay precision for the detection of PCT in EDTA-plasma samples of patients was evaluated and corroborated with that of a commercial sandwich ELISA. Other evaluated bioanalytical parameters include interferences, inter-day and intra-day variability, fabrication reproducibility and storage stability. Table 1.

2. Materials and methods

2.1. Materials

3-Aminopropyltriethoxysilane (APTES, purity 98%, w/v), Tween 20, H_2O_2 (30%, v/v), stop solution, PrA, protein G (PrG), protein A/G (PrA/G), EDC, *N*-hydroxysulfosuccinimide (sulfo-NHS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, while 2-(*N*-morpholino)ethane sulfonic acid (MES, pH 4.7) was from Thermo Fischer Scientific. The components of human PCT IA comprising the Duoset PCT kit, consisting of mouse anti-human PCT capture Ab, biotinylated sheep anti-human PCT detection Ab, recombinant human PCT standard, and streptavidin-HRP, were purchased from R&D Systems. The nonspecific control proteins, such as recombinant human serum albumin (HSA), CRP, human fetuin-A (HFA), human lipocalin 2 (LCN2), interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α, were obtained from RnD Systems. All buffers and solutions were prepared in 18 MΩ Milli-Q ultrapure water (UPW) filtered through a 2 μm filter. The SPR Au chips (SIA kit), CM5 dextran (CMD)-functionalized Au chips, ethanolamine hydrochloride (1 M, pH 8.5), HBS-EP (0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v surfactant P20), and glycine-HCl (10 mM, pH 2.0) were procured from GE Healthcare. The running buffer for BIAcore and all sample dilutions was HBS-EP. SPR was performed on the BIAcore 3000 from GE Healthcare. The conventional sandwich ELISA procedure was performed using the guidelines provided by the supplier. The anonymized left-over EDTA plasma samples of patients treated by the ICU at the University Hospital Ulm, Germany were provided by Dr. Eberhard Barth in order to validate the developed PCT IA. The PCT spiked human plasma and serum samples were prepared by spiking various concentrations of PCT in a fixed dilution (1:10) of human plasma. The EDTA-plasma samples from the patients were diluted 1:10 using the standard sample preparation guidelines for clinical IA. This enables the unknown PCT concentration within the samples to fall within the detection range of the developed IA.

2.2. Immobilization of capture anti-PCT Ab

The immobilization procedure involves the induction of hydroxyl groups on the SPR Au chip by treatment with 90 μL of 1% (w/v) KOH for 5 min and washing extensively with UPW. 990 μL of PrA (200 μg mL^{-1}) was mixed with 10 μL of EDC (4 mg mL^{-1} in 0.1 M MES, pH 4.7) and incubated for 15 min at RT. The resulting EDC-activated PrA was diluted in 1% (v/v) APTES and subsequently, 90 μL of the resulting solution was dispensed onto the hydroxylated SPR chip and left incubated for 30 min at RT in a fume hood (Fig. 1). The PrA-functionalized chip was washed extensively with HBS and docked into the BIAcore 3000 system. The chip was primed and 50 μL of anti-human PCT capture Ab (100 μg mL^{-1}) was injected at 10 μL min^{-1}, enabling the oriented Ab immobilization onto the PrA-functionalized SPR chip. This was followed by the injection of 20 μL of 2% (w/v) BSA at 10 μL min^{-1}, to block non-specific protein binding sites on the chip. For comparison, the PrG and PrA/G-functionalized SPR chips were prepared by the same procedure except that PrA was replaced by PrG and PrA/G, respectively. The details of various Ab immobilization procedures used for the

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Table 1
Clinical relevance of human procalcitonin (PCT).

Clinical relevance of PCT	References
Biomarker for bacterial infections	[5–7,13]
Biomarker for sepsis	[8,9]
Discrimination of infectious from non-infectious fever in febrile disease	[10]
Identification of low-risk febrile infants	[11]
Biomarker for pneumonia	[14–19]
Biomarker for early graft failure after heart transplantation	[20]
Associated with prognosis of acute heart failure	[21,22]
Predictor of mortality in patients admitted to the hospital	[23]
Guide for antibiotic therapy	[24,25]
Biomarker for lower respiratory tract infections	[26]
Predictor of short-term mortality in ICU patients	[27]
Predictor of mortality in critically ill neurological patients	[28]

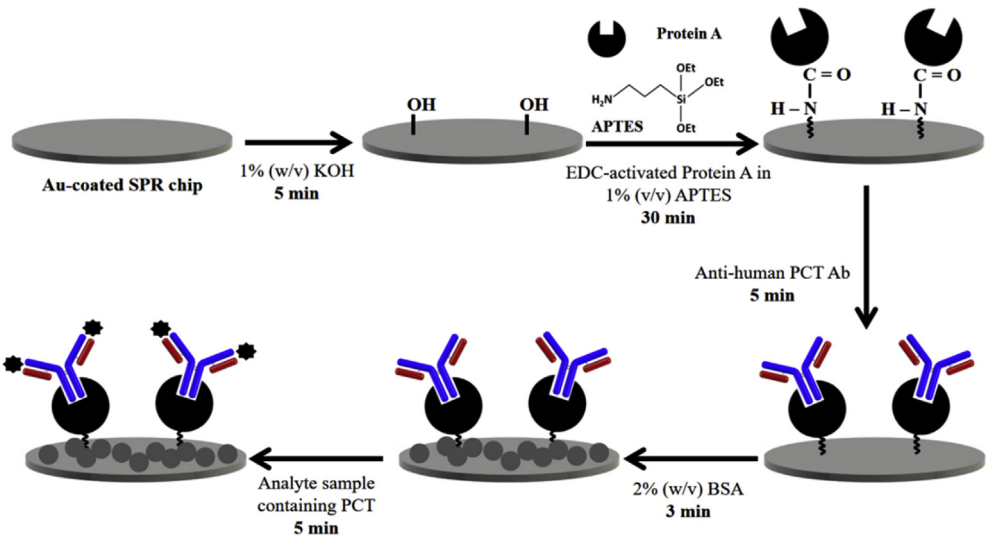

Fig. 1. Schematic demonstrating the bioanalytical procedure of the developed SPR based human PCT IA. It involves the induction of hydroxyl groups on the SPR Au chip by treatment with 1% KOH. This is followed by dispensing EDC-activated Pr A in 1% APTES onto the chip and incubating it at RT for 30 min. The alkoxy groups of APTES bind the hydroxyl groups on the chip while the free amino groups of APTES are crosslinked to the carboxyl groups of EDC-activated PrA. This is followed by the oriented binding of anti-human PCT Ab to PrA-coated chip, blocking with 2% BSA, and detection of CRP.

comparative assessment of the developed Ab immobilization procedure are provided in the supplementary section. A simplified Ab immobilization procedure is adapted from immobilization chemistry described by Vashist et al. [42,43] by adding PrA together with APTES during the immobilization step towards the realization of the oriented binding of Ab.

2.3. Detection of PCT

The detection of PCT was performed by passing PCT (50 μL, 4 ng mL^{-1}) through three detecting flow cells (FCs) and a reference FC of the SPR chip at 10 μL min^{-1}, and determining the SPR response units (RU) for all FCs. After each PCT IA, the PrA-bound SPR chip was regenerated by treatment with 20 μL of 10 mM glycine-HCl, pH 2.0 at 10 μL min^{-1}. Thereafter, the regenerated chip was used for the detection of other PCT concentrations (12, 36, 108 and 324 ng mL^{-1}) after binding to the capture anti-human PCT Ab. The RU value, corresponding to a given PCT concentration, was determined by subtracting the RU of the reference FC from the average RU value of three detecting FCs. The analyte loss due to its nonspecific adsorption on sample vials was minimized by preparing the dilution of PCT sample in BSA-blocked glass vials, which were prepared by treatment with 2% (w/v) BSA for 30 min at RT [44]. SigmaPlot version 11.2 software was employed for plotting the SPR-based PCT IA curves using a four-parameter logistic equation (eqn (1)) [39,45] to provide the desired critical bioanalytical parameters of maximum half-effective concentration (EC_{50}), R^2 and the Hillslope.

$$y = \frac{x_{min} + (X_{max} - X_{min})}{\left(1 + \left(\frac{X}{EC50}\right)^{-Hillslope}\right)} \quad (1)$$

The LOD and LOQ were determined as 3 × (standard deviation of the blank/slope) and 10 × (standard deviation of the blank/slope), respectively [43]. Fabrication reproducibility of the developed IA procedure was evaluated by comparing the bioanalytical performance of ten PrA-coated SPR Au chips for the detection of 36 ng mL^{-1} of PCT.

3. Results and discussion

3.1. Development of SPR-based PCT IA

The developed SPR-based PCT IA has the dynamic detection range of 4–324 ng mL^{-1} (R^2 = 0.999) with linearity from 12 to 324 ng mL^{-1}. The LOD and LOQ of the assay are 4.2 ng mL^{-1} and 9.2 ng mL^{-1}, respectively (Fig. 2A–B), with EC_{50} of 64.4 ng mL^{-1}.

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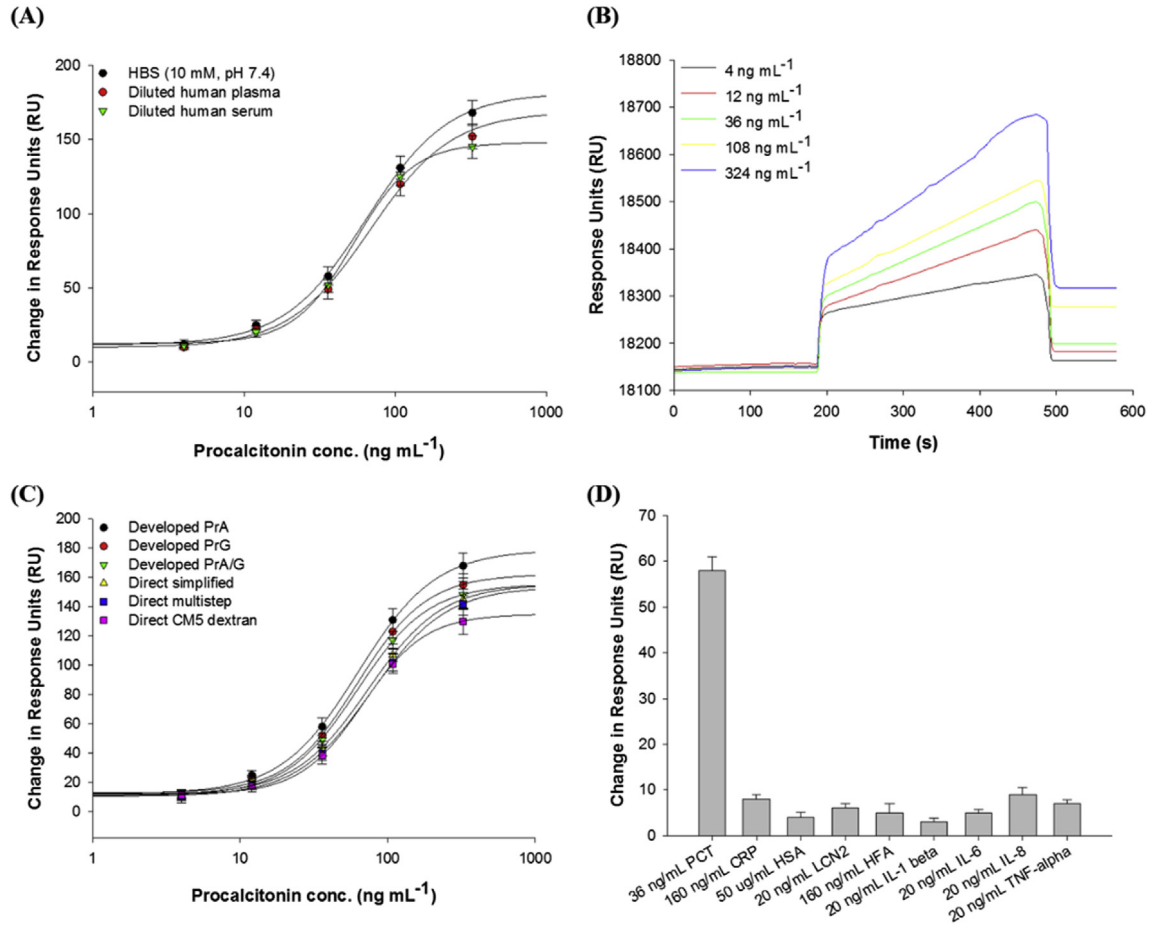

Fig. 2. The bioanalytical performance of the developed SPR-based human PCT IA. (A) Detection of HFA spiked in HEPES-buffered saline (HBS, 10 mM, pH 7.4), diluted human serum and diluted human plasma. (B) A typical sensorgram for the detection of various PCT concentrations in HBS. (C) Comparison of the SPR PCT IAs based on the use of various Ab immobilization strategies. (D) Evaluation of the specificity of the developed SPR IA for the detection of human PCT w.r.t. various non-specific control proteins: C-reactive protein (CRP), human serum albumin (HSA), human lipocalin-2 (LCN2), human fetuin-A (HFA), interleukin (IL)-1 beta, IL-6, IL-8 and tumor necrosis factor (TNF)-alpha. All experiments were performed in triplicate with the error bars representing the standard deviation.

The intra-day and inter-day variabilities of the IA were determined by five IA repeats (in triplicate) on a single day and five assay repeats (in triplicate) on five consecutive days, respectively. The intra-day variability ranged from 1.3 to 5.6% for various PCT concentrations in the IA while the inter-day variability was between 1.5 and 7.8%. Therefore, the variability was well within the acceptable range of less than 20%, as suggested by the bioanalytical method guidelines provided by the United States Food and Drug Administration.

The high analytical sensitivity of the IA is attributed to the higher Ab immobilization density and the oriented Ab immobilization via the intermediate fragment crystallizable (Fc) binding protein i.e. PrA. The higher Ab immobilization density is a result of the higher crosslinking of PrA to the APTES-functionalized SPR Au chip. PrA binds the constant Fc binding region of the Ab and orientates the Ab so that its antigen-binding sites on the fragment antigen-binding (Fab) region are free and accessible for binding to PCT.

The developed Ab immobilization procedure involves the covalent crosslinking of EDC-activated PrA to the KOH-treated SPR Au chip in just 30 min using only a single step. The EDC-activated PrA binds covalently the free amino groups of APTES in solution while the ethoxy groups of APTES bind simultaneously to the hydroxyl groups induced on the KOH-treated SPR chip. Subsequently, the PrA-bound chip enables the oriented immobilization of anti-human PCT capture Ab. The PrA immobilization procedure employs the optimal APTES concentration of 1% (v/v) with a corresponding incubation period of 30 min, as shown in Figs. S1A and S1B, respectively (Supplementary Information). The PrG and PrA/G bound SPR chips, used for comparison, were prepared by the similar procedure. The direct Ab bound SPR chip was prepared by the direct binding of EDC-activated anti-human PCT Ab, diluted in APTES, to the KOH-treated chip. The developed simplified oriented Ab immobilization procedure was compared to the multistep immobilization procedure based on the binding of EDC-activated PrA to the APTES-functionalized SPR chip [39].

All IAs were performed under identical conditions using the same IA components, thereby obviating any experimental variability. The PrA-based Ab immobilization procedure provided the highest Ab immobilization density compared to the other Ab immobilization schemes, (Table 2). The Ab immobilization densities were in the order of developed PrA > developed PrA/G > developed PrG > direct simplified > direct CM5 dextran > direct multistep. Correspondingly, the number of PCT molecules detected was in the order of developed PrA > developed PrG > developed PrA/G > direct simplified > direct multistep > direct CM5 dextran (Table 2) (Fig. 2C). The higher Ab immobilization density does not always result in higher analyte detection as the Ab may not be bound in the functionally active orientation, i.e., the antigen-binding sites of the Ab is not free for binding to analyte molecules [39]. This is the rationale behind the varying orders of Ab

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Table 2
Determination of molecular densities of anti-human PCT Ab bound and PCT detected by various SPR IA formats using different Ab immobilization strategies.

Ab immobilization procedure	Immobilization of anti-human PCT Ab			Detection of PCT[c]			EC_{50} (ng mL^{-1})
	ΔRU	Mass density[a] (ng cm^{-2})	Molecular density[b] (molecules cm^{-2})	ΔRU	Mass density[a] (ng cm^{-2})	Molecular density[b] (molecules cm^{-2})	
Developed PrA	1788.0 ± 8.7	178.8 ± 0.9	(7.2 ± 0.04) x10^{11}	58.0 ± 4.6	5.8 ± 0.5	(2.7 ± 0.2) x10^{11}	63.1
Developed PrG	1720.0 ± 7.5	172.0 ± 0.8	(6.9 ± 0.03) x10^{11}	54.0 ± 4.8	5.4 ± 0.5	(2.5 ± 0.2) x10^{11}	62.0
Developed PrA/G	1736.0 ± 6.5	173.6 ± 0.7	(7.0 ± 0.03) x10^{11}	52.0 ± 3.7	5.2 ± 0.4	(2.4 ± 0.2) x10^{11}	62.3
Direct simplified	1570.0 ± 9.4	157.0 ± 0.9	(6.3 ± 0.04) x10^{11}	41.0 ± 5.0	4.1 ± 0.5	(1.9 ± 0.2) x10^{11}	73.6
Direct multistep	1490.0 ± 11.9	149.0 ± 1.2	(6.0 ± 0.05) x10^{11}	38.0 ± 3.2	3.8 ± 0.3	(1.8 ± 0.1) x10^{11}	77.6
Direct CM5 dextran	1530.0 ± 8.8	153.0 ± 0.9	(6.1 ± 0.04) x10^{11}	36.0 ± 4.4	3.6 ± 0.4	(1.7 ± 0.2) x10^{11}	67.6

ΔRU: Change in resonance units (RU) caused by binding.
[a] Calculated using the commonly used conversion factor i.e. 1000 RU = 100 ng cm^{-2} [55–59].
[b] Calculated by [Mass density (ng cm^{-2})/Molecular weight (in ng)]. The molecular weight of anti-PCT antibody and PCT are 150 kDa and 13 kDa, respectively. In order to calculate molecular weight in SI units, the conversion factor 1 kDa = 1000 Da = 1.66 × 10^{-21} g was used. The molecular weight of anti-human PCT Ab and PCT are 24.9 × 10^{-11} ng and 2.16 × 10^{-11} ng, respectively.
[c] Calculations were performed for the detection of 36 ng mL^{-1} of PCT, i.e. the concentration near the EC_{50}.

immobilization densities and the number of PCT molecules detected. Nevertheless, the developed PrA-based Ab immobilization procedure has the highest Ab immobilization density and detects the highest number of PCT molecules. Such results confirm that it enables the most functionally effective immobilization of Ab on the SPR chip that results in greater detection sensitivity. (see Table 3).

The SPR-based IA using the developed PrA immobilization strategy outperformed the use of PrG and PrA/G, possibly due to the strong binding affinity of PrA to anti-PCT Ab in comparison to PrG. Therefore, PrA/G, a recombinant protein formed by the fusion of PrA and PrG, exhibits stronger binding affinity than PrG but comparatively lesser affinity in comparison to PrA. The SPR IA, using the PrA based Ab immobilization, is better than the direct simplified Ab immobilization due to the oriented binding of Ab that results in higher detection sensitivity. The direct simplified Ab immobilization is better than the multistep covalent Ab immobilization, which is mainly due to the highly efficient binding of APTES in solution to EDC-activated Ab and KOH-treated SPR Au chip [39]. The conventional CM5 dextran-based Ab immobilization procedure did not show comparable bioanalytical performance, plausible owing to the binding of Ab by its amino-terminal groups that are located near their antigen-binding sites. Therefore, the crosslinking of Ab by their amino terminal groups might cause partial masking of its antigen-binding sites, resulting in decreased detection sensitivity (Table 2).

The oriented Ab immobilization strategy is highly prospective for the development of IAs due to its higher simplicity, critically reduced duration, a lesser number of steps and higher detection sensitivity (Fig. S1). The results are in agreement with the literature findings, which recognize that the oriented and functionally-effective immobilization of Ab plays a critical role in improving the bioanalytical performance of an IA [46–51]. Due to the availability of numerous oriented Ab immobilization strategies [40,52–54], the screening of an appropriate strategy is an essential preliminary step towards the development of analytically-superior biosensors, *in vitro* diagnostics, lab-on-a-chip, and other miniaturized point-of-care IA formats.

Despite the excellent shelf life of PrA-bound SPR chip, it is more desirable to prepare the PrA-bound chip just before the IA to obviate the refrigeration of SPR chips, any undesirable storage effects, and high costs. The standard bioanalytical testing guidelines in healthcare and industrial settings do not recommend the use of PrA- or Ab-prebound chips/platforms after 2–4 weeks for most assays. Therefore, the developed rapid bioanalytical procedure for preparing PrA-functionalized chips within 30 min just prior to use is more appealing and cost-effective.

As mentioned previously, a microcontact imprinted SPR biosensor for PCT has been developed by Sener et al. [33] with a LOD of 9.9 ngmL^{-1}and a required duration of 1 h. Such a procedure involved the immobilization of PCT onto an APTES activated glass support. The surface was then brought into contact with a solution of 2- hydroxyethyl methacrylate and ethylene glycol dimethacrylate, followed by polymerization initiated by ultraviolet (UV) light at room temperature. After the removal of the PCT molecules, the specific molecular recognition sites were demonstrated, using PCT solutions and simulated blood plasma at different concentrations. Albeit several steps are required to fabricate such an imprinted recognition surface, this technique is amenable to mass production and the imprinted recognition polymer is more cost effective and robust, compared to its natural antibody counterpart. Considerable efforts are also needed to ensure the fabrication of such imprinted surfaces with excellent reproducibility. Nevertheless, the use of the anti-PCT antibody as described in our study enables a rapid detection of PCT in just a few minutes using a highly simplified bioanalytical procedure with high detection sensitivity.

Table 3
Determination of PCT in the EDTA plasma samples of patients with the developed SPR IA and the commercial sandwich ELISA.

Samples	Conventional	Developed	
	Determined conc. (ng mL^{-1})	Determined conc. (ng mL^{-1})	Percentage recovery
1	34.8	35.0	98.9
2	45.2	44.9	98.2
3	63.1	62.8	98.6
4	81.7	82.2	102.2
5	130.2	132.1	102.6
6	225.2	226.6	99.2
7	247.8	246.9	99.6
8	275.2	277.6	102.5

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3.2. Determination of bioanalytical parameters

The developed SPR IA detects PCT spiked in diluted human whole serum and plasma (Fig. 2A), which are the routinely used sample matrices for clinical IAs. There was a minor matrix effect, obviously due to the varying nature of the sample matrices. For the determination of PCT in the EDTA-plasma samples of patients, the results were correlated with those obtained by the conventional sandwich ELISA. (Table 2), thereby demonstrating its high precision. There was no significant interference from various non-specific control proteins such as CRP, LCN2, HFA, HSA, IL-1β, IL-6, IL-8 and TNF-α (Fig. 2D). These tested proteins are usually associated with PCT in patients with infections and inflammatory disorders.

The fabrication reproducibility for the preparation of the PrA-bound SPR Au chip was determined by preparing ten PrA-coated chips, binding them to anti-human PCT capture Ab, and analyzing the SPR RUs for the detection of PCT at 36 ng mL^{-1}. A high fabrication reproducibility is attributed to functionally uniform oriented Ab immobilization that leads to reproducible analyte detection (Fig. 3A). The storage stability of anti-human PCT Ab-bound SPR chips was also evaluated by storing ten freshly prepared Ab-bound chips at 4 °C for 10 weeks and using the SPR chip after every week for the detection of 36 ng mL^{-1} PCT. There was no decrease in the SPR RUs for up to 5 weeks (Fig. 3B), attesting the functionally stable covalent binding of PrA to the SPR Au chip and the leach-proof oriented immobilization of anti-human PCT Ab to PrA. However, there was a steady decrease in the functional activity of Ab-bound SPR chips after 5 weeks that led to 14% decrease in SPR response after 10 weeks. This makes the developed IA ideal for routine bioanalytical settings that employ the Ab-prebound SPR chips for rapid analyte detection. The end-user can prepare the Ab-bound SPR chip just prior to their intended use as it takes just 30 min. Further, the PrA-coated SPR Au chip was completely regenerated after an IA by treatment with 20 μL of 10 mM glycine-HCl, pH 2.0 at 10 μL min^{-1}, which is a standard regeneration procedure for the BiaCore-based SPR IA. The regeneration solution is available commercially from GE Healthcare. The PrA-coated SPR Au chip was used for 40 repeated IAs to detect 36 ng mL^{-1} PCT by regenerating the chip after each IA (Fig. 3C). In brief, there was no significant decrease in the PCT response for 32 regeneration cycles, but it gradually decreased thereafter to ~65% of the initial response after 40 regeneration cycles. This is very critical for resource-limited settings as the same PrA-coated chip can be used for the detection of other targets. However, the bioanalytical guidelines for clinical diagnostics do not recommend the reuse of the same diagnostic

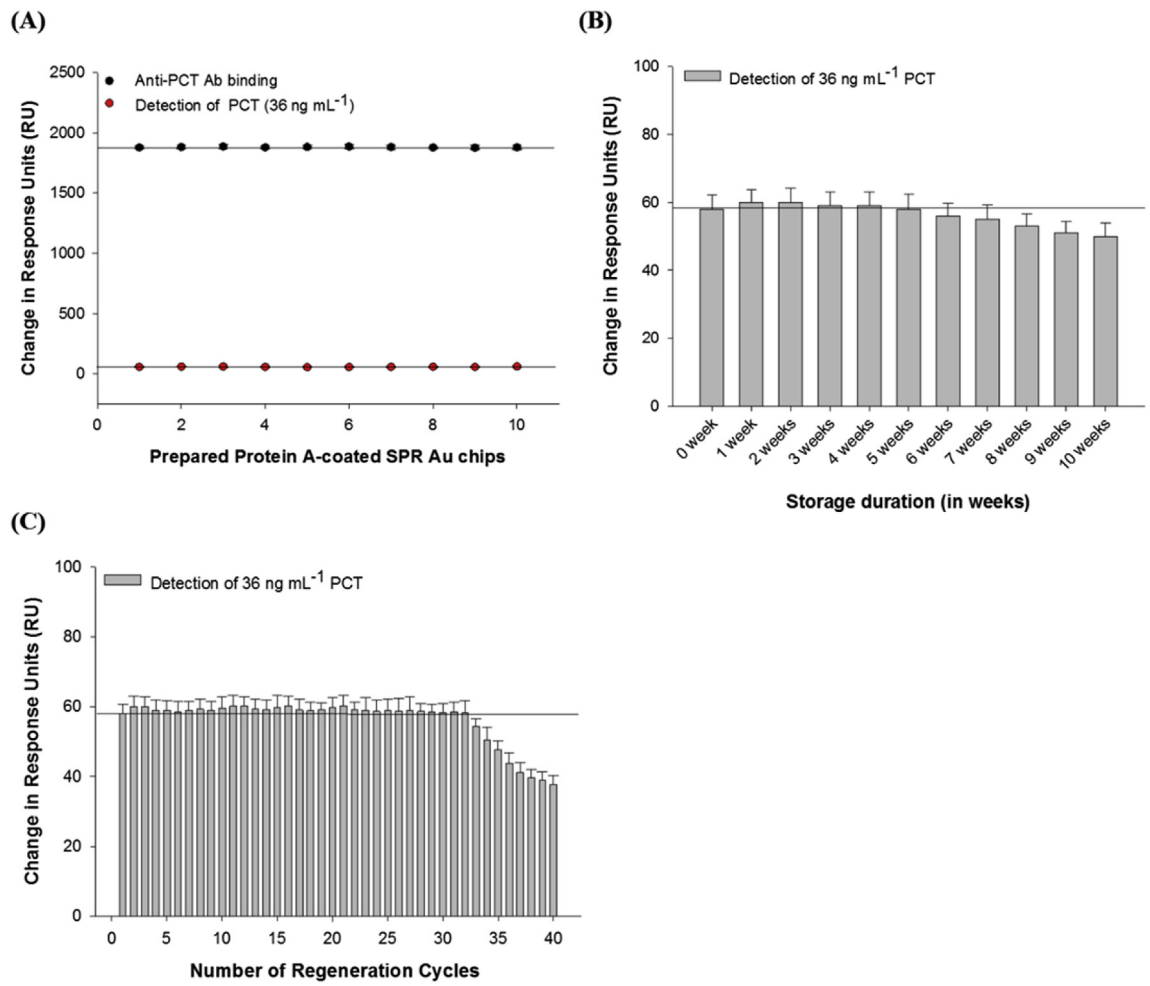

Fig. 3. (A) Fabrication reproducibility of the PrA-coated SPR Au chips prepared by the developed procedure. The reproducibility was assessed by determining the SPR responses of 10 freshly prepared PrA-coated chips for the binding of anti-human PCT Ab and PCT (36 ng mL^{-1}). (B) Stability of anti-human PCT Ab-bound SPR chips stored at 4 °C for 10 weeks, as determined by the detection of 36 ng mL^{-1} PCT. (C) Efficiency of regenerating the PrA-bound SPR Au chip, as determined by the use of a particular SPR chip for 40 repeated IAs to detect 36 ng mL^{-1} PCT by regenerating the chip after each IA. All experiments were performed in triplicate with the error bars representing the standard deviation.

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platform (SPR chip) for multiple patient samples and clearly specify the disposal of used platform after each IA."

The developed SPR IA procedure has superior bioanalytical performance, high reproducibility, prolonged stability, rapid response time, high simplicity, cost-effectiveness and good correlation with established sandwich ELISA. Moreover, being generic in nature, it can be employed for the detection of other analytes and disease biomarkers.

4. Conclusion

The SPR-based IA enables the highly sensitive detection of PCT in the clinically relevant range in less than 20 min using a rapid and simplified bioanalytical procedure based on the oriented immobilization of capture Ab facilitated by PrA. Detection specificity for PCT was confirmed by no significant interference from the control proteins. This cost-effective procedure employs just bare SPR Au chips, which are much less expensive than their pre-functionalized counterparts. Moreover, it obviates the need for complex process steps and additional chemicals that are required for prevalent Ab immobilization chemistries. The results obtained for the determination of PCT in EDTA plasma samples of patients correlate well with those obtained by the commercial sandwich ELISA kit. Therefore, the developed SPR-based IA could be reliably employed in bioanalytical settings for the detection of PCT.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.08.007.

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