Evaluation of PAMAM dendrimers (G3, G4, and G5) in the construction of a SPR-based immunosensor for cardiac troponin T

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Abstract

A SPR-based immunosensor was developed using a SAM of an alkanethiol associated with dendrimers PAMAM(G4) to enhance the sensitivity for troponin T detection in blood samples. The feasibility of using three-dimensional platforms based on dendrimers for the development of immunosensors was demonstrated by evaluating three different generations of these dendrimers (G3, G4, and G5) to detect troponin T. The results showed the efficiency of these 3D platforms in anchoring biomolecules, amplifying the detection of troponin T. The sandwich assay showed good performance for troponin T detection, using secondary monoclonal antibodies, in the concentration range of 5-300 ng mL⁻¹ (0.14 - 8.67 nmol L⁻¹), R²=0.991, with a LOD of 3.6 ng mL⁻¹.

The sandwich assay’s applicability was demonstrated by evaluating a secondary polyclonal antibody’s performance in the concentration range of 3-30 ng mL⁻¹, R²=0.998, with a LOD of 0.98 ng mL⁻¹. The immunosensor was applied to determine troponin T in blood plasma samples from healthy patients, with an average recovery of 88% to 104%. The performance of the SPR-based immunosensor indicates reliable results, contributing to the rapid diagnosis of heart attack, with reduced costs.

Keywords: Surface plasmon resonance; Immunosensor; PAMAM dendrimer; Acute myocardial infarction; Cardiac Troponin T.
Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally, estimating 18 million deaths each year (31% of all deaths worldwide), according to the World Health Organization\(^1\). Among CVD deaths, acute myocardial infarction (AMI) is one of the leading causes of mortality, and the risk of fatality is higher in the first hours after the event. The inadequate diagnosis can cause people’s hospitalization without AMI and the dismissal of patients affected by the problem. Thus, rapid and accurate diagnosis is critical to initiate appropriate treatment and increase survival chances.\(^2,3\)

The safest strategy for diagnosis at the very beginning of AMI is to quantify cardiac markers released into the bloodstream immediately after the onset of AMI. These cardiac biomarkers must have high specificity, rapidly released to enable rapid diagnosis, and be quantifiable since misdiagnosis can result in fatalities. Several cardiac biomarkers have been identified over the years, such as myoglobin, creatine kinase, cardiac troponins, and lactate dehydrogenase. Among them, troponins T (35 kDa) and I (24 kDa) are the most used in diagnosis due to the excellent specificity and sensitivity for detecting AMI.\(^3-7\)

These troponins are released even before the onset of symptoms, remaining in the bloodstream for 10 days, with a peak concentration 1-2 days after cell necrosis. Heart cell necrosis occurs 2-4 hours after the onset of AMI, and troponin levels are only measurable after this period. Normal blood troponin T levels are on the order of 0.1-1.0 ng mL\(^{-1}\), and values above these are already considered for the diagnosis.\(^4,8,9\)
Thinking about devices that provide sensitive and selective detection with low reagent consumption with the possibility of miniaturization highlights the biosensors. The biosensors involve an interdisciplinary field that combines biological material selectivity with the transducer’s sensitivity that converts the signal (chemical or biological) to a measurable qualitative and/or quantitative information. When antigen-antibody interaction is used in the recognition, the biosensors are called immunosensors. Immunosensors are based on the strong and selective binding of biomolecules, such as antibodies, to a target substance (antigen, protein), forming stable complexes, producing a measurable signal.\textsuperscript{10-14}

In the immunosensor construction, several steps must be considered to ensure the test’s stability and specificity, such as antibodies immobilization, type of assay (direct, sandwich or competitive), detection method, and blocking of nonspecific sites. All these factors are directly related to the employed transducer (electrochemical, piezoelectric, and optical). Among them, highlight the optical, in which a signal is generated (color) or a change in the optical properties (reflectance or refractive index) occurs after the recognition event. A photodetector collects this signal, converting an electrical signal, and processing it by the user.\textsuperscript{15,16} The main optical immunosensors stand out the colorimetric,\textsuperscript{17-19} such as paper-based tests, and the SPR-based ones.\textsuperscript{20-22}

One of the most critical aspects of the immunosensor construction is the immobilization methods employed for capture antibody. The self-assembled monolayer (SAM) is quite well employed, promoting the molecule's immobilization by forming organic films with high stability.\textsuperscript{23,24} However, even using SAM, a two-dimensional system, the SPR-based sensor's sensitivity may be limited depending on the purpose. In
this context, other biomolecule anchoring elements may be used to enhance response, such as chitosan, polyaniline (PANI), poly-l-lysine (PLL), 3D gels, dextran, etc.

Nevertheless, these layers have limitations in developing a stable, dense, and well-organized biosensor matrix. Besides, the leaching of bioreceptor molecules results in more considerable variations in signal intensity, in the case of 3D gels. In this point, three-dimensional macromolecules, such as dendrimers, have been proposed to overcome the mentioned limitations. Among these dendrimers, the most promising candidates for using a biosensor matrix are poly(amidoamine) dendrimers (PAMAM). These dendrimers are formed by well-designed branched polymers that mimic the three-dimensional structure of biomacromolecules with internal cavities and numerous surface amino-terminal groups. They are also biocompatible, making them excellent candidates for biosensor preparations. Therefore, combining PAMAM dendrimers and SAM may increase the sensitivity and stability of SPR-based immunosensors.

Currently, surface plasmon excitation associated with PAMAM dendrimers and SAM to evaluate surface processes has attracted much attention to enhance the sensitivity. The SPR technique is one of the main tools for assessing biological events, motivating the construction of SPR-based immunosensors. In the present paper, the effect of PAMAM dendrimer generations (G3, G4, and G5) on the response of an SPR sensor was evaluated to detect cardiac troponin T in blood plasma samples for rapid diagnosis of AMI.

**Experimental**

*Reagents and chemicals (Supporting Information)*
**Apparatus (Supporting Information).**

**Immunosensor construction**

Before forming a SAM of 3-MPA, the gold substrate was cleaned using piranha solution (1:3 mixture of 30% H$_2$O$_2$:H$_2$SO$_4$ conc.) for approximately 3-5 minutes, following the immersion in acetone (5 minutes) and isopropyl alcohol (5 minutes). Afterward, the substrate was washed with deionized water and dried under a stream of nitrogen. The functionalization of the gold surface for the immunosensor construction was performed by following the steps described below, as shown in Fig. 1.

**Figure 1**

Step 1 – SAM formation: The SAM was formed by the adsorption of 3-MPA on the gold surface from an ethanolic solution (1.0 mmol L$^{-1}$) overnight. The substrate was copiously washed with ethanol and acetate buffer solution (ABS) 10 mmol L$^{-1}$ pH 4.8 and dried with pure nitrogen. The terminal carboxylic groups of 3-MPA were activated by EDC (0.10 M)-NHS (0.15 M) in ABS for about 10 minutes.

Step 2 – PAMAM(G4) immobilization: The PAMAM(G4) (0.1 mmol L$^{-1}$) was spotted onto activated MPA/Au and allowed incubating for 30 minutes. After that, PAMAM(G4)/MPA/Au was washed three times with PBS to remove unbound dendrimers and once with ABS. Different generations (G3, G4, and G5) and concentrations of PAMAM dendrimers were evaluated in this step.

Step 3 – Capture antibody immobilization: Terminal carboxylic groups of capture antibody (Ab – 20 µg mL$^{-1}$) were activated by the addition of EDC (0.10 mol L$^{-1}$),
followed by spotted on PAMAM(G4)/MPA/Au using ABS, incubating for 40 minutes. Then, the Ab/PAMAM(G4)/MPA/Au was washed three times with ABS to remove unbound antibodies. In this step, different concentrations of Ab were evaluated (5, 10, 20, 30, and 40 \(\mu\)g mL\(^{-1}\)).

Step 4 – Blocking of non-specific adsorption sites: on the non-specific adsorption sites on the sensor surface were blocked by adding 30 \(\mu\)g mL\(^{-1}\) BSA in ABS for 5 minutes (500 \(\mu\)L to cover the disk), followed by the addition of 1M ethanolamine at pH 8.5 for 5 minutes (500 \(\mu\)L to cover the disk). This step is necessary to minimize or avoid non-specific binding that may occur during the assay.

The best buffer solution (ABS pH 4.8 or PBS pH 7.4) was evaluated for each immobilization in these steps.

*Optimization of experimental conditions (Supporting Information)*

*Sandwich assay for signal amplification (Supporting Information)*

*Samples assays*

Measurements demonstrated the applicability of the proposed immunosensor on blood samples from healthy patients. An optimized dilution factor was determined for the samples, and troponin T was added along with the secondary antibody to the samples. After stirring, this sample was added to the surface of the immunosensor to measure the angle variation.
Results and Discussion

Effect of generations and concentrations of the dendrimer

Firstly, it evaluated the effect of different generations of PAMAM dendrimers to be used in the immunosensor construction and the optimal concentration of each one. From the third generation, these dendrimers adopt a spherical shape, which increases the exposure of the terminal amino groups.\textsuperscript{33} Previously, the terminal carboxylic groups of 3-MPA (SAM) were activated via EDC/NHS for 10 minutes. Thus, PAMAM dendrimers generations 3, 4 and 5, were immobilized on the SAM, and the variations of SPR angle (\(\Delta \theta_{\text{SPR}}\)) of the species were observed.

Fig. 2 shows the effective \(\Delta \theta_{\text{SPR}}\) obtained for the immobilization step of PAMAM dendrimers G3 (Fig. 2a), G4 (Fig. 2b), and G5 (Fig. 2c), for different concentrations. It can be noted that the immobilization of PAMAM dendrimers have been a distinct maximum of \(\Delta \theta_{\text{SPR}}\) for each generation: PAMAM(G3) at 0.25 mmol L\(^{-1}\), PAMAM(G4) at 0.10 mmol L\(^{-1}\), and PAMAM(G5) at 0.05 mmol L\(^{-1}\), indicating that at these concentrations there is maximum coverage of the sensor surface. Concentrations higher than those mentioned promoted a decrease in \(\Delta \theta_{\text{SPR}}\), which may be related to the favoring the formation of the clusters, making it difficult to bind to the 3-MPA functional groups. Another relevant observation is that as the size of these PAMAM dendrimers increases (from G3 to G5), a lower concentration is required to achieve a maximum surface coverage, suggesting that the size of these three-dimensional structures directly affects the organization of this layer on the surface.

To optimize the best PAMAM dendrimer to be employed in the immunosensor construction, three immunosensors were constructed, with 0.25 mmol L\(^{-1}\) of
PAMAM(G3), 0.10 mmol L\(^{-1}\) of PAMAM(G4), and 0.05 mmol L\(^{-1}\) of PAMAM(G5). After that, the capture antibody was immobilized using a solution in a concentration of 20 µg mL\(^{-1}\). All obtained sensors were submitted to interact with troponin T (concentration range: 2.5 to 20.0 µg mL\(^{-1}\)). These results can be seen in Fig. S1 (Supporting Information), which shows a linear response (Δθ\(_{\text{SPR}}\)) as a function of the logarithm of Troponin T concentration. From analytical curves, it is possible to observe the viability of using PAMAM dendrimers, regardless of generation, to construct the immunosensors. The PAMAM(G4) was chosen as the dendrimer to be used in immunosensor construction due to its spherical form\(^{34}\) compared to G3, which may favor a better immobilization of antibodies, with less steric hindrance. Compared to PAMAM G5, the excess of unbound functional amino groups may hinder blockade in later stages. In addition, the effective Δθ\(_{\text{SPR}}\) obtained for PAMAM(G4) immobilization at 0.1 mmol L\(^{-1}\) (Fig. 2b) was the highest, indicating better surface coverage, with this dendrimer generation.

Dendrimers molecules form a dense film on the surface, which allows these molecules to bind strongly to proteins through a higher number of terminal groups. Thus, the number of terminals (generation) groups on a dendrimer is expected to influence the sensitivity.\(^{25}\) However, increasing the number of terminal groups increases the size of the molecule, and, the further away from the surface, detection occurs, the lower the sensor's sensitivity.

**Figure 2**

*Optimization of the capture antibody concentration (Supporting Information)*
Optimization of the blocking agents on non-specific adsorption sites

Currently, almost all immunosensors use BSA as a blocking agent for uncoated surface sites or possibly exposed reactive groups.\textsuperscript{33,38} Other blocking agent is ethanolamine (EA), a small molecule capable of blocking unreacted carboxylic groups on the sensor.\textsuperscript{39} The combination of these two blocking agents, under the right conditions, can contribute to inactivating the non-specific adsorption sites, making the sensor less susceptible to interferences, especially for the detection of smaller molecules in complex samples.

Thus, the blockage of non-specific sites in the proposed immunosensor was evaluated, employing a solution of \(30 \mu g \text{mL}^{-1}\) BSA for 5 minutes and \(1.0 \text{mol L}^{-1}\) EA at pH 8.5 for 5 minutes. The blocking step efficiency was verified with standard samples of \(5 \mu g \text{mL}^{-1}\) troponin T (positive sample), and \(5 \mu g \text{mL}^{-1}\) BSA (control sample)\textsuperscript{40}, and the \(\Delta \theta_{\text{SPR}}\) values obtained in this step can be seen in Table S1 (Supporting Information).

Table S1 (Supporting Information) shows the effective \(\Delta \theta_{\text{SPR}}\) values for immunosensors without blocking and with blocking, for the addition of \(5 \mu g \text{mL}^{-1}\) troponin T (positive) and \(5 \mu g \text{mL}^{-1}\) BSA (control) solutions. The effective variation decreases considerably after the blocking step. The BSA+EA blocker’s efficiency was demonstrated by observing the signal obtained with the control sample, which showed no evidence of effective variation. Presumably, BSA blocked the terminal amino groups of PAMAM(G4), and ethanolamine promoted the blocking of the remaining terminal carboxylic groups of SAM. As it is a small molecule, ethanolamine probably penetrated the layers of the immunosensor and promoted the blockage of difficult access groups.
After establishing the blocking strategy, the proposed immunosensor was constructed in real-time to observe all the optimized stages. The obtained sensorogram can be seen in Fig. S3 (Supporting Information), and it can be noted that in approximately 4 hours, it was possible to construct an immunosensor, which responds to troponin T, as expected. It is noteworthy that despite the relatively long time of platform construction, the interaction time with the protein of interest can be considered short, about 30 minutes, which is interesting to apply in clinical samples.

**Regeneration, stability, and precision of the proposed immunosensor**

The most favorable solution for the regeneration was 10 mM sodium acetate buffer at pH 4.8 with 0.1% Tween 20 for 5 minutes (Fig. S4 (Supporting Information)), which promoted the complete removal of troponin T from the binding site, favoring a new interaction of the protein. The second regeneration was not possible. As the isoelectric point (pI) of troponin T is approximately 5.0, slightly acidic conditions (pH < pI) were shown to be more advantageous. Possibly the conformation adopted by the protein in these conditions promotes the shutdown of the antibody recognition site.

The stability of the proposed immunosensor was studied by measuring the effective Δθ_{SPR} for a fixed concentration every 4 days of storage at 4 °C. The immunosensor was stable for 24 hours, decreasing 9% in relation to the initial response, indicating that the preparation of several immunosensors can be performed to be used for two days without significant variations. After 48 hours of the first test, the signal loss was 33.6%, which shows that despite the sensitivity of the proposed immunosensor, stability is still a point to be considered concerning commercial device construction.
The performance of the SPR-based immunosensor in intra- and inter-day precision assays were evaluated for one concentration of troponin T (5 µg mL\(^{-1}\)) in PBS 10 mM pH 7.4. The intra-day assay was evaluated from four successive measurements (n=4) performed on the same day using the 3-MPA/PAMAM(G4)/Ab immunosensor, obtaining a relative standard deviation (RSD) value of 8.7%. For the inter-day assay, the analysis was performed on six different days (n=6) using six different 3-MPA/PAMAM(G4)/Ab immunosensors, obtaining an RSD value of 4.7%. The RSD values for intra- and inter-day were lower than 10% for both cases, so the developed immunosensor presented good precision in the troponin T determination.

Besides these evaluations, the linear range obtained with the proposed immunosensor for cardiac troponin T detection in the sandwich assay was 5-300 ng mL\(^{-1}\) using a secondary monoclonal antibody (mAb) and 3.0-30 ng mL\(^{-1}\) using a secondary polyclonal antibody (pAb). These assays will be discussed in detail in the next section.

**Detection of Troponin T with the immunosensor**

The performance of the proposed SPR immunosensor for the detection of cardiac troponin T was evaluated in a sandwich assay, firstly with 10 µg mL\(^{-1}\) of a mAb. Fig. S5 (Supporting Information) shows the sensorgrams obtained for each troponin T concentration that previously interacted with mAb. The analytical curve (R\(^2\)=0.991) derived from the data in Fig. S5 (Supporting Information) can be seen in Fig. 3, showing the linear relationship obtained to troponin T concentration in ng mL\(^{-1}\). In Fig S5 (Supporting Information), the total time of approximately 50 minutes can also be seen considering the stabilization of the baseline before the addition of troponin T and
after washing. However, the assay time for the interest protein was 30 minutes. From Fig. 3, it was obtained a LOD of 3.6 ng mL$^{-1}$, which was calculated similarly to the method employed in potentiometry, based on a linear relationship between the potential and the logarithm of concentration. Thereby, the LOD value was calculated according to the IUPAC guidelines from the intersection of extrapolating linear segments of the calibration graph$^{41-43}$ (Fig. S6 (Supporting Information)).

The concentration of troponin T in the blood within a few hours of the onset of AMI symptoms starts in the order$^5$ of 1.0 ng mL$^{-1}$ and increases reaching a troponin concentration peaks after 24 hours of about 50 ng mL$^{-1}$. The LOD value obtained with the sandwich assay is very close to the cut-off of positive tests for troponin T. So, the proposed immunosensor could quantify changes in blood troponin T levels, assisting a rapid diagnosis of a heart attack.

**Figure 3**

The same test was performed with other secondary antibodies, pAb, to verify the sandwich assay's applicability. In this case, the capture antibody binds specifically between amino acids 171 to 190 of protein.$^{45}$ The pAb has chosen to be tested binds between amino acids 60 to 85$^{46}$, so there is no overlap of regions, reducing steric hindrance.

Thus, similarly to the previous test, a few minutes before the analysis, 10 µg mL$^{-1}$ of pAb was added to the troponin T solution to be monitored, and, as before, lower concentrations promoted considerable variations in the SPR angle for different troponin T concentrations. This assay's total time was the same for the mAb test, approximately
30 minutes, for troponin T interaction. The obtained analytical curve \( R^2 = 0.998 \) can be seen in Fig. 4, confirming the linear response of troponin T in the concentration level of ng mL\(^{-1}\), but for a narrower linear response range. The LOD estimated for this second sandwich assay with polyclonal antibody was 0.98 ng mL\(^{-1}\) of troponin T.

**Figure 4**

Observing the LOD values obtained with the monoclonal and polyclonal antibodies, the value was slightly smaller for pAb (polyclonal) than mAb (monoclonal). However, the sandwich assay with mAb presented higher sensitivity (Fig. 3B), probably due to the more specific interactions between the protein and the antibody. Thus, higher effective \( \Delta \theta_{\text{SPR}} \) was obtained even at lower concentrations (less than 5.0 ng mL\(^{-1}\)). The sandwich assays employing monoclonal antibodies provide more reproducible results and higher sensitivity values than those obtained with polyclonal ones, presumably because monoclonal antibodies are highly specific.\(^{47}\)

The comparison of the proposed immunosensor (3-MPA/PAMAM(G4)/Ab) with earlier sensors reported for cardiac troponin T is summarized in Table 1. The LOD achieved here is lower or comparable to those described in the literature. It is important to stress that the proposed immunosensor uses a less antibody than some of these sensors and dendrimers PAMAM to anchor the antibodies. Thus, the proposal to use three-dimensional macromolecules, as PAMAM dendrimers, to promote anchor and orientation of specific antibodies to troponin T is a novelty in the construction of SPR-based immunosensors for the diagnosis of this disease.
All sensors shown in Table 1 are based on SPR and have the advantages already mentioned. However, the method traditionally used to detect troponin T in serum samples is the electrochemiluminescence immunoassay. These immunoassays are based on the optical emission from the excited states of a luminophore, usually, ruthenium complexes, produced by electrochemical reactions in the solution.\textsuperscript{51,52} This method has many analytical applications, as it is highly sensitive and selective. However, the costs of automated equipment, which will carry out all the steps of a traditional immunoassay (immobilizations, reactions, and washes) standardized, and the generation of luminescence and analysis are considered a great challenge to overcome. Thus, methods for analyzing biological reactions, such as SPR, still have their place to contribute to clinical analysis.

Application of the proposed immunosensor for the determination of cardiac troponin T

The performance of the proposed SPR-based immunosensor for cardiac troponin T was applied in blood plasma from a healthy patient, fortifying with a concentration of troponin T (10 ng mL\textsuperscript{-1}), as a preliminary test. The determination of troponin T was performed by interpolation in the respective analytical curve: mAb (64.4 mº) and pAb (26.5 mº). The assays time was the same as that established for obtaining the calibration curves, of approximately 30 minutes for the interaction between troponin T and capture antibody.
By analyzing the results presented in Table 2, the recoveries ranged from 88% to 104% for this analyte in simulated blood plasma. From these values, it can be noticed that the recovery for the sandwich test indicates good accuracy in the developed assay. Also, it can be considered that the proposed immunosensor presented a reliable alternative for troponin T determination in samples of blood plasma and can be applied in patients affected by AMI, assisting in rapid and accurate diagnosis.

**Conclusions**

Different generations of PAMAM dendrimers were investigated to develop a SPR-based immunosensor for troponin T detection, in blood plasma, with high detectability. As described in this work, the PAMAM dendrimer generation 4 has shown better results than the others for cardiac troponin T quantification. Despite this, each system has its particularities, and different generations can also be employed in sensor construction. The regeneration strategy was developed to enable the reuse of immunosensor and promote a reagent economy in the calibration step. Besides, the RSD values for intra- and inter-day assays were lower than 10%, indicating a good precision in the troponin T determination. The sandwich assay was satisfactorily applied to quantify troponin T with secondary antibodies (monoclonal and polyclonal) in about 30 minutes. The LOD obtained were 3.6 and 0.98 ng mL$^{-1}$, respectively, which allowed the application in blood plasma samples. The dissociation constant, KD, was found to be 8.3x10$^{-7}$ M, indicating a high affinity between troponin T and the employed capture antibody. The developed dendrimer-based SPR immunosensor was successfully applied to detect troponin T in blood plasma, showing a reliable alternative to assist in diagnosing acute
myocardial infarction in hospital emergency triage with results in about 30 minutes. Our results suggest that the SPR-based immunosensor using PAMAM dendrimers present an excellent potential for practical applications, mainly in clinical diagnosis, and these results adding knowledge to development easy, fast, low-cost and sensitive diagnoses sensors.

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**Appendix A. Supporting Information**

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Table 1 Comparison of different SPR-based sensor for detection of cardiac troponin T

| SPR-based sensor          | Response range     | LOD       | Refs. |
|--------------------------|--------------------|-----------|-------|
| OEG/MHDA/Ab (10 µg mL\textsuperscript{-1}) | 100 ng mL\textsuperscript{-1} – 50 µg mL\textsuperscript{-1} | 100 ng mL\textsuperscript{-1} | [48] |
| MUDA/Ab (50 µg mL\textsuperscript{-1}) | 25 – 1000 ng mL\textsuperscript{-1} | 25 ng mL\textsuperscript{-1} | [9] |
| AuNP-Ab (sandwich) | 5 – 400 ng mL\textsuperscript{-1} (sandwich) | 5 ng mL\textsuperscript{-1} |     |
| AuNP-Ab (sandwich) | 0.5 – 40 ng mL\textsuperscript{-1} (AuNP-sandwich) | 0.5 ng mL\textsuperscript{-1} |     |
| MIP/PDA | 20 – 300 nM | 14.8 nM | [49] |
| MIP/PDA | (~500 ng mL\textsuperscript{-1}) |     |     |
| Dextran/biotin-streptavidin/Ab (120 µg mL\textsuperscript{-1}) | 0.03 – 6.5 ng mL\textsuperscript{-1} | 0.01 ng mL\textsuperscript{-1} | [50] |
| 3-MPA/PAMAM(G4)/Ab (20 µg mL\textsuperscript{-1}) | 5 – 300 ng mL\textsuperscript{-1} (mAb) | 3.6 ng mL\textsuperscript{-1} | Present |
| 3-MPA/PAMAM(G4)/Ab (20 µg mL\textsuperscript{-1}) | 3 – 30 ng mL\textsuperscript{-1} (pAb) | 0.98 ng mL\textsuperscript{-1} |     |

\(a\) Dextran: carboxymethylated dextran; OEG: Oligo (ethylene glycol); MHDA: Mercaptohexadecanoic acid; MUDA: 11-Mercaptoundecanoic acid; AuNP-Ab: Gold nanoparticles conjugated to detector antibodies; MIP: Molecularly imprinted polymer; PDA: Polydopamine.
Table 2 Results obtained in the determination of troponin T in blood plasma by the proposed SPR-based immunosensor

| Assay                  | Added     | Found$^a$         | Recovered (%) |
|------------------------|-----------|-------------------|---------------|
| Sandwich (monoclonal)  | 10 ng mL$^{-1}$ | $8.8 \pm 0.7$ ng mL$^{-1}$ | $88 \pm 7$ |
| Sandwich (polyclonal)  | 10 ng mL$^{-1}$ | $10.4 \pm 1$ ng mL$^{-1}$ | $104 \pm 10$ |

$^a n=3$
Figure Captions

Fig. 1 Scheme illustrating the steps involved in the construction of the immunosensor and its application in the detection of troponin T. Gold substrate coated with 3-MPA (structure shown in lower zoom), PAMAM(G4) dendrimers (structure shown in upper zoom), and capture antibodies, with non-specific sites blocked with BSA and ethanolamine.

Fig. 2 Effective $\Delta \theta_{\text{SPR}}$ for the different concentrations immobilization of PAMAM dendrimers (a) G3, (b) G4, and (c) G5 (Fig. 2c), on the SAM of 3-MPA.

Fig. 3 Analytical curve obtained by the effective $\Delta \theta_{\text{SPR}}$ as a function of the logarithm of protein concentration ($R^2=0.991$, $\Delta \theta_{\text{SPR}} = -42.321 + 113.089 \log \text{[Troponin T]})$.

Fig. 4 The analytical curve was obtained by the effective $\Delta \theta_{\text{SPR}}$ as a function of the logarithm of protein concentration using 10 $\mu$g mL$^{-1}$ of polyclonal secondary antibodies (pAb) ($R^2=0.998$, $\Delta \theta_{\text{SPR}} = 2.177 + 24.341 \log \text{[Troponin T]})$. 

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Fig. 1
Fig. 2

A  
\[ \Delta \theta_{\text{SPR}} / \text{m}^2 \]

PAMAM(G3) concentration / mmol L\(^{-1}\)

B  
\[ \Delta \theta_{\text{SPR}} / \text{m}^2 \]

PAMAM(G4) concentration / mmol L\(^{-1}\)

C  
\[ \Delta \theta_{\text{SPR}} / \text{m}^2 \]

PAMAM(G5) concentration / mmol L\(^{-1}\)
Fig. 3
Fig. 4
