Cardiac Troponin T capture and detection in real-time via epitope-imprinted polymer and optical biosensing

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Abstract

Millions of premature deaths per year from cardiovascular diseases represent a global threat urging governments to increase global initiatives, as advised by World Health Organization. In particular, together with prevention and management of risk factors, the development of portable platforms for early diagnosis of cardiovascular disorders appears a fundamental task to carry out. Contemporary assays demonstrated very good accuracy for diagnosis of acute myocardial infarction (AMI), but they are based on expensive and fragile capture antibodies. Accordingly, also considering the massive demand from developing countries, we have devoted our study to an affinity-based biosensor for detection of troponin T (TnT), a preferred biomarker of AMI. This combines a stable and inexpensive molecularly imprinted polymer (MIP) based on polydopamine (PDA) with surface plasmon resonance (SPR) transduction. Herein we report the fast and specific answer upon TnT binding onto an epitope-imprinted surface that strongly encourages the further development toward antibody-free point-of-care testing for cardiac injury.

Keywords: Acute myocardial infarction; Troponin T; Surface Plasmon Resonance; Polydopamine; Molecularly Imprinted Polymer; Epitope-Imprinted Biosensor

1 Introduction

According to estimates, cardiovascular diseases are responsible for 17.7 millions of worldwide deaths every year, mainly associated with heart attacks and strokes and mostly occurring in developing countries (Reed et al., 2017; Anderson and Morrow, 2017; WHO (World Health Organization), 2017~~WHO (World Health Organization), 2017).~~). However, early diagnosis of disorders of heart and blood vessels, together with prevention and control of major risk factors could dramatically reduce the steadily increasing mortality rate and the high cost from these adverse events. In this field, biomarkers detection is fundamental, since symptoms and electrocardiogram changes may be absent or nonspecific (Babuin and Jaffe, 2005; Jaffe et al., 2000). Currently, cardiac troponins (I or T) are the analytes of choice for the detection of acute myocardial infarction. These proteins confer greater sensitivity and higher specificity to the clinical assays with respect to measurement of traditional cardiac enzymes, such as creatine kinase and myoglobin (Babuin and Jaffe, 2005; Jaffe et al., 2000; French and White, 2004). In this framework, affinity-based biosensors are particularly suitable for the development of portable diagnostic platforms for cardiac injury due to mandatory demand of real-time detection of biomarkers with high specificity at the point of need (Sharma et al., 2015). Contemporary assays for troponins detection, for both commercial and research purposes, are solely based on capture monoclonal antibodies with several constraints linked to high cost and low stability (Apple et al., 2017; Abdolrahim et al., 2015; Sheng et al., 2017). However, in the last years, molecularly imprinted polymers (MIPs) have emerged as a robust and inexpensive alternative to naturally occurring receptors. A number of MIPs have already displayed excellent performances in the selective and sensitive recognition of small drugs, macromolecules, e.g. proteins and nucleic acids, and microorganisms (Mosbach and Ramström, 1996; Hussain et al., 2013; Xing et al., 2017; Dabrowski et al., 2018). Several successful applications include chemical separation processes, catalysis, and medical diagnostics (Jetzschmann et al., 2017; Boysen et al., 2017; Cheong et al., 2013). MIPs possess the advantage of higher resistance to elevated pressure, temperature and solvent composition compared to biological materials, and their synthesis mixture and conditions are simple in principle. Dopamine is an example of functional monomer used for self-polymerization, adhesion, and surface coating of organic and inorganic materials on the nanometer scale. It is inspired by adhesive mefp proteins in mussels that are rich in L-dopa, with pronounced redox functionality, and L~~-lysine~~-lysine aminoacids (Lee et al., 2007; Liu et al., 2014). Dopamine-template co-polymerization creates a three-dimensional network with cavities complementary to the original molecule in terms of dimension, shape, and noncovalent interactions useful for analyte detection (Mosbach and Ramström, 1996; Hussain et al., 2013; Xing et al., 2017). The main two strategies for protein imprinting require, alternatively, the whole biomolecule or small peptide fragments. The latter is the so-called epitope approach. It circumvents the use of expensive macromolecules and the difficulties related to template removal and/or reduced binding capability frequently encountered with large, complex, and conformational sensitive proteins (Boysen et al., 2017; Rachkov and Minoura, 2000, 2001). We present here an affinity-based biosensing of troponin T (TnT) for prospective real-time testing of cardiac injury. Literature survey shows

that very few experimental studies have been carried out on TnT imprinted polymers (Moreira et al., 2011; Karimian et al., 2013, 2014; Silva et al., 2016). In particular, these previous works describe the electro- or initiator-dependent polymerization of a functional monomer in presence of the whole TnT protein, and the subsequent electrochemical measurements of protein binding capability. Differently, we have used as recognition element a biocompatible MIP-based receptor obtained by the spontaneous self-assembly of dopamine in alkaline aqueous media (Lee et al., 2007; Liu et al., 2014), through the imprinting of short (10–15 aa) TnT-derived peptides. This produces a self-assembling adhesive imprinted nanofilm selective for TnT directly (Fig. 1a). Its growth, directly performed on the gold sensor surface in few hours, allows us to successfully bypass the formerly reported immobilization of antibodies (Dutra and Kubota, 2007; Dutra et al., 2007; Andersson et al., 2010). Thereafter, label-free molecular detection in real-time has been achieved monitoring the change in refractive index at the MIP film upon of analyte binding by using surface plasmon resonance (SPR), one of the most validated and assessed optical techniques (Bettazzi et al., 2017). Analysis of film selectivity has been performed using human serum albumin (HSA), which is the most abundant protein in human blood plasma and serum (Fanalia et al., 2012), instead of TnT. Furthermore, control experiments for protein/surface non-specific interactions have been made testing both HSA, TnT on not-imprinted polymer (NIP) of dopamine in place of MIP, and spiked human serum, as illustrated in Fig. 1b. The proven efficiency of our detection method could pave the way for the development of an antibody-free point-of-care (POC) platform for real-time monitoring of cardiac injury[7]. Additionally, we have identified the critical role of basic residues of the epitope for successful imprinting of peptides and proteins in PDA through favourable interactions of amino acid side-chains with quinone moiety. Therefore, the combination of the well-known universal adhesive capability of PDA (Lee et al., 2007; Liu et al., 2014) and the rational design of peptides for the PDA imprinting here described appears particularly promising for biosensors research and application.

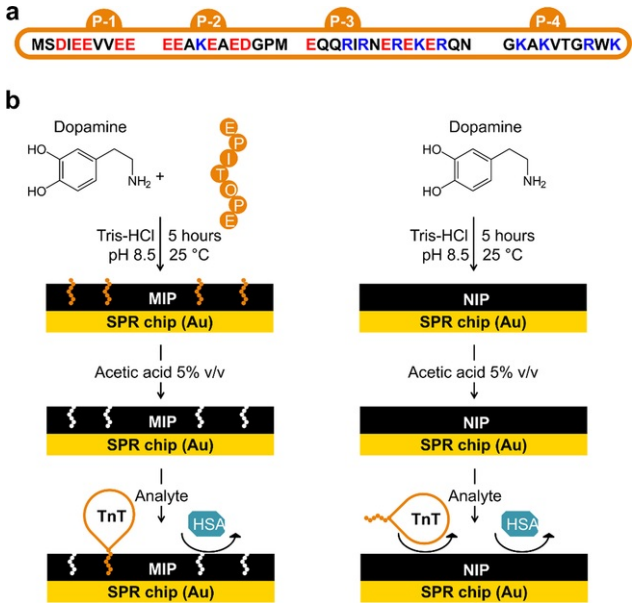

Fig. 1 (a) Primary sequence of synthetic peptide analogues of TnT used as template for PDA imprinting is reported with their approximated distribution along the TnT protein from the N-terminus (P-1) to the C-terminus (P-4). Positively charged residues (Arg and Lys) are reported in blue, whereas the negatively charged residues (Aps and Glu) are reported in red. (b) Schematic diagram representation of gold SPR chip surface coating with dopamine and templates (MIP), or dopamine alone (NIP) for the capture and detection of analytes.

alt-text: Fig. 1

2 Materials and methods

2.1 Reagents and chemicals

Dopamine hydrochloride, tris(hydroxymethyl)aminomethane hydrochloride (TRIS HCl), sodium hydroxide, sodium chloride, magnesium chloride, hydrocloric acid, acetic acid, human serum albumin (HSA), and Human Serum from human male AB plasma sterile-filtered were purchased from Sigma Aldrich (Italy). The synthetic peptides corresponding to TnT isoform 6; also known as TNT3 (The Universal Protein Resource ~~(UniProt),~~(UniProt), 2017); residues 1–10 (P-1: MSDIEEVVEE), 50–60 (P-2: EEAKEAEDGPM), 136–150 (P-3: EQQRIRNEREKERQN), and 279–288 (P-4: GKAKVTGRWK) were purchased from Genscript (U.S.A.). Recombinant human cardiac troponin T isoform 6 and Anti-

cTnT MAb 7G7 were purchased from HyTest Ltd. (Finland). All reagents were used without further purification. Water used for all preparations was obtained from a Milli-Q system. SPR measurements were carried out on Biacore X™, by using bare gold chips (General Electric Healthcare, Sweden).

2.2 Setup for surface plasmon resonance measurements

Polymeric films of dopamine were formed on clean gold-coated SPR surface by applying a solution of ~~5~~5 g L^{-1} of dopamine in ~~20~~20 mM TRIS HCl pH 8.5 at ~~25~~25 °C on the device surface for ~~5~~5 h in presence (MIP), or in absence (NIP), of protein TnT ~~(25~~(25 mg L^{-1}) or TnT-derived peptides ~~(1~~(1 g L^{-1}). Film deposition was followed by a washing procedure with acetic acid (5% v/v), H_2O, and running buffer ~~(10~~(10 mM Tris-HCl; ~~20~~20 mM NaCl; ~~2~~2 mM $MgCl_2$; pH 8.3). TnT, HSA, and peptides ~~(10~~(10 µL, from 10.0 to ~~0.625~~0.625 µg mL^{-1} in the running buffer, contact time: ~~2~~2 min) were injected on the NIP or MIP surfaces. Human serum ~~(20~~(20 µL) and human serum 10% v/v in PBS pH 7.4 ~~(100~~(100 µL) were injected on MIP-1234 surface. Measurements were conducted on the two cells available (flow rate: ~~5~~5 µL min^{-1}; temperature: ~~25~~25 °C), working in serial mode. The binding was followed by a regeneration step with ~~2~~2 mM sodium dodecyl sulphate (SDS) and/or ~~10~~10 mM HCl. Sensorgrams were elaborated by BIAevalutation 3.1 software.

3 Results and discussion

3.1 Evaluation of proteins interaction with whole protein-based MIP

Successful imprinting of TnT protein was previously achieved by using acrylamides (Moreira et al., 2011), *o*-phenylenediamine (Karimian et al., 2013, 2014), and pyrroles-carboxylic acids (Silva et al., 2016), as functional monomers. The reported results testify that the stability of troponin T to physicochemical characteristics of the environment is likely due to its conformational flexibility. In fact, structural analysis of TnT indicates that only the region responsible for the interaction with troponin C and I retains an α-helical conformation (residues 199–271), whereas the N- and C-terminus, through which the troponin complex interacts with the actin–tropomyosin, appear intrinsically disordered (Takeda et al., 2003; Katrukha, 2013). In the present study, the imprinting efficiency of PDA has been first tested on the entire protein TnT. A solution of Troponin T and dopamine has been left for 5 h onto gold surface of SPR chip to form a film with a presumable thickness of 20 nm in our conditions (Ball et al., 2012). The subsequent washing procedure has produced the MIP as biorecognition element (Fig. 1b). In our experiments, serial injections at increasing concentration of TnT solution, interspersed with a regeneration step, allowed the real-time sensing of protein binding onto the functionalized surface (MIP-TnT). The binding selectivity of MIP-TnT has been tested repeating the experiments with an analogous range of concentrations of HSA. Moreover, the interaction of Troponin T, and HSA, with NIP has been also investigated to take into account the non-specific interaction of proteins of interest with the non-imprinted film of polydopamine. Fig. 2 displays the response of the SPR chips to increasing TnT and HSA concentrations injected onto MIP (a) and NIP (b) surfaces in the range of 0–289 nM, exhibiting a fast (2 min) binding of TnT with MIP (Fig. 2a), with larger amount of TnT bound on MIP (b) in comparison with TnT on NIP (c), as well as the negligible interaction of HSA with MIP or NIP (b, c). The change in slope at the end of the analyte injections around 150 s in Fig. 2a is due to the running buffer after the end of analyte injection. The high stability of the signal during the washing stage demonstrates the excellent stability of the affinity complex. Moreover, Fig. 2d indicates that the biosensor responds linearly to the TnT concentrations in the range here explored with a sensitivity of 2.64 RU nM^{-1}. The estimated limit of detection (LoD) is 15.4 nM, and was inferred by the lowest TnT concentration giving a signal greater than three times the standard deviation of the noise level of baseline in buffer (3σ = 15 RU). Conversely, the interaction signals of HSA with MIP are small, almost irrespective of concentration of protein injected, and comparable to the responses diagrams from NIP. Altogether these results confirm the effective imprinting of TnT protein on PDA. Increasing standard deviations appear for high analyte concentration (Fig. 2a). This signal variability is likely due to non-specific binding with polydopamine film in presence of large TnT excess. This effect is confirmed by the consequent difficulty in achieving the complete analyte removal during the regeneration step. In fact, a similar behavior is displayed by the NIP surface for the same analyte concentration (Fig. 2c). However, this drawback seems not so limiting considering the perspective of a disposable POC test for TnT at low concentration through a signal amplification strategy (Lisi et al., 2017; O'Connor et al., 2016).

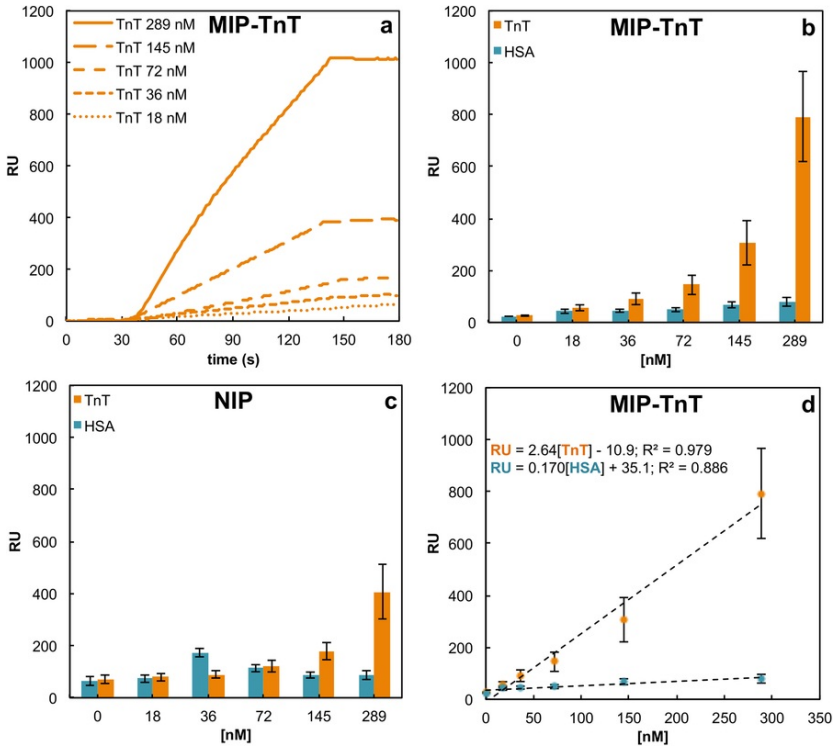

Fig. 2 (a) Representative sensograms for injections of TnT and HSA on MIP-TnT. Slope change indicates the end of TnT injection, i.e. complex stability in running buffer. (b) MIP-TnT and (c) NIP response of SPR biosensors at different concentrations of TnT and HSA. (c) Linear fitting of MIP response to TnT and HSA injections. We have analyzed four replicates for each analyte concentration, intersperse with a regeneration step, and reported the mean value and the standard deviation as error bars.

alt-text: Fig. 2

3.2 Rational selection of peptide fragments and analysis of TnT Epitope-imprinting

Although useful dopamine-based MIP has been here achieved and the use of capture-antibodies has been conveniently avoided, the whole protein here and previously exploited for surface imprinting still confers high cost to this strategy[~~21–24~~21–24]. In this regard, the so-called epitope-imprinting has emerged as a favourable approach to circumvent the use of expensive macromolecules for analyte detection (Rachkov and Minoura, 2001). This method requires the identification of small and surface-exposed peptide fragments (epitopes) of the protein used as templates for MIP preparation. However, to the best of our knowledge, this approach has been never used for TnT. The rational selection of our templates is based on the analysis of structural data and binding sites of commercially available antibodies for TnT. In detail, we have tested four short peptides corresponding to conformationally flexible and solvent exposed regions of TnT. In particular, they are analogous to the N-terminus (P-1), the C-terminus (P-4), and two epitopes recognized by commercially available TnT antibodies, namely 7G7 and 1C11, corresponding to peptides P-2 and P-3, respectively (Fig. 1a). Similarly to TnT-based MIP preparation, we have imprinted the above reported peptides in a film of PDA by using a solution containing the templates altogether at equal concentrations instead of TnT, and following the procedure already described for imprinting and templates removal (Fig. 1b). The SPR chip imprinted with four peptides (MIP-1234) has been evaluated for TnT and HSA binding efficiency, and the response diagrams for the TnT and HSA injections are reported in Fig. 3. Notwithstanding the reduced size and complexity of the epitopes in comparison to whole protein, MIP-1234 biosensor retains the linear response to the troponin concentrations here observed for MIP-TnT, with even larger sensitivity of 3.15 ~~RU nM~~RU nM^{-1}, lower LoD ~~(14.8~~(14.8 nM) and lesser non-specific interaction with HSA. These remarkable results for epitope-imprinting of TnT protein are unprecedented and move us closer to achieving the original intent, i.e. the development of affordable real-time antibody-free testing for cardiac injury.

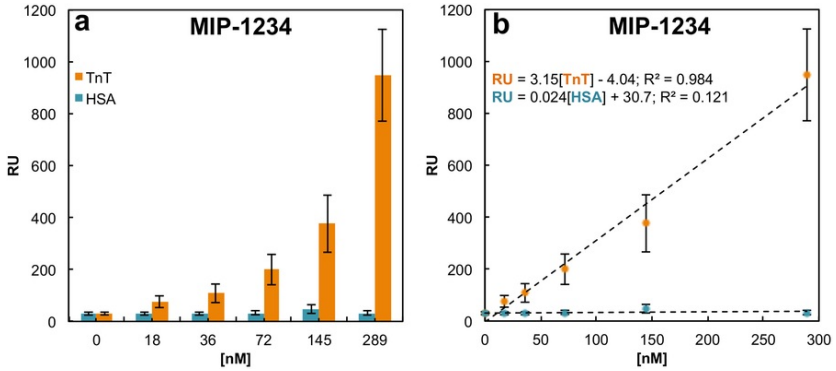

Fig. 3 (a) Response of MIP-1234 SPR biosensor at different concentrations of TnT and HSA. (b) Linear fitting of MIP response to TnT and HSA injections. We have analyzed four replicates for each analyte concentration, intersperse with a regeneration step, and reported the mean value and the standard deviation as error bars.

alt-text: Fig. 3

3.3 Investigation of MIP based on single TnT-epitope

Following the achievement described above, we have decided to look closer to this successful design, exploring the role of each epitope in template formation and consequent binding efficacy of MIP. Accordingly, we have tested the affinity of MIP-1234 described above for single synthetic peptide injected singularly on the sensor chip. Surprisingly, only P-4, corresponding to TnT C-terminus, appears to interact with both the imprinted surface and non-imprinted surfaces. Conversely, the other peptides have given negligible signals on MIP and NIP (Fig. S1). These results might indicate that only P-4 has been correctly imprinted with PDA, therefore allowing the capture of TnT, and suggesting that the imprinting of P1, P2 and P3 does not improve the sensitivity and specificity of our MIP-1234 with respect to MIP-4 here tested, and also likely the performance of MIP-14, MIP-24, and MIP-34 would be all very similar. Tentatively, we ascribe this outcome to the peculiar features of TnT C-terminus in terms of higher conformational mobility and larger basicity (P-4, pI = 11.8) in comparison to the other protein epitopes here explored. In particular, the molecular constraints of the inner regions of TnT could represent an obstacle to properly match the shape left by the linear peptides P-2 and P-3. Moreover, although the N-terminal region should not suffer from this drawback, the highly acid character of this peptide (P-1, pI = 2.89) could be unfavourable for the imprinting in dopamine polymer. The same for the subsequent rebinding, because of the electrostatic repulsion with the negatively charged PDA (Ma et al., 2015). In fact, P-4 is positively charged in the experimental conditions of surface imprinting. As previously showed for other proteins and peptides (Burzio and Waite, 2000; Wang et al., 1996), MIP formation could be thus also favoured by reversible nucleophile attack of lysine residues to quinones moiety of PDA, which are abundant in P-4 and absent in P-1. Finally, in order to exclude eventual hindrance among peptides in solution during MIP-1234 formation, we decided to produce a MIP surface for each epitope. Again, Troponin T is able to selectively and specifically bind only to MIP-4 (Fig. 4). Moreover, the MIP surface generated by using P-4 as template reproduces the sensitivity already observed for MIP-TnT and MIP-1234 (3.15 ~~RU nM~~RU nM^{-1}) in response to TnT injections although with a little higher LoD ~~(41.0~~(41.0 nM). Conversely, MIP-1, MIP-2, and MIP-3 give signals almost indistinguishable from NIP (Fig. S2).

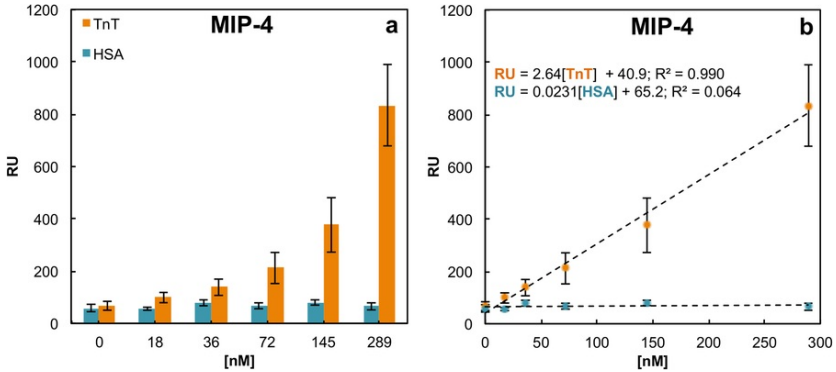

Fig. 4 (a) Response of MIP-4 SPR biosensor at different concentrations of TnT and HSA. (b) Linear fitting of MIP response to TnT and HSA injections. We have analyzed four replicates for each analyte concentration, intersperse with a regeneration step, and reported the mean value

and the standard deviation as error bars.

alt-text: Fig. 4

3.4 TnT detection from spiked human serum

Finally, we have tested sterile-filtered human sera samples injected on MIP-1234 biosensor, with and without the addition of TnT at the highest concentration of analyte here investigated. The response obtained from SPR was the same for pure human serum and samples spiked with TnT because of not-specific adsorption of molecules from this complex biological matrix (data not shown). However, the subsequent injection of a TnT-specific antibody (7G7 clone) has clearly and selectively recognized the target analyte, as shown in Fig. 5. This was successfully achieved both on 10% and undiluted serum. Furthermore, the surface of the coated sensor chip has been regenerated after each experiment, confirming the stability and reusability of the MIP surface. However, due to the complexity of the real matrix, a dedicated regeneration protocol has been performed, consisting in ~~2~~2 mM SDS followed by ~~10~~10 mM HCl, and deserves further improvement. However, for the perspective application of the method to point-of-care testing, regeneration aspects of this kind of low cost and biomimetic receptors result of minor importance respect to sensitivity and selectivity. Ongoing work is dedicated to the amplification of the detection limit by substituting the 7G7 antibody with a secondary synthetic receptor.

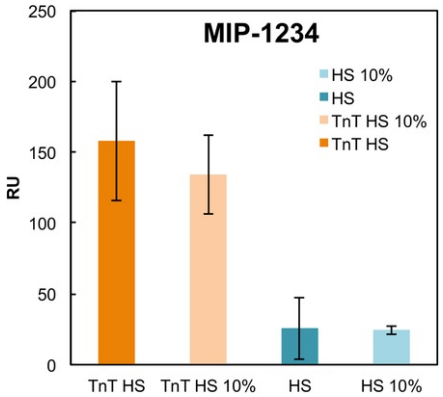

Fig. 5 Response of MIP-1234 SPR biosensor at ~~23~~23 mg L^{-1} of Anti-TnT 7G7 MAb after surface adsorption of human serum and human serum spiked with TnT at ~~289~~289 nM. The experiments have been repeated with serum diluted at 10% v/v in running buffer. We have analyzed four replicates for each analyte concentration, intersperse with a regeneration step, and reported the mean value and the standard deviation as error bars.

alt-text: Fig. 5

4 Conclusions

We have described here the first example of epitope-imprinted biosensor for cardiac biomarker troponin T (TnT), which is released from the cardiac tissue in the blood stream upon the damage of myocytes (French and White, 2004; Möckel et al., 2004). Contemporary assays are all based on monoclonal antibodies, and the clinical ones are specific to protein residues in the middle of the primary sequence for capture and detection of TnT. Conversely, we have developed here a stable and inexpensive polydopamine film imprinted with TnT-epitope, which exhibits a fast and specific capture of protein through its C-terminal region that, advantageously, should not be affected by proteolytic degradation of TnT recently detailed ~~in vivo~~in vivo (Cardinaels et al., 2013; Katrukha et al., 2017). Moreover, it leaves all the remaining part of protein available for molecular interactions with any specific antibody. The adhesive property of mussel foot proteins due to the recurrence of both L-dopa finds strict analogy with the same catechol functionality of dopamine, and basic residues media (Lee et al., 2007, 2011; Liu et al., 2014), and help us to advance some considerations. We hence attribute the effectiveness of TnT C-terminus in producing the imprinted film to the concurrence of flexibility and basicity of this template, characteristics also found in another peptide previously used for epitope imprinting of PDA for gp41 detection (Lu et al., 2012). Therefore, interconnected features of template, such as length, hydrophilicity, flexibility and uniqueness, as elsewhere described for the imprinting of acrylates polymers (Bossi et al., 2012) play undoubtedly a key role. Further, the presence of basic side-chains, which favour electrostatic and nucleophilic interactions with quinone moieties of PDA, appear equally crucial for rational selection of peptides for molecular imprinting onto dopamine polymers. This therefore may extend the field of application of this methodology well beyond the analytes and the sensory surface here investigated. Our MIP-based biosensor gives a linear SPR-response to the troponin concentration in the nanomolar range with a good sensitivity, however these values are above the picomolar level found for TnT in blood (French and White, 2004; O'Connor et al., 2016; Westermann et al., 2017). However, the necessity to detect much lower Troponin T concentration should overcome the disadvantageous increase of standard deviation encountered here for the highest analyte concentration, likely due to the increased non-specific binding of TnT with PDA. Nevertheless, as demonstrated by our preliminary experiments in spiked human serum, through a signal amplification strategy, and independently of transduction technique, this new

capture method for TnT opens the door to affordable real-time testing for cardiac injury at the point of need. Ongoing work is dedicated to the amplification of the detection limit by using molecular architectures built in a layer-by-layer fashion, biomolecules and nanostructures, as also recently reported (Lisi et al., 2017; O'Connor et al., 2016). ~~Uncited references(Uniprot; Who)~~

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.01.068.

References

Abdolrahim M., Rabiee M., Alhosseini S.N., Tahriri M., Yazdanpanah S. and Tayebi L., ~~*Analytical Biochemistry*~~*Anal. Biochem.* **485**, 2015, 1–10.

Anderson J.L. and Morrow D.A., ~~*The New England Journal of Medicine*~~*N. Engl. J. Med.* **376**, 2017, 2053–2064.

Andersson H., Kågedal B. and Mandenius C.-F., ~~*Analytical and Bioanalytical Chemistry*~~*Anal. Bioanal. Chem.* **398**, 2010, 1395–1402.

Apple F.S., Sandoval Y., Jaffe A.S. and Ordonez-Llanos J., ~~*Clinical Chemitry*~~*Clin. Chem.* **63**, 2017, 73–81.

Babuin L. and Jaffe A.S., ~~*Canadian Medical Association Journa*~~*Can. Med. Assoc. J.* **173**, 2005, 1191–1202.

Ball V., Del Frari D., Toniazzo V. and Ruch D., ~~*Journal of*~~ *J. Colloid Interface* ~~*Science*~~*Sci.* **386**, 2012, 366–372.

Bettazzi F., Marrazza G., Minunni M., Palchetti I. and Scarano S., ~~*Analytical Chemistry*~~*Anal. Chem.* **77**, 2017, 1–13.

Bossi A.M., Sharma P.S., Montana L., Zoccatelli G., Laub O. and Levi R., ~~*Analytical Chemistry*~~*Anal. Chem.* **84**, 2012, 4036–4041.

Boysen R.I., Schwarz L.J., Nicolau D.V. and Hearn M.T.W., ~~*Journal of Separation Science*~~*J. Sep. Sci.* **40**, 2017, 314–335.

Burzio L.A. and Waite J.H., *Biochemistry* **39**, 2000, 11147–11153.

Cardinaels E.P., Mingels A.M., van Rooij T., Collinson P.O., Prinzen F.W. and van Dieijen-Visser M.P., ~~*Clinical Chemistry*~~*Clin. Chem.* **59**, 2013, 1083–1090.

Cheong W.J., Yang S.H. and Ali F., ~~*Journal of Separation Science*~~*J. Sep. Sci.* **36**, 2013, 609–628.

Dabrowski M., Lach P., Cieplak M. and Kutner W., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **102**, 2018, 17–26.

Dutra R.F. and Kubota L.T., ~~*Clinica Chimica*~~*Clin. Chim. Acta* **376**, 2007, 114–120.

Dutra R.F., Mendes R.K., Lins da Silva V. and Kubota L.T., ~~*Journal of Pharmaceutical and Biomedical Analysis*~~*J. Pharm. Biomed. Anal.* **43**, 2007, 1744–1750.

Fanalia G., di Masi A., Trezza V., Marino M., Fasano M. and Ascenzi P., ~~*Molecular Aspects of Medicine*~~*Mol. Asp. Med.* **33**, 2012, 209–290.

French J.K. and White H.D., *Heart* **90**, 2004, 99–106.

Hussain M., Wackerlig J. and Lieberzeit P.A., *Biosensors* **3**, 2013, 89–107.

Jaffe A.S., Ravkilde J., Roberts R., Naslund U., Apple F.S., Galvani M. and Katus H., *Circulation* **102**, 2000, 1216–1220.

Jetzschmann K.J., Zhang X., Yarman A., Wollenberger U. and Scheller F.W., Label-Free MIP Sensors for Protein Biomarkers, Springer Series on Chemical Sensors and Biosensors (Methods and ~~Applications).~~Applications), 2017, Springer; Heidelberg, 1–31.

Karimian N., Turner A.P.F. and Tiwari A., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **59**, 2014, 160–165.

Karimian N., Vagin M., Zavar M.H.A., Chamsaz M., Turner A.P.F. and Tiwari A., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **50**, 2013, 492–498.

Katrukha I.A., *Biochemistry ~~(Moscow)~~(Mosc.)* **78**, 2013, 1447–1465.

Katrukha I.A., Kogan A.E., Vylegzhanina A.V., Serebryakova M.V., Koshkina E.V., Bereznikova A.V. and Katrukha A.G., ~~*Clinical Chemistry*~~*Clin. Chem.* **63**, 2017, 1094–1100.

Lee B.P., Messersmith P.B., Israelachvili J.N. and Waite J.H., ~~*Annual Review of Materials Research.*~~*Annu. Rev. Mater. Res.* **41**, 2011, 99–132.

Lee H., Dellatore S.M., Miller W.M. and Messersmith P.B., *Science* **318**, 2007, 426–430.

Lisi S., Scarano S., Fedeli S., Pascale E., Cicchi S., Ravelet C., Peryn C. and Minunni M., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **93**, 2017, 289–292.

Liu Y., Ai K. and Lu L., ~~*Chemical Reviews*~~*Chem. Rev.* **114**, 2014, 5057–5115.

Lu C.H., Zhang Y., Tang S.F., Fang Z.B., Yang H.H., Chen X. and Chen G.N., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **31**, 2012, 439–444.

Ma A., Xie Y., Xu J., Zeng H. and Xu H., ~~*Chemical Communications*~~*Chem. Commun.* **51**, 2015, 1469–1471.

Möckel M., Danne O., Schmidt A., Goldman M., Müller C., Dietz R. and Wu A.H.B., ~~*Clinica Chimica*~~*Clin. Chim. Acta* **342**, 2004, 83–86.

Moreira F.T.C., Dutra R.A.F., Noronha J.P.C., Cunha A.L., Goreti and Sales M.G.F., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **28**, 2011, 243–250.

Mosbach K. and Ramström O., ~~*Nature Biotechnology*~~*Nat. Biotechnol.* **14**, 1996, 163–170.

O'Connor E.F., Paterson S. and De La Rica R., ~~*Analytical and Bioanalytical Chemistry*~~*Anal. Bioanal. Chem.* **408**, 2016, 3389–3393.

Rachkov A. and Minoura N., ~~*Journal of Chromatography*~~ *J. Chromatogr. A* **889**, 2000, 111–118.

Rachkov A. and Minoura N., ~~*Biochimica et Biophysica*~~ *Biochim. Biophys. Acta* **1544**, 2001, 255–266.

Reed G.W., Rossi J.E. and Cannon C.P., *Lancet* **389**, 2017, 197–210.

Sharma S., Zapatero-Rodríguez J., Estrela P. and O'Kennedy R., *Biosensors* **5**, 2015, 577–601.

Sheng Q., Qiao X., Zhou M. and Zheng J., ~~*Microchimica*~~*Microchim. Acta* **184**, 2017, 1573–1585.

Silva B.V.M., Rodríguez B.A.G., Sales G.F., Sotomayor M.D.P.T. and Dutra R.F., ~~*Biosensors and Bioelectronics*~~*Biosens. Bioelectron.* **77**, 2016, 978–985.

Takeda S., Yamashita A., Maeda K. and Maeda Y., *Nature* **424**, 2003, 35–41.

~~Uniprot~~Uniprot.org/uniprot/P45379.

Wang S.X., Mure M., Medzihradszky K.F., Burlingame A.L., Brown D.E., Dooley D.M., Smith A.J., Kagan H.K. and Klinman J.P., *Science* **273**, 1996, 1078–1084.

Westermann D., Neumann J.T., Sörensen N.A. and Blankenberg S., ~~*Nature Reviews Cardiology*~~*Nat. Rev. Cardiol.* **14**, 2017, 472–483.

~~Who~~Who.int/cardiovascular_diseases/en/.

Xing R., Wang S., Bie Z., He H. and Liu Z., ~~*Nature Protocols*~~*Nat. Protoc.* **12**, 2017, 964–987.

Appendix A. Supplementary material

Multimedia Component 1

Highlights

- Troponin T is the most sensitive and selective biomarker of acute myocardial ~~infarction~~infarction.
- Emergency and clinical tests for TnT are still based on immuno-reactions and ~~antibodies~~antibodies.
- Here the first TnT biosensor based on epitope imprinting on polydopamine is ~~presented~~presented.
- Epitope imprinting with short peptides from TnT allowed low-cost and fast ~~preparation~~preparation.
- Direct assays in standard solutions and sandwich assays on spiked serum are ~~reported~~reported.

Queries and Answers

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