Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 1, 2023, 37
https://doi.org/10.33263/BRIAC131.037

Catechol Biosensor Design Based on Ferrocene-Derivatized 2,5-Dithienyl Pyrrole Copolymer with 3,4-Ethylenedioxythiophene

Ayhan Altun [1], Roxana-Mihaela Apetrei [2,3], Pinar Camurlu [3,*]

[1] Department of Chemistry, Gebze Technical University, 41400 Kocaeli, Turkey
[2] Dunarea de Jos' University of Galati, Galati RO-800008, Romania
[3] Department of Chemistry, Akdeniz University, 07058 Antalya
* Correspondence: pcamurlu@akdeniz.edu.tr (P.C);

Scopus Author ID 6507809815
Received: 6.11.2021; Accepted: 15.12.2021; Published: 23.01.2022

Abstract: Novel conducting platforms based on co(polymerization) of 2,5-dithienyl pyrrole-ferrocene and 3,4-ethylenedioxythiophene were employed for the first time in developing a catechol biosensor. The grafting of Fc moiety onto a hybrid thienyl pyrrole monomer for phenolics detection is proposed for the first time herein to enhance the efficiency of electrochemical reduction of quinone and, in turn, improve the stability of the biosensor in a 'reagentless' manner. Tyrosinase enzyme was immobilized by cross-linking onto the carbon nanotubes-enriched electrodeposited films, and catechol was determined with a low detection limit of 2.1 µM. Good operational stability (RSD 3.34 %) was observed during 20 consecutive measurements.

Keywords: catechol sensor; carbon nanotubes; 3,4-ethylenedioxythiophene; ferrocene; 2,5-di(thienyl)pyrrole; tyrosinase.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

New and improved methods have been widely researched because of the major relevance of phenolic detection for beneficial aspects in human physiology and toxicity concerns. Alongside classical analytical methods, electrochemical biosensors are highlighted for their attractive features (fast response time, no requirements for sample pre-treatment, possibility for miniaturization, and portability for on-field measurements) [1, 2]. At present, enzymatic phenol biosensors are highly relevant for the chemical, food, or pharmaceutical industry [3-6]. The operating principle is primarily based on Tyrosinase (Tyr) (E.C. 1.14.18.1, monophenol monooxygenase), which oxidizes monophenols and *o*-diphenols into corresponding *o*-quinones whilst reducing O_2 to water [7-9].

Conducting polymers (CPs) possess π-conjugated backbone, which confers good electronic properties [10, 11] and can also meet the requirements of biocompatibility [12, 13], efficient electron transfer (allowing both electronic and ionic transport) as well as facility in deposition on the desired type of electrode [14]. As some of the most researched materials in biosensing, the versatility in the synthesis of CPs has enabled immobilization of a wide range of biological moieties (enzymes, antibodies, whole cells, DNA, etc. [15]) and can provide a good option for stabilization of the desired bio-element [16, 17].

Considerable efforts have focused on immobilizing Tyr onto CP films for biosensing purposes, mainly PANI [18-20], PPy [21, 22], and PTh. [23]. Yet, the required deposition potential or processability of the final polymer structure can hinder the applicability; thus copolymerization of the monomers with hydrophilic polymers (polyvinyl alcohol [24], poly(ethylene glycol [25]), glycine methacrylate [26], or aminopropyl moieties [27]) has been employed. Functionalization of the monomer units has also been explored; however, insertion of functional groups onto PPy or PTh has proven unlucrative due to steric hindrances that reduce conjugation length and, in turn, to low conductivity [28]. Recently, a new class of conducting polymers (hybrid 2,5-thienylpyrroles) consisting of both pyrrole and thiophene units has been introduced and employed in a variety of fields from optoelectronics to biodevices. They provide a facility in synthesis with stable electrochemical behavior and tremendous potential for tailor-made functionality based on the N-substitution of the pyrrole fragment. The grafting of functional units onto a conducting matrix can be highly favorable in biosensors for maintaining proper conformation and orientation of the biorecognition element while providing accessibility and efficient mass transfer of analyte. The application of such tailor-made conducting structures in biosensor technology, albeit efficient, is still in the early stages of research, most notably employed in glucose detection [29]. Alternatively, poly 3,4-ethylenedioxythiophene (PEDOT) has been highlighted for its superior electrochemical features [23] and can enhance the performance of thiophene-pyrrole structures through copolymerization.

The current study proposes the polymer of ferrocene-derivatized 2,5-thienylpyrrole (further to be referred to as P(SNS-Fc)) and its copolymer with EDOT, for the first time, as immobilization supports for Tyrosinase (P(SNS-Fc)/Tyr and P(SNS-Fc-co-EDOT)/Tyr) in the development of catechol biosensors. Multi-wall carbon nanotubes (MWCNTs) were further introduced to enhance electron kinetics. The analytical characterization and stability investigations proved that the proposed concept represents a good alternative to reported analogs based on conducting matrices.

2. Materials and Methods

2.1. Materials.

Tyrosinase from mushroom (2687 Unit/mg solid) was purchased from Sigma (St. Louis, USA, www.sigmaaldrich.com). $LiClO_4$, $NaClO_4$, Multi-walled carbon nanotubes (MWCNT) (O.D. x L 6-9nm x 5µm, >95% (carbon)), sodium dodecyl sulfate (SDS), ethanol, acetonitrile were purchased from Sigma. All other chemicals were of analytical grade and purchased from Merck or Sigma.

2.2. Electrochemical polymerization and preparation of enzyme electrodes.

Synthesis of P(SNS-Fc) was performed as previously reported [30]. Shortly, P(SNS-Fc) films were prepared through potentiodynamic technique on Pt foil electrodes using 0.01 M monomer (SNS-Fc) in ACN/$LiClO_4$ solution. The potential was cycled between 0.0 and 0.9 V with a scan rate of 100 mV/s. A platinum wire and an Ag/Ag^+ electrode were used as the counter and reference electrodes, respectively, For P(SNS-Fc-co-EDOT), 5 µL of EDOT was introduced into the electrolysis cell, and the copolymer film was prepared by scanning the potential between 0.0 V and 1.0 V at a scan rate of 100 mV/s.

The addition of CNTs was performed by drop-casting 10 µL of 1 mg/mL CNTs-ethanol solution on the surface of the polymer-coated electrode. Immobilization of Tyrosinase (~40-100U) was done by cross-linking with different amounts of 1% glutaraldehyde (7.5 - 15 µL).

2.3 Instrumentation and principle of measurements.

All amperometric measurements were performed with the potentiostat GAMRY Ref 600 (GAMRY Instruments Inc., Pennsylvania, USA) in a three-electrode cell: Pt foil electrode (0.5 cm^2) - working electrode, Pt wire - counter electrode, Ag/AgCl (3M KCl) (BASI) - reference electrode. Optimized conditions (pH of buffer electrolyte and applied voltage) and controlled magnetic stirring were applied during amperometric measurements. The detection limit was calculated using 3Sb/m criteria, where m is the slope of the calibration curve, and Sb is the standard deviation of the responses at a minimal concentration (n = 10) [31].

3. Results and Discussion

3.1. Morphology characterization.

The synthesis and electrochemical characterization of the polymer platforms had been previously detailed in our reports [30, 32]. In the current study, particular attention has been given to the characterization of the (co)polymer system involving Tyr enzyme. Both polymer platforms were characterized by scanning electron microscopy (SEM) before and after the addition of CNTs and enzymes. The initial polymer films coated the electrode surface homogenously, and the copolymerization with EDOT led to an increase in the granular aspect of the surface (Figure. 1a,b), which should provide a large surface area, adequate for biomolecule immobilization, as seen in our previous study [32]. Subsequently, drop-casting of CNTs and enzyme immobilization resulted in typical fibrous structures [33], proving homogenous distribution and cross-linking, with increased porosity for the copolymer platform. The morphology proves similar to the one observed when the glucose oxidase enzyme was immobilized on this type of platform for glucose (bio)sensing [32].

3.2. Optimum biosensor design parameters.

The electrochemical characterization of the CP platforms has been recently disclosed when employed in glucose detection [32]. As such, herein, only design parameters related to catechol detection have been investigated. Operational parameters (potential, pH) for P(SNS-Fc)/CNTs/Tyr biosensor were optimized at -0.15 V vs. Ag/AgCl and pH 7.5 (Figure. S1a, S1b). Furthermore, optimum enzyme and cross-linker concentrations were established at 60 U and 10µL (Figure. S1c, S1d). The optimization experiment was performed identically for the copolymer platform P(SNS-Fc-co-EDOT)/CNTs/Tyr, showing very similar optimum parameters (Figure. S2). The results are in accordance with previous literature reports regarding Tyr biosensors based on the electrochemical reduction of quinone as the detection principle [34].

3.3. Analytical characterization.

Analytical characterization of both biosensor platforms (Figure. 2a-d,3a-d) also revealed very similar performance towards catechol detection as current response evolved linearly in the range 0.02 - 0.25 mM with the equation of y = -3.41x - 0.01 (R2 = 0.996) and y

= -2.5x - 0.001 (R2 = 0.995) for Pt/P(SNS-Fc)/CNT/Tyr and Pt/P(SNS-Fc-co-EDOT)/CNT/Tyr biosensor, respectively. The LOD value was calculated as 3.9 µM for the homopolymer-based sensor and it improved to 2.1 µM upon copolymerization of the ferrocene-tethered SNS monomer with EDOT.

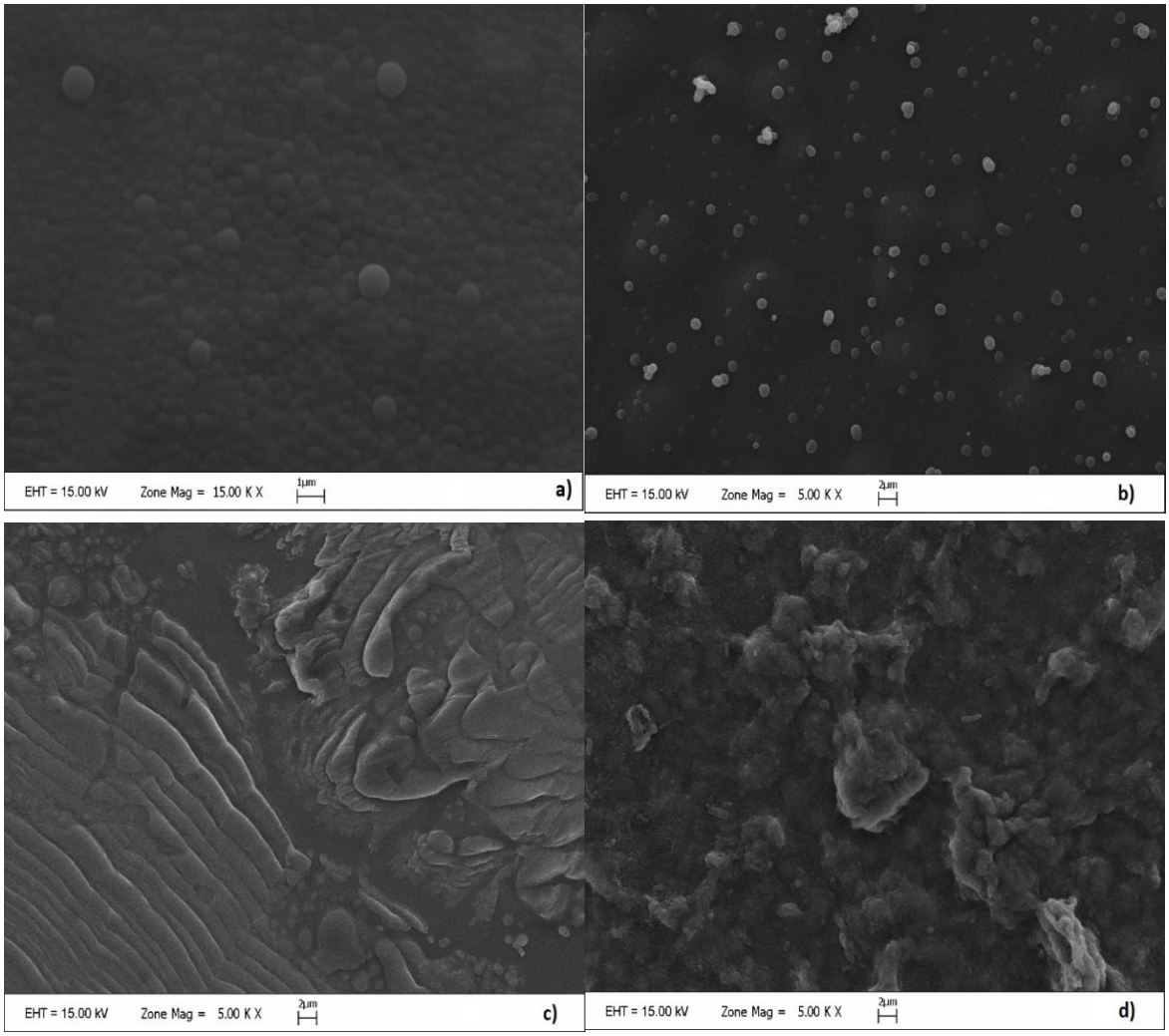


Figure 1. SEM characterization of (**a**) P(SNS-Fc); (**b**) P(SNS-Fc-co-EDOT); (**c**) P(SNS-Fc)/CNTs/Tyr; (**d**) P(SNS-Fc-co-EDOT)/CNT/Tyr.

K_m and I_{max} parameters were calculated (according to Lineweaver-Burk plots (Figure. 2d, 3d)) as 0.874 mM and 2.81 µA and 1.287 mM and 3.34 µA for Pt/P(SNS-Fc)/CNT/Tyr and Pt/P(SNS-Fc-co-EDOT)/CNT/Tyr, respectively. The slight difference observed in analytical data can be discussed considering both increased conductivity and decrease in the number of Fc moieties upon copolymerization, both factors counteracting each other, as proven by previous studies [32]. While the copolymerization increases the conductivity of the platform, the number of available Fc units is the more significant factor, a fact proven by publications concerned with the grafting of Fc moieties onto (non)conducting platforms for biosensing purposes [35, 36]. On this note, the motivation to employ an Fc-tethered CP platform in this study has been inspired by several reports on 'mediated' Tyr biosensors for phenol detection [37-40], which show that the electrochemical catechol recycling is amplified by the presence of a redox shuttle in the biosensing matrix. Our so-called 'reagentless mediated system' approach is more efficient since the redox component is not liable to leaking or diffusion barriers. Thus far, a single similar report on catechol detection is based on a hybrid thienyl pyrrole monomer derivatized with nitrophenol group and copolymerized with polypyrrole [41] that was employed as a spectrophotometric method for determination of analyte (Table 1).

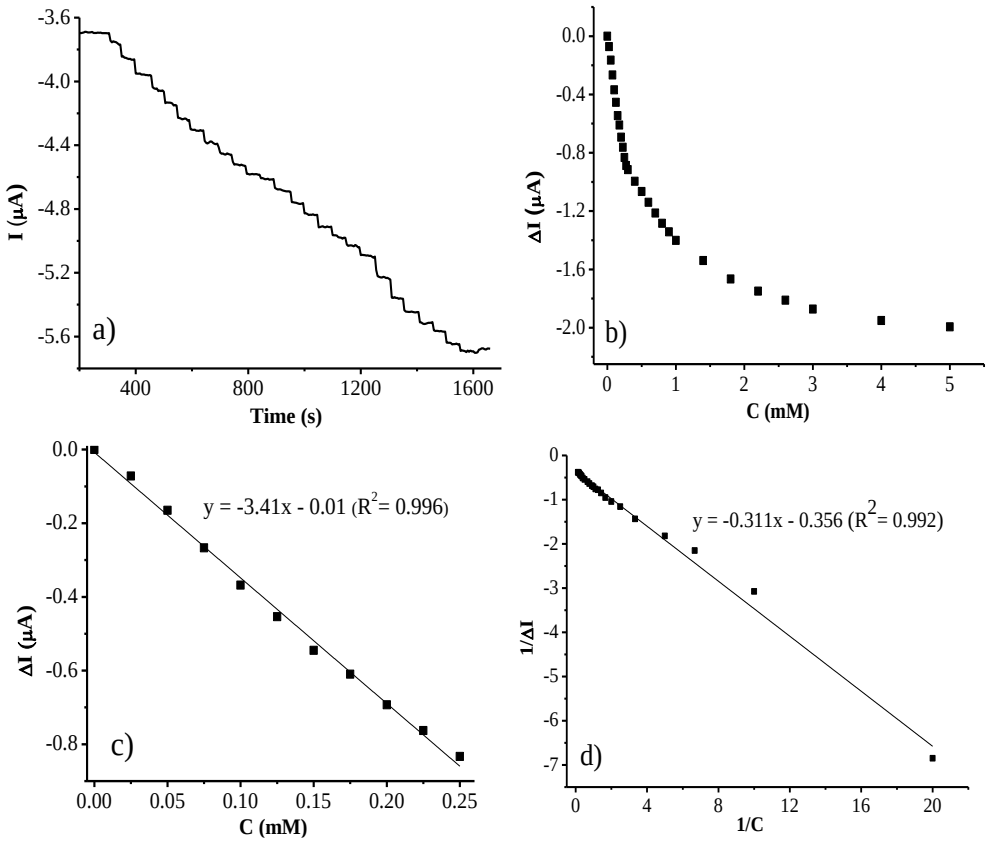


Figure 2. Calibration at -0.15 V potential for P(SNS-Fc)/CNT/Tyr: (**a**) amperometric response, (**b**) hyperbolic calibration curve; (**c**) linear fit, (**d**) Lineweaver - Burk plot; pH = 7.5; 0.1 M phosphate buffer, room temperature).

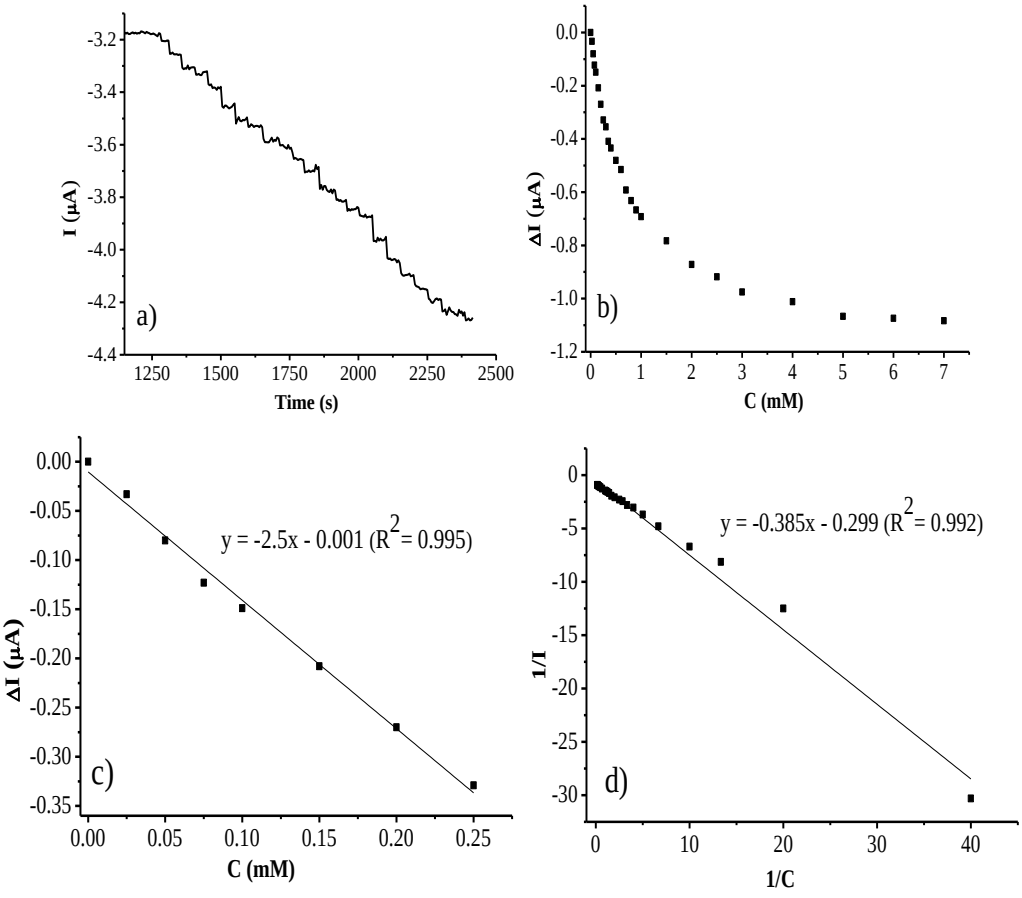


Figure 3. Calibration at -0.15 V potential for P(SNS-Fc-co-EDOT)/CNT/Tyr: (**a**) amperometric response, (**b**) hyperbolic calibration curves; (**c**) linear fit, (**d**) Lineweaver - Burk plot; pH = 7.5; 0.1 M phosphate buffer, room temperature).

3.4. Stability investigations.

Additional reasoning for grafting a mediator unit onto a conducting platform for developing a phenolic biosensor is given by the potential of improving the stability of analysis. Commonly, Tyr biosensors are liable to loss of activity due to the inherent instability and short lifetime of Tyr enzyme [42], but also due to electrode fouling by radical intermediates generated from the enzymatic catalysis [43]. In this context, if the electroreduction of the enzymatically generated quinone is mediated, thus, the reversibility is increased; it is thought that the fouling is reduced and, in turn, the stability of the sensor would increase [37]. The inhibition of the enzyme by its substrate (so-called 'suicide inactivation'[44]) may be delayed due to fast redox cycling [38].

Table 1. Summary of reported analogs.

Biosensor platform	Linear range (mM)	Sensitivity	LOD (µM)	Ref.
SPE/BSA-GA/Tyr	0.001–0.103	6.23 μAmM^{-1}	5.6	[40]
ITO/PAPCP/Tyr	0.0016 -0.1188	3.46 μAmM^{-1}	1.2	[27]
Pt/Os/Tyr	-	6.1 $nA\mu M^{-1}$	0.01	[38]
GCE/Ppy-MWCNTs/Tyr	0.003 - 0.05	8.0 $nA\mu M^{-1}$	0.671	[22]
SPE/MNP-MWCNTs-Tyr	0.01-0.12	4.05 $\mu AmM^{-1}cm^{-2}$	7.61	[42]
Pt/PANI-Tyr	0.005 - 0.14	-	0.05	[43]
Pt/PO_4^{-}Ppy/Tyr	0.01 - 0.12	0.047 $\mu A\mu M^{-1}$	0.84	[44]
Pt/[(SNS-NO_2)Ppy]/Tyr	0.05 - 0.5	0.0194	-	[41]
ITO/PVA-Silica/Tyr	0.01-0.2	5.7 μAmM^{-1}	10	[45]
GCE/PPy-PGA-P(GMA-co-Vfc)/Tyr	0.02-0.07	6.0 μAmM^{-1}	0.781	[46]
GC/pTN-GA/Tyr	0.001-0.3	5.04 μAmM^{-1}	6.0	[47]
Pt/P(SNS-Fc)/CNTs/Tyr	0.02 - 0.25	6.82 $\mu AmM^{-1}cm^{-2}$	3.9	
Pt/P(SNS-Fc-co-EDOT)/CNTs/Tyr	0.02 - 0.25	5.0 μAmM^{-1} cm^{-2}	2.1	This work

SPE: screen-printed electrode, BSA: bovine serum albumin, GA: glutaraldehyde, ITO: indium-tin oxide, PAPCP: poly (N-3-aminopropyl pyrrole-co-pyrrole), Os: Osmium complex, MNP: magnetic nanoparticles, PANI: polyaniline, SNS-NO2: 1-(4-nitrophenyl)-2,5-di(2-thienyl)-1H-pyrrole, Ppy: polypyrrole, pTN: poly(thionine), GMA: glycidylmethacrylate , Vfc: vinlyferrocene, PVA: polyvinyl alcohol.

Our study testifies to this, as the linear range reported herein for catechol detection (up to 0.25 mM) is slightly extended in comparison with literature (Table 1) data that reports availability for detection up to 0.103 mM [40], 0.12 mM [48] or 0.05 mM [22].

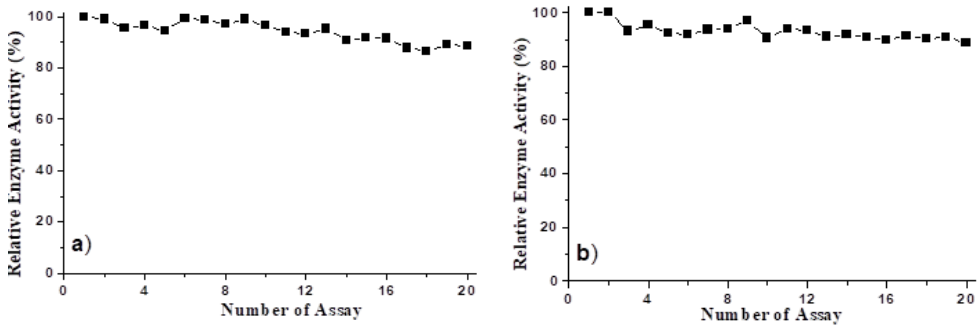


Figure 4. Operational stability of (**a**) P(SNS-Fc)/CNT/TYR and (**b**) P(SNS-Fc-co-EDOT)/CNT /TYR electrode (pH = 7.5; V=-0.15 V vs. Ag/AgCl; 0.1 M phosphate buffer, room temperature).

Relative deviations of 3.34% and 4.40% were obtained during 20 successive measurements for P(SNS-Fc)/CNT/Tyr and P(SNS-Fc-co-EDOT)/CNT/Tyr, respectively,

indicating good operational stability (~ 90% preserved activity, Figure. 4a, 4b) similar to recent reports based on Tyr enzyme [49, 50].

4. Conclusions

The current study aims to provide an alternative to conducting polymer-based matrices for catechol detection by introducing a ferrocene-tethered hybrid polymer type platform suitable for immobilization of Tyr enzyme. The incorporation of CNTs within the polymer P(SNS-Fc) and copolymer P(SNS-Fc-co-EDOT) matrices granted versatile properties for enzyme immobilization. As a result, adequate analytical characteristics were observed, accompanied by good stability given by the ability of the redox unit to facilitate electrochemical recycling of catechol at the electrode surface in a 'reagentless' manner. Thus, the work proposed herein has a twofold impact, common liabilities of mediated biosensor analysis are resolved, and stability issues regarding phenol detection through electrochemical reduction of quinone are improved.

Funding

Akdeniz University Research Funds.

Acknowledgments

The authors would like to acknowledge the financial support from Akdeniz University Research Funds.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bounegru, A. V.; Apetrei, C. Voltamperometric Sensors and Biosensors Based on Carbon Nanomaterials Used for Detecting Caffeic Acid. A Review. *Int. J. Mol. Sci.* **2020**, *21*, 9275, https://doi.org/10.3390/ijms21239275.
2. Harrad, L.; Bourais, I.; Mohammadi, H.; Amine, A. Recent Advances in Electrochemical Biosensors Based on Enzyme Inhibition for Clinical and Pharmaceutical Applications. *Sensors* **2018**, *18*, 164, http://dx.doi.org/10.3390/s18010164.
3. Da silva, W.; Ghica, M. E.; Ajayi, R. F.; Iwuoha, E. I.; Brett, C. M.A. Tyrosinase based amperometric biosensor for determination of tyramine in fermented food and beverages with gold nanoparticle doped poly(8-anilino-1-naphthalene sulphonic acid) modified electrode. *Food Chemistry* **2019**, *282*, 18–26, https://doi.org/10.1016/j.foodchem.2018.12.104.
4. Ibadullaeva, S. Zh.; Appazov, N. O.; Tarahovsky, Yu. S.; Zamyatina, E. A.; Fomkina, M. G.; Kim, Yu. A. Amperometric Multi-Enzyme Biosensors: Development and Application, a Short Review. *Biophysics* **2019**, *64*, 5, http://dx.doi.org/10.1134/S0006350919050063.
5. Min, K.; Park, G. W.; Yoo, Y. J.; Lee, J.-S. A perspective on the biotechnological applications of the versatile tyrosinase. *Bioresource Technology* **2019**, *289*, 121730, https://doi.org/10.1016/j.biortech.2019.121730.
6. Forzato, C.; Vida, V.; Berti, F. Biosensors and Sensing Systems for Rapid Analysis of Phenolic Compounds from Plants: A Comprehensive Review. *Biosensors* **2020**, *10*, 105, http://dx.doi.org/10.3390/bios10090105.
7. Alvarado-Ramírez, L.; Rostro-Alanis, M.; Rodríguez-Rodríguez, J.; Sosa-Hernández, J.E.; Melchor-Martínez, E.M.; Iqbal, H.M.N.; Parra-Saldívar, R. Enzyme (Single and Multiple) and Nanozyme Biosensors: Recent Developments and Their Novel Applications in the Water-Food-Health Nexus. *Biosensors* **2021**, *11*, 410, https://doi.org/10.3390/bios11110410.

8. Fritea, L.; Tertis, M,; Sandulescu, R,; Cristea, C. Enzyme–Graphene Platforms for Electrochemical Biosensor Design With Biomedical Applications. *Methods in Enzymology* **2018**, *609*, 295-328, http://dx.doi.org/10.1016/bs.mie.2018.05.010.
9. Shaikshavali, P.; Reddy, T. M.; Gopal, T. V.; Venkataprasad, G.; Narasimha, G.; Narayana, A. L.; Hussain, O. M. Development of carbon-based nanocomposite biosensor platform for the simultaneous detection of catechol and hydroquinone in local tap water. *J. Mater Sci: Mater Electron* **2021**, *32*, 5243–5258, https://doi.org/10.1007/s10854-021-05256-3.
10. Iqbal, S.; Ahmad, S. Recent development in hybrid conducting polymers: Synthesis, applications and future prospects. *Journal of Industrial and Engineering Chemistry* **2018**, *60*, 53–84, http://dx.doi.org/10.1016/j.jiec.2017.09.038.
11. Sharma, S.; Sudhakara, P.; Omran, A.A.B.; Singh, J.; Ilyas, R.A. Recent Trends and Developments in Conducting Polymer Nanocomposites for Multifunctional Applications. *Polymers* **2021**, *13*, 2898, http://dx.doi.org/10.3390/polym13172898.
12. Liu, Y.; Turner, A. P.F.; Zhao, M.; Mak, W. C. Processable enzyme-hybrid conductive polymer composites for electrochemical biosensing. *Biosensors and Bioelectronics* **2018**, *100*, 374–381, http://dx.doi.org/10.1016/j.bios.2017.09.021.
13. Ramanavicius, S.; Ramanavicius, A. Conducting Polymers in the Design of Biosensors and Biofuel Cells. *Polymers* **2021**, *13*, 49, http://dx.doi.org/10.3390/polym13010049.
14. Lakard, B. Electrochemical Biosensors Based on Conducting Polymers: A Review. *Appl. Sci.* **2020**, *10*, 6614, http://dx.doi.org/10.3390/app10186614.
15. Naseri, M.; Fotouhi, L.; Ehsani, A. Recent Progress in the Development of Conducting Polymer-Based Nanocomposites for Electrochemical Biosensors Applications: A Mini-Review. *Chem. Rec.* **2018**, *18*, 599–618, https://doi.org/10.1002/tcr.201700101.
16. Malhotra, B.D.; Chaubey, A.; Singh S. Prospects of conducting polymers in biosensors. *Analytica Chimica Acta* **2006**, *578*, 59-74, https://doi.org/10.1016/j.aca.2006.04.055.
17. Ahuja, T.; Mir, I.A.; Kumar, D. Biomolecular immobilization on conducting polymers for biosensing applications. *Biomaterials* **2007**, *28*, 791-805, https://doi.org/10.1016/j.biomaterials.2006.09.046.
18. Sasikumar, R.; Sethuraman, V.; Sathish, V. Electrochemical sensor for catechol based on a polyaniline-tyrosinase recognition element. *Journal of Bionanoscience* **2018**, *12*, 772-779, https://doi.org/10.1166/jbns.2018.1603.
19. Li, X.; Sun, C. Bioelectrochemical response of the polyaniline tyrosinase electrode to phenol. *Journal of Analytical Chemistry* **2005**, *60*, 1073–1077, https://doi.org/10.1007/s10809-005-0241-4.
20. Wang, P.; Liu, M.; Kan, J. Amperometric phenol biosensor based on polyaniline. *Sensors and Actuators B: Chemical* **2009**, *140*, 577-584, https://doi.org/10.1016/j.snb.2009.05.005.
21. Ameer, Q.; Adeloju, S.B. Development of a potentiometric catechol biosensor by entrapment of tyrosinase within polypyrrole film. *Sens Actuators B* **2009**, *140*, 5–11. https://doi.org/10.1016/j.snb.2009.03.056.
22. Ozoner, S.K.; Yalvac, M.; Erhan, E. Flow injection determination of catechol based on polypyrrole–carbon nanotube–tyrosinase biocomposite detector. *Current Applied Physics* **2010**, *10*, 323-328, https://doi.org/10.1016/j.cap.2009.06.017.
23. Védrine, C.; Fabiano, S.; Tran-Minh, C. Amperometric tyrosinase based biosensor using an electrogenerated polythiophene film as an entrapment support. *Talanta* **2003**, *59*, 535-544, https://pubmed.ncbi.nlm.nih.gov/18968938/.
24. Yildiz, H.B.; Sahmetlioglu, E.; Boyukbayram, A.E.; Toppare, L.; Yagci, Y. Immobilization of tyrosinase and alcohol oxidase in conducting copolymers of thiophene functionalized poly(vinyl alcohol) with pyrrole. *International journal of biological macromolecules* **2007**, *41*, 332-337, https://doi.org/10.1016/j.ijbiomac.2007.04.006.
25. Akbulut, H.; Bozokalfa, G.; Asker, D.N.; Demir, B.; Guler, E.; Odaci, Demirkol D.; Timur, S.; Yagci, Y. Polythiophene-g-poly(ethylene glycol) with lateral amino groups as a novel matrix for biosensor construction. *ACS Applied Materials & Interfaces* **2015**, *7*, 20612-20622, https://doi.org/10.1021/acsami.5b04967.
26. Ozoner, S.K.; Yilmaz, F.; Celik, A.; Keskinler, B.; Erhan, E. A novel poly (glycine methacrylate-co-3-thienylmethyl methacrylate)-polypyrrole-carbon nanotube-horseradish peroxidase composite film electrode for the detection of phenolic. *Current Applied Physics* **2011**, *11*, 402-408, https://doi.org/10.1016/j.cap.2010.08.010.

27. Rajesh, Takashima W.; Kaneto, K. Amperometric phenol biosensor based on covalent immobilization of tyrosinase onto an electrochemically prepared novel copolymer poly (N-3-aminopropyl pyrrole-co-pyrrole) film. *Sensors and Actuators B: Chemical* **2004**, *102*, 271-277, https://doi.org/10.1016/j.snb.2004.04.028.
28. Zotti, G.; Martina, S.; Wegner, G.; Schlüter, A.D. Well-defined pyrrole oligomers: Electrochemical and UV/vis studies. *Advanced Materials* **1992**, *4*, 798-801, https://doi.org/10.1002/adma.19920041206.
29. Apetrei, R.-M.; Camurlu, P. Review—Functional platforms for (Bio)sensing: thiophene-pyrrole hybrid polymers. *Journal of The Electrochemical Society* **2020**, *167*, 037557, https://doi.org/10.1149/1945-7111/ab6e5f.
30. Bicil, Z.; Camurlu, P.; Yucel, B.; Becer, B. Multichromic, ferrocene clicked poly(2,5 dithienylpyrrole)s. *Journal of Polymer Research* **2013**, *20*, 228, https://doi.org/10.1007/s10965-013-0228-z.
31. Shrivastava, A.; Gupta, V. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron Young Scientists* **2011**, *2*, 21–25, https://doi.org/10.4103/2229-5186.79345.
32. Altun, A.; Apetrei, R.-M.; Camurlu, P. Reagentless amperometric glucose biosensors: ferrocene-tetheringand copolymerization. *Journal of The Electrochemical Society* **2020**, *167*, 107507, https://doi.org/10.1149/1945-7111/ab9c81.
33. Ayranci, R.; Kirbay, F.O.; Demirkol, D.O.; Ak, M.; Timur, S. Copolymer based multifunctional conducting polymer film for fluorescence sensing of glucose. *Methods and Applications in Fluorescence* **2018**, *6*, 035012, https://doi.org/10.1088/2050-6120/aac519.
34. Karim, F.; Fakhruddin, A. Recent advances in the development of biosensor for phenol: a review. *Reviews in Environmental Science and Bio/Technology* **2012**, *11*, 261-274, https://doi.org/10.1007/s11157-012-9268-9.
35. Şenel, M. Construction of reagentless glucose biosensor based on ferrocene conjugated polypyrrole. *Synthetic metals* **2011**, *161*, 1861-1868, https://doi.org10.1016/j.synthmet.2011.06.025/.
36. Şenel, M.; Abasıyanık, M.F. Construction of a novel glucose biosensor based on covalent immobilization of glucose oxidase on Poly(glycidyl methacrylate-co-vinylferrocene). *Electroanalysis* **2010**, *22*, 1765-1771, https://doi.org/10.1002/elan.200900644.
37. Hedenmo, M.; Narváez, A.; Domínguez, E.; Katakis, I. Improved mediated tyrosinase amperometric enzyme electrodes. *Journal of Electroanalytical Chemistry* **1997**, *425*, 1-11, https://doi.org/10.1016/S0022-0728(96)04966-2.
38. Yildiz, H.B.; Castillo, J.; Guschin, D.A.; Toppare, L.; Schuhmann, W. Phenol biosensor based on electrochemically controlled integration of tyrosinase in a redox polymer. *Microchimica Acta* **2007**, *159*, 27-34, https://doi.org/10.1007/s00604-007-0768-1.
39. Kranz, C.; Wohlschläger, H.; Schmidt, H.L.; Schuhmann, W. Controlled electrochemical preparation of amperometric biosensors based on conducting polymer multilayers. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis* **1998**, *10*, 546-552, https://doi.org/10.1002/(SICI)1521-4109.
40. Montereali, M.R.; Vastarella, W.; Della, S. L.; Pilloton, R. Tyrosinase biosensor based on modified screen printed electrodes: Measurements of total phenol content. *International Journal of Environmental Analytical Chemistry* **2005**, *85*, 795-806, https://doi.org/10.1080/03067310500149775.
41. Tuncagil, S.; Varis, S.; Toppare, L. Design of a biosensor based on 1-(4-nitrophenyl)-2,5-di(2-thienyl)-1H pyrrole. *Journal of Molecular Catalysis B: Enzymatic* **2010,** *64*, 195-199. https://doi.org/10.1016/j.molcatb.2009.06.002.
42. Pérez-López, B.; Merkoçi, A. Magnetic nanoparticles modified with carbon nanotubes for electrocatalytic magnetoswitchable biosensing applications. *Advanced Functional Materials* **2011**, *21*, 255-260, https://doi.org10.1002/adfm.201001306/.
43. Tan, Y.; Kan, J.; L,i S. Amperometric biosensor for catechol using electrochemical template process. *Sensors and Actuators B: Chemical* **2011**, *152*, 285-291, https://doi.org/10.1016/j.snb.2010.12.021.
44. Apetrei, C.; Rodríguez-Méndez, M.; De Saja, J. Amperometric tyrosinase based biosensor using an electropolymerized phosphate-doped polypyrrole film as an immobilization support. Application for detection of phenolic compounds. *Electrochimica Acta* **2011**, *56*, 8919-8925, https://doi.org/10.1016/j.electacta.2011.07.127.
45. Oriero, D.A.; Gyan, I.O.; Bolshaw, B.W.; Cheng, I.F.; Aston, D.E.: Electrospun biocatalytic hybrid silica-PVA-tyrosinase fiber mats for electrochemical detection of phenols. *Microchem. J.* **2015**, *118*, 166–175, https://doi.org/10.1016/j.microc.2014.09.005.

46. Dursun, F.; Ozoner, S.K.; Demirci, A.; Gorur, M.; Yilmaz, F.; Erhan, E. Vinylferrocene copolymers based biosensors for phenol derivatives. *J. Chem. Tech. Biotechnol.* **2012**, *87*, 95–104, https://doi.org/10.1002/jctb.2688.
47. Lu, Z.; Wang, Y.; Zhang, Z.; Shen, Y.; Li, M. Tyrosinase Modified Poly(thionine) Electrodeposited Glassy Carbon Electrode for Amperometric Determination of Catechol. *Electrochemistry* **2017**, *85*, 17–22, https://doi.org/10.5796/electrochemistry.85.17.
48. Streffer, K.; Vijgenboom, E.; Tepper, A.W.; Makower, A.; Scheller, F.W.; Canters, G.W.; Wollenberger, U. Determination of phenolic compounds using recombinant tyrosinase from Streptomyces antibioticu. *Analytica Chimica Acta* **2001**, *427*, 201-210, https://doi.org/10.1016/S0003-2670(00)01040-0.
49. Li, J.; Gao, J.; Guo, T.; Huang, X.; Zhang, X.; Xu, C.; Xue, H. Hierarchically porous copolymer film as immobiliation matrix for phenol biosensor with high sensitivity. *ACS Applied Polymer Materials* **2019**, *1*, 3148-3156, https://doi.org/10.1021/acsapm.9b00795.
50. Stoytcheva, M.; Zlatev, R.; Velkova, Z.; Gochev, V.; Ayala, A.; Montero, G.; Valdez, B. Diazirine-functionalized nanostructured platform for enzymes photografting and electrochemical biosensing. *Electroanalysis* **2019**, *31*, 1526, https://doi.org/10.1002/elan.201900086.

Supplementary data

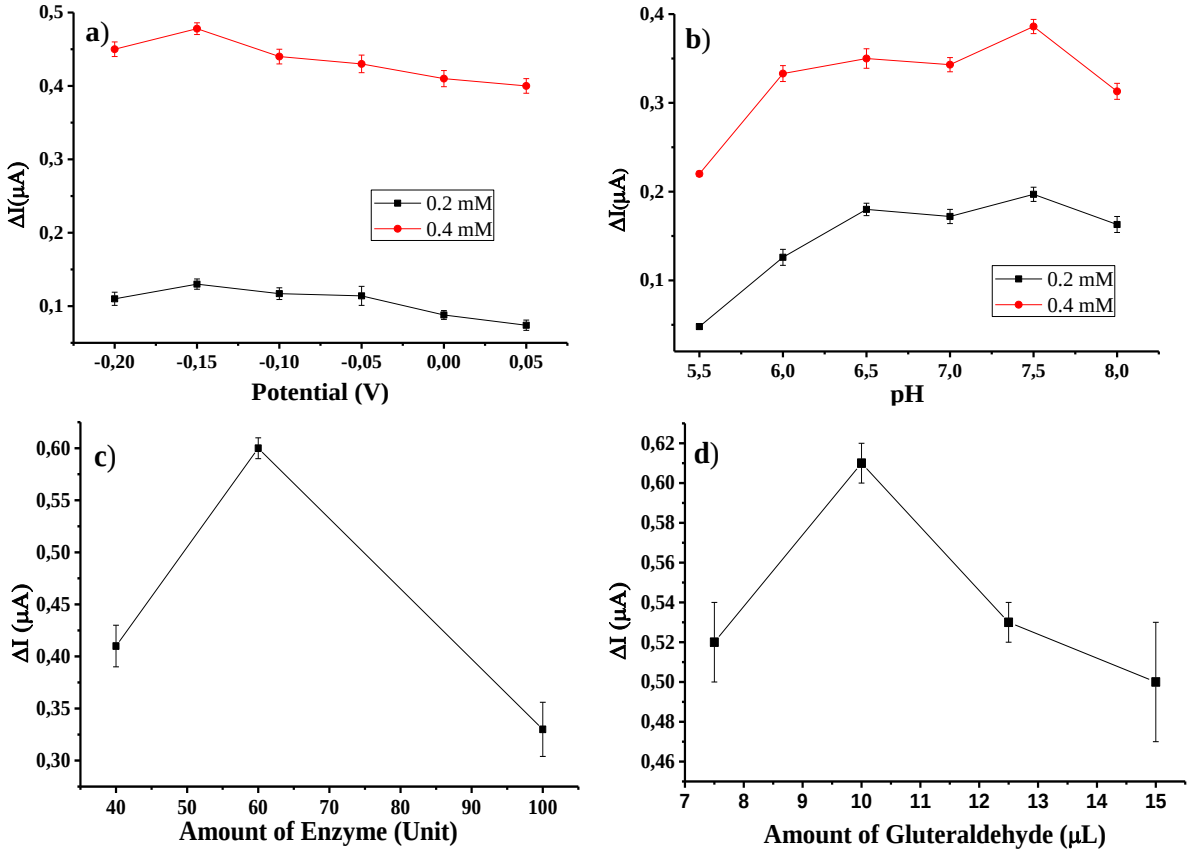


Figure S1. Optimum working parameters for Pt/P(SNS-Fc)/CNT/Tyr: (a) at the variation of potential; (b) at the variation of pH; (c) at the variation of amount of enzyme; (d) at the variation of amount of glutaraldehyde; room temperature, additions of catechol in 0.1 mM PBS.

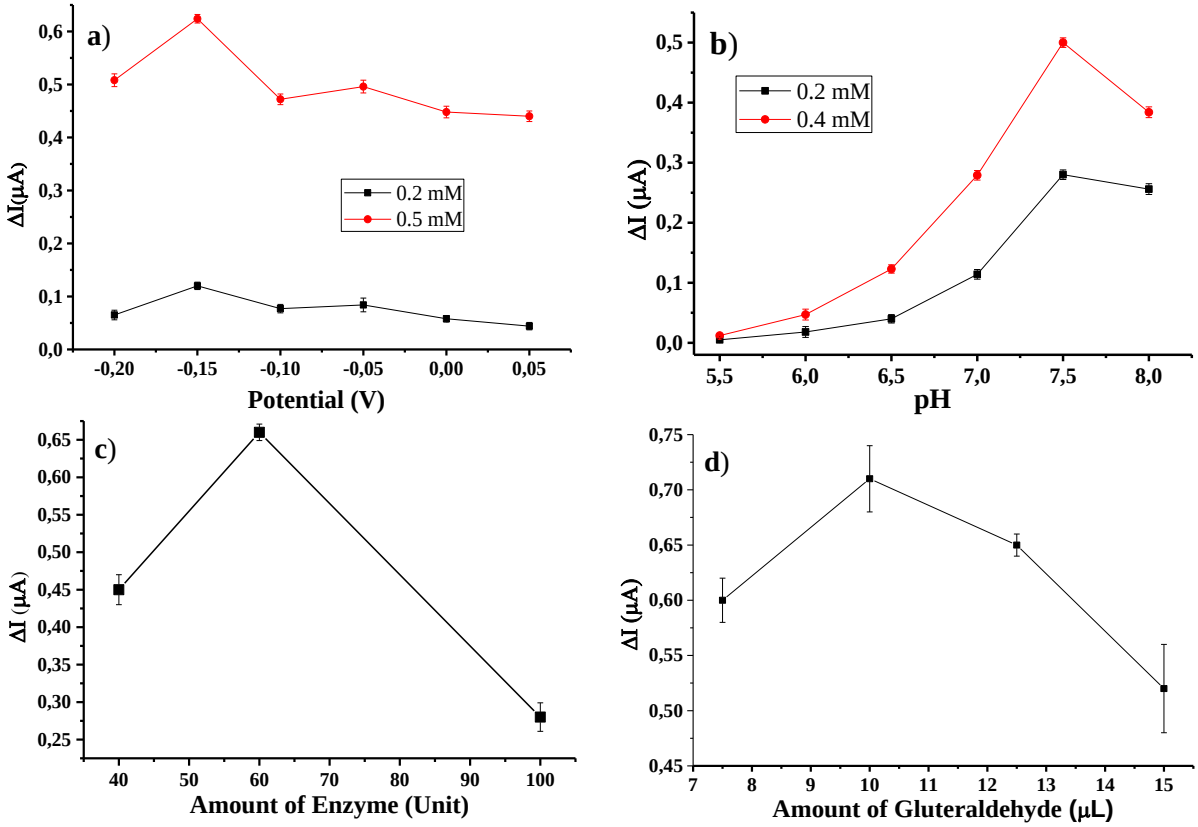


Figure S2. Optimum working parameters for Pt/P(SNS-Fc-co-EDOT)/CNT/Tyr: (a) at the variation of potential; (b) at the variation of pH; (c) at the variation of Amount of Enzyme; (d) at the variation of Amount of Glutaraldehyde; room temperature, additions of catechol in 0.1 mM PBS.