Direct exposure of dry enzymes to atmospheric pressure cold plasmas

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Abstract: Dry enzyme deposits were exposed to dielectric barrier discharges (DBDs) fed with pure helium, helium/oxygen and helium/ethylene mixtures, to reproduce the main plasma processes relevant to surface engineering, i.e., plasma treatment, etching and deposition, respectively. Results show that plasma-enzyme interaction leads to considerable decrease of enzyme activity only in case of oxygen-containing DBDs, for long exposure times. In contrast, enzymes fully retain their functionality when exposed to DBDs during deposition processes.

Keywords: dielectric barrier discharge, enzyme catalysis, plasma-enzyme interaction, enzyme immobilization.

1. Introduction

Enzyme immobilization received enormous attention in the last decades and still continues nowadays to attract growing interest in various applicative fields (e.g., analytical chemistry, biotechnology, synthetic chemistry) [1,2]. It has been demonstrated so far that enzyme immobilization onto solid supports enables the easy recovery and multiple reuse of enzymes, improving, in some cases, enzyme stability compared to enzymes in solution [1,2].

Plasma processes have been widely exploited to modify the surface of solid supports in order to allow enzyme immobilization through, for instance, physical adsorption and covalent bonding [3,4]. Interestingly, a novel plasma-based immobilization strategy has been recently proposed [5,6]. This strategy involves the injection of the aerosol of an enzyme solution in an atmospheric pressure cold plasma during a deposition process, to obtain in a single step the biomolecule entrapment in the plasma-deposited thin film [5,6].

The aim of our work is to achieve enzyme immobilization by overcoating with a plasma-deposited polymer layer. The proposed immobilization strategy consists of a two-step procedure in which enzymes are deposited by drop-casting onto a glass slide and, after drying, overcoated by a thin film deposited in a dielectric barrier discharge (DBD) at atmospheric pressure. The enzymes are expected to be entrapped under the polymer coating that should prevent enzyme leaching, while allowing the passage of substrate and product molecules. Since this approach involves the direct exposure of enzymes to the atmospheric pressure cold plasma, we have undertaken a comprehensive study of the effects of DBDs on enzymes functionality.

The following two enzymes were selected for this study:
- Glucose oxidase (GOx), catalyzing the oxidation of glucose to gluconic acid;
- Tyrosinase (Tyr), catalyzing the first two steps of melanin biosynthesis.

Dry deposits of glucose oxidase and tyrosinase were therefore exposed to DBDs fed with pure helium, helium/oxygen and helium/ethylene mixtures, to reproduce the main plasma processes relevant to surface engineering, i.e., plasma treatment, plasma etching and plasma-enhanced chemical vapour deposition (PE-CVD), respectively. Results enabled the identification of the experimental conditions that allow preserving or destroying enzyme functionality.

2. Materials and methods

Plasma processes were carried out using a parallel plate DBD reactor, as described elsewhere in full detail [7]. The DBD was generated at the excitation frequency of 20 kHz and applied voltages of 0.85 and 1.1 kV_{rms}. The discharge was fed with pure helium, helium/oxygen ($[O_2]$ = 1%) and helium/ethylene ($[C_2H_4]$ = 0.1 - 1%) mixtures. Process duration was varied between 10 min and 60 min.

5 μL drops of a solution of the selected enzyme were deposited on glass slides and dried [8]. By varying the enzyme concentration in the drop-casted solution it was possible to change both the amount of dried enzyme and the thickness of the enzyme deposit exposed to the DBD [8]. After the plasma process, the enzyme deposit was redissolved in buffer solution and its activity was spectrophotometrically assayed [8-10]. For selected plasma processes, the Michaelis-Menten plots (i.e., initial rate vs substrate concentration) were also obtained to determine the Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}) [8].

White light vertical scanning interferometry (WLVSI) was used for the three-dimensional non-contact imaging of enzyme deposits, and to evaluate enzyme deposit thickness before and after plasma processes. X-ray photoelectron spectroscopy (XPS) analyses were carried out to investigate the surface chemistry of the dry enzyme deposits before and after plasma exposure.

Results

First, GOx and Tyr dry deposits were exposed to DBDs fed with pure He and with a He/O_2 mixture ($[O_2] = 1\%$). As reported in **Table 1**, in case of a 100 µg dry deposit of GOx, after 30 min exposure to the He/O_2 DBD the enzyme residual activity decreases to 85% in comparison with the untreated sample (100%). GOx residual activity remains constant for longer exposure times [8]. In contrast, the He DBD does not appreciably affect enzyme functionality (>95% residual activity after 60 min exposure). WLVSI measurements showed a reduction of the thickness of the GOx deposit after exposure to an oxygen-containing DBD. Interestingly, very similar trends were observed for both the GOx residual activity and the residual thickness of the GOx deposit as a function of the exposure time to the He/O_2 DBD (**Table 1**). These evidences suggest the removal of the protein deposit through a mechanism of plasma etching. The etching rate seems to decrease with the exposure time and, eventually, vanishes after a characteristic time. XPS analyses showed that the interaction of the protein with the He/O_2 DBD mainly leads to the increase of the oxygen surface atomic concentration and, consequently, to a reduction of the carbon and nitrogen atomic percentages. This modification of the chemical structure of the GOx deposit surface seems to be responsible of the decrease of the etching rate with the exposure time [8].

The two enzymes respond differently to He/O_2 DBD exposure. GOx appears more prone to plasma etching. In contrast, Tyr exhibits strong resistance to etching; on the other hand, it suffers a certain inactivation upon plasma exposure. **Table 1** shows, in fact, that the residual activity of Tyr (5 µg deposit) after 60 min in He/O_2 DBD is ~60%, while the residual thickness of the protein deposit is 90%.

Table 1. Residual activity and residual thickness of glucose oxidase and tyrosinase exposed to a DBD fed with a He/O_2 mixture ($[O_2] = 1\%$) for different times.

Glucose Oxidase (100 µg deposit)		
Exposure time (min)	Residual activity (%)	Residual thickness (%)
0	100	100
30	85	88
60	85	82
Tyrosinase (5 µg deposit)		
Exposure time (min)	Residual activity (%)	Residual thickness (%)
0	100	100
30	67	94
60	64	90

In the second part of this study, DBDs fed with helium/ethylene mixtures were utilized to deposit a uniform polyethylene-like coating on the dry enzyme deposits [7,8]. After deposition, the coating was scratched, allowing the release and solubilization of the trapped enzyme in the buffer solution. The spectrophotometric assays showed that the He/C_2H_4 DBDs have only a slight impact on enzymes activity. In particular, in case of GOx, the residual activity is slightly reduced to 90% when an applied voltage of 1.1 kV_{rms} is used. GOx activity can be fully preserved by reducing the applied voltage to 0.85 kV_{rms}. On the other hand, the deposition time seems to be a critical parameter in case of tyrosinase. The exposure to a He/C_2H_4 DBD for 30 min leads, in fact, to a Tyr residual activity of 80%, regardless of the applied voltage and the ethylene concentration utilized in the PECVD process. Tyr activity can be fully retrieved by decreasing the deposition time to 10 min.

3. Conclusion

Enzyme dry deposits were exposed to dielectric barrier discharges generated under typical experimental conditions utilized for etching, treatment and thin film deposition.

Results reveal that plasma-enzyme interaction leads to considerable decrease of enzyme activity only in case of oxygen-containing plasmas, for long exposure times (>30 min). The optimized conditions for PECVD processes using helium/ethylene DBDs are friendly for enzymes and allow preserving their functionality.

This study enlightens a high stability of dry enzymes upon plasma exposure and opens the way to enzyme immobilization by overcoating with a plasma-deposited thin film.

4. References

[1] A. Abolpour Homaei, R. Sariri, F. Vianello, R. Stevanato, Journal of Chemical Biology, **6**, 85 (2013).

[2] A. Mallardi, V. Angarano, M. Magliulo, L. Torsi, G. Palazzo, Analytical Chemistry, **87**, 11337 (2015).

[3] M. M. Bilek, D. R. Mc Kenzie, Biophysical Reviews, **2**, 55 (2010).

[4] M. Ghasemi, M. J. G. Minier, M. Tatoulian, M. M. Chehimi, F. Arefi-Khonsari, Journal of Physical Chemistry B, **115**, 10228 (2011).

[5] P. Heyse, A. Van Hoeck, M. B. J. Roeffaers, J.-P. Raffin, A. Steinbüchel, T. Stöveken, J. Lammertyn, P. Verboven, P. A. Jacobs, J. Hofkens, S. Paulussen, B. F. Sels, Plasma Processes and Polymers, **8**, 965 (2011).

[6] F. Palumbo, G. Camporeale, Y.-W. Yang, J.-S. Wu, E. Sardella, G. Dilecce, C. D. Calvano, L. Quintieri, L. Caputo, F. Baruzzi, P. Favia, Plasma Processes and Polymers, **12**, 1302 (2015).

[7] F. Fanelli, F. Fracassi, R. d'Agostino, Plasma Processes and Polymers, **2**, 688 (2005).

[8] F. Fanelli, F. Fracassi, A. Lapenna, V. Angarano, G. Palazzo, A. Mallardi, Advanced Materials Interfaces, **5**, 1801373 (2018).

[9] S. J. Miskiewicz, B. B. Arnett, G. E. Simon, Clinical Chemistry, **19**, 253 (1973).

[10] E. Selinheimo, D. NiEidhin, C. Steffensen, J. Nielsen, A. Lomascolo, S. Halaouli, E. Record, D. O'Beirne, J. Buchert, K. Kruus, Journal of Biotechnology, **130**, 471 (2007).