Enzyme-free antifouling hydrogen peroxide biosensor for lab-on-chip and implant applications

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Abstract

Hydrogen peroxide (H_2O_2) is an important signaling molecule in biological organisms since it is a side product of various enzymatic reactions and a marker for local inflammation [1]. Many electrochemical sensors have been developed to monitor H_2O_2 *in vitro* and *in vivo* but their long-term use in contact with cells and tissues is limited due to sensor biofouling and degradation of the biorecognition layer (often based on peroxidases) [2].

In this work we attempted to replace biological peroxidases by an electrodeposited nanocomposite of Prussian blue (PB) and gold (Au) nanoparticles that act as a H_2O_2 catalyst [3]. The nanocomposite was coated with polydopamine (PDA) in order to increase its stability and the sensitivity towards H_2O_2 was verified. Lastly, we show that the PDA layers can be easily modified with sulfobetaïne methacrylate and methacrylic acid copolymer that demonstrates excellent antifouling behavior *in vitro*.

Sensor architecture

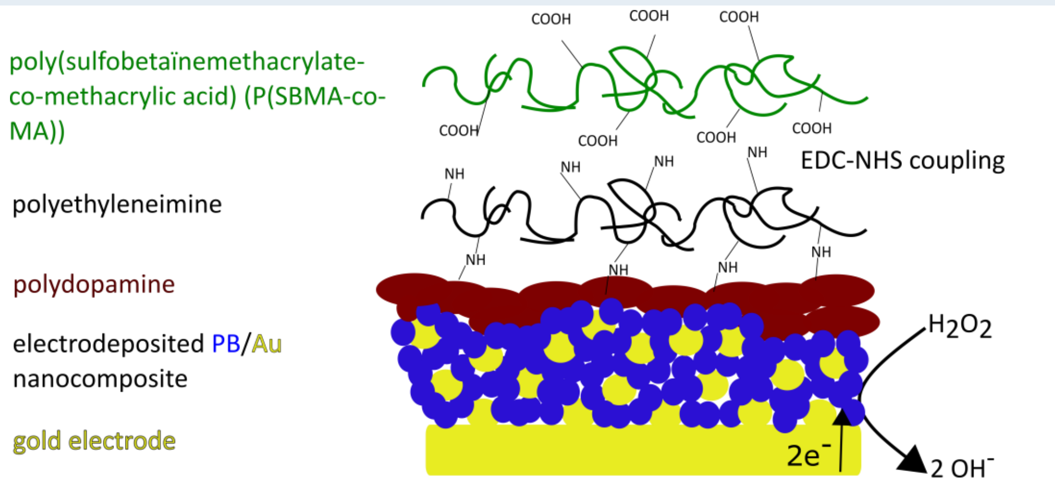

Figure 1. Envisioned sensor architecture

Electrodeposition and morphology

PB/Au layers are electrodeposited more slowly, are smoother and consist of smaller particles than PB layers.

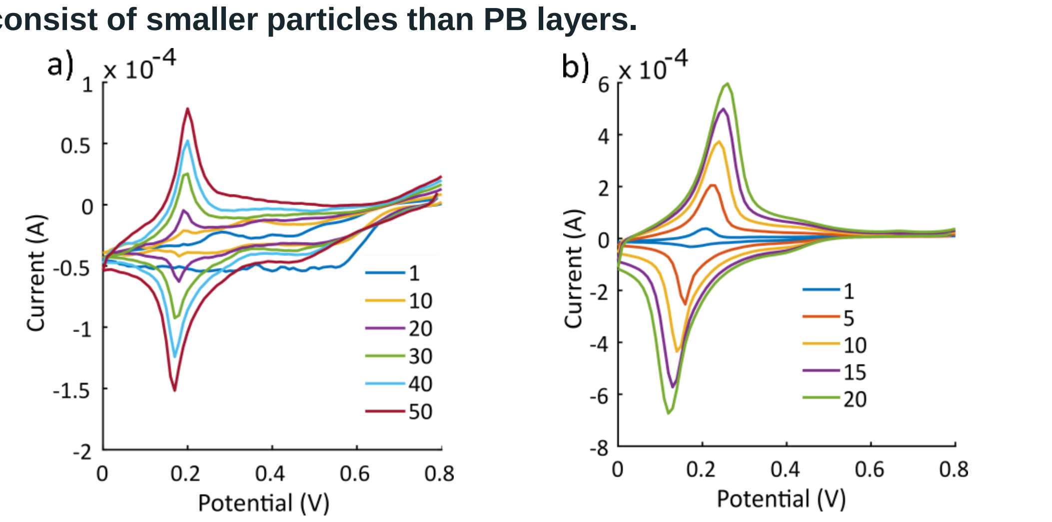

Figure 2. Electrodeposition of a) PB/Au and b) PB layers by cycling between 0 and 0.8 V from a solution containing $K_3Fe(CN_6)$, $FeCl_3$ and for PB/Au also $AuCl_3$. Steady growth of the PB redox peak at 0.2 V indicates layer growth. Electrode diameter is 4 mm^2 and numbers indicate cycles.

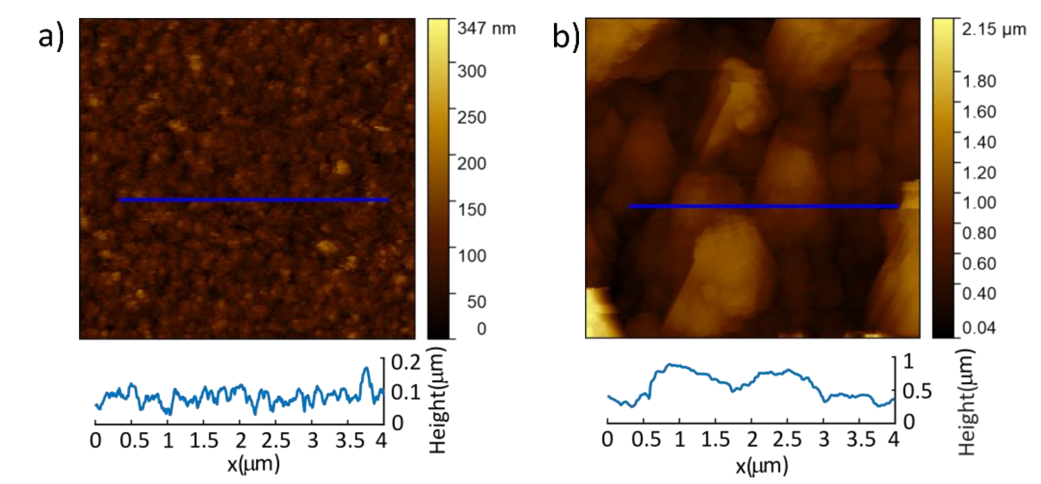

Figure 3. AFM scans of a) PB/Au and b) PB layers showing morphology. PB shows µ-sized particles and PB/Au layer consists of <100 nm particles.

Stability and sensitivity

PDA coating increases overall stability: PB/PDA has 98.5 % current retention after 50 cycles vs 91.5 % after 10 cycles for PB (cycling in pH 7 phosphate buffer, 0.1 M KCL). PB/Au/PDA layers have a higher sensitivity and stability in PBS compared to PB/PDA layers.

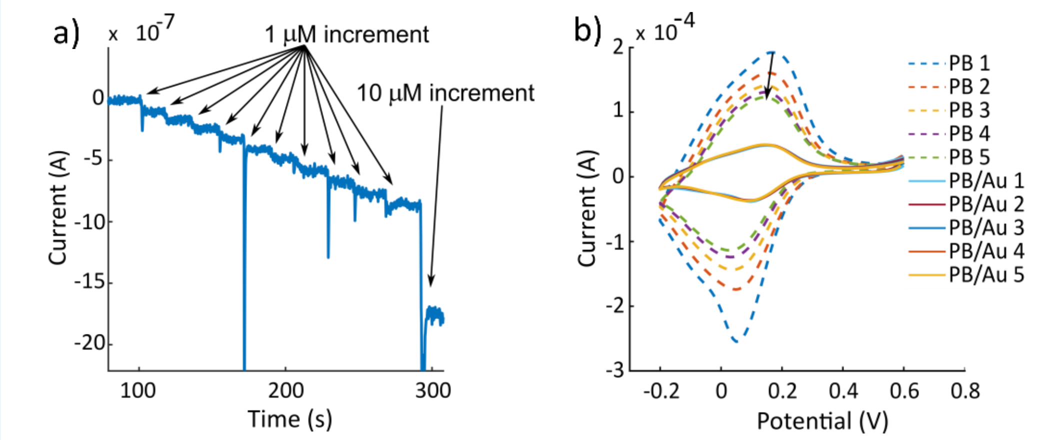

Figure 4. a) Successive additions of H_2O_2 for a PB/Au/PDA layer in PBS. Current was measured at 0 V vs Ag/AgCl. A **sensitivity of 670 vs 357 nA μM^{-1} cm^{-2}** for PB/PDA layer was found with an LOD of 0.58 μM vs 1.23 μM. b) Stability of PB/Au/PDA vs PB/PDA in PBS. Decrease in peak current indicates degradation.

Antifouling

Conjugating P(SBMA-co-MA) to PDA layer inhibits protein and fibroblast adhesion (for more than 9 days) *in vitro*.

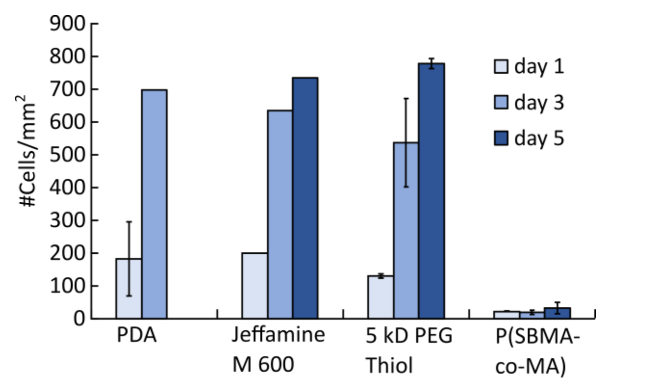

Figure 6. Cell adhesion on PDA functionalized with various antifouling molecules.

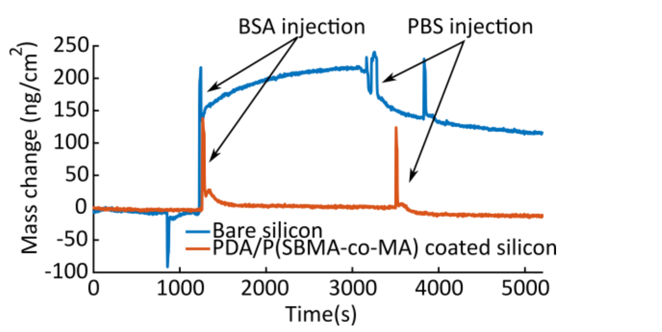

Figure 7. Adhesion of Bovine serum albumin (BSA) measured by QCM.

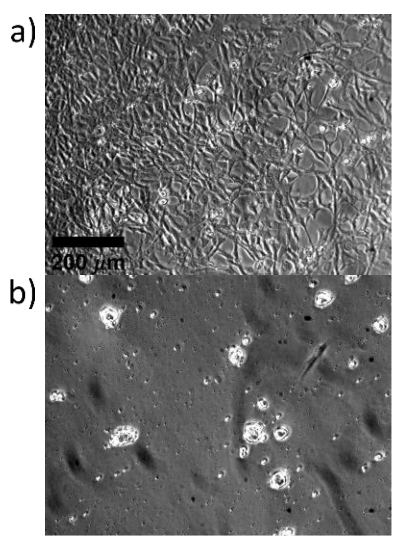

Figure 8. a) Confluent fibroblasts on a bare silica surface (4 days after seeding). b) Few fibroblast clusters on a PDA/P(SBMA-co-MA) coated surface (9 days after seeding).

Conclusion

The electrodeposited PB/Au has better sensitivity and stability than PB layers. Coating with polydopamine increases stability and allows for functionalization with antifouling polymers. Amongst these, P(SBMA-co-MA) has shown significant improvement over commonly used PEG brushes and inhibits fibroblast adhesion for more than 9 days.

References

[1] W. Chen et al. Analyst, 2012,137, 49-58
[2] G. Rong et al. ACS Sens. 2017, 2, 3, 327–338
[3] W. Wang et al. Analyst, 2014,139, 2904-2911

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