ChemPubSoc Europe

DOI: 10.1002/celc.201500080

CHEMELECTROCHEM Articles

Detection of Leukemic Cells by using Jacalin as the Biorecognition Layer: A New Strategy for the Detection of Circulating Tumor Cells

Juliana Cancino-Bernardi,* Valeria S. Marangoni, Henrique A. M. Faria, and Valtencir Zucolotto*[a]

The possibility of early cancer diagnosis has inspired the development of electrochemical systems that could improve both cell detection and activity monitoring, especially for circulating tumor cells. In this study, we propose an impedimetric leukemia sensor based on the use of immobilized jacalin, which is a lectin overexpressed in most types of human cancers. The biosensor is prepared through the chemical adsorption of (3-aminopropil)trimetoxisilano on indium tin oxide electrodes, followed by immersion in a jacalin solution. The performance of the jacalin-modified electrodes toward monocytic leukemic cells, THP-1, and myeloblastic leukemic cells, OCI-AMI3, was investigated through electrochemical impedance spectroscopy. Our results revealed that, upon using the jacalin biorecognition layer, the sensors were able to differentiate leukemic cells from healthy monocyte cells, even at low percentage ranges, with limits of detection of 4 ± 1 cells mL^{-1} for OCI-AMI3 and 3 ± 1 cells mL^{-1} for THP-1.

1. Introduction

The development of new diagnosis systems capable of improving cell detection as well as activity monitoring is a relevant topic in medicine, mainly for clinical diagnostics, toxicity monitoring, and life-science research.[1] In particular, the accurate qualitative detection of cells represents a critical step in cancer diagnosis. The development of efficient sensor systems may improve the early detection of circulating tumor cells, which has been shown to be a key mechanism related to the development of metastasis.[2] Several techniques for the detection of cancer cells have been developed, including flow cytometry and polymerase chain reaction (PCR) analyses.[2,3] A disadvantage, however, is that these methods are expensive or need advanced instrumentation. Flow cytometers are sophisticated instruments that require highly trained operators. Moreover, the cost of sheath fluid, fluorophores, replacement parts, and analysis software also contribute to the high costs regarding flow cytometry analyses.

Electrochemical sensors have attracted attention because of their simplicity and rapid response.[4] For example, Feng et al.[3a] developed an aptamer/graphene-based electrochemical sensor for the detection of cancer cells. The authors used a DNA aptamer with high binding affinity to nucleolin, which is overexpressed in the plasma membrane of tumor cells. Liu et al.[5] proposed a cancer-cell sensor by using folic acid molecules immobilized on the electrode surface as the outmost layer to selectively recognize folic receptor-enriched HeLa cells. To produce sensors with a high affinity for specific cancer cells, Moscovici et al.[6] and Maltez-da Costa et al.[7] immobilized EpCAM antibodies, which are overexpressed in a variety of tumor cells, to detect prostate cancer cells and circulating tumor cells, respectively.

One important characteristic that has been discussed in the literature is the anomalous glycosylation of human cancers cells.[8] Recent studies indicate that some, if not all, aberrant glycosylation is a result of initial oncogenic transformation, and it has been suggested as a key event in the induction of invasion and metastasis.[8a,9] The early detection of this glycosylation process, or even its monitoring, could be an important way to increase the chances of success in the treatment of cancer. Consequently, as lectins bind mono- and oligosaccharides reversibly with high specificity,[10] these proteins may be good candidates for glycosylation detection in cells.

Jacalin is a galactose-specific lectin from the seeds of jackfruit (*Artocarpus integrifolia*), which has a tetrameric form with a weight of 66000 Da at neutral pH.[10,11] This protein has been shown to be highly promising for biomedical applications, because of its ability to specifically recognize tumor-associated T-antigenic disaccharide Galβ1-3GalNAc[11,12] overexpressed in most of human carcinomas.[9,13] Recently, our group has reported the development of jacalin-conjugated gold nanoparticles as specific markers for leukemia cells.[14]

Few papers in the literature have used lectin as a selective layer for cancer-cell detection. Recently, a lectin-based biosensor was developed by Zhang et al.[15] for detection of cancer cells, using concanavalin A and *Sambucus nigra* agglutinin,

[a] Dr. J. Cancino-Bernardi,[+] V. S. Marangoni,[+] H. A. M. Faria, Prof. Dr. V. Zucolotto
Nanomedicine and Nanotoxicology Group
Physics Institute of São Carlos, University of São Paulo
Avenida Trabalhador São Carlense, 400, CEP13560970 (Brazil)
Phone: (+55) 16 3373 8656
E-mail: jcancino@ursa.ifsc.usp.br
zuco@ifsc.usp.br

[+] These authors contributed equally

which are specific for mannose and sialic acid, respectively. The authors showed that mannose exhibited high expression levels in both normal and cancer cells, whereas sialic acid was more abundant in cancer cells compared to normal controls.[15]

In this study, we developed an impedimetric leukemia biosensor based on jacalin as a selective biorecognition layer to identify leukemic cells. We show that the use of a lectin layer is an efficient alternative for cancer-cell sensor fabrication, improving selectivity towards the detection of cancer cells and/or circulating tumor cells.

2. Results and Discussion

Leukemia represents a type of cancer, for which early diagnosis is not straightforward,[16] which is mainly attributed to the complex variety of cells and metabolites contained in human fluid samples. As a strategy to improve the efficiency of leukemia-cell sensors, several studies have focused on the ability of glycan molecules to detect specific cancer cells.[17] Here, jacalin has been applied as a selective biorecognition layer for the detection of circulating tumor cells in blood fluids.

Electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM) were used to evaluate the functionalization of the electrodes. Figure 1 A displays the Nyquist plots of the (3-aminopropil)trimetoxisilano (APTS)-functionalized indium tin oxide (ITO) electrodes (ITO-NH_2) after incubation with jacalin (ITO-NH_2-jacalin) for 12 h at 4 °C. The zeta potential of jacalin at pH 7.4 was estimated to be +2.28 mV and, for this reason, the interaction of jacalin with APTS probably occurred through hydrogen bonds instead of electrostatic interactions. The latter was also observed in our previous study regarding the conjugation of jacalin with polyamidoamine dendrimer-coated gold nanoparticles.[14]

To verify whether the protein maintained its sugar-binding activity and to show that jacalin is selective towards sugar binding, the ITO-NH_2-jacalin electrode was immersed into a D-galactose solution and EIS measurements were performed in the absence and presence of D-galactose, as shown in Figure 1 A (ITO-NH_2-jacalin + D-gal).

EIS results, as shown in Figure 1 A, revealed that the modification of the ITO-NH_2 with jacalin increased the charge-transfer resistance from $\Delta R_{CT} = 596 \pm 20\ \Omega$ for ITO-NH_2 electrode to $2070 \pm 14\ \Omega$ for the ITO-NH_2-jacalin electrode, an increase of 1474 Ω in the charge resistance, which is approximately 3.5 times the resistance, indicating that the lectin was efficiently immobilized on the modified electrode.

The morphologies of the modified electrodes, ITO-NH_2 and ITO-NH_2-jacalin, were evaluated using AFM. Figure 1 B revealed an increase in the roughness values of the ITO-NH_2-jacalin electrode (continuous, red line) in comparison to ITO-NH_2 electrodes (dashed black line). The surface morphologies of ITO-NH_2 and ITO-NH_2-jacalin electrodes are shown in Figures 1 C and Figure 1 D, respectively. From Figure 1 D, we can see that jacalin molecules were distributed uniformly on the ITO-NH_2 sur-

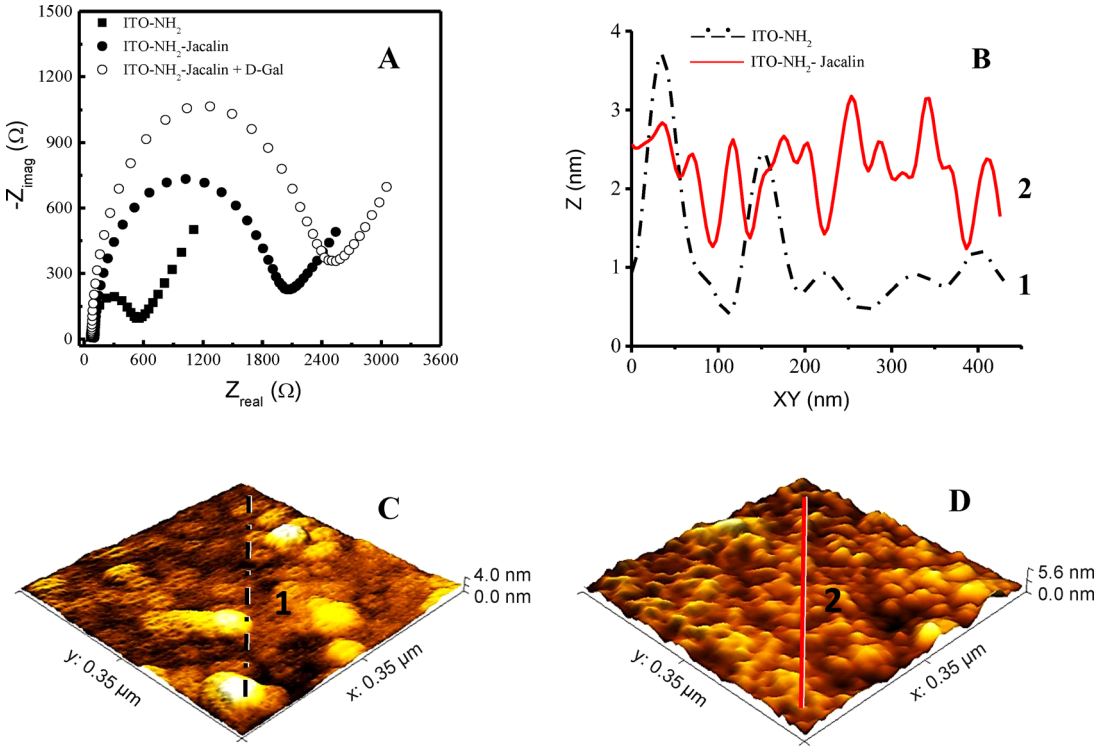


Figure 1. A) Nyquist plots obtained from EIS measurements when using modified electrodes after each modification process: aminosilane functionalization (ITO-NH_2), jacalin adsorption (ITO-NH_2-jacalin), and detection of D-galactose by using jacalin-modified electrodes. AC perturbation voltage of 0.01 V in the frequency range from 0.1 Hz to 10 kHz and in a buffer solution (PBS, pH 7.4) containing 4 mM $[Fe(CN)_6]^{3-/4-}$. B) AFM roughness profile of the ITO-NH_2 electrode (dashed black line) and ITO-NH_2-jacalin electrode (continuous red line), acquired from the AFM images shown. AFM topography of C) the ITO-NH_2 electrode and D) the ITO-NH_2-jacalin electrode. AFM operating in tapping mode and recorded in air.

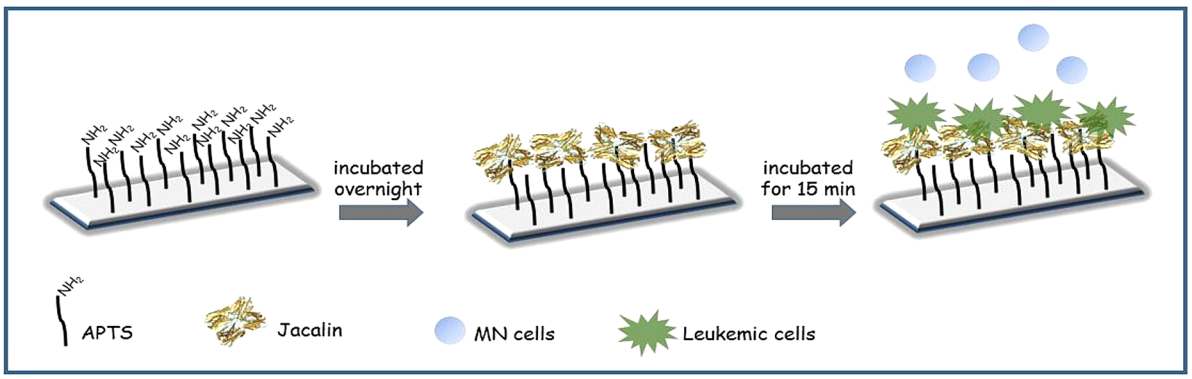


Figure 2. Schematic representation of the detection of leukemic cells when using the ITO-NH_2-jacalin biosensors. Images not to scale.

face. These AFM analyses corroborated the EIS measurements, confirming jacalin immobilization onto ITO-NH_2 electrodes.

The maintenance of the sugar-binding activity of jacalin is one of the most important features after the adsorption process, which may indicate the success of the biosensor in cancer-cell detection. From Figure 1 A, it could be observed that the ITO-NH_2-jacalin electrodes—containing immobilized jacalin—were able to detect D-galactose after only 15 min of incubation, indicating that the binding activity of the jacalin molecule has not been affected by immobilization.

The metastatic process of circulating tumor cells (CTCs) has been reported to be driven by certain carbohydrate residues presenting in the cells, which allows for specific interaction with other cells.[17a] The use of ITO-NH_2-jacalin electrodes may be advantageous in detecting CTCs, as the jacalin bioreceptor layer can specifically interact with such carbohydrate residues, promoting fast detection with lower detection limits.

THP-1 and OCI-AMI3 leukemic cell lines were chosen as a CTC model to be used in the detection tests with EIS measurements. Healthy monocyte (MN) cells have also been used for comparison. Figure 2 depicts a schematic representation of the detection of leukemic cells when using the ITO-NH_2-jacalin biosensors.

The detection ability of jacalin-modified electrodes was revealed through EIS experiments, as shown in Figure 3. According to Figure 3 B, for 1×10^2 OCI-AMI3 cells, $\Delta R_{CT}=1611\pm33\ \Omega$, whereas for 1×10^3 OCI-AMI3 cells, $\Delta R_{CT}=2354\pm29\ \Omega$, and for 1×10^5 OCI-AMI3 cells, $\Delta R_{CT}=3223\pm37\ \Omega$, representing an increase of 4.7, 4.1, and 4.2 times the charge-transfer resistance for the electrode in the absence of cancer cells, respectively. The same was observed for THP-1 leukemic cells. From Nyquist plots of Figure 3 C, it is possible to observe an increase in the charge-transfer resistances of 5.5, 4.9, and 4.6 times for 1×10^2, 1×10^3, and 1×10^5 cells, respectively. Although normal MN cells have also been detected at different concentrations (Figure 3 A), the difference in the charge-transfer resistance was not significantly higher compared to OCI-AMI3 and THP-1 leukemia cells under the same conditions. The latter is related to the ability of the sensors to specifically bind to sugar molecules overexpressed in the leukemia cells in comparison to nonspecific interactions with healthy MN cells. The variations in the ΔR_{CT} values are summarized in Table 1.

Table 1. Variation of ΔR_{CT} values calculated from the Nyquist plots collected after incubating the ITO-NH_2-jacalin electrodes in healthy MN, THP-1, and OCI-AMI3 cell lines at different concentrations.

Concentration [cells mL^{-1}]	ΔR_{CT} [Ω] MN	THP-1	OCI-AMI3
1×10^2	345 ± 17	1939 ± 25	1611 ± 33
1×10^3	577 ± 36	2908 ± 36	2354 ± 29
1×10^5	775 ± 24	3638 ± 41	3223 ± 37

The EIS analyses presented in Figure 3 revealed that the impedance increased with increasing cell concentration, resulting in a linear relationship between R_{CT} and the logarithmic of cell concentrations in the range of 1×10^2–1×10^6 cells mL^{-1}. The latter allowed us to estimate the limit of detection (LOD) of the modified electrodes, which was 4 ± 1 cells mL^{-1} for OCI-AMI3 and 3 ± 1 cells mL^{-1} for THP-1, with correlation coefficients (R) of 0.9617 for OCI-AMI 3 and 0.9882 for THP-1 cells. The LOD for MN cells was also estimated as 16 ± 3 cells mL^{-1} with $R=0.9182$, according to the LOD calculated from the $3\alpha/b$ ratio, where α is the standard deviation calculated from ten blank samples and b refers to the slope of the calibration curve. The performance of our biosensor was better in terms of LOD in comparison to some related biosensors in the literature. For example, a LOD of 10 cells mL^{-1} was found by Chen, using a glassy carbon electrode modified with graphene and concanavalin A.[18] The LOD for DU145 cells detection when using concanavalin A as a selective layer was found to be 125 cells per sensor[6] and 50 cells mL^{-1} for K562 cells.[19]

Also, it is important to mention that ITO-NH_2-jacalin biosensors were able to detect 12 ± 3 and 8 ± 2 cells mL^{-1} for OCI-AMP3 and THP-1, respectively, according to the limit of quantification calculated by the $10\alpha/b$ ratio. The low quantification limit exhibited by our sensor systems implies that smaller amounts of cells are needed for diagnosis. This may represent an advantage, especially for early diagnosis, as flow cytometry methods require approximately 1×10^5 cells mL^{-1} for diagnosis. Flow cytometry is the standard technique used to distinguish between healthy and cancer cells in routine analyses. Usually, patients suffering from leukemia present more than 15 000 cells mm^{-3}, and thousands of millions of cells are obtained from tissue biopsies.[7a,20]

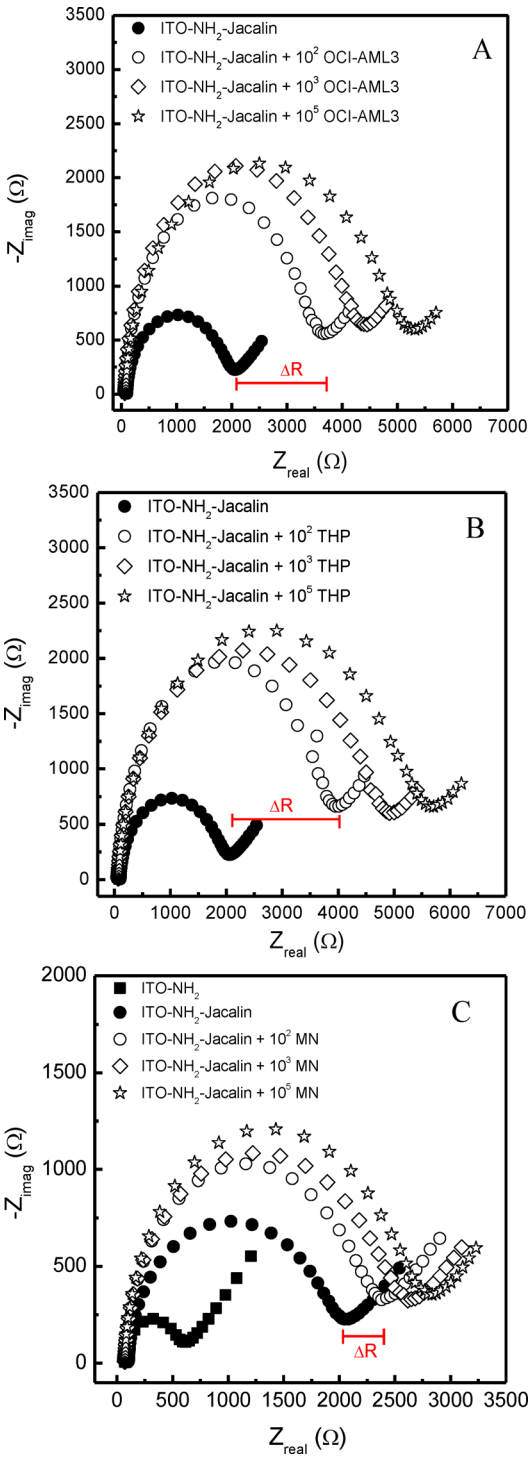


Figure 3. Nyquist plots collected after incubating the ITO-NH_2-jacalin electrodes in A) OCI-AMI3, B) THP-1, and C) MN cell lines at different concentrations (10^2, 10^3, and 10^5 cells mL^{-1}). Before each EIS measurement, the modified electrodes were incubated for 15 min to interact with each cell line. AC perturbation voltage of 0.01 V in the frequency range from 0.1 Hz to 10 kHz in a buffer solution (PBS, pH 7.4) containing 4 mM $[Fe(CN)_6]^{3-/4-}$.

Few studies have used lectins as selective layers for cancer detection. Concanavalin A is the most popular molecule used for this purpose. For example, concanavalin A has been functionalized with carbon nanotubes and used for the recognition of specific cancer cells. The electrodes exhibited a linear response from 1×10^4 to 1×10^7 cells mL^{-1} with a LOD of 50 for individual K562 cells. However, healthy cells have not been tested to check the selectivity and sensitivity of the sensor.[19]

A number of other biosensors for cancer-cell detection based on different materials as the biorecognition layer have been reported. In many cases, the use of nanomaterials has been reported as an efficient strategy to improve cancer-cell detection. For example, Feng et al.[3a] developed a graphene-functionalized electrochemical aptasensor for cancer-cell detection. The authors observed a significant increase in the signal upon incubation with cancer cells. This behavior was also reported by Ivanov et al.[21] in the detection of circulating cancer cells by using a chip-based nanostructured sensor. Our results are comparable to those in the latter reports, in which aptamers or RNA sequences have been used; however, the easy and low-cost extraction of jacalin represents an advantage for producing disposable modified electrodes for CTC detection.

To test the selectivity of the ITO-NH_2-jacalin biosensors, detection measurements were performed in mixtures containing only 10% leukemia cells (THP-1 and OCI-AMI3) and 90% of healthy MNs, with a total concentration of 10^3 cells mL^{-1}. Figure 4 shows the Nyquist plots (Figure 4a) and the ΔR_{CT} histogram (Figure 4b) obtained through EIS measurements.

As observed in Figure 4, the ITO-NH_2-jacalin biosensor was able to detect leukemic THP-1 and OCI-AMI3 cells from a solution mixture. As shown in Figure 4B, the changes in the charge-transfer resistance were $\Delta R_{CT}=384\pm36\ \Omega$ for 100% of MN cells, $1573\pm28\ \Omega$ for 100% THP-1 cells, and $1025\pm46\ \Omega$ for 10% THP + 90% MN cells. Based upon the latter results, it is possible to infer that THP-1 exhibited a higher affinity for the jacalin-modified electrodes compared to the healthy MN cells. This is related to the higher number of sugar molecules present in the THP-1 cell surface.

To verify the applicability of the jacalin-modified electrodes in real samples, two samples containing leukocytes cells collected from leukemia-diagnosed patients (P1 and P2) were centrifuged and re-suspended in pH 7.4 phosphate-buffered saline (PBS) solution containing 10^2, 10^3, and 10^5 cells mL^{-1} for detection experiments. The variation of ΔR_{CT} provided by the Nyquist plots for independent modified electrodes incubated with the patient's cells and their mixtures at a ratio of 90% of healthy MNs are shown in Figure 5.

Figure 5 revealed that the ITO-NH_2-jacalin biosensors can detect leukemic cells from real samples, even in the presence of healthy MN cells. For P1, an increase in the electrode resistance was observed. The resistance varied from $\Delta R_{CT}=202\pm19\ \Omega$ for 100% of healthy MN cells to $\Delta R_{CT}=441\pm45\ \Omega$ for 100% leukemic sample from patient P1. A similar behavior was observed for P2 cells, but with less difference in the resistance compared with the MN cells. To analyze the real samples, some points should be taken into account. Although the real simples had a pretreatment, these samples have more biomolecules than leukemic cells, for example, antibodies or proteins from the total blood. Moreover, these patients were detected to have leukemia, and it is unknown if the leukemia is from THP-1 or OCI-AMI3 cells, or another kind of leukemic cell such

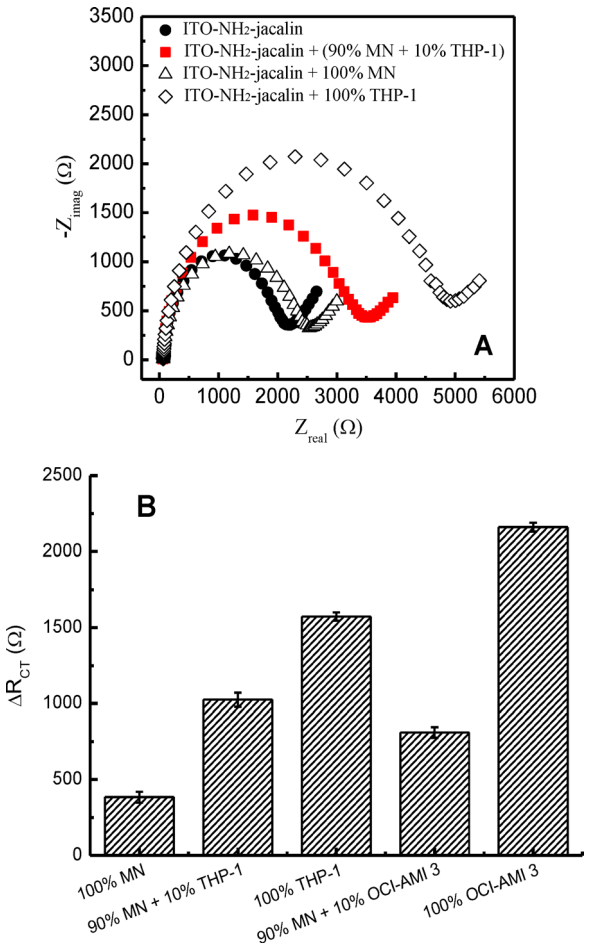


Figure 4. A) Nyquist plots obtained through EIS measurements collected after incubating the ITO-NH_2-jacalin electrode in 10% of THP-1 and 90% of MN mixtures of cells at a total concentration of 10^3 cells mL^{-1} as well as in 100% of each cell for 15 min. AC perturbation voltage of 0.01 V in the frequency range from 0.1 Hz to 10 kHz in a buffer solution (PBS, pH 7.4) containing 4 mM $[Fe(CN)_6]^{3-/4-}$. B) Variation of ΔR_{CT} after incubating independent electrodes with MN, THP-1, and OCI-AMI3 cell lines and their mixtures 90% MN/10% of each leukemic cell. AC perturbation voltage of 0.01 V in the frequency range from 0.1 Hz to 10 kHz in a buffer solution (PBS, pH 7.4) containing 4 mM $[Fe(CN)_6]^{3-/4-}$.

as K562 or NB4 leukemic cells. The number of cells, that is, the concentration of the leukemic cells in the blood, depends on the stage of the disease. It is important to emphasize that, despite the fact that our biosensor was able to detect leukemic cancer cells, specific and conventional techniques including flow cytometry or PCR should be applicable for a more detailed investigation/confirmation.

3. Conclusions

We developed a jacalin-modified biosensor with a high potential for application in the detection of leukemic and circulating tumor cells. As a key innovation, our system comprised a jacalin-based biorecognition layer, which was able to detect and differentiate between leukemic and healthy cells. The results show that, for THP-1 and OCI-AMI3 leukemic cells, the resistance values obtained in the EIS experiments were at least five

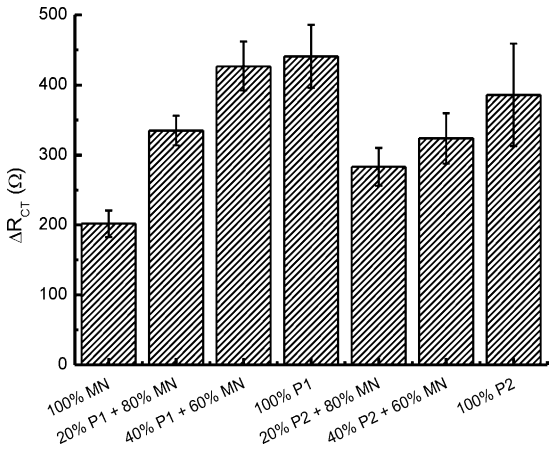


Figure 5. Variation of ΔR from the Nyquist plots of the sensor recorded after incubating independent electrodes with healthy MN cells from leukemia patients (P1 and P2) and their mixtures in a ratio of 90% of healthy MNs to 10% leukocyte cells of each patient's sample. AC perturbation voltage of 0.01 V in the frequency range from 0.1 Hz to 10 kHz in a buffer solution (PBS, pH 7.4) containing 4 mM $[Fe(CN)_6]^{3-/4-}$.

times higher than those in healthy MN cells. The LOD of our biosensor was 4 ± 1 cells mL^{-1} for OCI-AMI3 and 3 ± 1 cells mL^{-1} for THP-1, with quantification limits of 12 ± 3 and 8 ± 2 cell mL^{-1} cancer cells, respectively. These values revealed the high performance and ability of the ITO-NH_2-jacalin biosensor to differentiate normal from cancer cells, even at low concentrations and in real blood samples. Therefore, this sensor may represent a simple, alternative method for leukemic cell detection and/or their activity monitoring as circulating tumor cells.

Experimental Section

Materials

All solutions were prepared with Milli-Q water (18 MΩ cm^{-1}) from a Millipore system and all reagents were used as received. D-Galactose and APTS were acquired from Sigma–Aldrich. Buffer solutions were prepared by using potassium phosphate monobasic, sodium phosphate dibasic, and sodium chloride from Sigma–Aldrich.

Jacalin was obtained from the crude extract derived from seeds of *Artocarpus integrifolia* (jackfruit) and purified through affinity chromatography (Sepharose D-galactose-immobilized resin) and molecular exclusion chromatography, according to methods described in the literature.[14,22]

Biosensor Fabrication

ITO electrodes with a resistance of 8–12 Ω (Delta Technologies, Loveland, CO, USA) were washed in acetone, isopropyl alcohol, ethanol, and Milli-Q water for 5 min in each solution by using an ultrasonic bath to remove impurities and dried with nitrogen gas. The surface modification of the ITO electrodes with the aminosilane (ITO-NH_2 electrode) was performed by immersing the electrodes in a 0.12 mmol L^{-1} solution of APTS in ethanol for 4 h. ITO-NH_2-modified electrodes were incubated in a 0.4 mg mL^{-1} jacalin solution overnight (at least 12 h) at 4 °C. The electrodes were washed

in PBS solution to remove non-adsorbed molecules before being used in the electrochemical experiments. The electrodes were analyzed with EIS during all steps of the modification process, using 4 mmol L^{-1} potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$) in PBS pH 7.4 as a probe. AFM was employed to evaluate the morphology of the ITO-NH_2 and ITO-NH_2-jacalin electrodes by using a NanoSurf Flexa atomic force microscope (Nanosurf, Switzerland) in tapping mode with a resonant frequency of 300 KHz and vibration amplitude of 40.2 mV. The images were recorded in air under humidity control. The Gwyddion software was used for treatment of the images.

Cell Culture

Leukemic cell lines THP-1 (monocytic leukemia cell) and OCI-AMI3 (myeloblastic leukemic cell) were kindly supplied by Prof. Dr. Eduardo M. Rego from the Center for Cell-Based Therapy, Medical School of Ribeirao Preto, University of Sao Paulo, Brazil. Non-adherent cells were cultured in RPMI 1640 medium (Vitrocell, Campinas, Brazil) supplemented with 10% fetal bovine serum (FBS; Vitrocell, Campinas, Brazil) and maintained in a 5% CO_2 incubator at 37 °C. Cells were seeded at an initial density of 60000 cells mL^{-1} and 2 days later, or at 80% confluence, the cells were collected and separated from the medium by centrifugation at 2000 *g* for 10 min and re-suspended in pH 7.4 PBS solution containing 10^2, 10^3, or 10^5 cells mL^{-1} for experimentation. PBS was used as the electrolyte because of the isotonic and nontoxic environment provided for the cells during the measurements. This solution has been used for a variety of procedures in cell cultures, including washing of the cells before dissociation, transporting of cells or tissue, diluting of cells for counting, and so forth.

Peripheral blood mononuclear cells (PBMCs) were collected from healthy adult female and male volunteers, excluding pregnant women and tobacco or medication users. The subjects were informed of all legal procedures. The study was approved by the Ethics Committee of the Federal University of São Carlos in accordance with the principles in the Declaration of Helsinki. Mononuclear cells were isolated by using Ficoll–Hypaque 1077 (Histopaque, Sigma–Aldrich) fractionation. PBMCs were collected carefully, washed twice in PBS (240 g for 10 min), and suspended in RPMI 1640 medium with 10% FBS. Cell viability was investigated immediately before the assays (data not shown), showing viability higher than 95%, according to the trypan blue (Sigma–Aldrich, USA) exclusion assay. They were collected and separated from the medium by centrifugation at 2000 *g* for 10 min.

MN cells were separated from PBMC by using the adhesion technique. Cells (1×10^5 monocytes per well) were plated in 24-well culture plates after they were counted in neutral red solution (Sigma–Aldrich, USA) to adhere to RPMI 1640 medium without FBS for 2 h at 37 °C in a 5% CO_2 atmosphere. Next, the supernatant and the non-adhered cells were collected and discarded. The wells containing the MN cells were recharged with RPMI 1640 containing 10% FBS at 37 °C in a 5% CO_2 atmosphere and cultured for 24 h. Finally, MN cells were centrifuged and re-suspended in pH 7.4 PBS solution containing 10^2, 10^3, and 10^5 cells mL^{-1} for experimentation.

Two real samples, containing leukocytes, from patients diagnosed with leukemia (assigned as P1 and P2) were kindly provided by Prof. Dr. Eduardo M. Rego from the Center for Cell-Based Therapy, Medical School of Ribeirao Preto, University of Sao Paulo, Brazil. The cells were centrifuged and re-suspended in pH 7.4 PBS solution containing 10^2, 10^3, or 10^5 cells mL^{-1} for experimentation.

Electrochemical Analyses

Electrochemical measurements were carried out by using ITO electrodes with a geometric area of 0.6 cm^2 as the working electrodes. A Pt foil (2 cm^2 geometric area) and Ag/AgCl (saturated with 3 mol L^{-1} KCl) electrode were used as auxiliary and reference electrodes, respectively, and were employed in all experiments in a three-electrode one-compartment electrochemical cell with a polytetrafluoretilene (PTFE) cover. Electrochemical experiments were performed by using a PGSTAT40 Autolab electrochemical system (Eco Chemie, Utrecht, Netherlands) equipped with PGSTAT-12 and GPES/FRA 4.9 software (Eco Chemie, Utrecht, Netherlands). EIS was carried out in 0.1 mol L^{-1} PBS solution (pH 7.4) containing 4 mmol L^{-1} potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$). The experiments were performed in a frequency range from 10 KHz to 0.1 Hz with an amplitude of 0.01 V. The EIS spectra were represented as Nyquist plots ($-Z_{imag}$ vs. Z_{real}). The measurements were performed by using an open circuit.

The detection measurements were carried out by immersing the electrodes in the buffer solutions containing different cell concentrations for an optimized incubation time of 15 min at room temperature. All measurements were performed in duplicate in at least three independent experiments.

Acknowledgements

The authors gratefully acknowledge Prof. Dr. Eduardo M. Rêgo from the Center for Cell-Based Therapy, Medical School of Ribeirão Preto, University of São Paulo, Brazil who kindly provided the blood cells from leukemia patients. Financial support from FAPESP (the São Paulo research foundation) is also gratefully acknowledged.

Keywords: biosensors · circulating tumor cells · jacalin · leukemia · nanomedicine

[1] C. Hao, F. Yan, L. Ding, Y. D. Xue, H. X. Ju, *Electrochem. Commun.* **2007**, *9*, 1359 – 1364.
[2] P. Paterlini-Brechot, N. L. Benali, *Cancer Lett.* **2007**, *253*, 180 – 204.
[3] a) L. Y. Feng, Y. Chen, J. S. Ren, X. G. Qu, *Biomaterials* **2011**, *32*, 2930 – 2937; b) C. C. Kang, C. C. Chang, T. C. Chang, L. J. Liao, P. J. Lou, W. Xie, E. S. Yeung, *Analyst* **2007**, *132*, 745 – 749.
[4] a) A. C. Perinoto, R. M. Maki, M. C. Colhone, F. R. Santos, V. Migliaccio, K. R. Daghastanli, R. G. Stabeli, P. Ciancaglini, F. V. Paulovich, M. C. F. de Oliveira, O. N. Oliveira, V. Zucolotto, *Anal. Chem.* **2010**, *82*, 9763 – 9768; b) C. A. S. Ballesteros, J. Cancino, V. S. Marangoni, V. Zucolotto, *Sens. Actuators B* **2014**, *198*, 377 – 383; c) B. C. Janegitz, J. Cancino, V. Zucolotto, *J. Nanosci. Nanotechnol.* **2014**, *14*, 378 – 389.
[5] J. Liu, Y. Qin, D. Li, T. Wang, Y. Liu, J. Wang, E. Wang, *Biosens. Bioelectron.* **2013**, *41*, 436 – 441.
[6] M. Moscovici, A. Bhimji, S. O. Kelley, *Lab Chip* **2013**, *13*, 940 – 946.
[7] a) M. Maltez-da Costa, A. de La Escosura-Muniz, C. Nogues, L. Barrios, E. Ibanez, A. Merkoci, *Small* **2012**, *8*, 3605 – 3612; b) M. Maltez-da Costa, A. de La Escosura-Muniz, C. Nogues, L. Barrios, E. Ibanez, A. Merkoci, *Nano Lett.* **2012**, *12*, 4164 – 4171.
[8] a) S. Hakomori, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10231 – 10233; b) M. N. Christiansen, J. Chik, L. Lee, M. Anugraham, J. L. Abrahams, N. H. Packer, *Proteomics* **2014**, *14*, 525 – 546.
[9] G. Poiroux, M. Pitie, R. Culerrier, B. Segui, E. J. M. Van Damme, W. J. Peumans, J. Bernadou, T. Levade, P. Rouge, A. Barre, H. Benoist, *Photochem. Photobiol.* **2011**, *87*, 370 – 377.
[10] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637 – 674.
[11] R. Sankaranarayanan, K. Sekar, R. Banerjee, V. Sharma, A. Surolia, M. Vijayan, *Nat. Struct. Biol.* **1996**, *3*, 596 – 603.

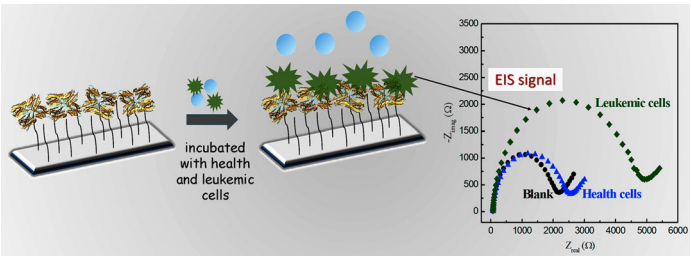
[12] S. K. Mahanta, M. V. K. Sastry, A. Surolia, *Biochem. J.* **1990**, *265*, 831 – 840.
[13] a) A. A. Jeyaprakash, P. G. Rani, G. B. Reddy, S. Banumathi, C. Betzel, K. Sekar, A. Surolia, M. Vijayan, *J. Mol. Biol.* **2002**, *321*, 637 – 645; b) S. Kabir, *J. Immunol. Methods* **1998**, *212*, 193 – 211.
[14] V. S. Marangoni, I. M. Paino, V. Zucolotto, *Colloids Surf. B* **2013**, *112*, 380 – 386.
[15] X. A. Zhang, Y. Q. Teng, Y. Fu, L. L. Xu, S. P. Zhang, B. He, C. G. Wang, W. Zhang, *Anal. Chem.* **2010**, *82*, 9455 – 9460.
[16] a) R. T. Calado, J. N. Cooper, H. M. Padilla-Nash, E. M. Sloand, C. O. Wu, P. Scheinberg, T. Ried, N. S. Young, *Leukemia* **2012**, *26*, 700 – 707; b) J. Hasford, M. Pfirrmann, A. Hochhaus, *Leukemia* **2005**, *19*, 497 – 499.
[17] a) T. Lange, T. R. Samatov, A. G. Tonevitsky, U. Schumacher, *Carbohydr. Res.* **2014**, *389*, 39 – 45; b) C. M. Ferrer, M. J. Reginato, *Cancer Discovery* **2014**, *4*, 275 – 277.
[18] X. J. Chen, Y. Z. Wang, Y. Y. Zhang, Z. H. Chen, Y. Liu, Z. L. Li, J. H. Li, *Anal. Chem.* **2014**, *86*, 4278 – 4286.
[19] Y. D. Xue, L. Bao, X. R. Xiao, L. Ding, J. P. Lei, H. X. Ju, *Anal. Biochem.* **2011**, *410*, 92 – 97.
[20] C. V. Cox, H. M. Martin, P. R. Kearns, P. Virgo, R. S. Evely, A. Blair, *Blood* **2007**, *109*, 674 – 682.
[21] I. Ivanov, J. Stojcic, A. Stanimirovic, E. Sargent, R. K. Nam, S. O. Kelley, *Anal. Chem.* **2013**, *85*, 398 – 403.
[22] D. Gupta, N. Rao, K. D. Puri, K. L. Matta, A. Surolia, *J. Biol. Chem.* **1992**, *267*, 8909 – 8918.

Received: February 18, 2015
Published online on ■■ ▮▮, 2015

J. Cancino-Bernardi, V. S. Marangoni, H. A. M. Faria, V. Zucolotto**

■■ – ■■

Detection of Leukemic Cells by using Jacalin as the Biorecognition Layer: A New Strategy for the Detection of Circulating Tumor Cells



Jacalin and Hyde: An impedimetric leukemia sensor based on the use of immobilized jacalin is proposed. The performance of jacalin-modified electrodes toward monocytic leukemic cells, THP-1, and myeloblastic leukemic cells, OCI-AMl3, is investigated through electrochemical impedance spectroscopy. The sensors are able to differentiate leukemic cells from healthy monocyte cells, even in low percentage ranges.