DEVELOPMENT OF DIAGNOSTIC AMPEROMETRIC AND PIEZOELECTRIC IMMUNOSENSORS BASED ON HUMAN OPEN-tTG FOR RECOGNITION OF ANTI-tTG ANTIBODIES IN CELIAC DISEASE

Anita Manfredi - Eleonora Umiltà - Marco Giannetto - Monica Mattarozzi - Maria Careri
Dipartimento di Chimica
Università di Parma
anita.manfredi@studenti.unipr.it

In this work, novel amperometric and piezoelectric immunosensors based on the immobilization of tissue transglutaminase in its open conformation (Open-tTG) were developed. Both immunosensing strategies allowed to obtain a reliable model for the fabrication of diagnostic devices for the determination of anti-tTG antibodies in serum of celiac patients

Sviluppo di immunosensori amperometrici e piezoeletrici per la rivelazione di anticorpi anti-tTG nella diagnosi della malattia celiaca
Il presente lavoro riguarda la realizzazione di immunosensori a trasduzione amperometrica e piezoelettrica, basati sull'immobilizzazione della transglutaminasi tissutale in forma aperta (Open-tTG). Entrambe le strategie hanno permesso di ottenere un modello affidabile per la messa a punto di dispositivi diagnostici per la determinazione di anticorpi anti-tTG in siero di pazienti celiaci.

Celiac disease (CD) is an autoimmune disorder that occurs in genetically predisposed people after the ingestion of gliadin, a prolamin found in wheat and other common grains, such as barley and rye. The rapidly increasing prevalence of CD, now recognized as one of the most common chronic disease in the world, has focused research interest towards its early and reliable diagnosis.

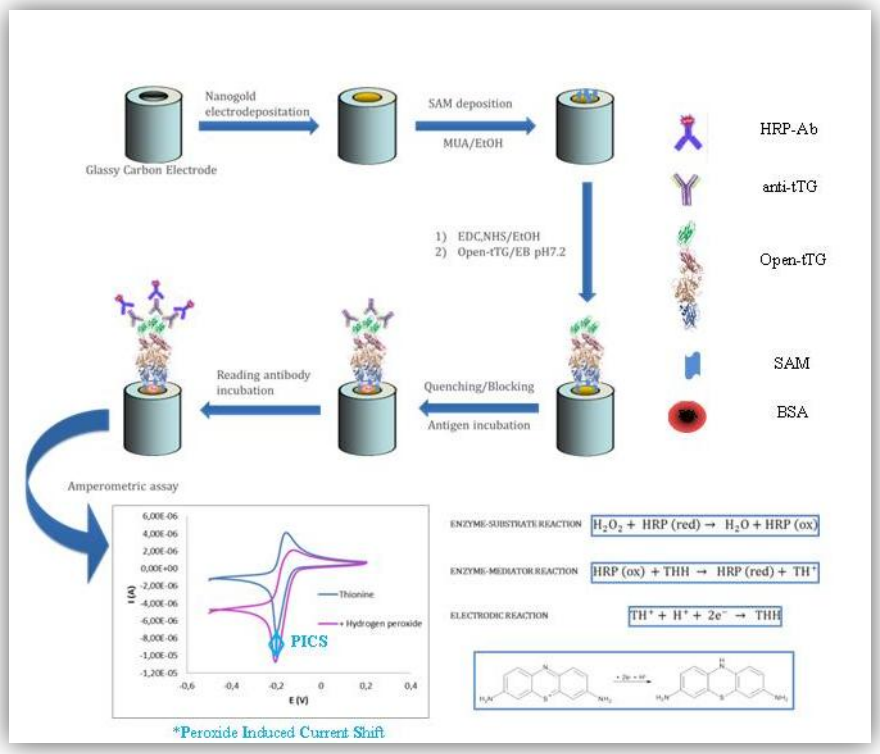

Fig. 1
Schematic depiction of the amperometric immunosensors set-up. The models are not in scale

Although duodenal biopsy remains the definitive approach for unequivocal diagnosis, serological blood tests of specific antibodies are the first-line investigation required. Some biomarkers[1] are involved in this disease and specific autoantibodies, such as anti-tissue transglutaminase (anti-tTG), anti-endomysium (anti-EMA) and anti-deamidated gliadin peptide antibodies, are used for the diagnosis. In particular, assays based on detection of anti-tTG, which is present at high concentration in celiac patients, have been demonstrated very sensitive and specific[2]. The diagnostic protocol specific for CD involves as first step the determination of IgA anti-tTG, in order to assess the occurrence of deficiency of this immunoglobuline isotype. However, it has to be taken into account that in about 2% of cases an IgA deficiency occurs, leading to false negative results, so that IgG anti-tTG antibodies screening should be performed[3]. In doubtful situations, in which the dosage of anti-tTG antibodies may give false negative results, the search for EMA antibodies and/or a small intestinal biopsy could be carried out.
Due to the high prevalence of CD, the development of rapid, sensitive, simple and reliable analytical methods for early diagnosis and follow-up are required as a valid alternative to classic colorimetric assay, such as ELISA. In this context, biosensors offer a powerful alternative to traditional methods for new devices with diagnostic application, in which a more accurate, fast, low cost and especially *in situ* analysis is needed.
Recently, it has been demonstrated that tTG enzymatic activity is tightly regulated, requiring in particular the presence of Ca^{2+} ions as well as an intra-molecular disulfide bond to induce tTG structural changes from close to open conformation. Thus, the extended structure exposes the site involved in the catalytic activity, so increasing autoantibody binding[4,5,6]. Actually, the open and active tTG presents a higher diagnostic accuracy with respect to the close conformation: the latter form is generally present in healthy tissue, whereas the extended one is more prominent during inflammation. Some studies concerning the fabrication of electrochemical and optical immunosensors for the detection of anti-tTG antibodies have been published[7], but until now no studies deal with the immobilization of the Open-tTG as receptor.
In this work we proposed new amperometric and piezoelectric immunosensors based on covalent immobilization of the Open-tTG for the detection of IgG and IgA antibodies in human sera by exploiting nanostructured materials in order to develop biomedical devices as high and sensitive diagnostic tools[8,9].

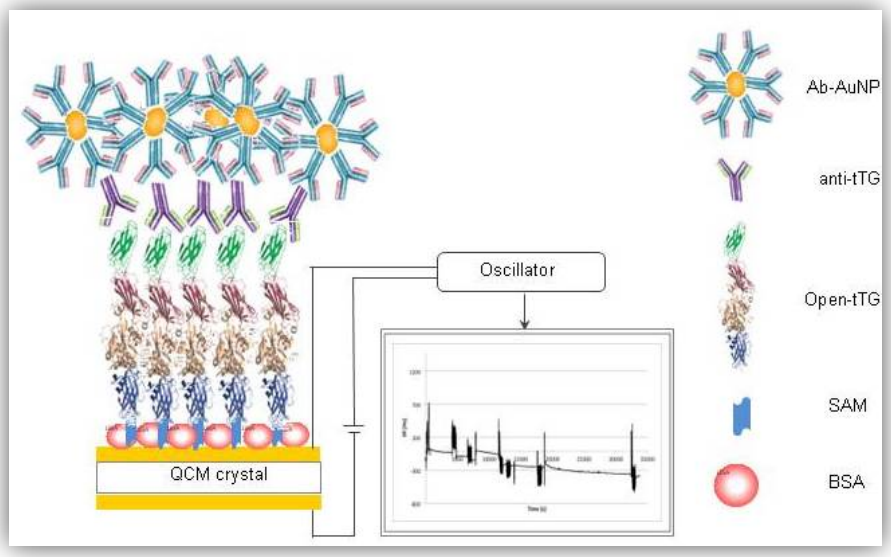

Fig. 2
Schematic depiction of the piezoelectric immunosensors set-up. The models are not in scale

Amperometric determination
The electrochemical immunosensing strategy combines the advantages taken from immunochemical assays with the high sensitivity of electrochemical transduction. The glassy carbon electrodic substrate was electrochemically functionalized with gold nanoparticles leading to amplification of the immunological reaction. In order to obtain a robust and repeatable functionalization of the transducer surface, we used a self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (MUA), which allows the covalent binding of the bioreceptor through its amino groups. In addition, particular attention was paid to chemometric experimental design-based optimization that

allows to identify the best conditions for the quantification of both human IgA and IgG antibodies. The presence of human antibody analytes in diluted human serum was recorded using horseradish peroxidase labeled anti-human antibodies that provided an enzyme based electrochemical signal (Fig. 1). After sensor validation, its reliability was investigated by using a commercial ELISA kit to confirm the achieved results, proving that the devised sensor has a lower threshold value and is able to discriminate between negative and positive samples.

Quartz crystal microbalance (QCM) determination

The QCM-based sensors are gaining an increasing interest as alternative devices for biosensing application and clinical bioassay in liquid phase, due to high sensitivity, low-cost and suitability for real-time monitoring of the sensor set-up.

The piezoelectric transduction, using QCM liquid/flow cell, is based on the same immunosensing strategy, involving the immobilization of the Open-tTG on the batch gold surface (Fig. 2). For reliable QCM biosensor applications, the properties of liquid phase should be taken under strict control and should not significantly change during analysis. In addition, the use of a cell operating in laminar flow conditions allows to minimize mechanical perturbation and to stabilize the recorded signal. In this context, the liquid phase detection conditions were properly selected in order to have good signal stability both in dynamic and in static mode. Since the values of frequency shift recorded during sample incubation were not high enough to reach the requested sensitivity for accurate diagnostic purpose, the sensor response was enhanced by final exposition to ten nm-sized Ab-AuNPs. In that way we were able to obtain an enhanced mass variation, which was recorded as an improved frequency shift, proportional to the amount of immobilized analyte. Optimization of the operating conditions by experimental design allowed us to obtain a reliable immunosenor with high potential as diagnostic device.

Conclusions

The results show that both methodologies are in good agreement. The use of nanotechnologies allows to obtain devices combining multiple benefits in terms of miniaturization, low cost, simplified instrumentation and high reproducibility of the response.

The proposed sensors were demonstrated to be highly reliable, with a clinically relevant dynamic range and good sensitivity, suitable for diagnostic purpose.

REFERENCES

[1]F. Bianchi *et al., Anal. and Bioanal. Chem.,* 2014, **406**, 15.
[2]M.M.P.D. Neves *et al., Anal. and Bioanal. Chem.,* 2010, **397**, 1743.
[3]M. Vives-Pi *et al., J. Clin. Gastroenterol.,* 2013, **47**, 308.
[4]E.B. Roth *et al., Autoimmunity*, 2003, **36**, 221.
[5]D.M. Pinkas *et al., PLoS Biol.,* 2007, **5**, 2788.
[6]J. Stamnaes *et al., J. Biol. Chem.,* 2010, **282**, 25402.
[7]S.V. Kergaravat *et al., Biosens. Bioelectron.,* 2013, **48**, 203.
[8]A. Manfredi *et al., Sensor Actuat. B Chem.,* 2014, **201**, 300.
[9]M. Giannetto *et al., Biosens. Bioelectron.,* 2014, **62**, 325.