Development of a Urease Quantification Biosensor to Monitor Biocementation Processes in Soils

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Abstract

The most successful process for bio-cementation in soils and other materials used in Civil Engineering applications is Microbially Induced Carbonate Precipitation (MICP) using Sporosarcina pasteurii to produce calcium carbonate, aiming to improve soil shear strength and reducing its permeability. This work presents a biosensor for quantification of urease levels in a sample of fluid extracted from soil, since this enzyme is related with the carbonate formation. Competitive and sandwich immunoassays were first performed in gold substrates. These assays allowed for definition of conditions, concentrations and dosages, achieving more promising results for the sandwich approach. Overall, assays were studied including 250 nm magnetic nanoparticles (MNP) as magnetic markers, a crosslinker agent (Sulfo-LC-SPDP) and blocking agents (BSA and SH-PEG). This protocol was later assigned for a microfabricated biochip (at INESC-MN) which was fully integrated into a magnetoresistive (MR) chip-based platform from INESC-MN/INESC-ID, allowing for signal acquisition from magnetized nanoparticles. In a very preliminary stage, 10 mg/mL of urease (2.08 $\mu$M) was detected with a $(\Delta V_{binding}/V_{sensor})$ signal of 1.1 ± 0.37 mVrms/Vrms.

Keywords: Microbially Induced Carbonate Precipitation, Magnetoresistive sensors, Biochip, Chip-based platform, Magnetic nanoparticles.

1 Introduction

In Civil Engineering, it is fundamental to ensure the most suitable mechanical and hydraulic conditions for the ground. The technique this work proposes is biocementation that recurs to MICP process to improve both hydraulic and mechanical properties of different materials used in construction (such as natural stones, concrete and soils). The process increases the alkalinity of the pore fluid and induce calcite precipitation, filling pores, flaws and cracks of those materials, as illustrated in Fig. 1, which shows this process in a teated soil, comparing it with an untreated sample [1]. MICP can be uplifted when ureolytic bacteria are injected in the soil or medium to treat along with nutrients, increasing the production of the enzyme urease, and, thus, increasing the calcium carbonate that is precipitated [2]. The injected nutrients are calcium chloride ($CaCl_2$), used as a calcium source, and urea ($CO(NH_2)_2$), which provides energy and nitrogen. If in the presence of urease, urea is hydrolyzed to form into ammonium ($NH_4^+$) and calcium carbonate (Eq. 1 and Eq. 2). Work conditions are pH = 9 and temperature between 30 - 37 °C [3].

$$CO(NH_2)_2 + 2H_2O \xrightarrow{urease} 2NH_4^+ + CO_3^{2-}$$ (1)

$$Ca + CO_3^{2-} \rightarrow CaCO_3$$ (2)

This work presents a biosensor developed to monitor, in situ, the concentration of a protein, urease, in a sample of fluid extracted from soil previously treated with MICP method. The quantification of this enzyme is reflected on the quantity of calcium carbonate fills the voids while binding the grains, adapted of [1].

Fig. 1 – Untreated (A) and treated (B) soils with microbially induced carbonate precipitation process: precipitation of calcium carbonate fills the voids while binding the grains, adapted of [1].
carbonate that is precipitated, indicating whether biocementation is under progress, as well as if the calcium carbonate levels are in the expected range. In this preliminary approach, tests on sensor development were performed to detect urease from *Canavalia ensiformis*, which species was used to simulate the enzyme but from *S. pasteurii*.

2 Background

2.1 Biosensors

Over the past decades, research on biosensor technology has grown rapidly and sensors have emerged in everyday life of human being. A scheme of a biological sensor assembly is shown of Fig. 2. A sensor provides specific quantitative or semi-quantitative information using a biological recognition element (receptor, element 2 in the figure) that is retained in direct spatial contact with a electronic transduction element (1). The bioreceptor has to first recognize the target (sample/analyte, 3) and will then produce a physicochemical output that is converted by the transducer [4].

The biosensor developed in this work follows a quantum mechanical magnetoresistance effect. These sensors are based on alternate stacks, having one non-magnetic conductive layer between two ferromagnetic conductive layers, thereby creating changes in resistance [5].

2.2 Magnetoresistive biosensors

The “giant magnetoresistive” (GMR) effect was discovered when, in presence of a very high magnetic field, Gruenberg and Fert first observed a large electrical resistance when they forced two adjacent ferromagnetic layers, separated by a nonmagnetic spacer, to align; showing that the electron flew through the structure due to the relative orientation of magnetizations in the adjacent layers (Fig. 3). In the parallel magnetic configuration (A), one spin species easily flows through the device, leading to a low electrical resistance; while in the antiparallel magnetic configuration (B) the resistance is high. This is due to the electrical resistance being associated with the scattering of electrons inside a material [5].

Examples of GMR sensors are spin valves (SV), which have been used to implement biological applications using magnetic beads to label probes. Moreover, groups have reported MR-sensors integrated in fully controllable biochip platforms, including control and acquisition electronics and microfluidic systems for sample handling. These arise as lab-on-a-chip devices.

The device developed during this work has the same general concepts as those above, studied to detect urease as motorization of MICP process. Biochips for protein applications are still a novelty and these chips bring up some new challenges, mostly associated with the complex secondary and tertiary structures of proteins, resulting in a higher degree of precision required for successful recognition.

Comparing with the traditional techniques, biosensors have several advantages in general, including the following: small size, practical and portable, fast response time, high specificity, quick and continuous measurement, low unit cost, requiring fewer reagents and be user-friendly.

3 Competitive and Sandwich Immunoassays

With the ultimate goal of quantifying urease, two immunoassays strategies similar to Enzyme-Linked ImmunoSorbant Assay (ELISA) were considered and analyzed: competitive and sandwich. Compared to traditional assays, the optical labels (where the enzyme substrate typically forms a colored product) were replaced by magnetic tags. Therein, the sensors detect the stray field from the magnetic tag, relating it with the captured analytes.

3.1 Competitive ELISA

The operation principle of competitive ELISA relies on the capacity of the antibodies to bind to the antigen. Due their high affinity relationship, tagged antibodies (C) recognize the antigen (B), whether the latter is the immobilized (purified antigen) on the surface or the as a sample. After a washing
step, only the antibodies that have bonded to the immobilized urease (A) stand over the sensor while the rest of them are washed out. The antibodies are then quantified through the magnetic label: the higher the signal, the lower the concentration of analyte. These events are illustrated in Fig. 4 and detailed below.

Fig. 4 – Competitive ELISA: urease functionalization (A), recognition of urease by the antibodies (B) and labeling antibodies with magnetic nanoparticle (C).

A) Urease immobilization on gold surface
To functionalize gold with urease, Sulfo-LC-SPDP (Sulfosuccinimidyl 6-3'-(2-pyridyldithio)propionamido)hexanoate) crosslinker was proposed. This linker binds gold through the thiol (SH) group with one end and accessing amine groups of lysines present in urease with the other chain end.

B) Protein-Antibody interaction
The structure of antibodies is known to comprise one Fc (fragment crystallizable) and two Fab (fragment antigen binding) fragments. This latter region is essential for the formation of immune complexes, as they represent the variable active sites of the molecule that will react with antigens.

C) Magnetic labeling of antibodies
The immobilization of antibodies on the magnetic nanoparticles takes advantage of the high affinity between the biotin that is attached to the antibodies (by the amine groups) and the streptavidin that covers the magnetic beads. As a tetramer, streptavidin features four locals in which biotin can bind. Streptavidin has a high affinity with the biotin which is immobilized on the antibodies, thus generating the particle-antibody complexes.

3.2 Sandwich ELISA
Unlike competitive method, detection of the target (urease) in a sandwich ELISA is proportional to the signal given by the magnetic particles. The antigen is immobilized over a layer of antibodies (probe antibodies) which is firstly bounded to the surface (A). Then, the antigen is recognized by the second antibodies (detection antibodies) (B) which are labeled with nanoparticles (C) for signal detection. These interactions are shown in Fig. 5 and detailed below.

Fig. 5 – Sandwich ELISA: adsorption of probe antibodies (A), antigen recognition by the antibodies (B) and magnetic labeling of antibodies (C).

A) Immobilization of probe antibodies
To promote steadier and stronger attachment on the surface, Sulfo-LC-SPDP is also used in this assay. The linker binds to the antibody also through the primary amines, while accessing gold with thiol group. However, this strategy also tend to immobilize antibodies randomly. Generally, following antibody immobilization, the remaining nonspecific active sites is blocked by proteins such as bovine serum albumin (BSA).

B) Antigen recognition by both probe and detection antibodies
Contrary to competitive assay, sandwich method involves two biotinylated (target) and non biotinylated (probe) antibodies. The antigen recognition has already been described in 3.1.

C) Magnetic labeling of antibodies
This interaction is presented in 3.1.

4 Immunoassays on Gold Substrates
Before using biochips for urease detection, biological procedures were performed over gold substrates, to save resources, which mimic the surface of the sensor where biological attachment takes place.

4.1 Competitive Assay
A schematic representation of the overall steps of this detection assay is presented in Fig. 6, where the surface is firstly functionalized with urease, (1), then a solution containing labeled antibodies and urease (previously assembled in (2)) is added over the substrate, (3). Then, biological recognition takes place, (4). Between each step, the substrates are washed with a buffer solution, to remove unbound molecules. The signal is then ready to be detected and analyzed. Every step must be indi-
Fig. 6 – Overall process of competitive magnetic ELISA for urease detection.

vidually verified as its biology assembly is confirmed and concentrations fined, before accessing the final configuration of the immunoassay.

4.1.1 Materials

4.1.1.1 Biochemical reagents

All reagents were of analytical grade. Water was ultra-pure (milliQ water from Millipore®). Phosphate buffer (PB) 0.1 M consisted in a combination of NaH$_2$PO$_4$ and Na$_2$HPO$_4$, adjusting the pH to 7.4. PB/Tween20 was obtained adding 0.02% (v/v) of Tween 20 from Promega to the previous buffer. Sulfo-LC-SPDP and Bovine Serum Albumin (BSA) were acquired from Pierce. MNP were Nanomag®-D, 250 nm in diameter, 75 - 80% (w/w) magnetite in a matrix of dextran (40 kD), coated with streptavidin, were obtained from Micromod, Germany. These particles present a magnetic moment of $1.6 \times 10^{-16}$ Am$^2$ (under fields of 1.2 kAm$^{-1}$ ~ 15 Oe) and a susceptibility of $\chi \sim 5$.

Urease from $C$. ensiformis was obtained in Sigma-Aldrich, rabbit polyclonal biotin conjugated anti-$C$. ensiformis urease was acquired from Rockland and rabbit polyclonal biotin conjugated anti-$H$. pylori urease was purchased from Aviva Systems Biology.

4.1.1.2 Preparation of gold surface for biochemistry

The 500 Å-thick gold layer was obtained by magnetron sputtering (Alcated machine), using a 50Å-thick Chromium film as adhesion layer. Clean gold coated substrates were exposed to ultraviolet light/ozone (UV-O) plasma for 11 min, at 28 mWcm$^{-2}$, at 5 mm distance from the UV lamp inside an UV-O cleaner machine (from Jelight, USA).

4.1.2 General Procedure

Initial quantities were determined based on calculus to 1) have enough urease to cover the spotted area, 2) use the same concentration of MNP as other works and 3) have an excess of antibodies to cover those particles. The antibodies assure detection of one layer of urease in the spotted area.

4.1.2.1 Urease immobilization

A) Only-Urease immobilized

A urease solution was prepared dissolving this enzyme in PB, achieving a concentration of 5 mg/mL. 2 µL of this solution were dispensed over the surface of a substrate, by manual spotting and set for immobilization at RT and a humid atmosphere (HA). After 2 h, each gold substrate was washed with a PB solution in order to remove the unbound molecules.

B) Linker and Urease immobilized independently

A solution of Sulfo-LC-SPDP crosslinker dissolved in PB was prepared, achieving a concentration of 1 mg/mL. 2 µL of this solution were dispensed over each substrate, immobilizing for 2 h at RT and a HA. After removing unbound molecules with PB, 2 µL of 10 mg/mL urease solution were dispensed over the linker spot and set 2h, at RT and HA. The unbound urease molecules were then washed away with PB.

C) Linker and Urease immobilized in solution

A solution was prepared adding 1 µL of a 2 mg/mL of crosslinker solution to 1 µL of 10 mg/mL urease solution, resulting in 2 µL, carrying the same amount of linker and urease as in the previous strategy. This volume was manually dropped over the gold substrate and set for immobilization for 2 h at RT and a HA. After, unbound molecules were washed away.

To produce a negative control, Bovine Serum Albumin (BSA) was used to functionalize the surface. BSA was diluted in PB to achieve 0.01 M and 2 µL were dispensed on the substrate, and the unbound molecules were washed away.

4.1.2.2 Antibodies labeling

For each substrate, a final concentration of $4.9 \times 10^7$ nanoparticles/µL was achieved by collecting 1 µL of Nanomag®-D 250 nm and diluting them 10 times, obtaining a solution of 10 µL. Biotinylated antibodies were used in excess to cover all locals of recognition, using a concentration of 10 µg/mL (10$^{13}$ particles/µL). 1 µL of this solution was added previous and placed in a rotative wheel for 45 min at RT to promote assembly of the labeled antibodies. After this time, particles were again concentrated and supernatant was replaced.

4.1.2.3 Urease in solution for competition and its detection

Competitive ELISA demands a solution containing the analyte. As a preliminary phase, urease solutions were prepared to dispense over gold substrates, mimicking the sample of soil in which urease...
is quantified. For this propose, urease was diluted in PB to achieve concentrations of 1, 10 and 100 mg/mL. 2 µL of the prepared solution was added to the 10 µL of labeled antibodies and placed, one last time, in the rotative wheel promoting assembly of particle-antibody-urease complexes. The 12 µL were then directly pipetted over the surface of the gold substrate (previously functionalized with urease) and let for interaction during 45 min at RT in a HA. Finally, unbound and weakly bounds were removed, by washing the substrate with PB/Tween20 solution two times and once with deionized water.

4.1.3 Optical analysis of magnetic labeling on gold substrate

After the experiments, substrates were taken to microscope and its images analyzed in ImageJ, where each result was converted into percentage of particle surface coverage (PSC, Eq. 3), due to the brown colour owned by MNP, which remain over gold in case of positive recognition.

\[
PSC = \frac{\text{Spot signal} - \text{Outspot signal}}{\text{Spot signal}}
\]  

(3)

4.1.4 Results and Discussion

4.1.4.1 Urease detection

This is the most basic assay and tests the gold functionalization (A) and urease recognition (B) by using labeled antibodies (C). These tests don’t have any urease in solution for competitive assay and they are the simplest way of producing signal, Fig. 7.

![Fig. 7 – Assembly of Gold surface functionalization tests: (A) Immobilization of urease over the gold surface, (B) Urease recognition by the antibody, (C) Labeling the antibody.](image)

Fig. 7 – Assembly of Gold surface functionalization tests: (A) Immobilization of urease over the gold surface, (B) Urease recognition by the antibody, (C) Labeling the antibody.

![Fig. 8 – A) Optical image (40x) of assay testing antibodies from Canavalia ensiformis (right) and Helicobacter pylori (left); B) analytical results from optical microscopy, where errors bars represent the standard deviation to the mean values.](image)

Fig. 8 – A) Optical image (40x) of assay testing antibodies from Canavalia ensiformis (right) and Helicobacter pylori (left); B) analytical results from optical microscopy, where errors bars represent the standard deviation to the mean values.

4.1.4.2 Urease immobilization strategy

The next assay consisted on testing the different urease immobilization approaches described at subsection 4.1.2, which results are in Fig 9.

![Fig. 9 – Microscope image (40x) of gold surface functionalization test with urease immobilization, using different solutions: (A) rease, (B) Sulfo-LC-SPDP crosslinker and urease immobilized independently, (C) Sulfo-LC-SPDP linker and urease immobilized in a solution, (D) Negative control immobilizing BSA.](image)

Fig. 9 – Microscope image (40x) of gold surface functionalization test with urease immobilization, using different solutions: (A) rease, (B) Sulfo-LC-SPDP crosslinker and urease immobilized independently, (C) Sulfo-LC-SPDP linker and urease immobilized in a solution, (D) Negative control immobilizing BSA.

According to Fig. 9, frames A, B and C verifies the direct ELISA method as antibodies detect urease immobilized on the gold substrate. Comparing strategies with linker, frame B have a small increase of homogeneity, and better defined borders of the signal. The negative control given by D represents an important assay, as it shows no signal, indicating that these antibodies have no affinity with BSA.

4.1.4.3 Urease immobilization: quantities trial

Different concentrations of urease (0.5, 5, 10 and 20 mg/mL) were tested to verify if 10 mg/mL used in previous tests is an appropriated concentration, (Fig. 10). Tests were performed using linker in the solution of immobilization.

Signal increases with urease from 0.1 to 5 mg/mL, which is justifiable by the fact that more epitopes are becoming available for antibodies recognition.
For concentrations of urease higher than 5 mg/mL, the signal stabilizes due to saturation of urease in the surface meaning the number of disposable epitopes remain the same for higher concentrations. It was chosen to persist with the concentration of 10 mg/mL (corresponding to 2 mg/mL of linker), compromising between a stable signal of a monolayer and urease in excess.

4.1.4.4 Urease in solution for competition and antibody recognition

This test corresponds to the competitive assay, where antigen in solution is now introduced in the system. The more urease is added in solution (analyte), the lower is the expected signal; as the analyte is captured by the antibodies, making it impossible for these antibodies to bind to the immobilized urease. In this assay, the signal is therefore inversely proportional to the analyte concentration.

Concentrations of 0, 1, 10 and 100 mg/mL of analyte were used. The analyte was added to the complexes of particles and antibodies before assessing the gold surface (with urease already immobilized). Optical results were converted into % of PSC for easier and quantifiable, Fig. 11.

The small signal variations shown in the figure suggest that the MNP are being detected. The undesired signal can be justified by clusters of complexes that may be formed during the mixture of the three molecules present in the added solution (particles, antibodies and urease) and as at least one antibody is free, it will bind to the antigen immobilized on the gold surface, Fig. 12.

4.2 Sandwich Assay

This second approach is schematically represented in Fig. 13. Surface functionalization is this time accomplished with non-biotinylated antibodies, (1). Then, a solution containing the analyte is added over the layer of antibodies, (2). In the meantime, MNP are functionalized with biotinylated antibodies, (3), and these complexes are deposited over the substrate, where biological recognition takes place, (4). The final step consists in detecting the nanoparticles that are linked to urease after the unbound molecules being washed away, (5).

Experiment protocol followed the direct assay, with the exception of adding an extra step before immobilizing urease, since the surface is functionalized with antibodies. As optimized through the competitive assays tests, a linker, essential for biosensors, was used to support antibodies.

4.2.1 Materials and Methods

4.2.1.1 Biochemical reagents

Besides reagents reported subsection 4.1.1, SHPEG was acquired from Sigma while rabbit polyclonal purified IgG anti-urease was purchased from Agrisera Antibodies (probe antibody).
4.2.1.2 Sandwich assay

A solution of Sulfo-LC-SPDP was prepared achieving a concentration of 2 mg/mL. 1µL of this solution was dispensed over the cleaned substrate. After 2 h of immobilization at RT and HA, the substrate was washed with PB buffer and 1 µL of antibodies solution was spotted (concentrations from 0.2 µg/mL to 250 µg/mL were prepared). Antibodies were set to immobilize overnight at 4 °C, in a HA. Unbound antibodies were then removed by washing the substrate with PB. Urease was added dispensing 1 µL of different concentrations (1, 10 and 100 mg/mL). After 1 h of immobilization, unbound molecules were washed away with PB and a solution of particles-antibodies complexes (prepared as in the competitive assay) was dispensed over the substrate. To remove unbound molecules, substrates were finally washed two times with PB/Tween20 and once with water.

To prevent nonspecific signal, BSA or SH-PEG were used as blocking agents, where 1 µL of this solution was dispensed between antibody immobilization and target steps. BSA/SH-PEG was left for incubation for 1 h at RT in a HA.

4.2.2 Results and Discussion

4.2.2.1 Probe antibodies optimization

BSA (1% in PB) was used to block the surface against nonspecific bounds. The chart (Fig. 14) shows particle coverage for different concentrations of probe antibodies, using a fixed concentration of urease of 10 mg/mL, resulting from data analysis of the optical images.

![Fig. 14 – Study of probe antibodies for magnetic sandwich ELISA, using 1% of BSA as blocking agent.](image)

It suggests that 10 µg/mL of probe antibodies is the optimal concentration for this fixed amount of urease (10 mg/mL), besides 1 µg/mL and 5 µg/mL being also promising. However, the associated values of particle coverage percentages seem to be fairly low, probably due to the blocking agent.

In order to try to increase this signal, assays with other blocking agent, SH-PEG, were performed, restraining concentrations of probe antibodies to 1, 5 and 10 µg/mL, along with 1, 10 and 100 mg/mL concentration, Fig. 15. This figure also shows an optical microscope image of a double test where the concentration of the analyte was 1 mg/mL. The spot on the right exhibits a higher signal and corresponds to 5 µg/mL of immobilized probe antibodies while, the negative signal on the left refers to 10 µg/mL. From the plot, it is clear that the concentration of 10 µg/mL is not optimal, suggesting BSA also blocks the outspot, making the difference between spotted and outspotted areas larger. The negative signal is due to have a higher particle deposition outside the spot (due to nonspecific binds), than within the spot. Thus, concentrations of 1 µg/mL and 5 µg/mL of probe antibodies were taken into consideration.

A wide spectrum of concentrations of antigen was studied to obtain a calibrated curve, using concentrations of 1 µg/mL and 5 µg/mL as probe antibodies, Fig. 16 and 17, respectively.

![Fig. 15 – Study of probe antibodies for magnetic sandwich ELISA, no blocking agent, for concentrations of 1, 5 and 10 µg/mL of probe antibodies, and 1, 10 and 100 mg/mL of antigen.](image)

In both cases, the signal increases with the concentration of the analyte, urease, as supported by the theory. Considering the correlation coefficient, concentration of 1 µg/mL of probe antibody is the optimal option indicating a steady logarithmic relation between signal and analyte.

Having the biological assembly proven and the con-
centrations optimized, the assays may now proceed to be performed on biochip sensors.

5 MR-chip for detection of urease

5.1 MR-chip platform

An array of micro magnetoresistive (MR) sensors is integrated in a single device, biochip, which is read by a MR-chip based read-out platform (Fig. 18), developed by in [7], and is able to detect variations in the resistance of the sensor, when an external magnetic field is applied. The presence of MNP close to the surface of the sensor creates a fringe field, large enough to change the magnetization direction and, consequently, the resistance in the device, detecting nanoparticles.

5.2 Biochip

Each biochip measures 18 x 9 mm$^2$ and includes 30 MR sensors, consisting on two parallel-shaped spin valves of 2.6 x 46.6 $\mu$m$^2$ each. The sensors are aggregated into six groups of five: one biologically inert sensor (reference) and four bioactive sensors, Fig. 19.

5.2.1 Fabrication

All the fabrication process was assured by INESC-MN, Lisbon, Portugal.

The top-sinned spin valve stack was deposited by Ion beam (Nordiko 3000 machine), consisting in 20Å Ta / 28Å NiFe / 23Å CoFe / 23Å Cu / 33Å CoFe / 70Å MnIr / 100Å Ta. The sensors were contacted by 3000 Å aluminum leads and surrounded by focusing lines, defined by lift-off. After the deposition of a 4000Å-thick Si$_3$N$_4$ passivation layer by PECVD over the entire chip, the contact vias were opened by reactive ion etching, 50Å Cr and 400Å Au films were deposited over the bioactive sensors for biology and the excess removed with lift-off. After magnetic annealing, dies were cut into their final dimensions and wire bonded to a Printed Circuit Board (PCB) carrier and wired protected with silicone to prevent corrosion, Fig. 20-A.

In order to use each group of five sensors independently, a system that portraits 6 micro-channels was designed to be attached on the biochip surface, corresponding each channel to each group of sensors. This PDMS piece has 6 pairs of inlet and outlet holes where channels are 25 $\mu$m height for solutions to flow and is sealed after UV-O treatment of both surfaces, Fig. 20-B.

After this step, the biochip is ready to be implemented on the platform.

5.2.2 On-chip Immunoassay

Before assessing the platform for detecting signal, it is important to perform biological test over the chip, to assure that the biology still responds in such smaller gold area (each gold pad measures 35.4 x 12.9 $\mu$m$^2$).

Due to its simplicity, the most basic assay was performed over the biochips, without the PDMS.
channels. The protocol is presented in subsection 3.1. At the end of the experiment, chips were analyzed at optical microscopy.

5.2.3 Results and Discussion
Fig. 21 shows optical results of this assay consisting on the immobilization of urease and its recognition by the particles-antibodies complexes. In frame B, the crosslinker was pre-immobilized. Both images show the 4 bioactive sensor with a considerable amount of nanoparticles over the gold pads, confirming urease detection. On the other hand, the reference sensor (upper sensor in each image) came out clean. This is the expected result because gold was not deposited over this sensor and, since urease does not immobilize on the sensor, particles do not attach and are completely washed.

5.3 MR sensors
5.3.1 Electronic read-out of the sensors
A complete measurement usually follows the consecutive phases (Fig. 22): initially, the sensor defines a stable voltage which is baseline of the sensor ($V_{\text{sensor}}$). I. When MNP are added over sensors, II, the signal decrease because the sensor feel the variations in resistance and are left to settle and interact with the antigen for about 30 minutes until saturation is achieved, IV. At this stage, particles are washed, and unbound molecules are removed from each sensor, increasing the signal, V. One or two additional washes may be required to exclude all the nonspecific and weakly bonds, stabilizing the signal at $V_{\text{particles}}$. The signal correspondent to the remaining MNP ($\Delta V_{\text{binding}}$) is acquired and the differential voltage between the sensor baseline and the signal obtained at the end of the experiment was calculated ($V_{\text{sensor}} - V_{\text{particles}}$). After each experiment, the user still has access to all the data (voltage vs time), and can save it for further analysis.

5.3.2 Immunoassay on the platform
This assay is the same performed over the biochip, i.e., tagged antibodies are tested to recognize urease. However, the platform requires a system that introduces the solution in the PDMS channels. To this step, a syringe pump is used, allowing for a more controlled and constant volume rate flow. The sensors were biased with a 1 mA DC current, the magnetic drive was set to 30 Oe DC and 13.5 Oe rms AC at 211 Hz. These fields are enough to magnetize the nanoparticles, as obtained from VSM curves and susceptibility [9].

The first step consists on immobilizing urease on the sensors, injecting it carefully through one PDMS inlet with a syringe (1.5 µL, 10 mg/mL). To avoid evaporation, the biochip was held for 2h in a humid atmosphere, at RT. After that time, the biochip was introduced in the platform and with the help of a syringe pump and a washing step was done, flowing PB buffer at 10 µL/min. At that moment, the platform started to read-out the chosen sensors. The syringe continued to pump until the solution of MNP and antibodies (1 µL, 4.9 x 10^7 particles) entered in the system, where it was set to stop, during 30 m. After, the syringe started to pump at flow rates from 0.5 µL/min to 2 µL/min. A solution of PB/Tween20 was added was a final step at 5 µL/min.

5.3.3 Results and Discussion
By the end of this work period, only one channel was tested, thus 5 sensors were measured. Fig. 23 corresponds to the reference sensor, which is biologically inert, while Fig. 24 presents one of the bioactive sensors. The both plots the behaviour described in Fig. 22, decreasing the signal when the MNP solutions enters the system and returning to higher voltage values after removing the unbound molecules. Analyzing the reference sensor, Fig. 23, is clear the completely return of the signal to the base.
line, implying that all the MNP were removed from the sensor. This is the expected signal, once this is the reference sensor in which gold was not deposited. In this way, urease could not immobilize, and consequently, tagged antibodies were no able to find urease, being washed away. Also, no nonspecific interaction has occurred.

On the other hand, Fig. 24 gives one bioactive sensor which represents the all 4 bioactive sensors that were tested, since they produced a very similar outcome. As observed, the signal after the washing step stabilized at a value, which seems to be slightly smaller than the value of the baseline signal. A variation of these signals is due to the presence of MNP which got retained at the surface of the chip. By this result, a low number of particles captured the urease molecules. In the case of sensor 17, it was acquired a differential voltage of 7 µV rms.

Bioactive sensors present higher signal ($\Delta V_{\text{binding}}/V_{\text{sensor}}$) which was 1.1 ± 0.37 mV rms/V rms, comparing with 0.37 mV rms/V rms from the reference. The error in the bioactive sensors results of the standard deviation to the mean, considering the four bioactive sensors. The obtained signal was then corrected with the reference sensor ($\Delta V_{\text{binding}}/V_{\text{sensor}} - \Delta V/V_{\text{reference sensor}}$) to 0.73 mV rms/V rms.

Such small variation should increase if, instead of air, a solution is used to push the flow through the channels or/and increasing channels height.

6 Conclusions
This work addresses a biochip for detection of urease in samples of fluid extracted from sand soils to monitor a biocementation process. Preliminary tests were performed on gold substrates and a calibration curve was obtained using a sandwich immunoassay, using magnetic particles (250 nm), a crosslinker (Sulfo-LC-SPDP) and a blocking agent (SH-PEG). Tests over the biochip in open chamber confirmed urease detection in gold pad areas of the biochip, and, using the MR-chip platform, a signal for positive recognition of 10 mg/mL of urease (2.08 µM) was achieved with a ($\Delta V_{\text{binding}}/V_{\text{sensor}}$) signal of 1.1 ± 0.37 mV rms/V rms.

Regardless the small signal, this work arises as a proof of concept as the biological system is accomplished over the biochip and this system can be adapted to use to detect other proteins.

References