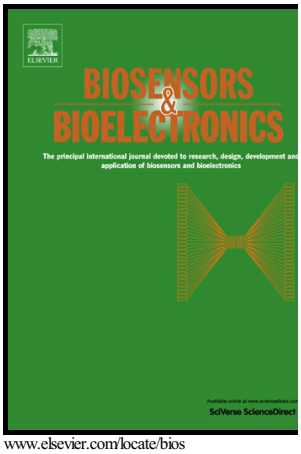

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Electrochemical magneto biosensor for CD4 count in AIDS diagnosis and monitoring

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Abstract

The counting of $CD4^+$ T lymphocytes is a clinical parameter used for AIDS diagnosis and follow-up. As this disease is particularly prevalent in developing countries, simple and affordable CD4 cell counting methods is urgently needed in resource-limited settings. This paper describes a magneto-actuated electrochemical biosensor for CD4 count in whole blood. The $CD4^+$ T lymphocytes were isolated, preconcentrated and labeled from 100 μL of whole blood by immunomagnetic separation with magnetic particles modified with antiCD3 antibodies. The captured cells were labeled with a biotinylated antiCD4 antibody, followed by the reaction with the electrochemical reporter streptavidin-peroxidase conjugate. The limit of detection for the CD4 counting magneto biosensor in whole blood was as low as 44 cells μL^{-1} while the logistic range was found to be from 89 to 912 cells μL^{-1}, which spans the whole medical interest range for CD4 counts in AIDS patients. The electrochemical detection together with the immunomagnetic separation confers high sensitivity, resulting in a rapid, inexpensive, robust, user-friendly method for CD4 counting. This approach is a promising alternative for costly standard flow cytometry and suitable as diagnostic tool at decentralized practitioner sites in low resource settings, especially in less developed countries.

Keywords: $CD4^+$ T lymphocyte, HIV, AIDS, CD4 counting, magnetic particle, magneto electrode, electrochemical biosensor, immunomagnetic separation

Introduction

The Human Immunodeficiency Virus (HIV) and the Acquired Immune Deficiency Syndrome (AIDS) affect the life of millions of people around the world, mainly in low- and middle-income countries. According to the World Health Organization (WHO), globally 35 million people were living with HIV in 2013. An estimated 0.8 % of adults aged between 15-49 years are living with HIV worldwide. However, sub-Saharan Africa remains most severely affected, with nearly 1 in every 20 adults (4.7 %) living with HIV and accounting for 68 % of the people living with HIV worldwide (UNAIDS, 2013). The HIV virus infects the cells of the immune system, primarily $CD4^+$ T lymphocytes decreasing CD4 levels from the normal values (ranging from 500 to 1,200 cells μL^{-1}), which weakens the immune system and causes the progression to AIDS and death from cancer or opportunistic infections (WHO, 2005). When the number of the CD4 cells falls below 200 cells $\mu L^{-1}$of blood, it is considered to have progressed to AIDS (WHO, 2005). AIDS it is also diagnosed with the emergence of one or more opportunistic illnesses regardless the CD4 count. Without treatment, people who progress to AIDS typically survive about 3 years. However, life-expectancy without treatment falls to about 1 year with the presence of opportunistic illness. Under antiretroviral treatment (ART) while maintaining a low viral load, a patient may enjoy a near normal life span without progression to AIDS. The $CD4^+$ T cell count is thus a critical parameter in monitoring HIV disease, since lower numbers of circulating $CD4^+$ T cells imply a more advanced stage of HIV disease and less competent defense mechanisms. In HIV infected patients, the $CD4^+$ T cell count is useful not only for assessing the degree of immune deterioration and speed of progression towards AIDS, but also for initiating ART, for deciding the timing for prophylaxis of opportunistic infections and, finally, for monitoring the efficacy of the treatment (WHO, 2004). The new recommendations encourage all countries to initiate the treatment in HIV infected adults with CD4 cell count down to 500 cells μL^{-1} when their immune systems are still strong, regardless of the presence or absence of clinical symptoms (WHO, 2013). Unfortunately, the areas mostly affected by the HIV epidemic are resource-limited countries, wherein the CD4 count is not available due to laboratory requirements and cost of the assay (Carinelli et al., 2015).

Flow cytometry is the gold standard technology for $CD4^+$ T cell count because of its accuracy, precision and reproducibility. This technology is also capable of high sample throughput. However, flow cytometry based CD4 counting is relatively complex, and therefore technically demanding and costly, requiring regular maintenance, reliable electric supply and

skilled personnel. Moreover, the instruments commercially available are significantly expensive (US$ 20-95 000) (WHO, 2004). These instruments are thus rather impractical and difficult to sustain in resource-scarce settings. Currently, there are only few cheaper alternatives to flow cytometer and they are based mostly on fluorescent labeling, requiring thus costly imaging equipment to achieve detection or, instead, based on manual counting with light microscopes (Boyle et al., 2012; Carinelli et al., 2015). For instance, the PointCare NOW™ and the CyFlow® miniPOC are modified flow cytometers. PIMA™ is based on a dual-fluorescence image analysis to count $CD3^+/CD4^+$ cells (Diaw et al., 2011). The Coulter® CD4Count Kit and the Dynal T4 Quant Kit are manual CD4 tests based on the use of beads for lymphocyte capturing, labeling and counting under light microscope (Lutwama et al., 2008). These manual counting methods are rapid but laborious and have a limited throughput. In order to minimize reader error, they are restricted to 10 samples per day to prevent technician fatigue and eyestrain (Rinke de Wit, 2007). An enzyme immunoassay was also developed, showing a limit of detection (LOD) for CD4 cells of 230 cells μL^{-1}, being thus rather impractical for AIDS and opportunistic diseases diagnosis due to lack of sensitivity (Carriere et al., 1999). Finally, microfluidic devices based mostly on optical (Cheng et al., 2007; Gohring and Fan, 2010; Moon et al., 2011), fluorescence (Thorslund et al., 2008) or impedance (Jiang and Spencer, 2010; Mishra et al., 2005; Whatkins et al., 2011) detection were reported. However, the cost of production of some microfabricated devices still constitute a bottleneck and may put them out of range for end users in the developing world (Moon et al., 2011).

To solve the urgent need for improving the diagnostic tools of AIDS, electrochemical biosensors offer an exciting alternative, especially in resource-scarce settings, as rapid, cost-effective devices that can be handled for unskilled personnel at the community and primary care level.

This paper addresses a magneto actuated electrochemical biosensor for $CD4^+$ T lymphocytes count in whole blood. In this work, CD4 cells are preconcentrated from whole blood by immunomagnetic separation based on the CD3 receptor and using magnetic particles modified with an antiCD3 antibody. The captured cells are at the same time labeled with a biotinylated antiCD4 antibody, followed by the reaction with an electrochemical reporter. As monocytes and macrophages also express the $CD4^+$ molecule, they are excluded from the absolute count: although a number of blood cell types may express either CD4 or CD3 antigens (for instance, monocytes, and thymocytes, respectively), only $CD4^+$ T lymphocytes express both CD4 and CD3 simultaneously. Due to the high sensitivity, the CD4 counting electrochemical magneto biosensor offers outstanding analytical performances and advantages compared with the gold standard flow cytometry method.

Experimental section

Instrumentation

Amperometric measurements were performed with a LC-4C amperometric controller (BAS Bioanalytical Systems Inc, USA). Voltammetric characterization was carried out using an Autolab PGSTAT Eco-chemie. A three-electrode setup was used comprising a platinum auxiliary electrode (Crison 52-67 1), a double junction Ag/AgCl reference electrode (Orion 900200) with 0.1 mol L^{-1} KCl as external reference solution and a working electrode (the magneto electrode, m-GEC) (Pividori and Alegret, 2005). The data were analyzed using the Graph Prism software (GraphPad Software, San Diego, CA).

Cell count was performed in Neubauer counting chamber using Nikon Eclipse TS100 microscope (Nikon Instrument, USA) or by flow cytometry using FACSCanto cytometer (BD Bioscience, USA).

The confocal images were taken with the TCS-SP5 Leica Microscope. The software used for the image process was Imaris X64 v.6.2.0 (Bitplane, Zurich, Switzerland).

The SEM images were taken with the scanning electron microscope Hitachi LTD S-570 (Hitachi LTD, Tokyo, Japan). For the sample treatment an E5000 Sputter Coater Polaron Equipment Limited metallizer and K850 Critical Point Drier Emitech (Ashford, UK) was used.

Chemicals and Biochemicals

Magnetic particles modified with antiCD3 antibody (antiCD3-MPs, Dynabeads® CD3 Prod. No. 111-51D) were purchased from Dynal Biotech ASA. Biotinylated antiCD4 antibody (Prod. No. 347321) was provided by BD Bioscience and the streptavidin–horseradish peroxidase conjugate (HRP, 1.11.1.7) came from Roche Diagnostics. Perfect count microspheres (Prod. No. CYT-PCM-100) used for absolute cell count by flow cytometry were purchased from Cytognos SL, Spain. The streptavidin labeled with cyanine 5 (Strep-Cy5) dye used in confocal microscopy was purchased from Life Technologies (Prod No. SA-1011).

All buffer solutions were prepared with milliQ water and all other reagents were in analytical reagent grade (supplied from Sigma and Merck). The composition of these solutions is described in Supp. data.

Cell culture

The $CD4^+$ T cell clone PB100.29 was obtained by cloning the infiltrating lymphocytes from a pancreatic donor organ (Xufré et al., 2013). The expansion method is described in detail in Supp. Data.

Confocal microscopy study of the immunomagnetic separation and labeling

Confocal microscopy was performed to characterize not only the distribution of CD4 and CD3 receptors on the lymphocyte surface but also their capability of being recognized by the antiCD3 antibody immobilized on the magnetic particles (MPs) and the biotinylated antiCD4antibody.

A solution of 1000 cells μL^{-1} was incubated with Hoechst (45 $\mu g\ mL^{-1}$) for 15 min at room temperature in darkness, to stain DNA of the living cells nuclei. The cells were then captured with the antiCD3-MPs (8×10^5 CD3-MPs per well) and labeled with biotinylated antiCD4 antibody (36 $ng\ mL^{-1}$), respectively, in one step procedure. Washing steps with PBS 0.1% BSA were performed. Finally, cells were incubated with strep-Cy5 (2 $\mu g\ mL^{-1}$) for 30 min at room temperature (RT) to achieve the fluorescence readout.

SEM study of the immunocaptured CD4 cells- on the electrode surface

The distribution of the antiCD3-MPs after the binding of the CD4 cells on the magneto electrode surface was evaluated by scanning electron microscopy (SEM). 500 cells μL^{-1} of $CD4^+$ T lymphocytes were captured with 8×10^5 antiCD3-MPs.

The CD4 cells were incubated with magnetic particles for 30 minutes while shaking at 4 °C followed by a washing step. The modified magnetic particles were then resuspended in 140 µL of PBS 0.5% BSA and captured by the m-GEC. The CD4 cells/antiCD3-MPs complexes captured on the magneto electrode surface were then fixed with fixation buffer (0.1 mol L^{-1} cacodylate, 1% glutaraldehyde, pH 7.4) for 1 hour at room temperature and rinsed in cacodylate buffer. Dehydration was carried out in ethanol solution from 50 to 95% for 5 min each at room temperature and finally twice at 100% ethanol for 10 min. The treated electrodes were coated with a thin layer of gold.

CD4 counting magneto biosensor in whole blood

The CD4 counting electrochemical magneto biosensor is based on the following four steps, as depicted in Figure 1: (A) Immunomagnetic separation of $CD4^+$ T cells and labeling. In this step, the antiCD3-MPs (8×10^5 MP per well) and the biotinylated anti-CD4 antibody (36 ng mL^{-1}) were simultaneously incubated with 100 µL of sample for 30 min with shaking at 4 °C, followed by three washing steps with PBS 0.1% BSA, (B) Incubation with the optical reporter streptavidin-HRP (30 µg mL^{-1}) for 30 min while shaking at RT, followed by two washing steps with PBS 0.1% BSA, (C) Magnetic actuation of the modified magnetic particles on the m-GEC electrode surface, and (D) Amperometric detection using the m-GEC electrodes polarised at –0.150 V (vs. Ag/AgCl), under enzyme saturation conditions with the substrate. The explanation of the amperometric detection using m-GEC electrodes as working electrodes, as well as the selection of the potential applied for the measurements is fully detailed in Supp. Data (Fig. S1 to S3). A steady-state current was obtained normally after 1 min upon the addition of hydroquinone (1.8×10^{-3} mol L^{-1}) and hydrogen peroxide (1.1×10^{-3} mol L^{-1}) in phosphate buffer (ePBS), as mediator and substrate for the enzyme HRP, respectively. This steady-state current was used for the electrochemical signal plotted in further results shown in Fig. 4.

Preferred position for Figure 1

After each incubation (steps A and B) or washing step, a magnetic separator was positioned under the tubes until pellet formation on the tube side wall, followed by supernatant separation. After the amperometric detection (step D), the surface of the m-GEC electrodes were renewed for further uses by the elimination of the modified magnetic particles with a simple polishing procedure, as detailed in Supp. Data.

Three different methods for the total depletion of lymphocyte from whole blood were tested with the CD4 counting magneto immunoassay, namely A) Ficoll-Paque depletion; B) IMS with Dynabeads® CD3; and finally, C) a combination of both (Ficoll-Paque/IMS), as detailed in Supp Data. The matrixes were evaluated by the CD4 counting magneto biosensor. By the

combination of Ficoll-Paque/IMS, a whole blood matrix almost clean from lymphocytes (quantified by flow cytometry in 4 cells μL^{-1}) was achieved. This lymphocyte depleted whole blood matrix was used in further studies for diluting the whole blood.

The calibration curve was prepared as follow: the exact concentration of the initial whole blood sample was firstly determined by flow cytometry. Serial dilutions of whole blood were performed using the optimized leukocyte-depleted whole blood matrix (Ficoll-Paque/IMS), to achieve the concentration range from 0 to 1200 cells μL^{-1}. The CD4 cell range was chosen to include the concentration of clinical interest for $CD4^+$ lymphocytes in AIDS diagnosis.

The recovery was determined by spiking low (200 cells μL^{-1}), medium (350 cells μL^{-1}), and high (500 cells μL^{-1}) concentration level of CD4 cells in the lymphocyte-depleted whole blood, covering all the whole clinical interest range.

The recovery was calculated as % R = 100. (P_1 / P_0), being P_0 the real number of cells spiked to the sample and P_1 the number of cells obtained by interpolating the signal in the standard curve.

Safety considerations

All the procedures involving the manipulation of human cells were handled using Biosafety Level 2 Laboratory (BSL-2) and containment. All works were performed in a Biosafety cabinet, and all material decontaminated by autoclaving or disinfected before discarding in accordance with U.S. Department of Health and Human Services guidelines for level 2 laboratory Biosafety (Chosewood and Wilson, 2009).

Results and discussion

Confocal microscopy study of the immunomagnetic separation and labeling

The distribution of CD4 and CD3 receptors on lymphocyte membrane was evaluated by confocal microscopy, as shown in Figure 2.

The typical dense nucleus of the lymphocytes is shown in blue due to Hoescht dye, a cell permeable DNA stain which is excited by ultraviolet light and emits blue fluorescence at 460 to 490 nm. The lymphocytes were immunocaptured by the antiCD3-MPs. As the particles are coated with polystyrene, they show green autofluorescence (Nurmukhametou et al., 2006). The CD4 receptors of the cells were labeled with the biotinylated antiCD4 antibody and Strep-Cy5 to achieve the fluorescence readout in red wavelength. Figure 2, panel A, shows the effectiveness of the selected system to achieve the CD4 counting, based on the magnetic

immunoseparation and the CD4 labeling. Single binding of CD4 cells to the antiCD3-MPs is shown in Figure 2, panel B. Figure 2, panel C shows the 3D binding pattern achieved by treating the confocal images with the Imaris X64 v.6.2.0 software. CD4 cells are captured by one or more magnetic particles. Some aggregates are also observed, due to multivalency of both antiCD3-MPs and CD4 cells.

Figure 2, panels D and F, shows single planes for the confocal images. The CD4 receptors (red dots) are distributed throughout the CD4 cell surface. The high expression of these receptors guarantees a high sensitivity and low limit of detection (LOD). Moreover, the binding of the CD4 receptor is not hindered by the antiCD3-MPs.

Preferred position for Figure 2

SEM study of the immunocaptured CD4 cells on the electrode surface

Scanning electron microscopy was used to evaluate the distribution on the magneto electrode surface of the magnetic particle bind to the CD4 cells after the magnetic actuation. 500 cells μL^{-1} of $CD4^+$ T lymphocytes were captured with 8×10^5 antiCD3-MPs.

The Figure 3, panel A shows, at a resolution of 20 μm, the distribution of the MPs on the electrode surface, following the pattern of magnetic fields. In some areas including the center, agglomerates of MPs are shown, while in others the bare surface of the electrode can be clearly identified. The bare areas of the m-GEC electrode allow the mediator to reach the surface, granting the electrochemical readout. Figure 2 also shows the SEM images at a resolution of 2 (panels B to D), and 1 μm (panels E and F). The pictures illustrate the T lymphocytes (7-15 μm of diameter) attached to the spherical magnetic particles (4.5 μm of diameter). Some aggregates were observed due to the binding of two or more different magnetic particles with many lymphocyte cells (panels B to D) as a result of multivalency. In most of the cases, the binding to the magnetic particles was achieved with more than one specific binding site on the lymphocyte, being the T lymphocytes surrounded by magnetic particles (panel E).

Preferred position for Figure 3

CD4 counting magneto biosensor in whole blood

The CD4 counting magneto biosensor in whole blood was firstly optimized by changing the amount of the electrochemical reporter (streptavidin-HRP conjugate) and the antiCD3-MPs and, as shown in Figure S6 and S7 (Supp data). Moreover, two different strategies were also performed by simplifying the labeling step into one to achieve analytical simplification, as schematically shown in Figure S8 (Supp. Data). Improved analytical performance was achieved by the simultaneous incubation of the CD4 cells with the antiCD3-MPs and the biotinylated antiCD4 antibody in one step, and doing in a consecutive step the incubation with the optical reporter (streptavidin-HRP). The results are presented in Figure S9. It seems that the binding of the biotinylated antiCD4 antibody to the CD4 cells is hindered by the electrochemical reporter, achieving thus better results when the incubation of the electrochemical reporter is performed in a separate step. A full discussion of the different procedures is presented in Supp. Info.

The electrochemical response of the CD4 counting magneto biosensor towards the CD4 cells (from 0 to 1150 cells μL^{-1}) in whole blood is shown in Figure 4, panel A. The CD4 cell range was chosen to include the concentration of clinical interest for $CD4^+$ lymphocytes in AIDS diagnosis. The CD4 counting magneto biosensor was fitted using a nonlinear regression (Four Parameter logistic Equation– GraphPad Prism Software) (R^2=0.9862), as shown in Figure 4, panel B. The LOD was estimated by processing the negative control samples (n=16) (optimized leukocyte-depleted whole blood matrix by Ficoll-Paque/IMS) obtaining a mean value of 0.191 μA with a standard deviation (SD) of 0.060. The cut-off value was then determined with a one-tailed t test at a 95% confidence level, giving a value of 0.297 μA (shown in Figure 4, as the dotted lines). The LOD was found to be 44 CD4 cells μL^{-1}, being the logistic range from 89 to 912 CD4 cells μL^{-1}, including the whole range of clinical interest of $CD4^+$ lymphocytes in AIDS diagnosis.

Preferred position for Figure 4

Spiked recovery was performed to assess the overall accuracy of the CD4 counting magneto immunoassay (Davies, 2001). This method incorporates variables in assay preparation as well as the regression analysis. In this case, the recovery was performed by spiking to the leukocyte-depleted whole blood, low (200 cells μL^{-1}), medium (350 cells μL^{-1}), and high (500 cells μL^{-1}) concentration of $CD4^+$ lymphocytes (quantified by flow cytometry) in the range of

clinical interest for AIDS diagnosis and follow up. Table 1 shows the spiked recovery values obtained for the CD4 cell concentrations, ranging from 89 to 109 %.

Preferred position for Table 1

The CD4 counting magneto biosensor in whole blood showed also an outstanding reproducibility and reusability, as shown in Figure S10 (Supp. Data). No matrix effect was observed when comparing phosphate buffer and whole blood as matrixes (Figure S11 in the Supp. Data), confirming thus that the immunomagnetic separation completely removed the red cells (Figure S12) as well as other interfering molecules, which are not able to bind the antiCD3-MPs. Although there are only few examples of emerging technologies for CD4 cell count (Carinelli et al., 2015), mostly of them based on cell counting by microscopy, the detection range was very narrow or only limited to few discrete values of CD4 cells (Cheng et al., 2007; Lutwana et al., 2008, Gohring and Fan, 2010, Moon et al., 2011; Thorslund et al., 2008), being thus these methods only useful for a specific stage of the disease. It is important to highlight the wider logistic range of detection for the CD4 counting magneto biosensor (from 89 to 912 CD4 cells μL^{-1}). This range ensures that this device can be used for the diagnosis of AIDS and the monitoring of the disease in any stage. For instance, 500 cells μL^{-1} is used not only for increasing the CD4 monitoring but also for initiating ART. Moreover, CD4 count thresholds are related to common HIV-associated-diseases, for instance pneumonia (<200 cells μL^{-1}), encephalitis (<100 cells μL^{-1}) or Mycobacterium (<50 cells μL^{-1}) coinfections, and should be taken into account for prophylaxis and treatment of associated-AIDS infection diseases (AIDSInfo, 2015).

Although good correlation with the gold standard flow citometry was obtained with some emerging technologies previously reported (Cheng et al., 2007; Whatkins et al., 2011), the CD4 counting magneto biosensor showed a lower LOD value (44 CD4 cells μL^{-1}), wider range of detection (from 89 to 912 CD4 cells μL^{-1}), higher specificity (because two receptors, CD4 and CD3, are used for the accurate identification of CD4 cells) and the advantage of being able to quantify CD4 cells. Moreover, the use of magnetic particles allows the detection in whole blood without any sample pretreatment. The analytical performance of other CD4 counting sensors platforms, in terms of i) Assay format, ii) Detection technique, iii) Test matrix, iv) Limit of detection (LOD), linear range are summarized in the Table S2 (Supp Data).

Conclusions

Recent guidelines published by WHO recommend that diagnostic devices for developing countries to be ASSURED being this acronym defined by (A) *A*ffordable, (SS) *S*ensitive, *S*pecific, (U) *U*ser-friendly, (R) *R*apid and *R*obust, (E) *E*quipment free, and (D) *D*eliverable to those who need it. Although there are many commercially available methods for point of care HIV diagnosis, there is still the need for novel affordable alternatives to flow cytometry for CD4 count in order to monitor the AIDS disease and the treatment in low resource settings. In order to meet these demands, a CD4 counting electrochemical magneto biosensor for whole blood, based on the magnetic separation of the CD4 lymphocytes is presented for the first time. The integration of the antiCD3 magnetic particles provides specificity to the biosensor. As not only CD4 T lymphocytes express CD4 receptor but also other immunological cells (for instance monocytes, macrophages, and dendritic cells), the immunomagnetic separation of the CD4 lymphocytes by the CD3 receptor avoids interferences of other expressing CD4 receptor cells. Moreover, the magnetic particles also enhance the performance of the immunological reaction. Due to the improved washing and separation steps, the matrix effect of whole blood was eliminated. Therefore, the analysis of samples performed on magnetic particles is easily achieved in whole blood without any purification or pretreatment steps such as lysis or centrifugation, which is normally necessary for the gold standard flow cytometry, being an analytical simplification.

As the CD4 count must be quantitative, associated instrumentation is required. The electrochemical readout can be achieved with instrumentation designed to be low maintenance, battery/solar energy operated, and low cost, to meet the demands for ASSURED diagnosis recommended by WHO.

The CD4 counting electrochemical biosensor shows a LOD as low as 44 cells μL^{-1}, being the logistic range from 89 to 912 CD4 cells μL^{-1}, including the whole range of clinical interest of $CD4^+$ lymphocytes in AIDS diagnosis and follow up.

The CD4 counting electrochemical biosensors presented in this work offers an exciting alternative, especially in countries with limited resources, as a rapid, cost-effective, analytical strategy that can be handled for unskilled personnel at the community and primary care level.

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Figure Captions

Figure 1. Schematic representation of the CD4 counting magneto biosensor (A) The $CD4^+$ T lymphocytes are captured from whole blood by the CD3-MPs (8×10^5 MPs per assay) and labeled in one step with antiCD4-biotin (36 ng mL^{-1}); (B) The incubation with the electrochemical reporter streptavidin-HRP (30 μg mL^{-1}) is then performed. (C) Finally, the electrochemical readout is achieved with H_2O_2 and hydroquinone as mediator.

Figure 2. Confocal microscopy images of the $CD4^+$ T lymphocyte (1×10^5) captured by antiCD3-MPs (8×10^5 magnetic particles) and labeled with biotinylated antiCD4 antibody/Strep-Cy5 system (36 ng mL^{-1} and 2 μg mL^{-1}). The nuclei of the CD4 cells were stained with the dye Hoechst in blue. Streptavidin conjugated to the fluorescent dye cyanine was used to visualize the biotinylated antiCD4 antibody attached to the CD4 receptor, as red dots. Magnetic particles are shown in green due to autofluorescent.

Figure 3. Scanning electron microphotographs of CD4 cells/antiCD3-MPs captured on the surface of m-GEC electrodes at different resolution levels. (A) 20 μm; (B) to (D), 2 μm; (E) and (F), 1 μm. In all cases, identical acceleration voltage (15 kV) was used.

Figure 4. Calibration curve for detection of CD4 cells with the CD4 counting magneto biosensor in whole blood. The reagent concentrations were: 8×10^5 antiCD3-MPs, 36 ng mL^{-1} biotinylated antiCD4 antibody, 30 μg mL^{-1} streptavidin-HRP conjugate. In all cases, n=3, except for the negative control (n=16).

Table 1. Recovery study for the CD4 counting magneto biosensor.

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Figure 1

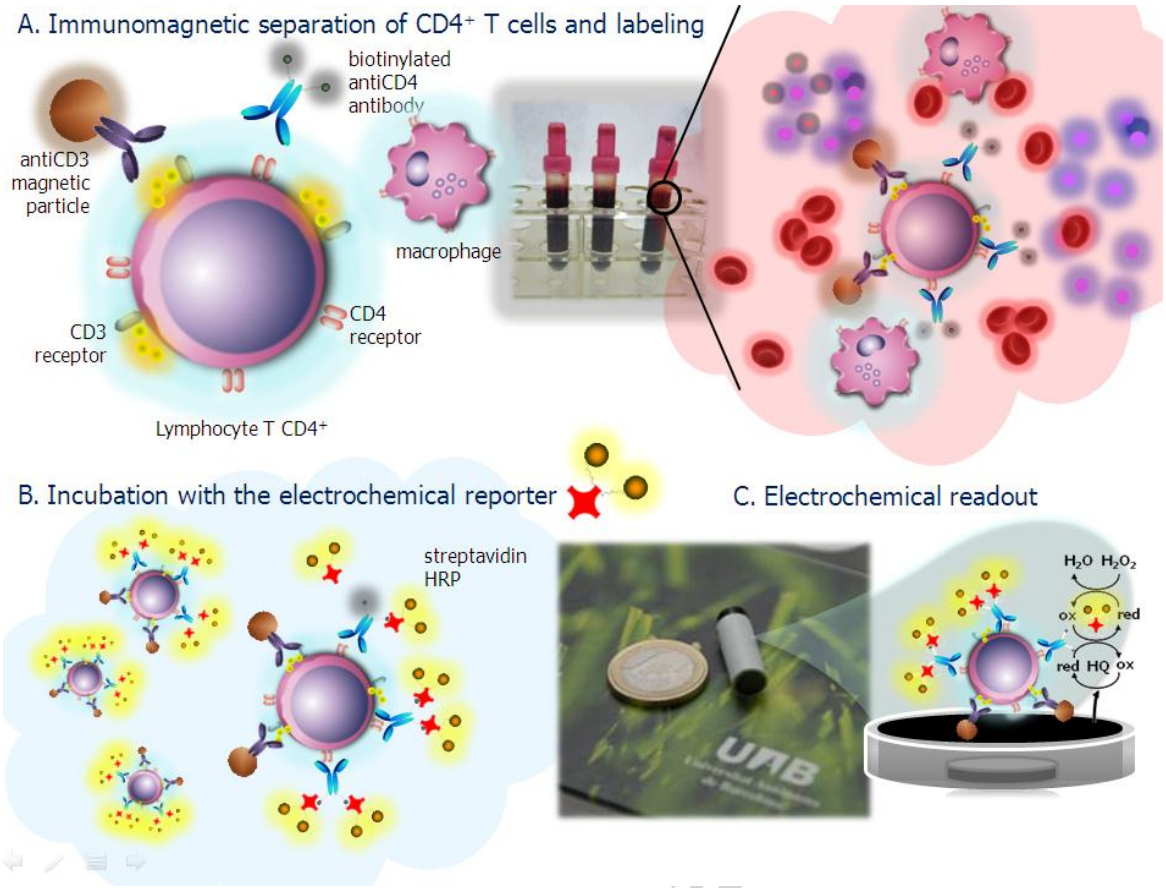

fig 1

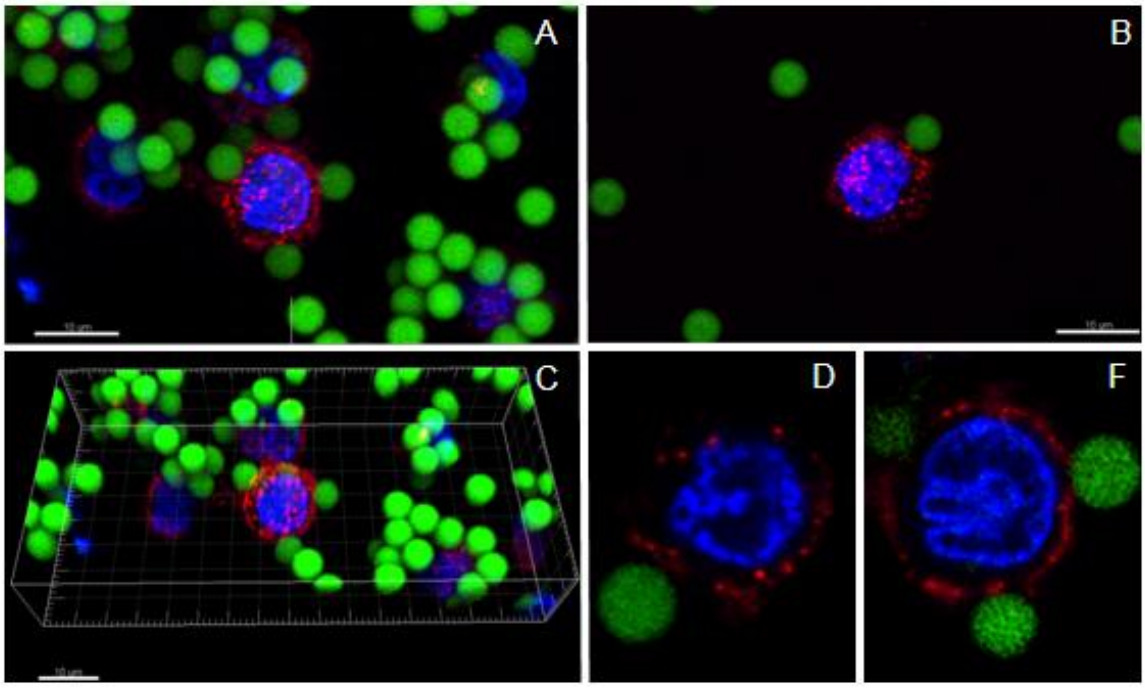

fig 2

Figure 3

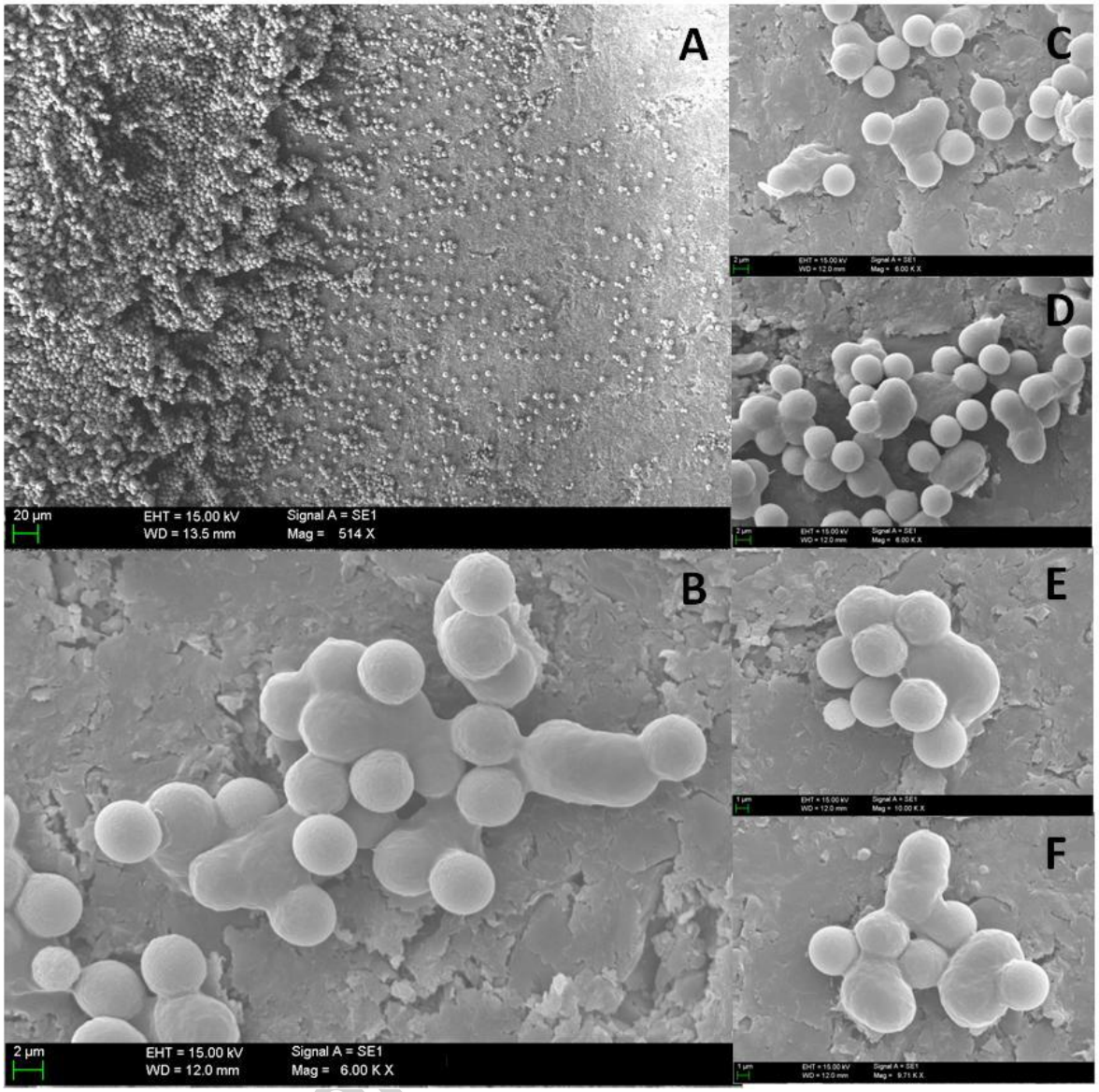

fig 3

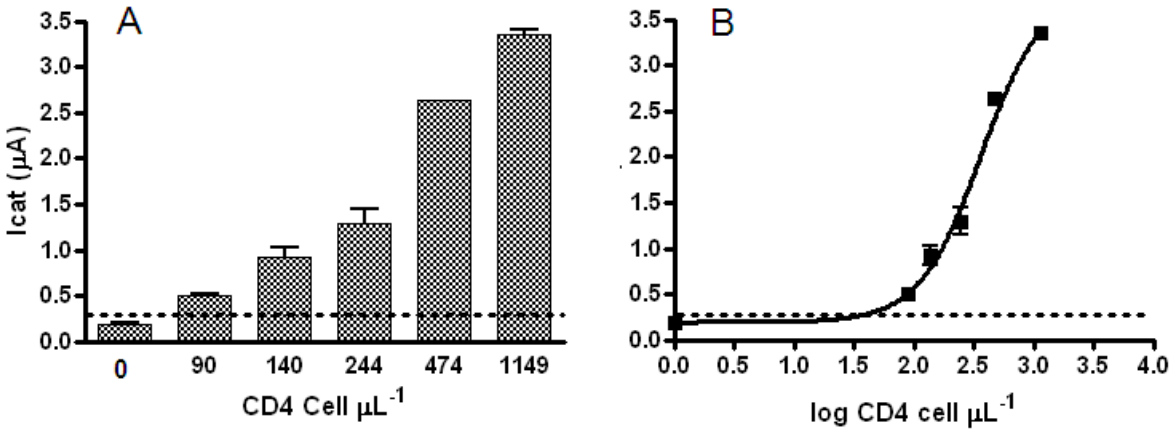

fig 4

Table 1

Actual concentration (cell μL^{-1}) by flow citometry	Recovered concentration (cell μL^{-1}) / (SD)	% Recovery
500	457 / (57)	**91**
350	311 / (41)	**89**
200	218 / (10)	**109**

Highlights

- There is an urgent need for novel affordable alternatives to flow cytometry for CD4 count in order to monitor the AIDS disease and the treatment in low resource settings.
- A magneto actuated electrochemical biosensor for CD4+ T lymphocytes count in whole blood is presented, with a LOD of 44 cells μL^{-1} and a logistic range including the whole range of clinical interest of CD4+ lymphocytes in AIDS diagnosis and follow up.
- The CD4 cells are preconcentrated from whole blood by immunomagnetic separation based on the CD3 receptor and using magnetic particles modified with an antiCD3 antibody. The captured cells

are labeled with a biotinylated antiCD4 antibody, followed by the reaction with an electrochemical reporter for further electrochemical biosensing.

- The electrochemical magneto biosensors offer an exciting alternative, especially in resource-scarce settings, as rapid, cost-effective devices that can be handled for unskilled personnel at the community and primary care level.