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Research paper

Antibody detection employing sol–gel immobilized parasites

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ABSTRACT

Immunofluorescence assay (IFA) and immunoperoxidase assay (IPA) are useful diagnostic techniques for specific antibody detection for different diseases. Both involve several alternatives for immobilization of cells, such as solvent or heat fixation. Non-covalent immobilization implies rigorous storage conditions at −20 °C to preserve the slides, and usually numerous cells are detached during the washing steps, which can lead to inconsistencies in the results. Sol–gel chemistry is usually used for coating different materials because of the mild conditions of the polymerization reaction and the ability to introduce functional groups to a wide variety of surfaces. We have developed a novel procedure for the attachment of *Trypanosoma cruzi* epimastigotes and *Leishmania guyanensis* promastigotes to a silicon oxide polymer covered glass surface. The film was prepared using standard microscope slides with tetraethoxysilane and 3-aminopropyl triethoxysilane as polymeric precursors. When acetone was used as the major coating solvent, the IFA showed the fluorescence of the attached parasites without matrix background interference. Similar results were observed when the IPA was evaluated. The sensitivity and specificity of the sol–gel immobilized parasite slides were comparable with the heat fixation technique. The performance of the coated slides was maintained for at least 2 months at 4 °C storage temperature. This immobilization method does not affect the molecular epitopes of the attached cells. Thus, homogeneous, ready to use, long lasting coated slides were obtained, which are appropriate for field conditions.

1. Introduction

Early detection of infectious diseases is of great importance for the implementation of efficient treatment. Although direct detection of pathogens is the definitive diagnosis, this may involve laborious and problematic experimental procedures. Particularly, direct parasitological tests such as hemoculture or xenodiagnosis for detection of *Trypanosoma cruzi* have proven to be highly specific, but are time consuming and the sensitivity of these techniques is very low (Rey, 1991; Chiari, 1999; Marcon et al., 2002). Thus, detection of antibodies elicited in response to infectious agents is usually used for the diagnosis of a broad range of diseases, applying immunodiagnostics in a useful, essential tool. Several indirect techniques are employed for antibody detection, including enzyme immunoassay (EIA), radiobinding immunoassay (RIA), immunofluorescence assay (IFA) and immunoperoxidase assay (IPA). In order to detect serum antibodies against pathogens, the different techniques employ immobilization of cells (i.e. bacteria or parasites) or antigens to different supports. Then the patient's serum is incubated in contact with fixed antigens and specific antibodies are recognized with species specific conjugated antibodies (Winkler et al., 1995; Messmer et al., 2001; Frank et al., 2003; Maple et al., 2004; Atik et al., 2006; Ankelo et al., 2007; Fernandes et al., 2007). Indirect diagnosis may be difficult to implement in areas endemic for parasitic diseases, where the infrastructure is usually inadequate and shipment or transport of serum samples to diagnostic centers may be difficult and costly. These facts emphasize the need to develop techniques that can be employed in such areas with minimal equipment and limited storage conditions.

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IFA and IPA involve different methods of immobilization of the cells on slides such as heat or solvent fixation (Czar et al., 1996; Yang et al., 1998; Villegas et al., 2002). These immobilization techniques have several drawbacks. Once the cells are fixed to the support they must be stored at −20 °C in order to diminish or avoid antigen deterioration. Another disadvantage is that cells usually detach from the support during storage and washing steps (Berthelot et al., 1995).

Sol–gel chemistry allows the coating of different materials because of the mild conditions of the polymerization reaction, which include room temperature operation and polymerization at physiological compatible pH (Iler, 1979). This had allowed the entrapment of proteins, bacteria and parasites and specific antigen recognition by the corresponding antibodies (Livage et al., 1996; Lan et al., 2000; Du et al., 2003; Zhong and Liu, 2004; Lee et al., 2005). In recent years, medicinal and bioanalytical applications of sol–gel technology have received extensive coverage (Coradin et al., 2006; Kandimalla et al., 2006). Presently, sol–gel chemistry combined with coating methods is used to obtain thin films (Brinker and Scherer, 1990). Chemical and mechanical stability is characteristic of the glass material surface coating when silicon oxide precursors are employed. The method generally includes an alkoxide precursor, usually tetraethoxysilane (TEOS), which is partially hydrolyzed in acid or basic medium by addition of a controlled amount of water in order to form the sol. When functional groups are required, an organically modified silicate (ORMOSIL) precursor containing the desired group can be used. The resulting doped sol–gel matrix is allowed to gel and polycondensation takes place.

The aim of this work was to generate a versatile method for covalent attachment of cells for the detection of antibodies present in the serum of infected patients, which can be used in the field under unfavorable infrastructure conditions. For this purpose, we have successfully immobilized *T. cruzi* epimastigotes and *Leishmania guyanensis* promastigotes on a silicon oxide polymer covered surface. This has been shown to be useful for the detection of specific serum antibodies and presents the advantage of homogenous distribution and maintenance of its properties over months of storage at 4 °C without losing immobilized cells.

2. Materials and methods

2.1. Chemicals

Tetraethoxysilane (TEOS) was purchased from Fluka (Buchs, Switzerland). 3-Aminopropyltriethoxysilane (APTES) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Glutaraldehyde was from Mallinckrodt Baker (Phillipsburg, NJ, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse and anti-human polyvalent immunoglobulins and peroxidase-conjugated anti-mouse and anti-human immunoglobulins, all developed in goat, were from Sigma (St Louis, MO, USA). All other reagents were of analytical grade.

2.2. Parasites

T. cruzi epimastigotes (Tulahuen, RA strain), and *L. guyanensis* promastigotes were grown in biphasic and liquid medium, as previously described (Chiari and Camargo, 1984; Jaffe et al., 1984). Parasites were harvested during the exponential growth phase by centrifugation at 5000 ×*g* for 15 min and washed 3 times with 0.1 M phosphate buffered saline solution (PBS, pH 7.2). The collected parasites were processed to obtain formalin-fixed epimastigotes or promastigotes as described previously (Zwirner et al., 1992, 1994).

Bloodstream trypomastigotes of *T. cruzi*, used in antisera generation, were isolated from acute infected BALB/c mice at the peak of parasitemia, as previously described (Andrews and Colli, 1982). Blood was centrifuged for 10 min at 100 ×*g*; after 1 h at 37 °C plasma was isolated and centrifuged for 10 min at 590 ×*g*. The parasite pellet was washed twice and resuspended in M-199 medium.

2.3. Sera from infected mice and humans

BALB/c mice were infected intraperitoneally with 50–100 bloodstream trypomastigotes of *T. cruzi*. Blood samples were collected 3–6 months after infection and stored at 4 °C. Sera were subsequently separated by centrifugation and aliquots were stored at −70 °C. The antibody levels in the sera were monitored by ELISA. Anti-*L. guyanensis* mice serum and human serum infected with *T. cruzi* or *L. guyanensis* were kindly provided by S.I. Cazorla and F.M. Frank.

All experiments using animals were carried out following the guidelines of the National Research Council (CONICET) of Argentina.

2.4. Sol–gel film slides

The film was made on standard microscope slides (75 mm × 25 mm) previously conditioned by sonication (30 min, 35 kHz), first in acetone and then in ethanol. The slides were then air dried.

A TEOS sol was prepared by sonicating (Transonic 540 sonicator, 35 kHz) a mixture of 1 ml of TEOS, 0.06 ml of 0.05 M HCl and 0.2 ml of water for 30 min at 20 °C. The optimum coating solvent mixture was evaluated by making a 20-fold dilution of the TEOS sol with several water/acetone percentages. One milliliter of a solution containing APTES (0.05 ml) in acetone (0.95 ml) was added to 9 ml of the diluted TEOS sol. The APTES content used was the maximum that did not interfere with the polymerization. One microliter was deposited over the slides in order to generate a circular spot.

Polymerization took place at 25 °C and then air dried. The slides were then immersed sequentially in 0.5 M potassium phosphate buffer (pH 7.5) solutions containing: (a) 0.25% glutaraldehyde; (b) 10^6 parasites ml^{-1}; and (c) blocking solution (1 mg ml^{-1} skim milk). The slides were incubated for 10 min with each solution and washed with 0.5 M potassium phosphate buffer (pH 7.5) between different incubation steps. Finally, the slides were air dried. Films were stored without the addition of any antimicrobial agent at 4 °C until used. Storing time ranged from 1 day to 2 months.

2.5. Infrared spectrum

ATR–FTIR transmission spectra were acquired in the range of 4000–650 cm^{-1}, using a Fourier transform infrared spectrometer (FT-IR) with flat-plate attenuated total reflectance (ATR) (Perkin Elmer, Spectrum One IR). All slides were