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Development of a colloidal gold nanoparticle-immunochromatographic strip for the detection of human insulin

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ABSTRACT

The monitoring of insulin is of great relevance for the management of diabetes, the detection of pancreatic islet-cell malfunction, the definition of hypoglycemia, and the diagnosis of insulinoma.

Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are the two conventional methods for determining insulin. However, these methods needed long procedure, specific machine for determination. The objective of this study is to develop an immunochromatographic strip for insulin using anti-insulin polyclonal antibodies (pAbs). This self-assembled immunochromatographic strip was convenient, rapid, low price and no machine needed in clinical diagnosis. The colloidal gold was made from the chloroauric acid ($HAuCl_4$) and labeled anti-insulin pAbs with 25 nm of colloidal gold. This insulin immunochromatographic strip prepared could determine for 0.01-100 μg/mL of insulin and no cross-reaction. Moreover, this strip could be applied to determine mouse serum samples, and the detection time was approximately 3-5 min.

INTRODUCTION

Diabetes is among the most prevalent and costly diseases in the world, affecting 16 million people in the United States and over 100 million people worldwide. Diabetes is caused by the insufficient release of insulin or less of insulin action at target tissues, which results in aberrant glucose and lipid metabolism. Diabetes is brought on by either too little insulin in the body (type 1 diabetes) or by the body's not responding to the effects of insulin (type 2 diabetes).

It is know that glucose is the primary regulator of insulin secretion, which has complex kinetics. The regulator of such dynamics is unclear; however, the understanding of this mechanism is very important for revealing the derangement that occurs in diabetes. For better management of diabetes, rapid detection of pancreatic islet-cell malfunction, a clear definition of hypoglycemia, precise diagnosis of insulinoma, and early prediction of trauma, there is a need to develop diagnostic devices for monitoring of insulin and glucose, or, hence, of the insulin/glucose ratio. Much attention has been given to the development of electrochemical biosensors for the continuous monitoring of glucose. However, the development of insulin sensing

needs more exploration.

The aim of this study was to study on the development immunochromatographic strip for insulin. This self-assembled immunochromatographic strip will be used for home care, food poisoning and point-of-care in sample diagnosis. The advantages of this self-assembled immunochromatographic strip was no pretreatment, low sample requirement, easy operation, rapid determination, low price, no cross-reaction, long-term preservation, no machine needed etc.

MATERIALS AND METHODS

Materials

Human insulin from recombinants (expressed in yeast) obtained from Sigma Co. St. Louis, U.S.A. Anti-insulin pAbs produced in quinea pig, whole antiserum obtained from Sigma Co. St. Louis, U.S.A..

Test Principle

The test principle of the newly developed immunochromatographic test (Fig.1) is based on the gold-labeled immunochromatography technology. Both anti-insulin pAbs as test line and goat anti-mouse pAbs as control line are immobilized on a nitrocellulose membrane. After colloidal gold nanoparticle-antibody conjugate and sample solution were mixed, the mixture was applied on the sample pad and moved forward on the membrane by capillary action. When insulin negative samples were tested, no color appeared on the test line and the control line. On the other hand, for insulin positive samples, complex of gold nanoparticle-antibody conjugate passed through the test line and reached to the control line. Since the complex was captured by the anti-mouse immunoglobulin, visible blue color appeared on the test line. Thus, the color intensity of the test line was proportional to the concentration of insulin in the sample. The reaction color could be read by naked eyes.

Preparation Process of Immunochromatographic Strip

Sample pad and nitrocellulose membrane were immersed in 10% CH_3OH solution for 30 min, and dried. Sample pad, absorbent pad and nitrocellulose membrane were fixed on plastic backing. Test line and control line were loaded on nitrocellulose membrane, and dried. The strip was blocked in casein buffer for 60 min, and dried.

Colloidal Gold Preparation:

0.5 mL of 1% $HAuCl_4$ was added into 50 mL deionized H_2O, and heating to boiling. Then, 1% tri-sodium citrate was added, heated to boiling, and refluxed 30 min. Cooling down, then adjusted to pH 7.0 by 0.2 M K_2CO_3.

Preparation of colloidal gold nanoparticles-insulin pAb conjugates :

The insulin pAbs was conjugated with colloidal gold microparticles by the

following method. In brief, 10 μg of the purified insulin pAb in colloidal gold microparticles (25nm diameter) to 1000 μL, and incubated for 5 min in order to conjugate the immunoglobulin with the colloidal gold microparticles. After centrifugation at 10,000 ×g for 15 min at room temperature, the colloidal gold nanoparticles-insulin pAbs conjugates were collected in the pellet form. The supernatant was removed, and the pellet was suspended in 100 μL casein buffer. Finally, The colloidal gold nanoparticles insulin pAb conjugates suspension was dispensed onto the conjugate pad.

RESULTS AND DISCUSSION

Fig.2.shows the optimum amount of the anti-insulin pAbs conjugated with the colloidal gold nanoparticles.the insulin pAbs. The results demonstrated 1mL of colloidal gold nanoparticle (diameter: 25 nm) added 10 μg/mL anti-insulin pAb was optimum.

Fig.3.shows the self-assembled insulin immunochromatographic strip could be determined to 0.01~100 μg/ mL insulin. Insulin positive samples, complex of gold nanoparticle-antibody conjugate passed through the test line and reached to the control line. Since the complex was captured by the anti-mouse immunoglobulin, visible blue color appears on the test line. Because anti-insulin pAbs produced in guinea pig and goat anti-mouse pAbs could not combine, no color appeared on the control line.

Fig.4.shows the self-assembled insulin immunochromatographic strip had no cross reaction with C-reactive protein and interferon- α . No color appeared on the test line and the control line.

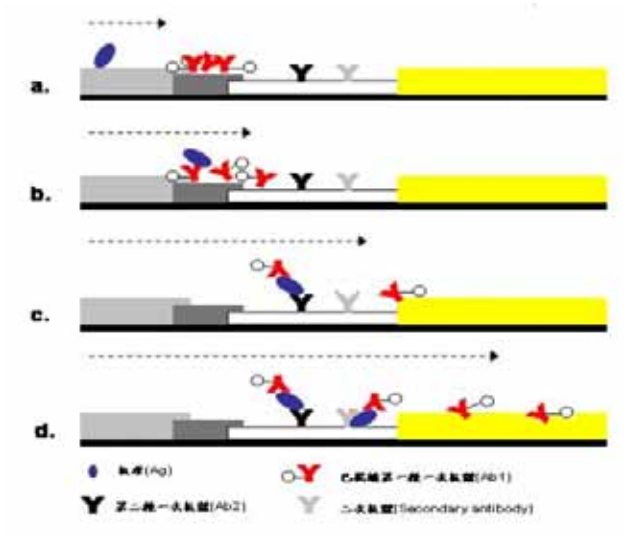

Fig.1.The test principle of immunochromatographic strip.

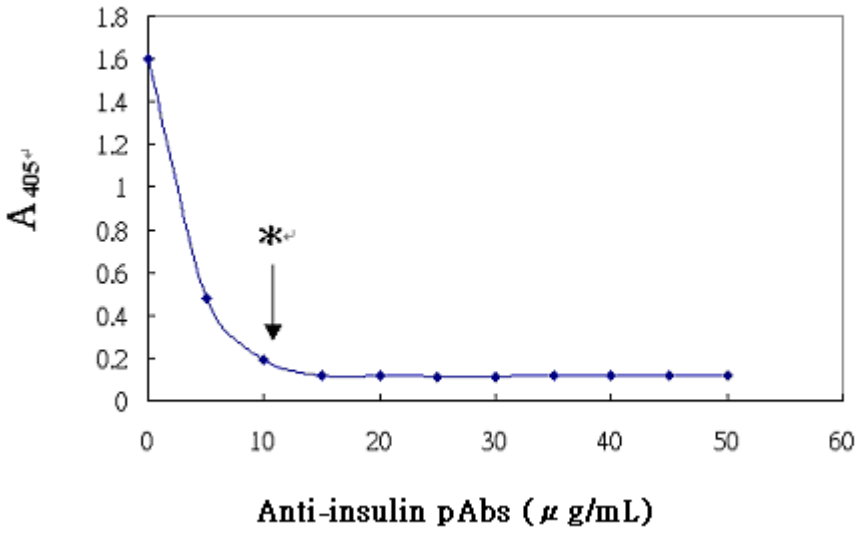

Fig.2. The optimum amount of the anti-insulin pAbs conjugated with the colloidal gold nanoparticles.

※ Colloidal gold：25 nm (diameter) /1mL

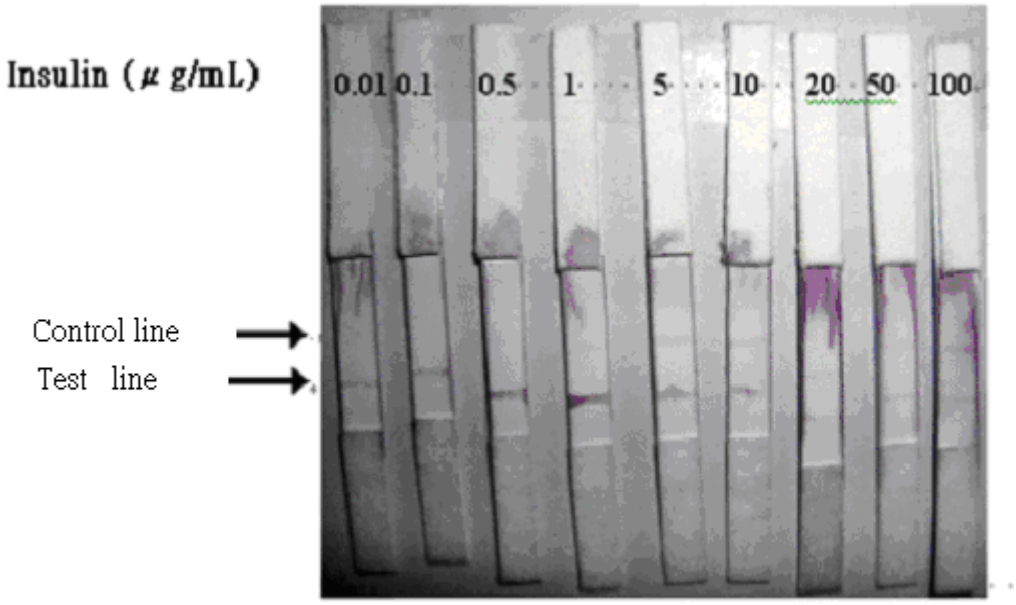

Fig.3. Determination of insulin with different concentrations in PBS using the self-assembled insulin immunochromatographic strip

(a) C-reactive protein

(b) Interferon-α

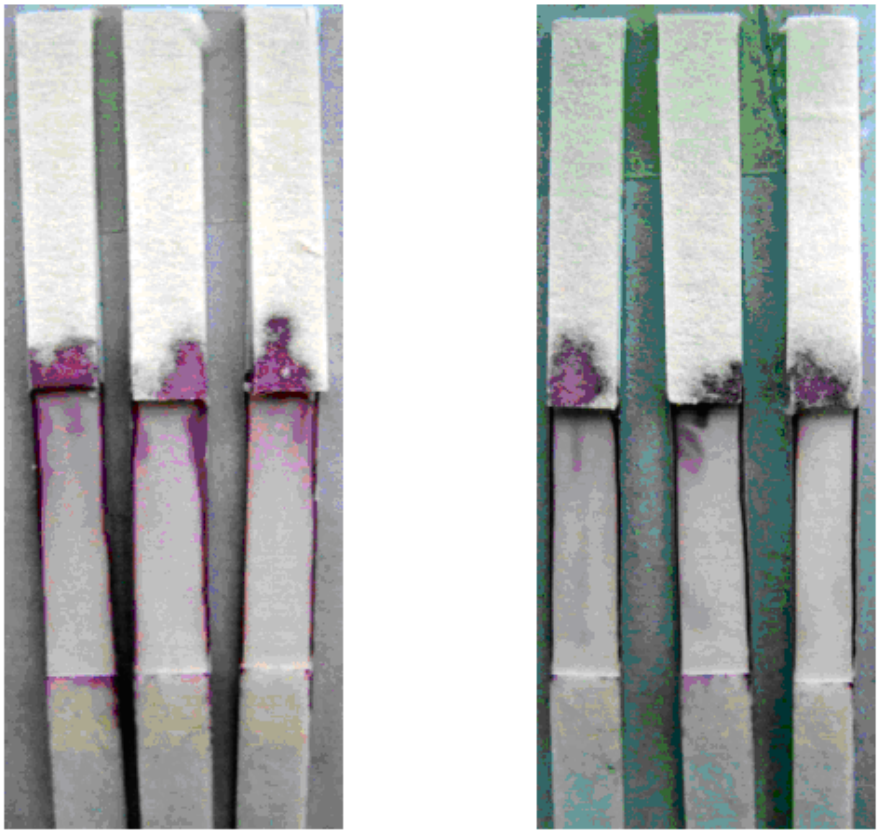

Fig.4. The cross reaction for C-reactive protein and interferon-α using the self-assembled insulin immunochromatographic strip

(a) 10 μg/mL of C-reactive protein

(b) 10 μg/mL of interferon-α

Each assay was performed with triple replicates.