A24 ABO Blood Group Antigens and Mucin-Like Proteins in Human Buccal Cells

Kabre'Shiya S. Austin, Oluseyi A. Vanderpuye, PhD*, and Dwayne Goolsby, BS*, Albany State University, Forensic Science, 504 College Drive, Hartnett Building, Room 118, Albany, GA 31705*

After attending this presentation, attendees will gain an understanding of previously undescribed polymorphisms in buccal cell proteins, their expression of ABO blood group antigens and their comparison to soluble salivary fluid proteins. Knowledge of salivary and buccal cell proteins may translate into applications for forensic testing in pathology and drug abuse.

This presentation will impact the forensic science community by showing how salivary components such as soluble proteins and buccal cells can be analyzed by fluorescence microscopy, microtiter plate assays, and lectin staining of electrophoresis gel transfers, and by informing attendants that saliva represents an easily accessible body fluid and is often present at crime scenes.

Buccal cells are a major component of saliva and are a major source of DNA for forensic and biomedical analyses. Little is known; however, about the protein components of these cells but such information could be used to identify biomarkers for forensic testing and for pathological conditions such as exposure to toxic chemicals and chronic drug abuse.

The characterization of buccal cell and cell-free salivary fluid proteins, their variations among individuals, and their reactivity with Ulex europaeus-I lectin was studied by different techniques. Buccal cells

were isolated from whole saliva and washed twice by centrifugation and resuspension in fresh buffer. The identification of blood group O-bearing glycoproteins was conducted by separating, buccal cell proteins by SDS gel electrophoresis and electrotransferred to nitrocellulose. These gel replicas were incubated with biotinylated UEA-I lectin which recognizes blood group O. The proteins bound by UEA-I were revealed by binding of streptavidin conjugated to alkaline phosphatase and a colorimetric substrate. The same approach was used to identify proteins bound by other lectins such as those from peanut agglutinin, Artocarpuis integrifolia and Vicia Villosa which recognize galactos and N-acetyl galactosamine terminated saccharides on glycoproteins.

In order to test for the presence of blood group O structures on intact buccal cells, buccal cells were examined by fluorescence microscopy after incubation with fluorescent UEA-I (UEA-I FITC) or biotinylated UEA-I followed by Alexa conjugated streptavidin.

The relative amounts of blood group O antigens on buccal cells and in cell free saliva from different donors were measured by binding of UEA-I to samples immobilized on microtiter plates.

After biotin-UEA-1 staining of nitrocellulose transfers of Laemmli SDS electrophoresis gels of 48 salivary fluid samples from different individuals, 42 had staining of the stacking gel. A 150 kDA band was present in 20 samples, a 75-100 kDA was found in 22 samples and six samples had UEA-1 staining in the region 40-50 kDa. Six out of nine buccal cell samples had UEA-I binding in the stacking gel of nitrocellulose transfers and differed in staining of 130kDa to 200 kDa proteins.

Five of eight salivary fluids and three of four buccal cell samples coated onto microtiter plates bound biotin-UEA-1 and the amount of binding varied among individuals for the same number of cells. By fluorescence microscopy, five out of seven buccal cell preparations from donors with different ABO blood groups bound UEA-1 and the staining intensity varied among individuals.

A number of novel findings were made in this study: (1) a high molecular mass glycoprotein with blood group O antigensthat bound UEA-I lectin was identified in human buccal cells; (2) buccal cells from different individuals varied in the amount of UEA-I lectin they could bind and in the molecular masses of UEA-I binding proteins; (3) the high molecular mass UEA-I binding glycoprotein only poorly bound to lectins such as PNA, JCA and VVL that characteristically recognize O-linked oligosaccharides; and (4) A lower molecular mass 120kDa-200kDA buccal cell protein did not bind UEA-I lectin but did bind to PNA, JCA and VVL lectins.

Saliva, Buccal Cells, Glycoproteins

 ** Presenting Author*